้ลักษณะสมบัติของยีสต์ดำ Aureobasidium spp. ซึ่งคัดแยกจากบริเวณซายฝั่งทะเลไทย



นางสาวเบญจวรรณ ยันต์วิเศษภักดี



บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์ตั้งแต่ปีการศึกษา 2554 ที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR) เป็นแฟ้มข้อมูลของนิสิตเจ้าของวิทยานิพนธ์ ที่ส่งผ่านทางบัณฑิตวิทยาลัย

The abstract and full text of theses from the academic year 2011 in Chulalongkorn University Intellectual Repository (CUIR) are the thesis authors' files submitted through the University Graduate School.

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต สาขาวิชาพฤกษศาสตร์ ภาควิชาพฤกษศาสตร์ คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปีการศึกษา 2557 ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

CHARACTERIZATION OF BLACK YEAST *Aureobasidium* spp. ISOLATED FROM THAI COASTAL AREA

Miss Benjawan Yanwisetpakdee



จุฬาลงกรณ์มหาวิทยาลัย Chulalongkorn University

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy Program in Botany Department of Botany Faculty of Science Chulalongkorn University Academic Year 2014 Copyright of Chulalongkorn University

Thesis Title	CHARACTERIZAT	ΓΙΟΝ	OF	BLA	ACK	YEAST
	Aureobasidium	spp.	ISOLAT	ED	FROM	THAI
	COASTAL AREA					
Ву	Miss Benjawan Ya	anwiset	oakdee			
Field of Study	Botany					
Thesis Advisor	Associate Profess	sor Huns	sa Punna	apaya	ak, Ph.D.	
Thesis Co-Advisor	Assistant Professo	or Pong	tharin Lo	otraku	l, Ph.D.	

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

Dean of the Faculty of Science

(Professor Supot Hannongbua, Dr.rer.nat.)

THESIS COMMITTEE

Chairman

(Assistant Professor Tosak Seelanan, Ph.D.)
Thesis Advisor

(Associate Professor Hunsa Punnapayak, Ph.D.)

_____Thesis Co-Advisor

(Assistant Professor Pongtharin Lotrakul, Ph.D.)

Examiner

(Professor Sirirat Rengpipat, Ph.D.)

Examiner

(Assistant Professor Sehanat Prasongsuk, Ph.D.)

Examiner

(Teerada Wangsomboondee, Ph.D.)

_____External Examiner

(Kamonchai Cha-aim, Ph.D.)



จุฬาลงกรณ์มหาวิทยาลัย Chulalongkorn University เบญจวรรณ ยันต์วิเศษภักดี : ลักษณะสมบัติของยีสต์ดำ *Aureobasidium* spp. ซึ่งคัดแยกจาก บริเวณซายฝั่งทะเลไทย (CHARACTERIZATION OF BLACK YEAST *Aureobasidium* spp. ISOLATED FROM THAI COASTAL AREA) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: หรรษา ปุณณะพยัคฆ์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: พงศ์ธาริน โล่ห์ตระกูล, 4 หน้า.

Aureobasidium spp. เป็นจุลินทรีย์ที่มีคุณสมบัติทนทานต่อภาวะรุนแรง มีความสำคัญที่ใช้ใน เชิงเทคโนโลยีชีวภาพ ราในกลุ่มนี้ผลิตผลิตภัณฑ์ที่มีมูลค่า ได้แก่ พอลิแซ็กคาไรด์; EPS (พูลลูแลนและเบต้า กลแคน) เอนไซม์ไซแลนเนส และสารต้านเชื้อรา ราในกลุ่มนี้มีความหลากหลายในสายพันธ์ และถกจัดจำแนก เพิ่มเติมออกมาอีก 4 สปีชีส์ ได้แก่ A. pullulans, A. melanogenum, A. namibiae และ A. subglaciale ใน การศึกษานี้ได้คัดแยกรา Aureobasidium spp.จากบริเวณชายฝั่งทะเล จากจำนวน 66 สายพันธุ์ที่ใช้ใน การศึกษา ประกอบด้วยสายพันธุ์ใหม่ 54 สายพันธุ์ และ 12 สายพันธุ์เปรียบเทียบ สายพันธุ์ใหม่ถูกจัดแยก ประเภทโดยอาศัยการวิเคราะห์ลำดับเบสหลายตำแหน่ง จำนวนทั้งสิ้น 3 ตำแหน่ง ได้แก่ ITS *TUB* และ *ELO* บน พื้นฐานของการวิเคราะห์ระบบพันธุ์ สายพันธุ์เหล่านี้ถูกจำแนกออกเป็น 12 กลุ่ม แสดงให้ถึงความหลากหลาย ของราสายพันธุ์ที่คัดแยกจากบริเวณซายฝั่ง อย่างไรก็ตาม พบราเพียง 2 ชนิด โดยพบ A. melanogenum มาก ที่สุดและพบ A. thailandense เพียงเล็กน้อย และพบสายพันธุ์ color-variant ที่มีความจำเพาะกับแหล่งอาศัยใน เขตร้อนหรือกึ่งเขตร้อน โดยราสายพันธุ์นี้ถูกจำแนกอยู่ในกลุ่มเดียวกับ A. melanogenum ดังนั้นลักษณะทาง สัณฐานวิทยา การผลิต EPS และแอคติวิตีของเอนไซม์ไซแลนเนสจึงถูกนำมาประเมินเพื่อบ่งชี้ลักษณะพิเศษของ สายพันธ์ในแต่ละกลุ่มเพื่อเปรียบเทียบกับสายพันธ์ที่คัดแยกได้จากบนบก ผลการศึกษาแสดงความแตกต่างของ สีอาหารเลี้ยงเชื้อ ชนิด EPS และราบางกลุ่มมีการผลิต EPS และ/หรือแอคติวิตีของเอนไซม์ไซแลนเนสในระดับ สูง นอกจากนี้ ฤทธิ์ต้านเชื้อรา และความเครียดจากสิ่งเร้า ได้แก่ การทนเค็ม การทนแรงดันออสโมติก การทน ความร้อน และการทนความเป็นกรดด่าง ถูกนำมาใช้ทดสอบเพื่อค้นหาสายพันธุ์ที่เป็นประโยชน์ หรือ ทนทานต่อ ภาวะรุนแรงเพื่อนำไปประยุกต์ใช้ในเชิงเทคโนโลยีชีวภาพ ผลการศึกษาแสดงให้เห็นว่าความสามารถดังกล่าว ขึ้นกับราแต่ละสายพันธุ์ เพื่อศึกษาความสัมพันธ์ระหว่างการทนเค็ม การทนแรงดันออสโมติก กับการผลิต EPS ้จึงคัดเลือกราจำนวน 3 สายพันธุ์มาศึกษา พบว่ารา A. melanogenum ที่ทนเค็มจะทนแรงดันออสโมติกได้ อย่าง มีนัยสำคัญ แต่ความสัมพันธ์ดังกล่าวไม่เกี่ยวข้องกับการผลิต EPS นอกจากนี้ เพื่อศึกษาศักยภาพของเอนไซม์ไซ แลนเนสในเชิงเทคโนโลยีชีวภาพ จึงคัดเลือกราเพื่อผลิตเอนไซม์ไซแลนเนสและนำไปผลิตไซโลโอลิโกแซ็กคาไรด์ (XOS) โดยสกัดไซแลนจากฐปฤาษี (*Typha angustifolia* L.) เพื่อใช้เป็นแหล่งคาร์บอน พบว่าผลิตภัณฑ์หลักที่ ้ได้จากการย่อยด้วยเอนไซม์ไซแลนเนสเป็นไซโลไบโอสที่มีไซโลสปะปน โดย XOS ที่ผลิตได้มีฤทธิ์ต้านอนุมูล อิสระเมื่อทดสอบด้วยวิธี 2,2-diphenyl-1-picrylhydrazyl (DPPH)

ภาควิชา	พฤกษศาสตร์	ลายมือชื่อนิสิต
สาขาวิชา	พฤกษศาสตร์	ลายมือชื่อ อ.ที่ปรึกษาหลัก
ปีการศึกษา	2557	ลายมือชื่อ อ.ที่ปรึกษาร่วม

5273825023 : MAJOR BOTANY

KEYWORDS: AUREOBASIDIUM / COASTAL / POLYSACCHARIDE / XYLANASE

BENJAWAN YANWISETPAKDEE: CHARACTERIZATION OF BLACK YEAST *Aureobasidium* spp. ISOLATED FROM THAI COASTAL AREA. ADVISOR: ASSOC. PROF. HUNSA PUNNAPAYAK, Ph.D., CO-ADVISOR: ASST. PROF. PONGTHARIN LOTRAKUL, Ph.D., 4 pp.

Aureobasidium spp. is polyextremotolerant microorganism of considerable biotechnological importance that thrives in a broad range of habitats worldwide. This fungus produces valuable products including exopolysaccharides; EPS (pullulan and β -glucan), xylanase, and antifungal agents. Recently, four varieties were separated into four species including A. pullulans, A. melanogenum, A. namibiae and A. subglaciale. A number of Aureobasidium spp. from coastal area was isolated. Among 66 isolates used in this study include 54 new isolates and 12 comparative strains. All new isolates were classified using multi locus sequence analysis from three loci including the rRNA ITS region, TUB, and ELO. Based on the phylogenetic analysis, they were classified into 12 clades, suggesting a vast diversity within the coastal area. However, only two species were found in this study and the dominant species in coastal area was found to be A. melanogenum whereas a few A. thailandense was also found. The color-variant strains that specific and found in only tropical or subtropical zone were obtained and they were located in A. melanogenum clade. Consequently, morphological characteristics, EPS production, and xylanase activity were determined for all isolates in an attempt to identify specific characteristics of each clade, and to compare with terrestrial isolates. The results exhibited different colors on different culture media, type of EPS, and some clades showed high levels of EPS production and/or xylanase activity. Moreover, antifungal activity and multiple abiotic stresses including halotolerance, osmotolerance, thermotolerance, and tolerance against various pH were observed in attempt to discover the useful isolates or extremotolerant for applying in biotechnology. The results showed their ability were strain dependence. To study associations among halotolerance, osmotolerance and EPS production, three strains with different tolerance and EPS production were selected. The results showed halotolerance in A. melanogenum was significantly associated with osmotolerance, but not vice versa. Halo- and/or osmotolerant strains produced low to moderate EPS yield. Moreover, to study the potential application of xylanase in biotechnology, a representative strain was selected and xylanase was produced for xylooligosaccharides (XOS) production. Xylan from Typha angustifolia L. was extracted and used as sole carbon source. The main hydrolysis products yield were xylobiose and small amount of xylose. XOS obtained in this study exhibited antioxidant activity when 2,2diphenyl-1-picrylhydrazyl (DPPH) assay was used for evaluation.

Department:	Botany	Student's Signature
Field of Study:	Botany	Advisor's Signature
Tield of Olddy.	Dotany	
Academic Year:	2014	Co-Advisor's Signature

ACKNOWLEDGEMENTS

I wish to express my deepest gratitude and sincerest appreciation to my advisor, Associate Professor Hunsa Punnapayak, for his excellent supervision, expert advice and guidance, and constant encouragement throughout my graduate course.

I wish to extend my deep and sincere appreciation and gratefulness to my co-advisor, Assistant Professor Pongtharin Lotrakul for his valuable recommendations and useful suggestions for the manuscript and full encouragement throughout the period of my study.

I would like to express my sincere gratitude to Assistant Professor Sehanat Prasongsuk and Assistant Professor Tosak Seelanant for their comments, useful suggestions for the manuscript and serving on the thesis committee.

I am indebted to Professor Sirirat Rengpipat, Dr.Teerada Wangsomboondee, and Dr.Kamonchai Cha-aim for their kind suggestions and serving on the thesis committee.

Special thanks also go to the Botany Department's faculty members for the encouragement, kind recommendations and friendship.

Sincere thanks go to all my friends for their kindness, generosity and friendship.

The Feeling of thankful appreciation belongs to my colleagues at the Plant Biomass Utilization Research Unit at the Department of Botany for their support.

Finally, I would like to express my sincere gratitude to my parents and my family for bringing me up with love, great care, encouragement and support. Without their love and advice I could never have completed my study.

CONTENTS

	Page
THAI ABSTRACT	iv
ENGLISH ABSTRACT	V
ACKNOWLEDGEMENTS	vi
CONTENTS	vii
REFERENCES	2
VITA	4



จุฬาลงกรณ์มหาวิทยาลัย Chulalongkorn University

CHAPTER I

1.1 Rationale

Aureobasidium pullulans (de Bary) G. Arnuad is ubiquitous yeast-like fungus classified in Ascomycetes by Cooke (1959). It is called black yeast due to the accumulation of black to olivaceous melanin pigmentation during growth. Its distinctive polymorphic forms are yeast-like cell, hyphae, pseudohyphae, swollen cells, and chlamydospore, depending on age, strain, and environmental conditions which has complicated its identification (de Hoog and Yurlova, 1994). It is cosmopolitan lives in a wide range of habitats both in temperate and tropical zone. In Thailand, *A. pullulans* from a wide range of terrestrial habitats were isolated and their physiological characters and phylogenetic relationships were studied (Lotrakul *et al.*, 2013; Manitchotpisit *et al.*, 2009; Prasongsuk *et al.*, 2005; Punnapayak *et al.*, 2003).

A. *pullulans* is industrially important due to it produces many valuable products. The most well-known product produces from *A. pullulans* is exopolysaccharide (EPS) called pullulan. This biopolymer is unique with many useful applications in biotechnology. Pullulan powders are white, non-hygroscopic and dissolve easily both in hot and cold water. It is colorless, tasteless, non-toxic, edible, and biodegradable (Leathers, 2003). Consequently, it used in many applications including food, pharmaceutical, agricultural, and chemical (Singh and Saini, 2008). Furthermore, the new applications related to human health resulting in its demand in commercial is increasing (Cheng *et al.*, 2011). In addition, *A. pullulans* produces a different EPS structure called aubasidan, a β -glucan which has α -1,4-D-, β -1,6-D and β -1,3-D-glucosidic bonds. β -glucan is known to be an immune activation system, enhancement of growth activation in probiotic bacteria, and is used in anti-cancer drugs or health-promoting foods (Lotrakul *et al.*, 2013).

It has been reported that different strains of *A. pullulans* isolated from different environments can produce hydrolytic enzymes including amylase, proteinase, lipase, cellulase, xylanase, mannanase, and transferases, etc. One of the most widely studied enzymes from *A. pullulans* is xylanase. Xylanases are hydrolyzed enzymes that degrade xylan, the second most abundant polysaccharide in plant cell wall. Xylanases have many applications in pulp and paper, fermentation and food industries, as well as in waste water treatment (Chi et al., 2009b). In particular, xylanase produced from color variant strains that have been isolated only in tropical or subtropical regions, were reported for secreting high levels of xylanase (Leathers, 1986). The color variant strains produce brilliant pigments of pink, yellow, and purple instead of typical black or olivaceous melanin. Furthermore, these strains also produce pullulan in relatively higher amount than that of the typical pigmented strains (Leathers et al., 1988). A. pullulans has been considered as an effective biocontrol due to its strong antagonistic activity against other microorganisms. A. pullulans is used for the production of antifungal agent, aureobasidin that exhibited antifungal activities against Candida albicans, Saccharomyces cereviseae and some Aspergillus spp. Recently, the tropical A. pullulans strains were isolated and studied for their antifungal agent production (Lotrakul et al., 2009; Prasongsuk et al., 2013). In addition, some strains of A. pullulans can produce antibacteria compound including exophilin A and liamocins (Price et al., 2013).

In recent years, *A. pullulans* has been recognized as a polyextremotolerant species that tolerate for several unfavorable environment conditions including elevated temperatures, low water content, oxidative stress, and others (Gostincar *et al.*, 2011). It can survive in hypersaline, acidic, basic, cold and oligotrophic conditions because of several physiological and molecular adaptations (Kogej *et al.*, 2005; Selbmann *et al.*, 2008). Based on the isolates of *A. pullulans* exhibit polymorphic forms, multilocus DNA sequences were used for identification and classification. In species level, the rDNA ITS (internal transcribed spacer) region is one of the most widely used, while analysis of more variable DNA locus was used for subspecific differentiation. As a result, the infraspecies relationship of *A. pullulans*, var. *melanogenum*, var. *namibiae* and var. *subglaciale* (Zalar *et al.*, 2008). The phylogenetic analysis of 45 tropical *A. pullulans* from Thailand was also showed high variation that at least 12 different clades were

obtained (Manitchotpisit *et al.*, 2009). Besides the result from that study leads to discover of *A. thailandense*, a new species was described from culture of material collected in Thailand (Peterson *et al.*, 2013). This suggested that *A. pullulans* strains from the tropical areas have a vast diversity within the species. Moreover in 2014, the four varieties were separated into four species: *A. pullulans* and the newly assigned *A. melanogenum*, *A. namibiae* and *A. subglaciale* based on genome comparison (Gostincar *et al.*, 2014).

From an ecological point of view, Thailand is an apparent source of genetically diverse of *A. pullulans* that is ubiquitous and widely distribute in several terrestrial habitats (Lotrakul *et al.*, 2009; Manitchotpisit *et al.*, 2009; Prasongsuk *et al.*, 2005; Punnapayak *et al.*, 2003). It has been reported that, marine fungi exhibit many interesting characters that might be the results of physiological adaptations toward the unique physicochemical environments of the sea (Gunde-Cimerman *et al.*, 2009; Torzilli, 1997). Additionally fungi from salt habitats have been proven to be of biotechnological significance. Especially, halophilic microorganisms possess many hydrolytic enzymes and are capable of functioning under conditions (Chi *et al.*, 2009a) a. Furthermore *A. pullulans* was promising to be an extremotolerant species that was propose for its ability in many industrial applications (Chi *et al.*, 2009b; Gostincar *et al.*, 2011; Wu *et al.*, 2012). However, only terrestrial strains of *A. pullulans* in Thailand were collected and studied while *A. pullulans* also found in hypersaline and coastal habitats (Gunde-Cimerman *et al.*, 2000). Consequently, it is interesting to investigate the differences between *A. pullulans* living in terrestrial and salt-stress environs such as coastal area.

The range of these studies is as follows. A number of tropical *Aureobasidium* spp. from Thai coastal area were isolated and characterized. The diversity and phylogenetic relationship among *Aureobasidium* spp. isolates were classified based on morphological and physiological characters together with DNA sequences using the rRNA ITS region, *TUB*, and *ELO*. Production of EPS, xylanase, and antifungal substance by each isolate were investigated. EPS was characterized and analysis with FT-IR and enzyme sensitivity. Additionally, xylanase was measured both quality and quantity method

with congo-red plate assay and enzyme activity determination. In vitro-antifungal activity of each isolate was considered using plate assay challenged with some Aspergilli. Furthermore, all isolates were studied for their tolerance against multiple stresses including halotolerance, osmotolerance, thermotolerance and tolerance against different pH. Moreover, the representative strains were selected to study their potential application in biotechnology.

1.2 Objectives of this study

- 1. To isolate Aureobasidium spp. from various coastal area in Thailand.
- 2. To characterize Aureobasidium spp. from coastal area focusing on morphological and physiological characters, DNA sequence comparisons and tolerance against multiple stresses.
- 3. To study the phylogenetic relationships among Aureobasidium spp. isolated from coastal area in Thailand and their terrestrial counterparts.
- 4. To explore the potential application of xylanase produced by Aureobasidium spp. from coastal area in biotechnology.

1.3 Key words

1.4

Aureobasidium spp., coastal, exopolysaccharide, xylanase

Anticipated benefits

- 1. Biodiversity and phylogenetic relationships among Aureobasidium spp. strains from coastal area and their terrestrial counterparts in Thailand will be classified.
- 2. Aureobasidium spp. strains with potential in diverse industrial applications will be obtained.

CHAPTER II

LITERATURE REVIEW

2.1 Aureobasidium spp.

2.1.1 Taxonomy

The genus *Aureobasidium* is ubiquitous yeast-like fungus, commonly known as black yeast. It is a member of Dothideales that comprises of 27 taxa. The most recently described species of *Aureobasidium* are performed by (Li *et al.*, 2015). It has been divided into three species, *A. pullulans* (de Bary) G. Arnuad, *A. leucospermi* Crous and *A. proteae* (Joanne E. Taylor & Crous) Joanne E. Taylor & Crous. The newest species in this genus is *A. thailandense* S.W. Peterson, Manitchotpisit & Leathers that was isolated from wood surfaces in Thailand (Peterson *et al.*, 2013).

A complex species, *A. pullulans* was described firstly as *Dematium pullulans* by de Bary in 1866 (Cooke, 1959). *A. pullulans* was redefied and suggested it has four varieties (Zalar *et al.*, 2008). *A. pullulans* var. *pullulans* was exhibited its characters by pinkish cultures and rapidly expanding. It can develop the dark brown sectors on its colony due to the presence of melanized hyphae and tolerate salt stress up to 17% NaCl (w/v). *A. pullulans* var. *melanogenum* was recognized by melanin production referred to cultures colony that become black or dark olivaceous-green, the conidia forming. It is oligotrophic, occurs in the watery habitats including marine water and can grow at 37°C while the other three species can only grow to 35°C. *A. pullulans* var. *namibiae* was isolated from marble in Namibia that showed the specifically structure of leathery hyphae of the colonies. Finally, *A. pullulans* var. *subglaciale* was isolated from metabolize under extreme conditions in Arctic glaciers.

2.1.2 Morphology

The polymorphic nature of *A. pullulans* has been recognized, and it varies depending on environmental conditions. *A. pullulans* grow easily on potato and malt glucose agar that obtained a colony diameter of 35-45 mm in range, within 7 days at room temperature. Colonies color is creamy or pale pink at first, then usually becoming black throughout except the margin. Young colonies are flat, smooth and slimy. Mature colonies develop to velvety texture and dark brown or black with grayish fringe. Colonies sometimes is irregularly developing in radial or sectors at marginal areas (Cooke, 1959).

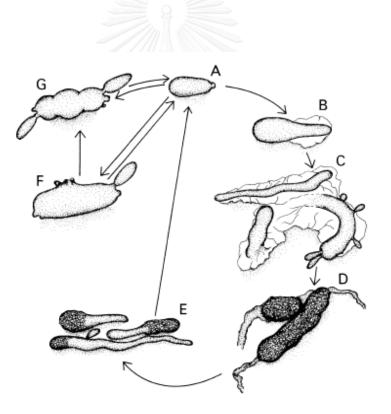


Figure 2.1 Diagram of polymorphic forms of *A. pullulans*. The development stages are as follow (A) blastospore, (B) germinating blastospore, (C) initial hyphae, (D) chlamydospores, (E) germinating chlamydospores, (F) swollen cell, (G) septate swollen cell (Pechak and Crang, 1977).

It is initially yeast-like cell or blastospore in culture, later developing a mycelium with budding conidia, and producing chlamydospores in the late stage (Figure 2.1). In addition, there are reports that one cell type could develop into another, suggesting by chlamydospores can develop from swollen cells or septate swollen cells by changing to a very thick wall and accumulating melanin on its surface (Pechak and Crang, 1977).

2.1.3 Classification

A. pullulans was classified in fungal kingdom as filamentous ascomycetes. Its affinity with relatives to the order Dothideales, family Dothideaceae based on the characters of conidiogenesis, growth expansion, and nutrients assimilation profile combined with 18s ribosomal sequencing data also confirms that it is an anamorph of a member of the Dothidealaes (de Hoog and Yurlova, 1994; Yurlova et al., 1996). Yurlova and de Hoog (1997) represented a new variety, A. pullulans var. aubasidani Yurlova as the strain producing aubasidan; glucans with α -1,4-D-, β -1,6-Dand β -1,3-D-glycosidic bonds. It had been found to differ in genotypic and physiological characters due to its structurally unique polysaccharide. Besides, A. pullulans var. aubasidani also distinguishes from A. pullulans var. pullulans by the absence of assimilation of methyl- α -D-glucoside and lactose. A. pullulans identity is nearly to a member of ascomycete by using data from ITS (Internal transcribed spacer) sequences that were able to distinguish species among fungi in the order Dothidealaes by using ITS1, 5.8S and ITS2 rDNA sequences de Hoog et al. (1999). Prasongsuk et al. (2005) also used ITS sequences to differentiate tropical A. pullulans and found that the similar results were obtained. Additionally, the infraspecies classification of A. pullulans were redefined by Zalar et al. (2008) and 2 new varieties were represented; A. pullulans var. namibiae and A. pullulans var. subglaciale, respectively. Besides, A. pullulans var. aubasidani which had been previously described was synonymised with A. pullulans var. pullulans. Various loci including ITS rDNA, intergenic spacer 1 (IGS), translation elongation factor-1 α , β -tubulin, and RNA polymerase II have been used to infer the taxonomy and phylogeny of the taxa in 45 tropical strains of *A. pullulans* (Manitchotpisit *et al.*, 2009).

In recent year, the order *Dothideales* accommodates only single family of *Dothideaceae*. Based on both morphology and phylogenetic analysis of LSU, SSU and ITS gene regions, a new family *Aureobasidiaceae* was proposed (Thambugala *et al.*, 2014). Besides, *Dothioraceae* is not recognized as a distinct family and is synonymized under *Dothideaceae*. Moreover, genome sequencing of the four varieties of *A. pullulans* was studied. The 25.43-29.62 Mb genomes of the four varieties of *A. pullulans* encode between 10266 and 11866 predicted proteins. The differences between them are large enough to justify their redefinition. Based on genome comparison, the four varieties were separated into four species including *A. pullulans* and the newly assigned *A. melanogenum*, *A. namibiae* and *A. subglaciale* (Gostincar *et al.*, 2014).

Refer to this data, *A. pullulans* and its varieties should now classified according to the following schedule:

Division Ascomycota

Class Dothidiomycetes

Order Dothideales,

Family Aureobasidiaceae

Genus Aureobasidium Species Aureobasidium pullulans Aureobasidium melanogenum Aureobasidium namibiae Aureobasidium subglaciale

(de Hoog and Yurlova, 1994; Gostincar *et al.*, 2014; Thambugala *et al.*, 2014; Yurlova *et al.*, 1996; Zalar *et al.*, 2008)

2.1.4 Habitat

A. pullulans widely distributes in diverse habitats. It is cosmopolitan that common in temperate zones however it has been isolated in other areas ranging from the Arctic to Africa (Deshpande et al., 1992). A. pullulans has been suggested that the widespread distribution of the fungus is contributed by the differences in its genetic and phenotypic forms (Leathers, 2003). It is often report as a plant pathogen due to its found often in phyllosphere and aerial parts of plant, as an epiphyte or endophyte. Moreover, A. pullulans was also found in soil, wood, fresh water, fruit, leather, plastics, surfaces, and indoor environments. Recently, more A. pullulans isolates have been found in coastal and hypersaline habitats including mangrove sediments, sea water and sea sediments (Wu et al., 2010). These A. pullulans isolates from salt-water environs also exhibited different morphological and physiological characteristics, compared to those of the terrestrial isolates (Torzilli et al., 1985; Torzilli, 1997; Urzì et al., 1999). Recently, A. pullulans has also been proposed to be a polyextremotolerant species that can resist the unfavorable physicochemical parameters such as elevated temperatures, low water content, oxidative stress, and others. Some of habitats are particular unusual including glacial ice, frozen, polluted water, salt-preserved and dried food (Gostincar et al., 2014; Kogej et al., 2005). It has been found to cause of disease in humans, and infections were reported even in systemic infections.

During the past decade, a number of tropical *A. pullulans* have been isolated from various habitats in Thailand as airborne spores (Punnapayak *et al.*, 2003), plant leaves, painted wall (Prasongsuk *et al.*, 2005), and bathroom surfaces (Lotrakul *et al.*, 2009). In the most recent study, 45 terrestrial isolates of *A. pullulans* from Thailand were isolated from various habitats in Thailand (Manitchotpisit *et al.*, 2009). Some of these isolates showed the characteristics of the so-called color variant strains suggested previously that specific to tropical or subtropical habitats. Such color variant strains produced brilliant pigments of red, yellow, orange, or purple instead of the off-white to black color of the typically pigmented strains (Wickerham and Kurtzman, 1975). It was

classified into 12 clades by using multilocus phylogenetic analyses suggesting a vast diverse in genetic background.

2.2 Bioproducts

A. pullulans has been reported as of significant industrial yeast due to its capability of producing exopolysaccharide (EPS) called pullulan which is commercially exploited in industrial and biotechnological applications. Additionally, the biological potential of *A. pullulans* is also found in the production of hydrolytic enzymes, antimicrobial, poly (β -L-malic acid), and siderophores (Chi *et al.*, 2009b). Consequently different strains of *A. pullulans* have many uses in different fields.

2.2.1 Exopolysaccharides

2.2.1.1 Pullulan

A. pullulans is of biotechnological importance and has been widely studied for potential industrial applications that most well-known for its pullulan. Pullulan was first reported by Bauer in 1938 and named by Bender *et al.* in 1959 (Leather, 2003). It is a neutral, water-soluble biopolymer that synthesized as cellsurface attached material. It is linear α -D-glucan link of maltotriose units connected with α -1,6-D-glycosidic and α -1,4-D-glycosidic bonds (Figure 2.2). This unique linkage pattern of pullulan leads to its structural flexibility, adhesive ability and solubility polymer (Leather, 2003). This EPS is colorless, tasteless, non-toxic, edible, and biodegradable. Consequently, pullulan is of industrial importance that used in many applications. Furthermore, due to the new applications related to human health resulting in its demand in commercial is increasing (Cheng *et al.*, 2011).

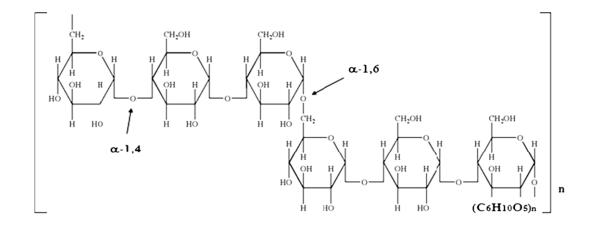


Figure 2.2 Structure of pullulan (Cheng et al., 2011).

The mechanism of pullulan biosynthesis is still little understood. As a result, present studies of pullulan concerning the basically findings of a pullulanproducing strains, pullulan synthesis, and genetic regulations, and also pullulan production and applications (Leathers, 2003; Singh and Saini, 2008). Pullulan is intracellular synthesized and secreted as extracellularpolymer mixed in the media. One of undesirable problem occurred with the production of pullulan included the dark pigment appears in the medium that resulting in high cost associated with pullulan recovery process, and the inhibitory effects caused by high sugar concentration in culture broth (Youssef *et al.*, 1999).

HULALONGKORN UNIVERSITY

Due to more advantages for economy process, saving the solvent during recovery step of pullulan yield are concerned, increasing of pullulan concentration in production process by utilizing the high concentration of sugar are important. Consequently, the efficiency of the strains is determined by their ability to utilize sugar substances, sugar tolerance capacity, and pullulan production capacity of the strains. Pullulan production can be limited by high sugar concentration used as the carbon source in the culture broth (above 5 % (w/v)) (Cheng *et al.*, 2011). It is more economic for pullulan production if a high concentration of sugar can be used since it would reduce the volume of solvent used during recovery. Recently, an osmotolerant strain of *A. pullulans* was studied for pullulan production from sucrose and yielded

pullulan at 60.7 gl⁻¹ from 100 g sucrose (Cheng *et al.*, 2011). Similarly, pullulan production by another osmotolerant *A. pullulans* RBF-4A3 isolated from a nectarous flower yielded 66.79 gl⁻¹ of pullulan from 150 g glucose (Choudhury *et al.*, 2011).

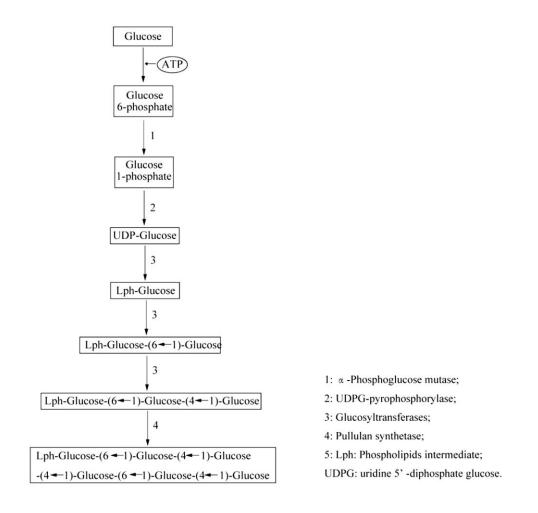


Figure 2.3 The proposed pathway of pullulan biosynthesis from glucose (Li et al., 2015).

The attempt to investigation on biochemical mechanisms of pullulan synthesis have been studies so far, it is relatively little understood. In 1982, Catley and McDowell have proposed the order of the biochemical events preceding pullulan formation (Chi *et al.*, 2009a). They reported that the size of UDP-glucose pool and glucosyltransferase activity in the cells of *A. pullulans* Y68 may be correlated with high pullulan production. The result from that studied was clearly found that more pullulan is produced and the cells have higher activities of α -phosphoglucose mutase, UDPGpyrophosphorylase, and

glycosyltransferase when grown *A. pullulans* in glucose containing medium. Consequently, based on the result that they obtained, a pathway of pullulan synthesis in *A. pullulans* Y68 was proposed (Figure 2.3). If the pullulan biosynthesis and regulation in *A. pullulans* are elucidated, it will be very easy to enhance pullulan yield using molecular methods (Li *et al.*, 2015).

2.2.1.2 Beta-glucan

A. pullulans produced another type of EPS called aubasidan, a group of glucans with α -1,4-D-, β -1,6-D- and β -1,3-D-glycosidic bonds. Based on the structure of this EPS, nutrition assimilation profile, and some molecular evidences, *A. pullulans* var. *aubasidani* was proposed to be a new variety (Yurlova and de Hoog, 1997). However, the results from multilocus phylogenetic analysis demonstrated the type strain of *A. pullulans* var. *aubasidani* within the same clade as *A. pullulans* var. *pullulans* var. *aubasidani* within the same clade as *A. pullulans* var. *pullulans* var.

In general, β-glucan is the most widely distributed polysaccharides in the cell walls of fungi. The synthesis of β -glucan in *A. pullulans* was stimulated by laminaribiose and sodium nitrate is suitable for production of aubasidan (Yurlova and de Hoog, 1997). It has been confirmed by Lotrakul et al. (2009), the nutrient assimilation profile of two strains of A. pullulans (NRRL58539 and NRRL 58543) exhibited that NRRL58539 and NRRL 58543 preferred sodium nitrate as the sole nitrogen source than the others. The preparation of purified β -glucan by pullulanase is difficult and has high cost. Therefore, a mutant strain produce pure β -glucan was creating. The pullulan synthetase gene (pul) of A. pullulans IMS822KCTC11179BP was disrupted and A. pullulans NP1221 was constructed. The β -glucan yield of mutant NP1221 was 2.3 fold (9.2 g l^{-1}) greater than that of wild-type (Kang *et al.*, 2010). However, it has not been completely known about its synthesis pathway and secretion system. It might be followed the pullulan biosynthesis (Li et al., 2015). It has been reported about biological activities from β -glucan derived from yeasts and mushrooms.

It was used to enhance the mammal immune system, to lower blood cholesterol levels (Kang *et al.*, 2010), and growth of probiotic bacteria (Lotrakul *et al.*, 2009). Furthermore, the anti-tumor, anti-infectious disease and anti-allergic activities of the β -(1-3), (1-6)-D-glucan produced by *A. pllulans* have been reported (Muramatsu *et al.*, 2012).

2.2.2 Hydrolytic enzymes

Different strains of *A. pullulans* can produce different enzymes. It produces protease, amylase, lipase, cellulase, xylanase, mannanase, and transferases, which have been reported for their potential applications in biotechnology. As a result, *A. pullulans* has become important industrial yeast.

<u>Xylanase</u>

One of the most studied enzymes from *A. pullulans* is xylanase. Xylan is a complex polysaccharide comprising a backbone of xylose residues linked by β -1,4glycosidic bonds. It is a major abundant polysaccharide in plant cell wall and an important renewable resource in the world. The chemical composition and structure of xylan backbone are various, depending on its source including wood, grass, and algae. Hardwood xylan is composed of 0-acetyl-4-0-methylglucuronoxylan while softwood is arabino-4-0-methylglucuroxylan. Xylan from marine algae, on the other hand is linked by β -1,3 or β -1,3, 1,4-glycosidic bonds (Dhiman *et al.*, 2008). In recent years, xylan has increased the interest of researchers for their applications in food industries.

The enzymatic degradation of xylan to xylose requires the catalysis of both endoxylanase (EC 3.2.1.8) and β -xylosidase (EC 3.2.1.37). Endoxylanase hydrolyzed main chain of xylan that linked by glycosidic bonds and released small unit of oligosaccharides. On the other hand, β -xylosidase removes single unsubstituted xylose moieties from the non-reducing ends of xylooligosaccharides (Chi *et al.*, 2009b). The properties of a cell-associated β -xylosidase from the strain ATCC 20524 differed from the extracellular enzyme previously reported. It showed an apparent M_r of 88.5 kDa and β -xylosidase activity was optimal at pH 3.5 and 70 °C (Ohta *et al.*, 2010).

The typical strain of *A. pullulans* was found to produce xylanase constitutively, and the color variants strain Y-2311-1 express relatively high levels of activity. Xylanase activity from this strain was induced by D-xylose, xylobiose, xylan, and arabinose, in contrast it was repressed by glucose (Leathers, 1986). Furthermore, two xylanases showed the similar molecular masses with 20 and 21 kD. The highly actives enzyme, APX-I and APX-II produced by *A. pullulans* were purified and characterized, the result suggested that both of them are encoded by the same gene (Li *et al.*, 1993).

A. pullulans produces hemicellulolytic enzymes with predominant xylanase and β -xylosidase activity and no cellulase activity when grown on xylose. Based on this ability it was used in pulp and paper industry. Eucalyptus pulp was treated by xylanase produced from *A. pullulans* that contains less xylan (48%) and glucomannan (15%) than the untreated reference of dissolving pulp (Christov and Prior, 1996).

Moreover, glycoside hydrolase (GH) family-10 and -11xylanases from *A. pullulans* var. *melanigenum* strain ATCC 20524 was purified and cloned the respective encoding genes. It exhibited acidophilic character that optimal at pH 2.0 and 50°C. In addition, phylogenetic tree showed that xylanases from this fungus are closely related with those enzymes from *Aspergillus* and *Penicillium* (Ohta *et al.*, 2001).

Manitchotpisit *et al.* (2009) have been reported using multilocus, pullulan production and xylanase activity as characters to analyze the relationship of tropical isolates of *A. pullulans* in Thailand. Most isolates were collected from leaves and the data exhibited that isolates within each clade shared many similarities include xylanase activity profile. The color variant strains were also observed and the high level of xylanase activity was reported. However xylanase production in the *A. pullulans* var. *pullulans* has not been studied.

2.2.3 Antifungal agent

Many strains of *A. pullulans* are used as biocontrol agents, especially in post-harvest diseases of fruits and vegetables. Two strains of *A. pullulans* (SL250 and SL36 has been found to control *Penicillium digitatum* on grapefruit, *Botrytis cinerea*, *Rhizopus stolonifer*, and *Aspergillus niger* on table grape and *B. cinerea* and *R. stolonifer* on cherry tomato (Mounir *et al.*, 2007). Bencheqroun *et al.* (2007) suggested a main mechanism of biocontrol activity of *A. pullulans* strain Ach1-1 may be due to its ability to compete with *P. expansum*.

Takesako *et al.* (1991) has been reported a new antifungal antibiotics, aureobasidins that were isolated from fermentation broth of *A. pullulans* R106. Aureobasidins are cyclic depsipeptide with molecular weight ranging from 1,070 to 1,148. These antibiotics showed high in vitro antifungal activity against *Candida albicans*.

Structure of aureobasidin A was reported by Ikai *et al.* (1991). It is a cyclic depsipeptide consisting of eight α -amino acid units and one hydroxyy acid unit. Aureobasidin A produced by *A. pullulans* play a key role in the strong antagonistic effect against *Candida* species. This antibiotic shown to inhibit the phosphatidylinositol:ceramide phosphoinositol transferase that is involved in sphingolipid synthesis

The production and antifungal activity of aureobasidin produced from tropical *A. pullulans* have been reported. Four isolates of *A. pullulans* collected from bathroom surfaces in Thailand were found to produce aureobasidin A. Antifungal activities against four selected *Aspergillus* species were tested. Cell extracts from isolates BM1, KT1,

HKW1 and HKW2 inhibited *A. terreus*. KT1 and BM1 extracts inhibited *A. fumigatus*, whereas BM1 extract inhibited *A. flavus* (Lotrakul *et al.*, 2009).

Prosongsuk *et al.* (2013) has been reported the effects of carbon and amino acid supplementation on antifungal activity produced by *A. pullulans* NRRL 58536. Glucose was able to induce the production of antifungal activity. Although amino acids supplementation with different combinations increased the antifungal activities but it varied between *Aspergillus* species and amino acid combinations.

2.2.4 Siderophores

Siderophores are low molecular weight, iron-chelating agents that have high potential in biotechnology. Due to its role as iron scavenging compounds, it can affect to microorganisms in the environments. In general, many fungal species were found to be able to produce siderophore. It has been reported for production of siderophores by yeast cell and only hydroxamate type compound was found. In contrast with bacteria, it produces hydroxamate type as well as catecholate siderophores (Chi *et al.*, 2009b). They have been reported to act as antimicrobials so its applications in medical and environmental were applied including to remediation from polluted environments. *A. pullulans* strain HN6.2 was isolated from marine habitat and found to be a siderophore producer. Under optimal conditions, it produces 1.1 mgml⁻¹ of the siderophore. Siderophore production was enhanced by L-Ornithine while Fe³⁺ was found to inhibit it production. Antimicrobial activity of siderophore produce by *A. pullulans* HN6.2 showed that it could inhibit cell growth of *Vibrio parahaemolyticus* (Wang *et al.*, 2009).

2.2.5 Heavy oils

Some strains of *A. pullulans* have been found to be able to produce heavy oils. It was found that in the survey of more than 50 various strains of *A. pullulans* 21 of them produced extracellular heavy oils. It colors are bright yellow and malachite.

The surface active of this oil suggested it functions as a biosurfactant. It was reported for the inhibition of mammalian cancer cell lines. Oils produced from NRRL Y-12974 were found to inhibit non-cancerous African green monkey kidney cells, whereas oils from CU 43 was non-cytotoxic and exhibited small cell lung cancer (Manitchotpisit *et al.*, 2011). The results suggesting the heavy oils from different strains have different effects (Li *et al.*, 2015).

2.2.6 Poly (β -L-malic acid)

Poly (β -L-malic acid) or PMA is natural water soluble polyester that has pharmaceutical applications as a drug carrier. In 1992, Nagata *et al.* first reported of PMA production by *A. pullulans*. It was needed to discover and develop promising the second generation biomaterials. Based on it is biocompatible, degradable, water soluble, and easily chemicals modified, the related applications including the production of detergents, biodegradable plastics and biomaterials could be more applied (Liu and Steinbüchel, 1996). PMA was produced from simple sugars, particularly glucose or sucrose. Recently, it was produced by agricultural biomass substrates including corn fiber and wheat straw (Leathers and Manitchotpisit, 2013).

> จุฬาลงกรณิมหาวิทยาลัย Chulalongkorn University

CHAPTER III

MATERIALS AND METHODS

3.1 Isolation and Identification of coastal isolates of *Aureobasidium* spp.

3.1.1 Aureobasidium spp.

Leaf samples (3 leaves per plant) with no visible sign of disease were collected from plants growing at various coastal habitats including mangroves and beachfront gardens in Thailand (Guimarães *et al.*, 2011). Sterile cotton swabs were smeared on the rock surfaces in tidal zone (3 cotton swabs per place). Sediment samples were collected at 0-5 cm from the surface, during low tide (Wu *et al.*, 2010). All samples were kept in the fridge prior to further isolation.

Leaves were aseptically cut and placed on half strength malt extract agar (MEA) containing Chloramphenicol (50 mg/L) and 0.01% (w/v) Rose Bengal (Fischer Scientific, Pittsburgh, PA, USA) were added to the medium to delay bacterial and fungal contamination (Prasongsuk *et al.*, 2005). Cotton swabs were streaked on the same medium. *Aureobasidium pullulans*-like colonies were transferred to new medium until pure cultures were obtained. All cultures were maintained on MEA and stored at 4°C. For longterm storage, all cultures were kept in 20 % (v/v) glycerol or freeze-dried. All freezedried isolates were deposited in culture collection of the Plant Biomass Utilization Research Unit at Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok, Thailand. The formula of medium used in this study was shown in Appendix A.

3.1.2 Morphological identification

Colony morphology was observed. A single colony of each isolate was stabbed onto potato dextrose agar (PDA), MEA and yeast malt extract agar (YMA) and incubated at 30±2°C for 7 days. Colony morphology was recorded with a CANON IXUS digital camera. For microscopic characters, a single colony of each isolate growing on YMA was inoculated into YM broth and incubated at 30±2°C for 1-5 days with agitation at 150 rpm. Cell morphology was observed as described by Cooke (1959) and photomicrographs recorded (Model Olympus BX51/DP70).

3.1.3 Physiological identification

Carbon and Nitrogen assimilation were observed using the protocols described by Kurtzman *et al.* (2011). The ability to grow on various carbon sources in an agar medium was done by replica plating method. Each plate containing one carbon source or nitrogen source in basal agar medium was prepared. Fresh and young of a single colony was transferred onto the template by spotting with a needle. The masterplate was incubated for 24-48 hours and used for inoculums starter. Each of carbon or nitrogen source test plate was done. A sterile velveteen cloth was attached to the end of a cylinder and pressed onto the master plate and then, in turn, onto each of the test plates. The results were read by inspecting the plates and comparing the colonies of a negative control provided by a plate containing the basal medium without a carbon source or nitrogen source.

3.1.4 DNA amplification, sequencing and phylogenetic analysis

For DNA isolation, each isolate was cultured in YM broth overnight at $30\pm2^{\circ}$ C with agitation at 150 rpm. Cells were harvested by centrifugation (4,025xg, 5 min). DNA was extracted by the phenol-chloroform method (Sambrook *et al.*, 1989).

According to the multilocus analysis of Zalar et al. (2008), a high level of support was evident for the clade containing Aureobasidium spp. together with Selenophoma mahoniae, three loci (ITS, TUB, and ELO) were amplified by using different primers and conditions (Table 3.1). The ITS region was amplified by PCR using the primers ITS5 and ITS4 (White et al., 1990) while β -tubulin (TUB) (Glass and Donaldson, 1995) was amplified by using the primers Bt2a and Bt2b with thermocycles described by Manitchotpisit et al. (2009). For amplification and sequencing of the partial elongase gene (ELO), the primers ELO2-F and ELO2-R were used with conditions described by Zalar et al. (2008). DNA sequencing was performed by dideoxy termination method at Macrogen Korea Corp. (Seoul, Korea) and GENEWIZ, Inc. (North Brunswick, NJ). Multiple sequence alignment was performed by using ClustalW (Larkin et al., 2007) and a phylogenetic tree was constructed by using MEGA 6 v 5.10 (Tamura et al., 2013). Selenophoma mahoniae (CBS 242.64) were included as the outgroups. For the neighbor-joining analysis, distances between the sequences were calculated based on Kimura's two-parameter model (Kimura, 1980), supporting the confidence limits for branching topologies with bootstrap analysis (1000 replicates).

Table 3.1 Primers used for PCR and sequencing.

Target DNA	Primer ²	Sequence 5' - 3'	Cycling reaction	Approximately PCR product	Source
region				(dq)	
ITS	ITS5 (F)	GGAAGTAAAGTCGTAACAAGG	95°C, 20 s	550	White et al. (1990)
	ITS4 (R)	TCCTCCGCTTATTGATATGC	56°C, 30 s		White et al. (1990)
			72°C, 1 min		
TUB	Bt2a (F)	GGTAACCAAATCGGTGCTGCTTTC	95°C, 30 s	450	Glass and Donaldson
	Bt2b (R)	ACCCTCAGTGTAGTGACCCTTGGC	58 ° C, 1 min		(1995)
			72 ° C, 1 min		Glass and Donaldson
					(1995)
ELO	ELO2-F (F)	CACTCTTGACCGTCCCTTCGG	94°C, 15s	200	Zalar <i>et al.</i> (2008)
	ELO2-R (R)	GCGGTGATGTACTTCTTCCACCAG	58°C, 15 s		Zalar <i>et al.</i> (2008)
			72°C, 45 s		
			94°C, 15s		
			56°C, 15 s		
			72°C, 45 s		

^a F and R in the parentheses mean forward and reverse primers, respectively

3.2 Characterization by phenotypic analysis

Aureobasidium isolates were characterized for EPS, xylanase, and antifungal substance production. Three reference strains of *A. pullulans* (NRRL 58560, NRRL 58561, and NRRL Y-12974) from Plant Biomass Utilization Research Unit, Department of Botany, Faculty of Science, Chulalongkorn University were used for comparative characterization and phylogenetic study.

3.2.1 EPS production and analysis

3.2.1.1 EPS Production

For seed culture preparation, one full loop of each fresh colony growing on YMA (2-3 days) was transferred to 20 ml of YMB in 50 ml Erlenmeyer flasks and grown overnight at $30\pm2^{\circ}$ C with 150-rpm agitation. Cell density was adjusted to 2.5x10⁷ cells/ml before being transferred at 1 % (v/v) to 100 ml of production medium (PM) containing (all w/v) sucrose (5%), (NH₄)₂SO₄ (0.06%) , peptone (0.06%), K₂HPO₄ (0.5%), MgSO₄ ·7H₂O (0.04%), NaCl (0.1%), and yeast extract (0.04%), in 200 ml Erlenmeyer flasks and grown under the same conditions for 7 days (Prasongsuk *et al.*, 2005). The EPS was recovered from supernatant and EPS yield was measured as gram per liter of the medium. The production efficiency (% conversion) was calculated as percentage of gram of EPS produced per gram of sugar supplied (Youssef *et al.*, 1999). Strains with equal to more than 40, <40-30, <30-20, <20-10 and <10-0 % conversion were considered high, relatively high, moderate, relatively low and low EPS production, respectively. The test was performed in triplicate.

3.2.1.2 EPS analysis

Enzyme sensitivity test was performed. EPS was digested by pullulanase (from *Klebsiella pneumoniae*, Sigma, USA), and β -glucanase (from *Trichoderma longibrachiatum*, Sigma, USA). One 1 mg of EPS was suspended into 1ml of 50mM sodium acetate buffer at 0.1 U/ml, and incubated under the optimal conditions. Released reducing sugars were measured using the dinitrosalicylic acid (DNS) method. Sensitivity to the specific enzyme digestion in percentage (%) was calculated incomparison to the value obtained from each control enzyme-digested substrate (pullulanase on pullulan (Sigma, USA) as substrate and β -glucanase on β -glucan (produced by the strain CBS 100524 as substrate). Experiments were carried out in triplicate.

Pullulan content in EPS will be determined as described by Lotrakul*et al.* (2013). Fourier transform infrared (FT-IR) spectra was measured with a Perkin Elmer-Spectrum RX1 spectrometer (32 scans; resolution, 4 cm-1) over KBr pellet. EPS (2 mg) was blended with 60 mg of KBr powder, and then desiccated overnight at 50°C to under reduced pressure prior to FT-IR measurement at Scientific and Technological Research Equipment Centre, Chulalongkorn University.

3.2.2 Multiple stress tests

The ability of *Aureobasidium* strains growing on different stresses were investigated (Kane and Summerbell, 1987; Kurtzman *et al.*, 2011; Selbmann *et al.*, 2008). The result was analyzed and reported as relative growth. The test was performed in triplicate.

3.2.2.1 Halotolerance test

Halotolerance was determined by growing each strain on PDA containing 5, 10, and 15 % (w/v) NaCl at $30\pm2^{\circ}$ C. Colonies with a diameter of > 2 mm were considered as growing. Colony diameter was measured at day 7 in comparison to that of the strain growing on PDA without NaCl addition.

3.2.2.2 Osmotolerance test

Osmotolerancewas determined by growing each strain on YMA containing 5, 30, and 50 % (w/v) glucose at $30\pm2^{\circ}$ C. Colony diameter was measured at day 7in comparison to that of the strain growing on YMA with 1% (w/v) glucose.

3.2.2.3 Thermotolerance test

Thermotolerance was determined in 3 levels of temperature (35, $37, 40^{\circ}$ C) by incubating each strain on 2 % MEA for 7 days and the diameter of each colony was recorded.

3.2.2.4 Tolerance against different pH value

The ability of *Aureobasidium* strains growing at different pH values (3, 5, 7, 9) were tested by using 2% MEB. *Aureobasidium* cultures were incubated at 30°C in agitation at 70 rpm periodically for one month. Growth will be determined as cell dry weighed.

3.2.3 Associations among halotolerance, osmotolerance, and EPS production

3.2.3.1 Effects of sucrose concentration

The promising strain with different halotolerance, osmotolerance and EPS production was selected and studied for effects of sucrose concentration on growth and EPS production by growing selected strains in PM containing sucrose ranging from 5 to 20 % (w/v) under standard conditions as previously described. Cell and EPS dry weights were measured 5 days after inoculation. Relative growth and EPS conversion were calculated in comparison to values obtained in PM containing 5 % (w/v) sucrose. The test was performed in triplicate. Relative growth was calculated by using the following equation: Relative growth (%) = (cell dry weight at desired % (w/v) of sucrose concentration/cell dry weight at 5 % (w/v) of sucrose x 100). EPS conversion was calculated by using the following equation: EPS conversion (%) = (EPS yield (gL⁻¹)/the amount of provide sugar (gL⁻¹) x 100).

3.2.3.2 Detection of intracellular osmolyte

Intracellular osmolyte was extracted using the method described by Managbanag and Torzilli (2002) with minor modification. Cells grown in PM with a range of concentrations of sucrose were harvested by centrifugation (4,025xg, 5 min) and suspended in 5 mL of sterile deionized H_2O . An equal volume of sterile glass beads (0.2 mm) was added and cells were broken for 15 rounds of vortexing, each round comprised three cycles of 30 s each. The extracts were kept on ice for 15 s between cycles. Cell debris was removed by centrifugation (5 min at 1500xg) and the supernatant stored at -20°C. To detect the osmolyte, the samples were spotted onto Silica Gel 60 F524 TLC plates (Merck, Darmstadt, Germany) and separated using butanol-pyridine-water (15:30:20, v/v) as the mobile phase. Spots were developed by dipping the plates in 0.5 % (w/v) KMnO₄ in 1 N NaOH. Mannitol (Merck, Darmstadt, Germany) and glycerol (Sigma) prepared at 2 % (w/v) were used as standards.

3.2.3.3 Associations among halotolerance, osmotolerance, and EPS production

Associations among halotolerance, osmotolerance, and EPS production were determined as paired data (halotolerance and osmotolerance, halotolerance and EPS production, osmotolerance and EPS production) using Fisher's exact test. The analysis was performed by using IBM SPSS Statistics for Windows Version 22 (IBM Corp., USA). Significances of differences between relative growths among strains and at different sugar concentrations and differences between relative EPS production among strains and at different sugar concentrations were determined by one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) using SPSS 17.0 software package (SPSS Inc., USA). Differences at P < 0.05 were considered significant.

ขุพาสงกรรรมทางทยาล

3.2.4 Screening of antifungal activity

Antifungal activity was screened by visual agar plate assay (Hua *et al.*, 1999) with minor modification. Each strain of *Aureobasidium* was grown in a flask containing 20 ml of PDB for 24 h at $30\pm2^{\circ}$ C with shaking at 150 rpm and used for inoculation. Each strain was streaked at the center line of a petri dish containing PDA and incubated for 72h. Plate of *Aspergillus niger* and *A. fumigatus* were prepared. Dish was cut at 1 cm of fungal edge and inoculated 1 dish of each fungal at the side of the PDA agar. Screen plates were incubated at $30\pm2^{\circ}$ C for 10 days and assessed visually for antifungal phenotypes. Antifungal activity was assessed by comparing the

zone of fungal growth inhibition in fungus co-cultured with *Aspergillus* as tests, in comparison with control plates which were inoculated only with the *Aureobasidium*.

3.2.5 Production and characterization of xylanase activity

3.2.5.1 Xylanase production

The initial screening of xylanase was determined on agar plate containing Beechwood xylan (1% w/v) and was assayed by Congo red staining (Christov and Prior, 1993). The colony with clear zone of xylan hydrolysis was observed and the ratio of the clear zone diameter to that of colony was determined.

Xylanase production was examined by culture inoculated in 50 ml Erlenmeyer flask with 10 ml of basal medium, consisting of 0.67 % (w/v) yeast nitrogen base, 0.2 % (w/v) asparagine, 0.5 % (w/v) KH_2PO_4 , and 1 % (w/v) glucose and incubated at 30±2°C for 3 days with agitation at 200 rpm. The culture was transferred to xylanase production medium, replacing glucose with 1% (w/v) purified beechhwood xylan (Sigma, St. Louis, MO). Xylanase activity was assessed at 50°C for 10 min in 50 mM Na-acetate buffer (pH 5.0) by modification of the DNS method. The absorbance was measured at 540 nm. One unit of xylanase activity was defined as the amount of enzyme produced 1 μ mol of xylose equivalent per minute under specified conditions.

3.2.5.2 Characterization of xylanase activity

To observe optimum pH and temperature, xylanase from each strain was characterized in various pH and temperature. The optimum pH and temperature on the reaction was determined by using 50 mM Na-acetate buffer (pH 3.0 to 6.0) and 50mM Na-phosphate buffer (pH 7.0 to 8.0). For optimum temperature, each enzyme was incubated under standard assay conditions with different temperatures in

the range of 30 to 90°C. The best strain was selected for further characterization. The thermostability of xylanase activity was monitored by incubating the enzyme sample for 60 min at various temperatures between 30 to 80°C in 50 mM Na-acetate buffer (pH 5.0). To test the pH stability, the crude enzyme was incubated for 60 min at 50°C in different pH range at 3.0 to 8.0. The enzyme was then assayed by pH 5.0 as described previously. The effect of salt concentration for crude xylanase was determined in 50 mM Na-acetate buffer (pH 5.0) containing various concentrations of NaCl (5–15% w/v). For enzyme stability, crude xylanase was incubated in Na-acetate buffer (pH 5.0) with salinity in the same range as above for 24 hours at 25°C. The effect of various additives such as solvents and detergents were determined by incubating each additive (1% final concentration) with the crude enzyme for 1 h at 50°C.

3.3 Potential of xylanase for xylooligosaccharide production

The strain PBUAP58 was selected for the study on XOS production and effect of XOS on antioxidant activity.

3.3.1 <u>Xylan preparation</u>

The xylan substrate was prepared from a whole plant of cattail. Dry materials were chipped and ground, then sieved into size of less than 1mm. The delignified material was extracted with minor modification method of Yoon *et al.* (2006) and Chapla *et al.* (2012). Five gram dry weight of each lignocelluloses material was stirred with 80 ml of 1.25 molL⁻¹ NaOH for 15 min. The mixture was shaken for 3 h on a horizontal shaker with300 rpm at 37°C and centrifuged at 16,270g for 20 min. The supernatant fraction (hemicellulose fraction) was acidified to pH 5.0 with concentrated HCI. The supernatant was precipitated with three volumes of ethanol, and separated by filtration

through a filter paper. The precipitated material was freeze dried and used as substrate for enzymatic hydrolysis experiments.

3.3.2 Enzyme hydrolysis

The experiments were conducted in 50ml Erlenmeyer flasks, each containing 1% (w/v) of xylan obtained from the hemicelluloses material and mixed with 25 Ug⁻¹ (Bian *et al.*, 2013) of crude xylanase. The mixture was incubated at 50°C on a horizontal shaker at 300 rpm for 1, 4, 6, 12, 16, 24 h. After incubation for the desired time, 0.2 mL of XOS-containing liquids was withdrawn from the incubation mixture and centrifuged at 1200xg for 5 min. Three volumes of ethanol were added to precipitated unhydrolyzed hemicelluloses and the XOS-containing liquor was filtrated. Ethanol was removed from the filtrate by rotary evaporation under reduced pressure at 45°C. The solid fraction was freeze dried.

3.3.3 XOS analysis

The hydrolyzed products were quantified by measuring the reducing sugar content with DNS method and expressed as milligrams per milliliter (mg/ml). At the desired time intervals, hydrolyzed products of each hemicellulose biomass were analyzed using Thin Layer Chromatography (TLC). The samples were spotted onto Silica Gel 60 F524 TLC plates (Merck, Darmstadt, Germany) and developed with a solvent system of chloroform-acetic acid-water (6:7:1, v/v/v). The sugars were detected by heating the plates to over 105°C for few minutes after dipping them with ethanol and sulfuric acid mixture (19:1, v/v). Xylose, xylobiose, Xylotriose, and Xylotetraose (Megazyme, Ireland) were mixed to XOS standard (Kallel *et al.*, 2015). FT-IR spectra was measured with a Perkin Elmer-Spectrum RX1 spectrometer (32 scans; resolution,

4 cm⁻¹) in the range of 4000–600 cm⁻¹ at a resolution of 8 cm⁻¹. All samples were performed at Scientific and Technological Research Equipment Centre, Chulalongkorn University.

3.3.4 Antioxidant activity

Antioxidant activity of XOS was measured by the effect of scavenging 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals according to Veenashri and Muralikrishna (2011) and Bian *et al.* (2013) and reported as percentage of inhibition. The XOS powder was dissolved in distilled water, an aliquot of sample was added to DPPH solution (1:1 dilution). The mixture was shaken vigorously and incubated for 120 min in the dark at 25° C. The absorbance was measured at 517 nm using spectrophotometer. The control was carried out by replacing the sample with water, while ethanol was used as blank. The ability of the sample to scavenge the DPPH radicals was calculated using the following equation: DPPH radical scavenging activity (%) = (1 - absorbance of sample/absorbance of control) x 100.

าลงกรณ์มหาวิทยาลั

3.3.5 <u>Statistical analysis</u>

Significances of differences between XOS yield (mg/ml) at desired time and inhibition (%) at different XOS concentrations were determined by one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) using SPSS 20 software package (SPSS Inc., USA). Differences at P < 0.05 were considered significant.

CHAPTER IV

RESULTS

4.1 Identification of Aureobasidium spp. isolated from coastal area

4.1.1 Aureobasidium spp.

Between 2010 and 2012, 54 strains of Aureobasidium pullulans-like isolates were obtained from a variety of coastal habitats at different geographical locations (Table 4.1), both the mainland and islands, covering both Gulf of Thailand (South China Sea) and the Andaman Sea (Indian Ocean). Isolates were collected from Bangkok, Chonburi, Chumphon, Krabi, Phetchaburi, Prachuap Khiri Khan, Samut Sakhon and Songkhla. Most strains were isolated from living leaf samples including seven species of mangrove plants (Acanthus ilicifolius L., Avicennia marina (Forssk.) Vierh., Avicennia officinalis L., Azima sarmentosa (Blume) Benth. & Hook.f., Rhizophora mucronata Lam., Sonneratia alba Sm. and Sonneratia caseolaris (L.) Engl.), five species of sandy beach plants (Casuarina equisetifolia L., Hibiscus tilliaceus L., Ipomoea pes-caprae (L.) R. Br., Thespesia populnea (L.) Sol. ex Corrêa and Thespesia populneoides (Roxb.) Kostel.), one species of plant commonly found on man-made salterns (Suaeda maritima (L.) Dumort.) and ten species of plants that do not specifically grow in salt water habitats (Acacia auriculiformis Benth., Calotropis gigantea (L.) Dryand., Conocarpus erectus L., Dimocarpus logan Lour., Diospyros sp., Ludwigia adscendens (L.) H.Hara, Pithecellobium dulce (Roxb.) Benth., Pterocarpus sp., Tamarindus indica L. and Terminalia catappa L.). All mangrove and saltern plants were exposed to brackish water directly during high tide period whereas the other plants were grown on beachfronts within the salt spray zone. On leaf surfaces soaked with salt water, once the water evaporated fine salt crystals were visible. One mangrove genus, Avicennia, foliar salt glands are present and salt is secreted out on the surface. Four strains were isolated from rock surfaces in the intertidal zone. Despite several attempts, isolation of *A. pullulans*-like colonies directly from marine water and sediment were unsuccessful, even when an enrichment protocol was employed.

Collection site	Geographic Coordinate
Bangkok (August 2010)	13° 30 [′] 08.7 [″] N, 100° 27 [′] 05.6 [″] E
Chonburi (December 2010)	13° 20 ′ 26.7 ″ N, 100° 55 ′ 32.9 ″ E
Chonburi (February 2012)	12° 55 ′ 32.5″ N, 100° 46 ′ 29.5″ E
Chumphon (May 2011)	9° 57 ′ 12.6″ N, 99° 09 ′ 28.1″ E
Krabi (April 2011)	7° 38' 37.4" N, 99° 01'13.7" E
Phetchaburi (July 2010)	12° 42 ′ 14.4″ N, 99° 57 ′ 28″ E
Prachuap Khiri Khan (August 2010)	12° 34 ′ 31.9 ″ N, 99° 57 ′ 29.1 ″ E
Samut Sakhon (May 2011)	13° 28 ′ 33.6″ N, 100° 06 ′ 13.9″ E
Songkhla (April 2010)	7° 09 ′ 23.2 ″ N, 100° 32 ′ 04.3 ″ E

 Table 4.1 Geographic coordinates of the sample collection sites.

All new isolates were deposited at culture collection of the Plant Biomass Utilization Research Unit (PBURU) and Fungal Section, Professor Kasin Suvatabhandhu Herbarium (BCU), Department of Botany, Faculty of Science, Chulalongkorn University. The strain accession number, source of isolation and habitats were shown in table 4.2.

Isolate	Accession number	number	Source of isolation	Place and date of isolation
AP4	PBUAP4	BCU011	Thespesia populnea (L.) Sol. ex Corrêa	Songkhla (April 2010)
AP5	PBUAP5	BCU012	Hibiscus tilliaceus L.	Songkhla (April 2010)
AP5.1	PBUAP5.1	BCU013	Hibiscus tilliaceus L.	Songkhla (April 2010)
AP7.1	PBUAP7.1	BCU014	Rhizophora mucronata Lam.	Songkhla (April 2010)
AP9	PBUAP9	BCU015	Acanthus ilicifolius L.	Songkhla (April 2010)
AP13	PBUAP13	BCU016	Calotropis gigantea (L.) Dry and.	Songkhla (April 2010)
AP14	PBUAP14	BCU017	Ipomoea pes-caprae (L.) R.Br.	Songkhla (April 2010)
AP16	PBUAP16	BCU018	Terminalia catappa L.	Phetchaburi (July 2010)
AP17	PBUAP17	BCU019	Pithecellobium dulce (Roxb.) Benth.	Phetchaburi (July 2010)
AP20	PBUAP20	BCU020	Ipomoea pes-caprae (L.) R.Br.	Phetchaburi (July 2010)
AP22	PBUAP22	BCU021	Rhizophora mucronata Lam.	Bangkok (August 2010)
AP23	PBUAP23	BCU022	Rhizophora mucronata Lam.	Bangkok (August 2010)
AP24	PBUAP24	BCU023	Terminalia catappa L.	Songkhla (April 2010)
AP25	PBUAP25	BCU024	Sonneratia caseolaris (L.) Engl.	Bangkok (August 2010)
AP26	PBUAP26	BCU025	Avicenne officinalis L.	Bangkok (August 2010)

Table 4.2 Aureobasidium strains isolated from various habitats along Thai coasts.

solate	Accession number	nber	Source of isolation	Place and date of isolation
AP27	PBUAP27	BCU026	Ludwigia adscendens (L.) H.Hara	Prachuap Khiri Khan (August 2010)
AP29	PBUAP29	BCU027	Acacia auriculiformis Benth.	Chonburi (December 2010)
AP30	PBUAP30	BCU028	Acacia auriculiformis Benth.	Chonburi (December 2010)
AP31	PBUAP31	BCU029	Acacia auriculiformis Benth.	Chonburi (December 2010)
AP32	PBUAP32	BCU030	Acacia auriculiformis Benth.	Chonburi (December 2010)
AP33	PBUAP33	BCU031	Tamanindus indica L.	Chonburi (December 2010)
AP34	PBUAP34	BCU032	Tamarindus indica L.	Chonburi (December 2010)
AP35	PBUAP35	BCU033	Tamanindus indica L.	Chonburi (December 2010)
AP36	PBUAP36	BCU034	Sonneratia alba Sm.	Chonburi (December 2010)
AP37	PBUAP37	BCU035	Sonneratia alba Sm.	Chonburi (December 2010)
AP38	PBUAP38	BCU036	Sonneratia alba Sm.	Chonburi (December 2010)
AP39	PBUAP39	BCU037	Terminalia catappa L.	Krabi (April 2011)
AP40	PBUAP40	BCU038	Casuarina equisetifolia L.	Krabi (April 2011)
AP41	PBUAP41	BCU039	Diospyros sp.	Krabi (April 2011)
AP42	PBUAP42	BCU040	Diospyros sp.	Krabi (April 2011)

Table 4.2 (continued)

Isolate	Accession number	number	Source of isolation	Place and date of isolation
AP43	PBUAP43	BCU041	Diospyros Sp.	Krabi (April 2011)
AP44	PBUAP44	BCU042	Pterocarpus sp.	Krabi (April 2011)
AP45	PBUAP45	BCU043	Pterocarpus sp.	Krabi (April 2011)
AP46	PBUAP46	BCU044	Suaeda maritima (L.) Dumort.	Samut Sakhon (May 2011)
AP47	PBUAP47	BCU045	Suaeda maritima (L.) Dumort.	Samut Sakhon (May 2011)
AP48	PBUAP48	BCU046	Terminalia catappa L.	Chumphon (May 2011)
AP49	PBUAP49	BCU047	Terminalia catappa L.	Chumphon (May 2011)
AP50	PBUAP50	BCU048	Terminalia catappa L.	Chumphon (May 2011)
AP51	PBUAP51	BCU049	Azima sarmentosa (Blume) Benth. & Hook.f.	Samut Sakhon (May 2011)
AP53	PBUAP53	BCU050	Dimocarpus longan Lour.	Chonburi (February 2012)
AP55	PBUAP55	BCU051	Conocarpus erectus L.	Chonburi (February 2012)
AP58	PBUAP58	BCU052	Conocarpus erectus L.	Chonburi (February 2012)
AP59	PBUAP59	BCU053	Conocarpus erectus L.	Chonburi (February 2012)
AP61	PBUAP61	BCU054	Avicennia marina (Forssk.) Vierh.	Chonburi (February 2012)
AP62	PBUAP62	BCU055	Avicennia marina (Forssk.) Vierh.	Chonburi (February 2012)

Table 4.2 (continued)

О.
œ
×
=
<u> </u>
•••
0
Ο.
-
~
Ň
Ň
12
42
12
4
12
4
4
ble 4.
0 4
ble 4.

Isolate	Accession number	number	Source of isolation	Place and date of isolation
AP65	PBUAP65	BCU056	Rock surface	Chonburi (February 2012)
AP67	PBUAP67	BCU057	Rock surface	Chonburi (February 2012)
AP70	PBUAP70	BCU058	Rock surface	Chonburi (February 2012)
AP71	PBUAP71	BCU059	Rock surface	Chonburi (February 2012)
AP72	PBUAP72	BCU060	Thespesia populneoides(Roxb.) Kostel.	Chonburi (February 2012)
AP73	PBUAP73	BCU061	Diospyros sp.	Chonburi (February 2012)
AP75	PBUAP75	BCU062	Avicennia marina (Forssk.) Vierh.	Chonburi (February 2012)
AP76	PBUAP76	BCU063	Diospyros sp.	Chonburi (February 2012)
AP77	PBUAP77	BCU064	Azima sarmentosa (Blume) Benth. & Hook.f.	Chonburi (February 2012)

4.1.2 Morphology

Morphology identification of all strains was compared with *A. pullulans* NRRL 58560, NRRL 58561 and NRRL Y12974 obtained from the ARS Culture Collection, National Center for Agricultural Utilization Research, USDA, Peoria, IL, USA was also used for comparison.

Colony characteristic

The fungal characters based on cultures grown on MEA, PDA, and YMA at 30°C at day 7. Almost all strains rapidly grew on MEA, PDA, and YMA. Colonies morphology varied depending on the strains. Morphology on different plate agar was shown In Appendix B.

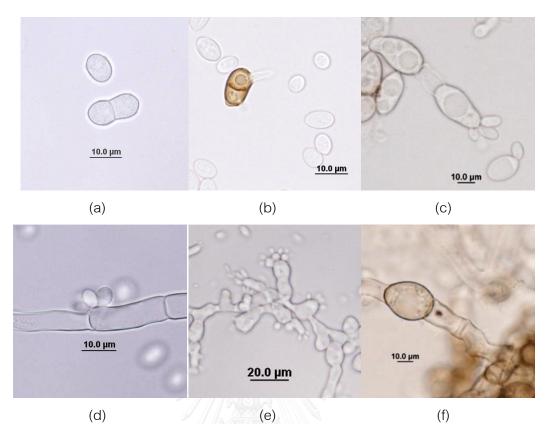
In general, colony on YMA was smooth, still remained pale pink, appearing slimy as yeast at the center of colony with entirely margin. The isolates attained 4-6 diameters in 1 week, with different on each media. After day 7, mature colonies on YMA developed a velvety texture while leathery was also found in some colonies. At the center of colony covered with slimy exudate. Colonies remained cream or pale pink for 3-4 days and became pigmented in 1 week on PDA and MEA due to sporulation. Mature colonies on MEA also developed dark septate hyphae that mostly observed after day 7.

Aerial mycelium was found and marginal areas of colonies were various, reverse from dark color peripherally to light. In some colonies, entirely white hyphae were found at the edge of colony. Most of the strains obtained in this study formed dark olivaceous to black colonies when aged. Five strains including PBUAP5, PBUAP5.1, PBUAP7.1, PBUAP55 and PBUAP58 were color variants that produced pink, yellow, and purple pigments instead of the usual dark melanin.

Microscopic examination

All strains produced polymorphic cells typical of *Aureobasidium* spp. ranging from blastospores, swollen cells, chlamydodpores, to hyphae and pseudohyphae (Figure 4.1). Generally, its morphology like yeast cells and hyphae which produce synchronous conidia when they are young. The conidia then fall off and depending on the nutritional condition they will germinate with yeast cells or with hyphae. Conidia hyaline, ellipsoidal shape (Figure 4.1a, b).

Unicellular budding originate from polar (Figure 4.1c). Vegetative hyphae hyaline, smooth, thin-walled, 6–15 µm wide, transversely septate (Figure 4.1d), in older cultures sometimes locally converted to dark-brown hyphae. Conidia produced synchronously in dense groups (Figure 4.1c, e) and conidia hyaline formed to dark brown in older culture. Hyaline conidia were one-celled, smooth, ellipsoidal, very variable in shape and size $(7-15 \times 15-35 \mu m)$. Budding of hyaline and dark brown conidia were frequently seen with the secondary conidia being smaller than the primary conidia. Conidia in old cultures transferred to globose, brownish structures of 10–15 µm diameter. Conidiogenous cells undifferentiated, lateral, intercalary or terminal conidia were originated directly from the hyaline mycelium (Figure 4.1c, d, e). Later stages of growth, dark brown conidia with thick walled (chlamydospores) were 1-2 cells, one cell 15–30 × 12–18 μ m, two cells slightly constricted at septum, 25–35 × 20–25 μ m (Figure 4.1f, b). It had a very thick wall which showed the presence of large amounts of melanin deposits over its surface and beaming to pseudohyphae (Figure 4.1g). Some hyphal strands which roduve chlamydosores become septate, thick walled, and cover with melanin (Figure 4.1f). Oil production was observed after 4 days in some strains, especially in the strain that produced melanin pigment (Figure 4.1h). Swollen cells was with extracellular secretion (Figure 4.1i).



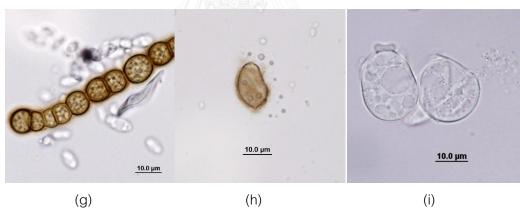


Figure 4.1 Polymorphic forms of *Aureobasidium* spp. grown in YM broth at 30 °C with agitation at 150 rpm. (a) blastospores (b) germinating chlamydospore with endoconidia (c) polar budding and budding conidia (d) intercalarychlamydospore (e) pseudohyphae with adhearing conidia (f) true hyphae with intercalary conidia (g) melanized hyphae /pseudohyphae (h) melanized spore with oil droplet (i) swollen cells with extracellular secretion

4.1.3 Physiology

The nutrients assimilation patterns of all strains comparison with *A. pullulans* NRRL 58560 were shown in Table 4.3 and 4.4 for carbon and nitrogen sources, respectively.

The diverse range of carbon and nitrogen sources were utilized. All strains utilized L-Arabinose, D-Cellobiose, D-Fructose, D-Glucose, β -Lactose, D-Mannitol, D-Mannose, D-Sucrose, and Xylitol that correlate with assimilation patterns of the control strains. The variation of strains and standard control was found in assimilation of α -Cellulose, D-Galactose, D-Glucosamine, Glycerol, Methyl- α -D-glucoside, L-Sorbose, D(+)Trehalose-2hydrate, and D-Xylose.

A range of nitrogen sources including amino acids were utilized. Nitrogen sources that were utilized included Ammonium acetate, Ammonium oxalate, Ammonium sulphate, Ammonium tartrate, L-Asparagine, L-Leucine, L-Lysine, Peptone, Potassium nitrate, Sodium nitrite, and Sodium nitrate, while varied in L-Glutamic acid, and Glycine. Almost strain utilized urea, except three strains including PBUAP17, PBUAP70, and PBUAP 77, whereas the strain PBUAP16 exhibited weak assimilation on urea test agar. Table 4.3 Assimilation profile on yeast nitrogen base for carbon assimilation tests of Aureobasidium spp. at 25 C unless noted

otherwise and incubation was for 7 days.

Carbon source	NRRL 58560	PBUAP4	PBUAPS	PBUAP5.1	FLAP7.1	PBUAP9	PBUAP13	PBUAP14	PBUAP16	PBUAP17
	÷	+	÷	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+
	•	•	•	•	•	•	•	•	M	W
	+	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	w	w
	+	+	+	+	+	+	+	+	+	+
D-Glucosamine	÷	+	•	•	•	+	+	+	+	+
	+	+	+	w	M	M	+	+	+	+
	+	+	+	+	+	+	+	+	+	+
	÷	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+
Methyl-0-D-glucoside	+	+	+	+	+	+	+	M	M	W
	.m.	+	AM.	M	M	÷	÷	÷		•
	+	+	+	+	+	+	+	+	+	+
D(+)Trehalose-2hydrate	+	+	+	+	+	+	+	+	M	•
	÷	+	+	+	+	+	+	+	AN .	M.
	÷	+	+	+	+	+	+	+	+	+

*Standard strain A. pullulans NRRL 58560, + = assimilation, w = weak, - = non assimilation

1		-	
	c	2	
	q)	
	2	э	
	c	2	
	ē		
	¢		
	c)	
	£	x	
•	2	٥	
		f	
	7	5	
		2	
		2	

Letratinget \cdot </th <th>Carbon source</th> <th>PBUAP20</th> <th>PBUAP22</th> <th>PBUAP23</th> <th>PBUAP24</th> <th>PBUAP25</th> <th>PBUAP26</th> <th>PBUAP27</th> <th>PBUAP29</th> <th>PBUAP30</th> <th>PBUAP31</th> <th>PBUAP32</th>	Carbon source	PBUAP20	PBUAP22	PBUAP23	PBUAP24	PBUAP25	PBUAP26	PBUAP27	PBUAP29	PBUAP30	PBUAP31	PBUAP32
Oblication +	L-Arebinose	+	+	+	+	+	+	+	+	+	+	+
Uldee ··	D-Cellobiose	+	+	+	+	+	+	+	+	+	+	+
Title + <td>0-Cellulose</td> <td>•</td> <td></td>	0-Cellulose	•	•	•	•	•	•	•	•	•	•	
Scote + + + + + + + + + + + + + + Cose ·<	D-Fructose	+	÷	+	+	+	+	+	+	+	+	+
Cole · · · · · · · · · · · · · · · · · · ·	D-Galactose	+	+	+	+	+	+	+	+	+	+	+
Construint +	D-Glucose	÷	+	+	+	÷	+	÷	÷	+	+	÷
ol +	D-Glucossmine	+	+	+	+	+	+	+	+	+	+	+
Use Use <td>Glycerol</td> <td>+</td>	Glycerol	+	+	+	+	+	+	+	+	+	+	+
Initial + </td <td>β-Lactose</td> <td>+</td> <td>+</td> <td>+</td> <td>+</td> <td>÷</td> <td>+</td> <td>÷</td> <td>÷</td> <td>+</td> <td>÷</td> <td>÷</td>	β-Lactose	+	+	+	+	÷	+	÷	÷	+	÷	÷
Indee ++ + +	D-Mannitol	+	+	+	+	+	+	+	+	+	+	+
-c·D-glucoside +	D-Mannose	+	+	+	+	+	+	+	+	+	+	+
006 -	Methyl-0-D-glucoside	+	+	+	+	+	+	+	+	+	+	+
Coee +	L-Sorbose	+	+	+	+	+	+	+	+	+	+	+
ehalose-2hydrose +	D-Sucrose	+	+	+	+	+	+	÷	+	÷	+	÷
	D(+)Trehalose-2hydrate	÷	+	+	+	÷	+	÷	+	÷	÷	+
* * * * * * * * *	D-Xylose	+	+	+	+	÷	+	÷	÷	÷	÷	÷
	Xylital	÷	+	+	+	÷	+	÷	+	÷	÷	+

-	
0	
(D)	
-	
-	
9	
9	
-	
3	
4	
Ð	
ō	
CD	
Ë.	

interfactore interfactore<	Carbon source	PBUAP33	PBUAP34	PBUAP35	PBUAP36	PBUAP37	PBUAP38	PBUAP39	PBUAP40	PBUAP41	PBUAP42	PBUAP43
Colore Colore<	L-Anabinose	+	+	+	+	+	+	+	+	+	+	+
Unlose ·· Ublose ·· Conserved ··	D-Cellobiose	+	+	+	+	+	+	+	+	+	+	+
Conservation · · · · · · · · · · · · · · · · · · ·	0-Cellulose	•	•	•	•	•	•	•	•	•	•	
ecces + <td>D-Fructose</td> <td>+</td>	D-Fructose	+	+	+	+	+	+	+	+	+	+	+
Cosenina Cosenina <td< td=""><td>D-Galactose</td><td>+</td><td>+</td><td>+</td><td>+</td><td>+</td><td>+</td><td>+</td><td>+</td><td>+</td><td>+</td><td>+</td></td<>	D-Galactose	+	+	+	+	+	+	+	+	+	+	+
Colamina	D-Glucose	+	+	+	+	+	+	+	+	+	+	+
Idl 1	D-Glucosamine	+	+	+	+	+	+	+	+	+	+	+
1000 1000	Glycerol	+	+	+	+	+	+	w	+	+	W	+
Milol Milol Mole Mole Mole<	B-Lactose	÷	+	+	÷	÷	+	+	+	+	+	+
Mocean Holose Holose<	D-Mannitol	+	+	+	+	+	+	+	+	+	+	+
-0-Dejucceide - -0-Dejucceide + -0-Dejucceide + -0.0 + + + + + + + + + + + + + +	D-Mannose	+	+	+	+	+	+	+	+	+	+	+
0000 0000 + +	Methyl-0-D-glucoside	÷	+	+	÷	+	+	w	+	+	<i>I</i> M	+
Ince Ince rehalces-2hydrase + * + <td>L-Sorbose</td> <td>+</td> <td>+</td> <td>+</td> <td>+</td> <td>+</td> <td>+</td> <td>+</td> <td>•</td> <td>+</td> <td>+</td> <td>•</td>	L-Sorbose	+	+	+	+	+	+	+	•	+	+	•
rehalose-Zhydrate + rehalose <	D-Sucrose	+	+	+	÷	+	+	+	+	÷	+	+
 	D(+)Trehalose-2hydrate	+	+	+	+	+	+	+	+	+	+	+
* * * * * * *	D-Xylose	+	+	+	+	÷	+	÷	+	+	+	+
	Xylinal	+	+	+	+	+	+	÷	÷	+	+	+

-	7
τ	3
q)
2	5
c	2
Ē	
è	
č	5
ĩ	۶.
2	٥
9	ŕ
~	5
	2
	2
4	

Carbon source	PBUAP44	PBUAP45	PBUAP46	PBUAP47	PBUAP48	PBUAP49	PBUAP50	PBUAP51	PBUAP53	PBUAP55	PBUAP58
L-A rebinose	+	+	+	+	+	+	+	+	+	+	+
D-Cellobiose	+	+	+	+	+	+	+	+	+	+	+
0-Cellulose	•	•	•	•	•	•	•	•	•	•	
D-Fructose	+	+	+	+	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	+	+	+	+	+	+
D-Glucose	+	+	+	+	+	+	+	+	+	+	+
D-Glucosamine	+	+	+	+	+	+	+	+	+	•	•
Glycerol	M	+	+	+	+	+	+	+	+	W	W
B-Lactore	+	+	+	+	+	+	+	+	+	+	+
D-M annitol	+	+	+	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+	+	+	+
Methyl-0-D-glucoside	+	+	+	+	+	+	÷	+	+	+	+
L-Sorbose	+	+	÷	÷	÷	÷	÷	+	÷	+	+
D-Sucrose	+	+	÷	÷	÷	÷	÷	+	÷	+	+
D(+)Trehalose-2hydrate	+	+	+	+	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+	÷	+	÷	+	+
Xylinal	+	+	+	+	+	+	+	+	+	+	+

_
-
0
00
-
-
-
0
ö
~
m
1.2
4
Ð
-
CD

Carbon source	PBUAP59	PBUAP61	PBUAP62	PBUAP65	PBUAP67	PBUAP70	PBUAP71	PBUAP72	PBUAP75	PBUAP76	PBUAP77
	÷	+	+	÷	÷	+	+	÷	+	÷	+
D-Cellobiose	+	+	+	+	÷	+	+	+	+	+	+
	•	•	•		•	w	•	w	•	•	w
	+	+	+	+	+	+	+	+	+	+	÷
D-Galactose	+	+	+	+	+	w	+	w	+	+	w
	+	+	+	÷	÷	÷	÷	÷	÷	+	÷
D-Glucosamine	+	+	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+	+
	+	+	+	÷	÷	÷	+	÷	÷	+	÷
	+	+	+	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+	+	+	+
Methyl-0-D-glucoside	+	+	+	+	+	w	+	w	+	+	w
	+	+	+	+	+	W	+	w	+	+	w
	+	+	+	+	+	÷	÷	÷	+	+	÷
D(+)Trehalose-2hydrate	÷	+	+	÷	÷	•	÷	•	÷	÷	•
	+	+	+	+	+	+	+	+	+	+	+
	÷	+	÷	+	÷	+	÷	÷	÷	÷	+

Table 4.4 Assimilation profile on yeast carbon base for nitrogen assimilation tests of Aureobasidium spp. at 25 C unless noted

otherwise and incubation was for 7 da/s.

Nitrogen source	NRRL 58560	PBUAP4	PBUAP5	PBUAP5.1	1'14MBd	6dWN8d	PBUAP13	PBUAP14	PBUAP16	PBUAP17
Armonium acetate	+	+	+	+	+	+	+	+	+	+
Ammonium oxaliate	+	+	+	+	+	+	+	+	+	+
Ammonium sulphate	+	+	+	+	+	+	+	+	+	+
Ammonium tartrate	+	+	+	+	+	+	+	+	+	+
L-A sparagine	÷	+	+	+	+	+	÷	÷	+	+
L-Glutamic acid	+	+	+	+	+	+	+	+	+	W
Glycine	+	+	+	•	•	M	+	+	w	W
L-Leucine	÷	+	+	+	+	+	+	+	+	+
L-Lysine	+	+	+	+	+	+	+	+	+	+
Peptone	+	+	+	+	+	+	+	+	+	+
Potassium nitrate	÷	+	+	+	+	+	÷	÷	÷	+
Sodium nitrite	÷	+	+	+	+	+	+	+	÷	+
Sodium nitrate	+	+	+	+	+	+	+	+	+	+
Urea	+	+	+	+	+	+	+	+	w	•

Table 4.4 (continued)

Nitrogen source	PBUAP20	PBUAP22	PBUAP23	PBUAP24	PBUAP25	PBUAP26	PBUAP27	PBUAP29	DEGMD30	18dWD8d	PBUAP32
Ammonium acetate	+	+	+	+	+	+	+	+	+	+	+
Ammonium oxelate	+	+	+	+	+	+	+	+	+	+	+
A mmonium sulphate	+	+	+	+	+	+	+	+	+	+	+
Ammonium tartrate	+	+	+	+	+	+	+	+	+	+	+
L-Asparagine	+	+	+	+	+	+	+	+	+	+	+
L-Glutamic acid	+	+	+	+	+	+	+	+	+	+	+
Glycine	÷	+	÷	+	÷	÷	+	+	+	+	÷
L-Leucine	+	+	+	+	+	+	+	+	+	+	+
L-Lysine	+	+	+	+	+	+	+	+	+	+	+
Peptone	+	+	÷	+	÷	÷	+	+	+	+	÷
Potassium nitrate	+	+	+	+	+	+	+	+	+	+	+
Sodium nitrite	+	+	+	+	+	+	+	+	+	+	+
Sodium nitrate	+	+	+	+	+	+	+	+	+	+	+
Urea	+	+	+	+	+	+	+	+	+	+	+

Table 4.4 (continued)

PBUAP43	+	+	+	+	+	+	W	+	+	+	+	+	+	+
PBUAP42	+	+	+	+	+	+	W	+	+	+	+	+	+	+
PBUAP41	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PBUAP40	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PBUAP39	+	+	+	+	+	+	W	+	+	+	+	+	+	+
PBUAP38	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PBUAP37	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PBUAP36	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PBUAP35	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PBUAP34	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PBUAP33	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nitrogen source	Ammonium acetate	Ammonium oxalate	Ammonium sulphate	Ammonium tartrate	L-Asparagine	L-Glutamic acid	Glycine	L-Leucine	L-Lysine	Peptone	Potassium nitrate	Sodium nitrite	Sodium nitrate	Urea

Table 4.4 (continued)

Nitrogen source	PBUAP44	PBUAP45	PBUAP46	PBUAP47	PBUAP48	PBUAP49	PBUAP50	PBUAP51	PBUAP53	PBUAP55	PBUAP58
Ammonium acetate	+	+	+	+	+	+	+	+	+	+	+
Ammonium oxalate	+	+	+	+	+	+	+	+	+	+	+
Ammonium sulphate	+	+	+	+	+	+	+	+	+	+	+
Ammonium tartrate	+	+	+	+	+	+	+	+	+	+	+
L-Asparagine	+	+	+	+	+	+	+	+	+	+	+
L-Glutamic acid	+	+	+	+	+	+	+	+	+	+	+
Glycine	M	M	+	+	M	+	+	+	W	+	+
L-Leucine	+	+	+	+	+	+	+	+	+	+	+
L-Lysine	+	+	+	+	+	+	+	+	+	+	+
Peptone	+	+	+	+	+	+	+	+	+	+	+
Potassium nitrate	+	+	+	+	+	+	+	+	+	+	+
Sodium nitrite	+	+	+	+	+	+	+	+	+	+	+
Sodium nitrate	+	+	+	+	+	+	+	+	+	+	+
Urea	+	+	+	+	+	+	+	+	+	+	+

_
ъ
æ
_
. =
+
-
0
()
_
-
-
-
•
d)
_
-
6
_
-

Nitrogen source	PBUAP59	PBUAP61	PBUAP62	PBUAP65	PBUAP67	PBUAP70	PBUAP71	PBUAP72	PBUAP75	PBUAP76	PBUAP77
Ammonium acetate	+	+	+	+	+	+	+	+	+	+	+
Ammonium oxalate	+	+	+	+	+	+	+	+	+	+	+
Ammonium sulphate	+	+	+	+	+	+	+	+	+	+	+
Ammonium tartrate	+	+	+	+	+	+	+	+	+	+	+
L-Asparagine	+	+	+	+	+	+	+	+	+	+	+
L-Glutamic acid	+	+	+	+	+	W	+	M	+	+	W
	+	+	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+	+	+
Potassium nitrate	+	+	+	+	+	+	+	+	+	+	+
Sodium nitrite	+	+	+	+	+	+	+	+	+	+	+
Sodium nitrate	+	+	+	+	+	+	+	+	+	+	+
	+	+	+	+	+		+	•	+	+	

4.1.4 DNA amplification, sequencing and phylogenetic analysis

DNA sequences determined in this study were used for phylogenetic analyses and shown in Appendix C. ITS sequences were deposited in Genbank under accession numbers KP965436- KP965489. The phylogenetic analyses of each loci and combined trees were shown in Figure 4.2-4.6.

Data from ITS sequences classified 54 strains in to two main clades. Although 50 strains were placed in a clade with *A. melanogenum*, but ITS sequences could not differentiate individual strains in this clade. Therefore only 3 clades were found in this main clade. Besides, the others 4 strains seemed to be more closely related to *A. thailandense* (Figure 4.2). The *TUB* sequences classified all strains into 8 main clades (Figure 4.3), while *ELO* sequences classified all strains into 11 main clades (Figure 4.3), while *ELO* sequences classified all strains into 11 main clades (Figure 4.4). Therefore both locus combined and three-locus combined trees were made. (Figure 4.5-4.6). Isolate PBUAP4 was located in the group of *Aureobasidium*, but differentiated out of the main clade that related with *A. melanogenum* in all tree. Isolate PBUAP47 was located in clade 12 in the *TUB* tree, but found in combined clade of 6 and 7 in the *ELO* tree. Isolate PBUAP53 was located in clade 4 of the *TUB* tree, but separated out of the main clade that related with *A. melanogenum*. It was located in the same clade with *A. thailandense* instead.

From 54 strains of *Aureobasidium*, the 12 clades were obtained from combined data sequences of three loci. The eleven clades were located in the same clade with *A. melanogenum*, whereas one clade was located in the clade related with *A. thailandense*. Tree generated from the individual loci either was not informative (ITS) or produced trees with the same terminal groups. A branch was considered strongly supported if the bootstrap proportion was 90-100%.

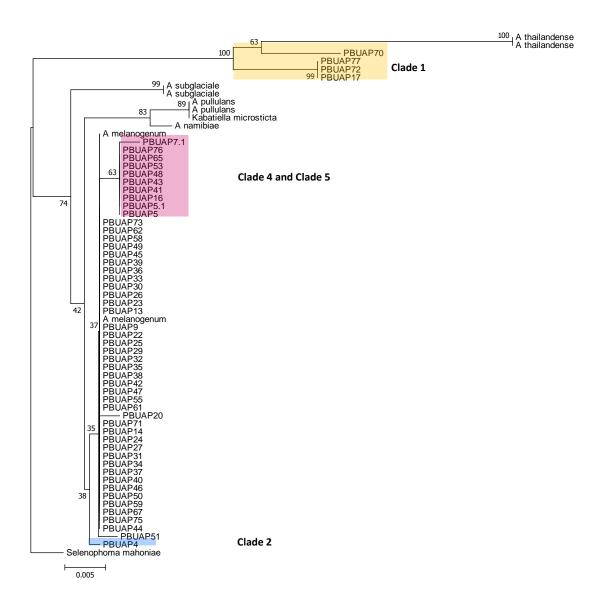


Figure 4.2 Neighbor-joining tree depicting the relationships based on the partial ITS sequences between the 54 *Aureobasidium* new strains and five standard *Aureobasidium* species. Only the branch leading to PBUAP17, PBUAP72 and PBUAP77 is strongly supported clade outside of the ingroup. Numbers on the nodes indicate bootstrap supports.

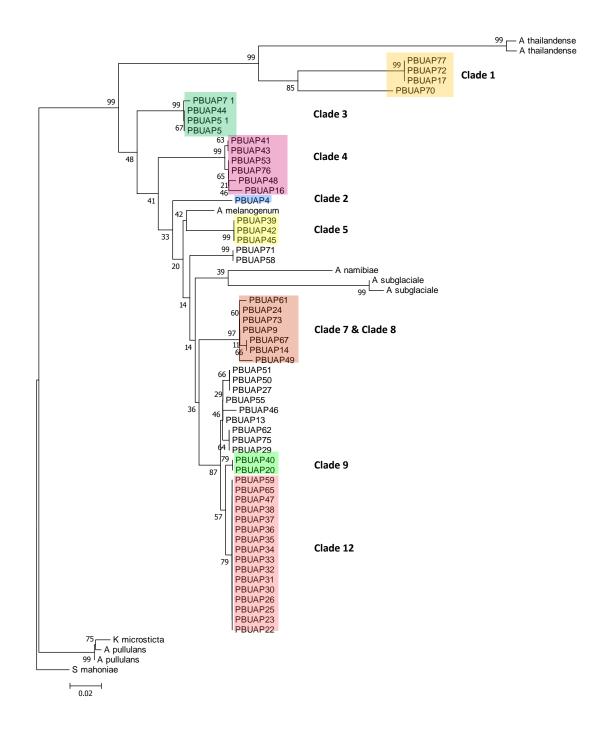


Figure 4.3 Neighbor-joining tree of *TUB* sequences between the 54 *Aureobasidium* new strains and five standard *Aureobasidium* species. Some of the terminal groups are strongly supported by statistic, deeper branches in the tree are mostly not statistically significant. Numbers on the nodes indicate bootstrap supports.

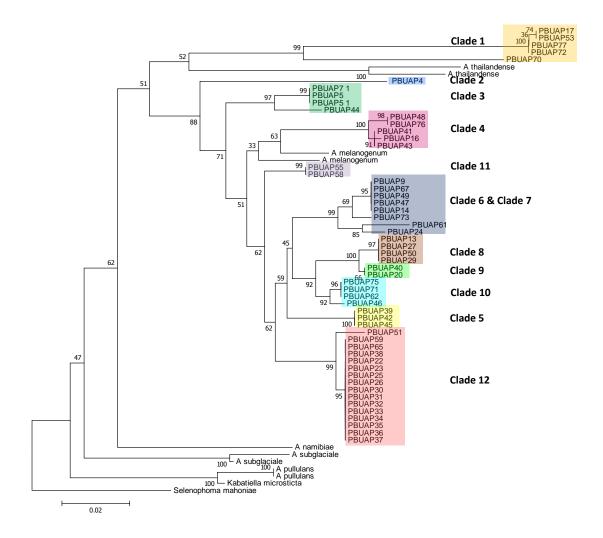


Figure 4.4 Neighbor-joining tree of *ELO* sequences between the 54 *Aureobasidium* new strains and five standard *Aureobasidium* species. All strains are formed ingroup, but related only 2 clades including *A. melanogenum* and *A. thailandense*. Most of the terminal groups are strongly supported by statistic, deeper branches in the tree are often not statistically significant. Numbers on the nodes indicate bootstrap supports.

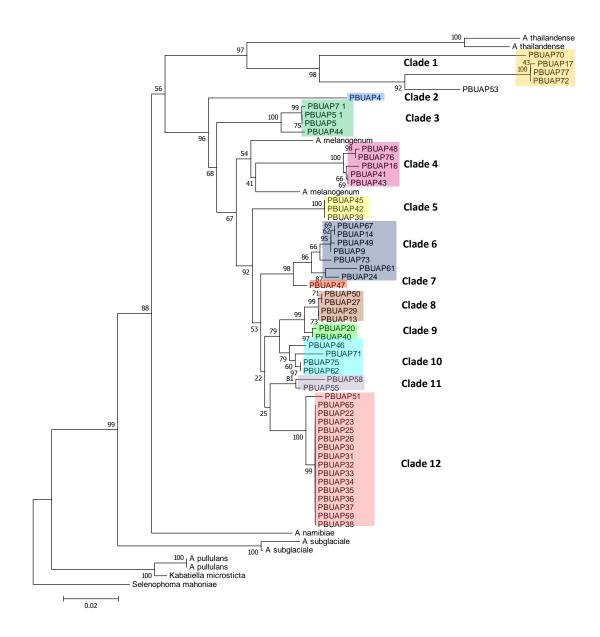
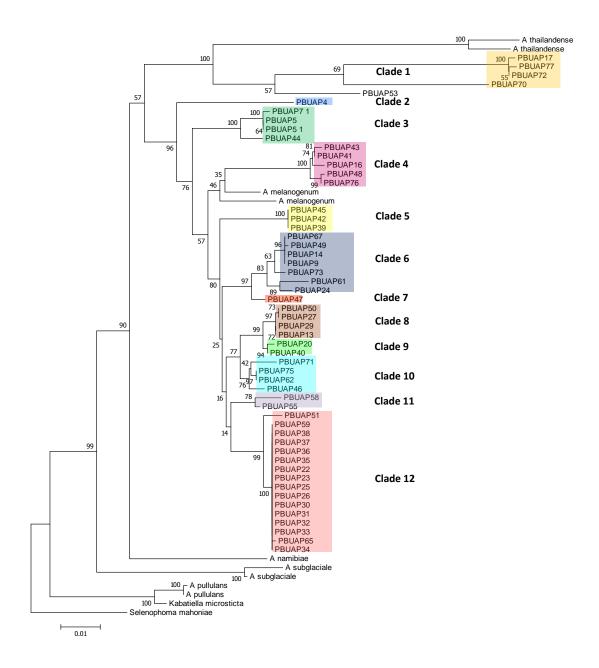
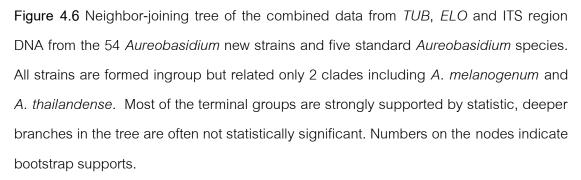


Figure 4.5 Neighbor-joining tree of the combined data from *TUB* and *ELO* sequences from the 54 *Aureobasidium* new strains and five standard *Aureobasidium* species. All strains are formed ingroup but related only 2 clades including *A. melanogenum* and *A. thailandense*. Most of the terminal groups are strongly supported by statistic, deeper branches in the tree are often not statistically significant. Numbers on the nodes indicate bootstrap supports.





The order of clades was derived from 3 locus combined tree, and 12 clades were obtained. The characters of each isolate, including morphology, EPS yield, and xylanase activity were grouped and explained the specific characters as followed.

Clade 1 (PBUAP17, PBUAP53, PBUAP70, PBUAP72, and PBUAP77) was related with *A. thailandense*. Although data from *ELO*, and combined tree showed PBUAP53 located in this clade but it was separated from the analysis from ITS or *TUB* tree. It produced reddish brown color on PDA. The strains in this clade produced β -glucan rather than pullulan.

Clade 2 (PBUAP4) consisted of only one strain. Data from all trees exhibited this strain was separated from the large group of *A. melanogenum* clade. It produced a moderate yield of pullulan and rather high activity of xylanase was detected. Its colony on MEA was white hyphae with olivaceous sporulation in the center of colony.

Clade 3 (PBUAP5, PBUAP5.1, PBUAP7.1, and PBUAP44) was a group of color variant, except PBUAP44. This clade showed color rings of pink, yellow, and orange when grown on PDA and YMA for 7 days. The color rings depended on diurnal cycles of day and night. The strains in this clade produced a high activity of xylanase, although they had low pullulan yields.

Clade 4 (PBUAP16, PBUAP41, PBUAP43, PBUAP48 and PBUAP76) with the exception of PBUAP43, represented white hyphae with dark pigment center on PDA, cream or brown color on YMA. The strains in this clade produced low levels of EPS with brown or dark color and low xylanase activity.

Clade 5 (PBUAP39, PBUAP42 and PBUAP45) represented yellow color on YMA. The production of dark pigment represented as color ring was found on PDA with white hyphae, low EPS yields was detected with black color. Xylanase activity varied depending on strains. Clade 6 (PBUAP9, PBUAP14, PBUAP49, PBUAP67, PBUAP73 and sub clade PBUAP24, PBUAP61) exhibited white hyphae with dark centers on MEA. Cultures in liquid PM were brown or dark brown, except PBUAP24 and PBUAP67 showed cream color in liquid PM instead. Nevertheless PBUAP73 produced very low EPS that could not recovered.

Clade 7 (PBUAP47) consisted of only one strain. Its colony on MEA was white hyphae with dark in the center of colony. It produced a moderate yield of pullulan and moderate xylanase activity. Cultures in liquid PM was orange and when EPS was precipitated with ethyl alcohol the supernatant exhibited a distinctive orange-red color.

Clade 8 (PBUAP13, PBUAP27, PBUAP29 and PBUAP50) exhibited white hyphae and pale pink on MEA and YMA. Only PBUAP13 represented dark centers on MEA. Xylanase activity and pullulan yield were varied. Cultures color in liquid PM were cream and white. Only PBUAP29 produced high level of pullulan with low melanin contamination, which could be beneficial in commercial pullulan production.

Clade 9 (PBUAP20 and PBUAP40) showed cream colony on YMA but dark center on PDA. Diverse results were found from xylanase activity and EPS production.

Clade 10 (PBUAP46, PBUAP62, PBUAP71 and PBUAP75) exhibited cream color on PDA, MEA and YMA. The colony color of PBUAP62 had olivaceous center on PDA. This clade produced high levels of xylanase activity. Only PBUAP46 showed the characters same as clade 7.

Clade 11 (PBUAP55 and PBUAP58) was distinctive from the others since they produced a purple-red (vinaceous) pigment on PDA, MEA and YMA. Cultures in liquid PM were orange and a high level of oil was produced. Relative high viscosity of liquid PM was obvious when culture supernatants were precipitated with ethanol. They also produced high levels xylanase activity. Clade 12 (PBUAP22, PBUAP23, PBUAP25, PBUAP26, PBUAP30, PBUAP31, PBUAP32, PBUAP33, PBUAP34, PBUAP35, PBUAP36, PBUAP37, PBUAP38, PBUAP51, PBUAP59 and PBUAP65) exhibited cream or pale pink color on MEA and YMA. The colony color on PDA had brown at the center of colony. Only PBUAP51 had dark pigment on PDA. However PBUAP59 and PBUAP65 exhibited cream and slimy with dark sector on YMA and MEA. This clade produced moderate to high levels of xylanase activity. Pullulan yield was varied depending on each strain.

4.2 Characterization by phenotypic analysis

4.2.1 EPS production and analysis

The result of the EPS produced by all strains and the analysis was shown in Table 4.5. Among 54 isolates, EPS yield ranged from 0.4 to 31.86 gl⁻¹ and ranked into three levels including high, moderate and low. EPS color and appearance varied depending on the strains. Both pullulan and β -glucan were found. Almost all isolates produced only pullulan or β -glucan, except PBUAP24, PBUAP38, PBUAP41, and PBUAP44 produced both EPS type. However, the strain PBUAP73 and PBUAP77 produced EPS in very small amount that noticeable but non detectable. Unidentified EPS was found and exhibited dark color of pigmentation in strain PBUAP14, PBUAP16, PBUAP39, PBUAP55, PBUAP58, and PBUAP67. PBUAP34 is the highest EPS producer (31.86±0.77 gl⁻¹) after cultured using 5% sucrose medium for 7 days at 30°C with agitation. The solubility ability of each EPS was different depending on strains and solvents.

When pullulan powder was tested, pullulanase activity was detected in the content of reducing sugars after pullulanase treatment, whereas non-detectable activity was detected from treatment with β -glucanase and *vice versa* the opposite result was found in β -glucan. Variable result was found in unidentified EPS and both pullulanse and β -glucanase activity were also found. The analysis of the structure of EPS by FT-IR spectroscopy exhibited the presence of α -configuration compared with pullulan

standard produced from *A. pullulan* NRRL 58560, with wavenumber at 850 cm⁻¹. On the other hand, β -glycosidic bond exhibited the presence of β -configuration compared with β -glucan produced from *A. pullulan* NRRL 58013, with wavenumbers at 890 cm⁻¹. For unidentified EPS, although the activity of pullulanase and β -glucanase were detected, both α and β -configuration of this EPS type were absent.



จุฬาลงกรณ์มหาวิทยาลัย Chulalongkorn University

Checks	EPS	EPS production	Sensit	Sensitivity (%)	Appearance	rance	Water solubility	NaOH solubility
otrain	Type	٩	Pullulanase	β-Glucanase	Color	Character	(25°C)	(0.1M)
PBUAP4	٩	15.71 ± 0.52	89.86	QN	white	hard	easily soluble	easily soluble
PBUAP5	٩	4.04 ± 0.46	79.75	QN	white	hard	insoluble	easily soluble
PBUAP5.1	٩	5.76 ± 0.64	61.89	13.5	white	hard	easily soluble	swells
PBUAP7.1	٩	4.03 ± 0.16	73.01	QN	White	hard	insoluble	insoluble
PBUAP9	٩	5.54 ± 0.40	75.26	QN	brown	hard-sticky	incompletely soluble	easily soluble
PBUAP13	٩	12.71 ± 0.91	87.62	Q	cream	fragile	easily soluble	easily soluble
PBUAP14	z	1.07 ± 0.05	64.03	9.6	brown	fragile	incompletely soluble	essily soluble
PBUAP16	z	1.00 ± 0.06	82.00	7.07	dark-grey	fragile	insoluble	easily soluble
PBUAP17	8	0.8 + 0.03	10.77	71.15	brown	fragile	insoluble	swells
PBUAP20	٩	6.98 ± 0.08	62.90	Q	cream	fragile	insoluble	easily soluble
PBUAP22	٩	0.8 ± 0.03	83.12	Q	dark-brown	fragile	incompletely soluble	easily soluble
PBUAP23	٩	15.07 ± 0.31	70.77	QN	dark-brown	fragile	incompletely soluble	essily soluble
PBUAP24	P&B	3.56 ± 1.01	80.88	9.6	cream	fragile	easily soluble	easily soluble
PBUAP25	٩	17.69 ± 0.60	83.12	QN	brown	fragile	easily soluble	easily soluble
PBUAP26	٩	20.2 ± 0.81	78.63	QN	brown	fragile	incompletely soluble	easily soluble
PBUAP27	٩	9.34 <u>+</u> 0.19	77.51	QN	white	fragile	insoluble	easily soluble
= 4 *	*P = pullulan,	$B = \beta$ -gluc	an, P&B = pu	ullulan and B-g	pullulan and B-glucan, N= unidentified EPS,	dentified EPS,	ND = non detectable	0

Table 4.5 EPS production and its properties from Aureobasidium spp.

atroit of	Ğ	EPS production	Sensit	Sensitivity (%)	Appearance	ance	Water solubility	NaOH solubility
ouall	Type	9,	Pullulanase	B-Glucanase	Color	Character	(25°C)	(0.1M)
PBUAP29	۹.	22.37 ± 0.77	86.49	QN	white	fragile	incompletely soluble	easily soluble
PBUAP30	٩	17.65 ± 0.44	80.88	QN	cream	fragile	easily soluble	easily soluble
PBUAP31	۹.	20.70 ± 0.58	84.25	QN	cream	fragile	easily soluble	easily soluble
PBUAP32	۹.	27.31 ± 0.56	78.63	Q	cream	fragile	easily soluble	easily soluble
PBUAP33	٩	29.65 ± 1.81	74.14	QN	dark-brown	fragile	incompletely soluble	easily soluble
PBUAP34	۵.	31.86 ± 0.77	83.12	QN	cream-brown	fragile	easily soluble	easily soluble
PBUAP35	٩	22.80 ± 0.10	77.51	QN	cream	fragile	easily soluble	easily soluble
PBUAP36	٩	22.95±0.79	79.75	QN	cream	fragile	easily soluble	easily soluble
PBUAP37	٩	16.55 ± 0.52	82.00	QN	cream	fragile	easily soluble	easily soluble
PBUAP38	P & B	22.50 ± 1.12	88.74	19.2	cream	fragile	easily soluble	easily soluble
PBUAP39	z	0.97 ± 0.04	61.78	23.1	black	fragile-fine	insoluble	incompletely soluble
PBUAP40	•	3.43 <u>+</u> 0.18	10.77	71.89	black	fragile-fine	insoluble	easily soluble
PBUAP41	P & B	0.85 ± 0.20	85.8	55.8	dark-brown	fragile	insoluble	easily soluble
PBUAP42	•	1.34 ± 0.12	11.78	93.8	black	fragile-fine	insoluble	insoluble
PBUAP43	٩	1.40 ± 0.28	76.96	Q	cream	fragile	incompletely soluble	incompletely soluble
PBUAP44	P & B	5.72 ± 0.07	66.27	23.1	cream	fragile	insoluble	easily soluble

Table 4.5 (continued)

Atomica	EPS	EPS production	Sensitivity	(%) (July	Appearance	allice	Water solubility	NaOH solubility
ouall	Type	<u>و</u>	Pullulanase	B-Glucanase	Color	Character	(25°C)	(0.1M)
PBUAP45	٩	0.80 ± 0.15	59.53	13.8	black	fragile-fine	insoluble	insoluble
PBUAP46	٩	16.01 ± 0.46	86.49	QN	orange-cream	fragile	easily soluble	easily soluble
PBUAP47	٩	15.80 ± 0.98	77.51	29.2	orange-cream	fragile	incompletely soluble	easily soluble
PBUAP48	٩	0.93 ± 0.03	65.15	QN	dark-brown	fragile-fine	insoluble	incompletely soluble
PBUAP49	٩	3.45 ± 0.24	69.64	QN	dark-brown	fragile	insoluble	incompletely soluble
PBUAP50	٩	14.55 ± 0.30	88.74	QN	cream	fragile	insoluble	easily soluble
PBUAP51	٩	13.17 ± 1.12	79.75	QN	Jce/	fragile	incompletely soluble	easily soluble
PBUAP53	•	0.40 ± 0.06	12.09	17.77	cream	fragile	easily soluble	incompletely soluble
PBUAP55	z	12.37 ± 0.36	68.52	29.92	cream-brown	fragile	insoluble	swells
PBUAP58	z	5.99 ± 0.23	65.15	12.5	grey-black	fragile	insoluble	swells
PBUAP59	٩	12.77 ± 0.68	77.51	QN	brown	hard	incompletely soluble	easily soluble
PBUAP61	٩	7.45 ± 0.82	91.10	QN	brown-grey	hard	incompletely soluble	easily soluble
PBUAP62	۵.	9.20 ± 0.54	80.88	QN	cream	hard	incompletely soluble	easily soluble
PBUAP65	٩	12.23 ± 1.22	67.40	Q	dark-brown	fragile-fine	insoluble	incompletely soluble
PBUAP67	z	1.62 ± 0.06	61.78	26.9	cream	hard-fine	incompletely soluble	easily soluble

ð
S
2
Ш
Z
S EPS,
ĕ
1
ē
<u>9</u>
5
Ľ
Ż
ć
ğ
3
Ō
d.
p
В
-
1
đ
П
- 0ă
ã
ć
g
ž
- Ö
à.
ш
8
B
- B
5
0
Ш
0

Table 4.5 (continued)

Type gl^{r1} Pullulanse β -Glucanse Color Character 25° CC 770 B 2.96 ± 0.64 11.78 82.88 cream-brown fragile-fine insoluble 10^{-1} 771 P $16.67 \pm$ 79.75 ND brown fragile-fine insoluble 10^{-1} 772 B 0.70 ± 0.05 18.41 84.23 black fragile-fine insoluble 10^{-1} 773 ND 0.00 0.00 0.00 16.27 80.00 16.24^{-1} 16.27^{-1} 16.27^{-1} 16.27^{-1} 16.27^{-1} 16.27^{-1} 16.27^{-1} 16.27^{-1} 16.27^{-1} 16.27^{-1} 16.27^{-1} 16.27^{-1} 116^{-1} 118^{-1} 118^{-1} 118^{-1} 118^{-1} 118^{-1} 118^{-1} 118^{-1} 118^{-1} 118^{-1} 118^{-1} 118^{-1} 118^{-1} 118^{-1} 118^{-1} 118^{-1} 118^{-1} 118^{-1} 118^{-1} 118^{-1}	Chain	Ξ	EPS production	Sensit	Sensitivity (%)	Appea	Appearance	Water solubility	NaOH solubility
B 2.96±0.64 11.78 82.88 cream-brown fragile-fine insoluble P 15.67± 79.75 ND brown fragile-fine insoluble 4 B 0.70±0.05 18.41 84.23 black fragile-fine insoluble 4 ND - 0.00 0.00 - - - - ND - 0.00 0.00 black fragile-fine insoluble 4 ND - 0.00 0.00 - - - - ND - 0.00 0.00 - - - - ND - 0.00 0.00 - - - - ND - 0.00 0.00 0.00 - - - ND - 0.00 0.00 - - - - ND - 0.00 0.00 - - - <td< th=""><th>OUBID</th><th>Type</th><th></th><th>Pullulanase</th><th>B-Glucanase</th><th>Color</th><th>Character</th><th>(25°C)</th><th>(0.1M)</th></td<>	OUBID	Type		Pullulanase	B-Glucanase	Color	Character	(25°C)	(0.1M)
P 15.67 ± 79.75 ND brown fragile insoluble B 0.70 ± 0.05 18.41 84.23 black fragile-fine insoluble ND - 0.00 0.00 - - - B 1.64 ± 0.12 16.27 80.00 black fragile insoluble ND - 0.00 0.00 brown hard-sticky insoluble ND - 0.00 0.00 0.00 - - - ND - 0.00 0.00 - - - - ND - 0.00 0.00 - - - - ND - 0.000 0.00 - - - - ND - 0.000 ND insoluble insoluble - ND 0.000 0.00 - - - - - P 2.08 ± 0.23 66.	PBUAP70	•	2.95 ± 0.64	11.78	82.88	cream-brown	fragile-fine	insoluble	insoluble
B 0.70 ± 0.05 18.41 84.23 black fragile-fine insoluble ND - 0.00 0.00 - - - B 1.64 ± 0.12 16.27 80.00 black fragile-fine insoluble P 3.45 ± 0.21 66.27 ND brown hard-sticky insoluble ND - 0.00 0.00 - - - ND 3.45 ± 0.21 66.27 ND brown hard-sticky insoluble ND - 0.00 0.00 - - - ND - 0.00 16.27 ND brown insoluble ND 2.0.8 ± 0.23 66.27 ND brown-grey fragile easily soluble P 2.08 \pm 0.240.51 100.00 ND indotegrey fragile P 2.08 \pm 0.210 100.00 ND indotegrey fragile easily soluble P 2.00 \pm 0.13 </td <td>PBUAP71</td> <td>٩</td> <td>15.67 ±</td> <td>79.75</td> <td>Q</td> <td>brown</td> <td>fragile</td> <td>insoluble</td> <td>easily soluble</td>	PBUAP71	٩	15.67 ±	79.75	Q	brown	fragile	insoluble	easily soluble
ND - 0.00 0.00 - - - B 1.64 ± 0.12 16.27 80.00 black fragile insoluble P 3.45 ± 0.21 66.27 ND brown hard-sticky insoluble ND - 0.00 0.00 - - - A P 33.76 ± 0.24 100.00 ND hard-sticky insoluble P 33.76 ± 0.64 100.00 ND hard-sticky hard-sticky insoluble P 23.76 ± 0.64 100.00 ND hard-sticky hard-sticky insoluble P 2.08 ± 0.23 66.27 ND hard-sticky fragile essily soluble P 2.08 ± 0.23 66.27 ND hard-steck fragile essily soluble P 2.08 ± 0.23 66.27 ND hard-steck fragile essily soluble P 2.08 ± 0.26 100.00 ND indoteck frag	PBUAP72	•	0.70 ± 0.05	18.41	84.23	black	fragile-fine	insoluble	insoluble
B 1.64 ± 0.12 16.27 80.00 black fragile insoluble P 3.45 ± 0.21 66.27 ND brown hard-sticky insoluble ND - 0.00 0.00 - - - A P 33.76 ± 0.64 100.00 ND ight-grey hard-sticky insoluble P 33.76 ± 0.64 100.00 ND light-grey hard easily soluble P 2.08 ± 0.23 66.27 ND brown-grey fragile incompletely soluble P 2.08 ± 0.23 66.27 ND brown-grey fragile incompletely soluble P 2.08 ± 0.23 66.27 ND brown-grey fragile incompletely soluble P 2.08 ± 0.201 100.00 ND brown-grey fragile incompletely soluble B 3.60 ± 0.57 100.00 ND indut-cream fragile incompletely soluble Illulan, $B = \beta$ -	PBUAP73	9		0.00	00:0				
P 3.45 ± 0.21 66.27 ND brown hard-stickly insoluble ND - 0.00 0.00 - - - A P 33.76 ± 0.64 100.00 ND light-grey hard easily soluble P 33.76 ± 0.64 100.00 ND light-grey hard easily soluble P 2.08 ± 0.23 66.27 ND brown-grey fragile incompletely soluble P 2.08 ± 0.23 66.27 ND brown-grey fragile incompletely soluble P 2.08 ± 0.201 100.00 ND light-cream fragile incompletely soluble B 3.60 ± 0.57 13.01 100.00 black fragile incompletely soluble MILah, $B = \beta$ -glucan, P& sulfulan and β -glucan, $N = unidentified EPS$, ND = non detectable	PBUAP75	•	1.64 ± 0.12	16.27	80.00	black	fragile	insoluble	insoluble
	PBUAP76	٩	3.45±0.21	66.27	QN	brown	hard-sticky	insoluble	insoluble
4P 33.76 ± 0.64 100.00NDlight-greyhardeasily solubleP 2.08 ± 0.23 66.27 NDbrown-greyfragileincompletely solubleP 25.00 ± 0.10 100.00NDlight-creamfragileeasily solubleB 3.60 ± 0.57 13.01100.00blackfragileincompletely solubleullulan,B = β -glucan,P& = pullulan and β -glucan,N= unidentified EPS,ND = non detectable	PBUAP77	9		0.00	00:0				
P 2.08 ± 0.23 66.27 NDbrown-greyfragileincompletely solubleP 25.00 ± 0.10 100.00 NDlight-creamfragileeasily solubleB 3.60 ± 0.57 13.01 100.00 blackfragileincompletely solubleullulan,B = β -glucan,P&B = pullulan and β -glucan,N= unidentified EPS,ND = non detectable	NRRLY12974	٩	33.76 ± 0.64	100.00	QN	light-gre/	hard	easily soluble	essily soluble
P25.00 ± 0.10100.00NDlight-creamfragileeasily solubleB 3.60 ± 0.57 13.01100.00blackfragileincompletely solubleullulan,B = β -glucan,P&B = pullulan and β -glucan,N= unidentified EPS,ND = non detectable	NRRL58561	٩	2.08 ± 0.23	66.27	QN	brown-grey	fragile	incompletely soluble	incompletely soluble
B 3.60 ± 0.57 13.01 100.00 black fragile incompletely soluble ullulan, B = β -glucan, P&B = pullulan and β -glucan, N= unidentified EPS, ND = non detectable	NRRL58560	٩	25.00 ± 0.10	100.00	QN	light-cream	fragile	easily soluble	essily soluble
	NRRL58013	•	3.60 ± 0.57	13.01	100.00	black	fragile	incompletely soluble	incompletely soluble
	*P = pull	ulan,	$B = \beta$ -glucan,	P&B = pullul	an and β-glucs	an, N= unider	ntified EPS,	ND = non detectable	

(continued)	
Table 4.5	

4.2.2 Multiple stress tests

For halotolerance test, all strains in standard condition (PDA at 30°C) occurred within 2 days with cream or light pink at the beginning, later they became darker, except color variant strains. All strains grew in the presence of NaCl but a different behavior among the strains exhibited with different salt concentrations. The percentages of reduction of colony diameter due to different concentrations of NaCl were shown in Figure 4.7. Some strains that grew on 15 % NaCl showed changes in the morphology with respect to their growth in PDA without addition of NaCl (data not shown).

Osmotolerance ability of *Aureobasidium* spp. was compared by using their growth on YMA supplemented with glucose. The comparison showed that all strains tolerate all concentration of glucose but slightly decreased in 30 and 50 %. In contrast, the highest relative growth was found in 5 % of glucose in comparison with the others concentration. The strain PBUAP55, PBUAP58 and PBUAP72 were only three strains that gave high relative growth (%) up more 100 in this concentration (Figure 4.8).

For thermotolerance test, growth at various temperatures (30, 35, 40° C) was tested and the result shown in Figure 4.9. After 7 days of incubation, all strains of *Aureobasidium* spp. grew optimally at 30° C and slightly decreased at 35° C. They formed visible colonies with smaller size on the agar medium at high temperature. In contrast, no growth was observed in all strain when the temperature was adjusted at 40° C.

Growth in 2% MEA at various temperatures exhibited diverse result depending on the strains. Most of the strains grew well in acidic conditions at pH 3 and 5. Among 54 strains, 14 strains were found to grow very well in pH 9 (Figure 4.10).

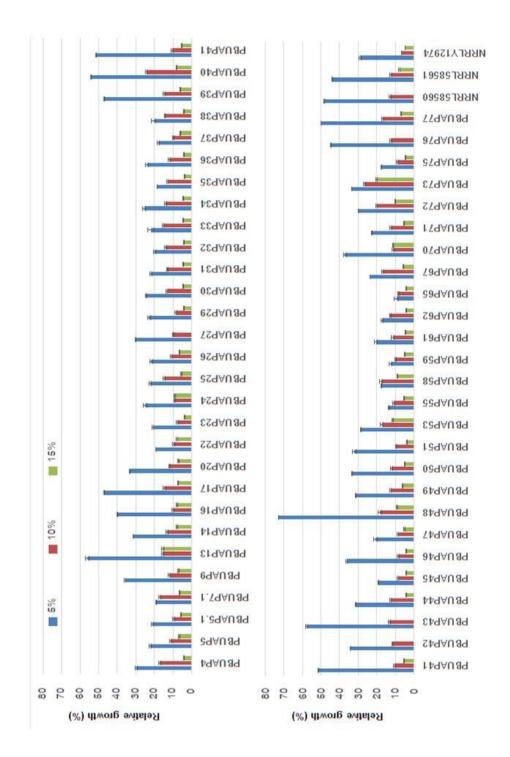


Figure 4.7 Relative growth (%) of 54 new strains of *Aureobasidium* spp. from coastal and three standard *Aureobasidium* species grown on PDA supplemented with different concentrations of NaCl (5 %, 10 %, 15 % w/v) at 30°C for 7 day, compared with the growth in PDA without addition of NaCl.

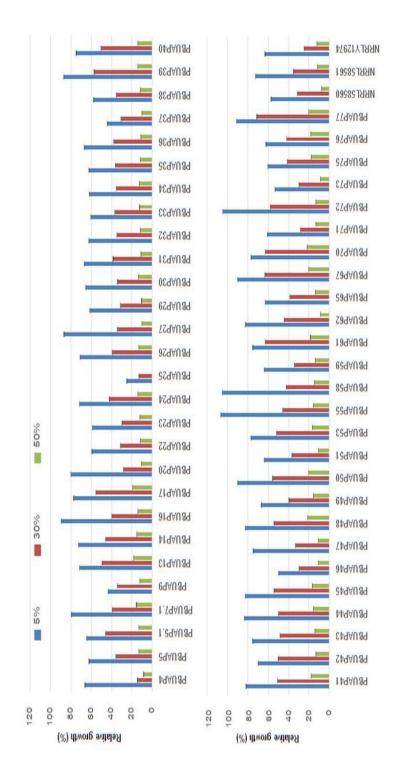


Figure 4.8 Relative growth (%) of 54 new strains of *Aureobasidium* spp. from coastal and three standard *Aureobasidium* species grown on YMA supplemented with different concentrations of glucose (5 %, 30 %, 50 % w/v) at 30° C for 7 day, compared with the growth on YMA with addition of 1% glucose.

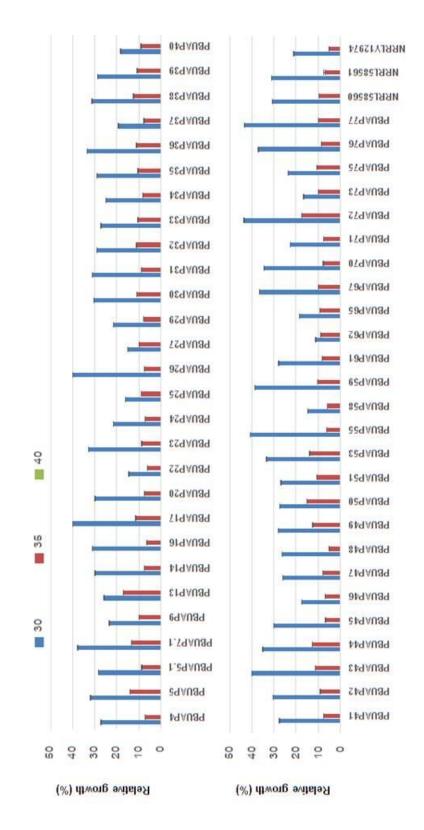


Figure 4.9 Relative growth (%) of 54 new strains of *Aureobasidium* spp. from coastal and three standard *Aureobasidium* species grown on 2% MEA incubated at various temperatures (30[°]C, 35[°]C, 40[°]C) for 7 day.

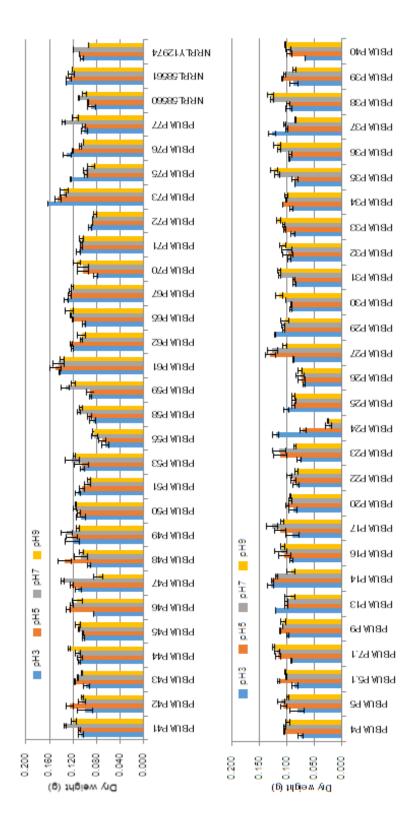


Figure 4.10 Relative growth (%) of 54 new strains of *Aureobasidium* from coastal and three standard *Aureobasidium* species grown in 2% MEB with different pH values (pH3, pH5, pH7, pH9) at 30^oC, 70 rpm, for 1 month.

4.2.3 Associations among halotolerance, osmotolerance, and EPS production

4.2.3.1 Effects of sucrose concentration

To investigate how A. melanogenum strains with different halotolerance, osmotolerance and EPS production would respond to elevating osmotic stress, i.e. sucrose concentration, three strains with different ability from Table 4.6 were selected, PBUAP13 (moderately halotolerant and moderately osmotolerant with moderate EPS production), PBUAP34 (relatively halo- and osmointolerant with high EPS production) and PBUAP50 (relatively halointolerant and moderately osmotolerant with moderate EPS production). Based on FT-IR analysis and enzyme sensitivity test, the EPS produced by these three strains was pullulan. Their EPS production was compared using the production medium and culture condition that were optimal for most Thai A. pullulans and A. melanogenum strains. The strains were grown in media containing sucrose 5 to 20 % (w/v). Responses to increasing osmotic stress were observed as relative growth (% of those grown in 5 % (w/v) sucrose) and relative conversion (% of those grown in 5 % (w/v) sucrose). Significantly higher growth (P < 0.05) were found in the moderately tolerant strains (PBUAP13 and 50) than the relatively intolerant strain (PBUAP34) at sucrose concentration of 15 % (w/v) and higher (Figure 4.11A). Similar changes in growth were found between the two moderately tolerant strains in that their cell dry weights increased when the sucrose concentration was raised from 5 % to 15 %(w/v). At 20 % (w/v) sucrose, a slight decrease in growth was observed in both tolerant strains, but the cell dry weights were still significantly higher than those at 5 % (w/v) On the contrary, significant growth inhibition occurred in the relatively sucrose. intolerant strain when the sucrose concentration reached 20 % (w/v). In contrast to growth, both moderately tolerant strains lost their EPS production efficiency very quickly when the sucrose concentration was increased higher than 5 % (w/v). At 20 % (w/v) sucrose, the conversion efficiency of PBUAP13 and 50 were 38.3±1.2 and 38.5±3.4 % of those at 5 % (w/v) sucrose, respectively (Figure 4.11B). The relatively intolerant strain also lost its EPS production, but not as drastically as the moderately tolerant strains. At 20 % (w/v) sucrose, the conversion efficiency of PBUAP34 was 45.0 ± 0.8 % of that at 5 % (w/v) sucrose (Figure 4.11). Significantly higher conversion efficiency was observed in the relatively intolerant strain than the two moderately tolerant strains at all sucrose concentrations higher than 5 % (w/v).

4.2.3.2 Detection of intracellular osmolyte

Cellular accumulation of mannitol was detected in all three strains tested, PBUAP13, 34 and 50 (Figure 4.12). In a medium without osmotic stress (1 % (w/v) sucrose), low amounts of mannitol were detected in all strains tested. The amount of mannitol accumulation in the moderately halotolerant and osmotolerant PBUAP13 was not visibly changed even when sucrose concentration was raised from 5 % to 20 % In the relatively halotolerant and osmointolerant PBUAP34, accumulation of (w/v). mannitol increased at 15 % (w/v) sucrose and higher. Accumulation of mannitol in the moderately osmotolerant but relatively halointolerant PBUAP50 was apparently a direct response to the increasing sucrose concentration. No glycerol was found in any strains and at any sucrose concentrations tested. The patterns of mannitol accumulated in all three strains were different, and it did not correlate with their tolerance properties. For example, although PBUAP13 was moderately tolerant to both salt and sugar, its mannitol accumulation did not change even when sucrose concentration reached 20 % (w/v). On the other hand, in the relatively intolerant strain PBUAP34, accumulation of mannitol increased at the highest concentration of sucrose (Figure 4.12). The other tolerance mechanisms must also contribute to the differences in halotolerance and osmotolerance among these strains.

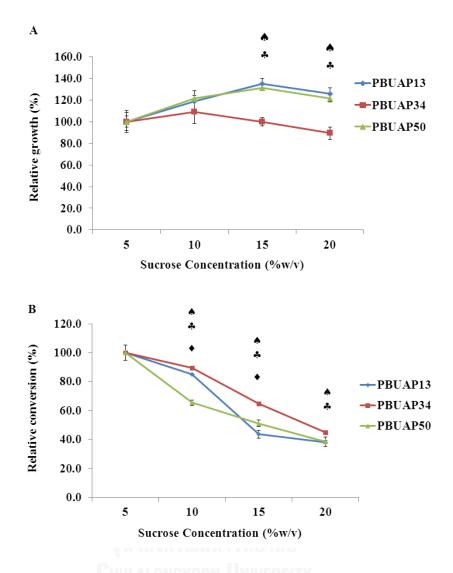


Figure 4.11 Effect of sucrose concentration on growth and comparison to its efficiency of conversion. (A) Relative growth (compared with growth in the medium containing 5% (w/v) of sucrose) and (B) relative conversion (compared with conversion in the medium containing 5% (w/v) of sucrose) of *A. melanogenum* strains PBUAP13, 34 and 50. All strains were grown in production medium containing sucrose at concentrations of 5 - 20% (w/v) at $30\pm2^{\circ}$ C with 150-rpm agitation for five days. The symbols: \bigstar indicates significant difference between PBUAP13 and 34, \clubsuit indicates significant difference between PBUAP13 and 50 and \bigstar indicates significant difference between PBUAP13 and 50.

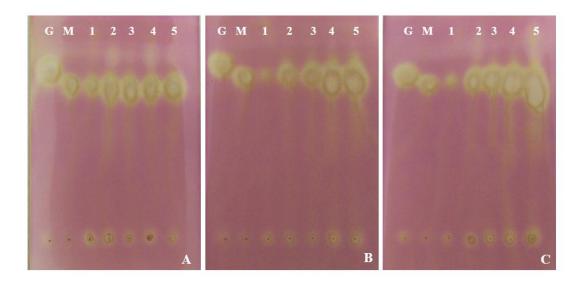


Figure 4.12 Cellular extracts of *A. melanogenum* analyzed by thin layer chromatography. *A. melanogenum* strains were grown in production medium containing various concentration of sucrose at $30\pm2^{\circ}$ C with 150-rpm agitation for five days. (A) PBUAP13, (B) PBUAP34 and (C) PBUAP50. Lane G: glycerol (0.01 mg), Lane M: mannitol (0.01 mg), Lanes 1-5: extracts of cells grown in media containing 1, 5, 10, 15 and 20 % (w/v) sucrose, respectively.

4.2.3.3 Associations among halotolerance, osmotolerance, and EPS

production

When 50 strains of *A. melanogenum* and four strains of *A. thailandense* were tested for their halotolerance, osmotolerance and EPS production, a wide variation among the three properties was observed (Table 4.6). Overall, the strains tested seemed to be less tolerant to ionic osmotic (salt) stress than non-ionic osmotic (sugar) stress as severe growth inhibition (less than 20 % relative growth) was observed at NaCl concentration of 10 % (w/v) (~1.7 M) whereas most strains retained more than 30 % relative growth when grown in a medium containing 30 % (w/v) (~1.67 M) glucose. At 50 % (w/v) glucose, growth of all strains was strongly inhibited with less than 22 % relative growth observed. A notably halotolerant strain PBUAP48 showed 70 % relative growth when grown in 5 % (w/v) NaCl whereas the highly osmotolerant strains

included PBUAP61, 67, 70 and 77 with more than 60 % relative growth in the medium containing 30 % (w/v) glucose. There was no apparent association (P = 0.249) between halotolerance and the direct exposure to salt water since some strains isolated from plant leaves and rock surfaces in the intertidal zone were relatively halointolerant. For EPS production, some strains were overproducers with more than 50 % conversion rate whereas many strains did not produce detectable EPS. To determine if there were associations among these three properties of the 50 A. melanogenum strains, Fisher's exact test was used and significant associations were found between halotolerance vs osmotolerance (P = 0.004), halotolerance vs EPS production (P = 0.049) and osmotolerance vs EPS production ($P \ll 0.001$). Highly to moderately halotolerant strains were found to be moderately osmotolerant. However, highly osmotolerant strains might or might not be halotolerant. Tolerant strains against either salt or sugar stress produced low to moderate EPS yields (less than 10 % to 30 % conversion). Strains relatively intolerant and intolerant to high salt and/or sugar concentration varied widely in their EPS production, exhibiting % conversion in a range of undetectable to more than 60 %. The four A. thailandense strains exhibited similar trend regarding associations among halotolerance, osmotolerance and EPS production. These four were too small a number to be statistically analyzed. Two of the A. thailandense-like strains (PBUAP17 and 77) were moderately halotolerant and highly to moderately osmotolerant (Table 4.6) whereas the other two strains (PBUAP70 and 72) were highly to moderately osmotolerant but relatively halointolerant (Table 4.7). All four strains produced EPS in very low amounts.

Table 4.6 Halotolerance, osmotolerance, and EPS production of *Aureobasidium* strains. Cultures were grown on agar media containing either 5% (w/v) NaCl or 30% (w/v) glucose, and in liquid EPS production medium containing 5% (w/v) sucrose, respectively.

Strain	Halotolerance	Osmotolerance	Conversion efficiency (%)
PBUAP48	++++	+++	L (1.9)*
PBUAP13	+++	+++	M (25.4)
PBUAP16	+++	+++	L (2.0)
PBUAP17	+++	+++	L (1.6)
PBUAP39	+++	+++	L (1.9)
PBUAP40	+++	+++	L (6.9)
PBUAP41	+++	+++	L (1.7)
PBUAP43	+++	+++	L (2.8)
PBUAP76	+++	+++	L (6.9)
PBUAP77	+++	++++	L (ND)
PBUAP4	++	+	RH (31.4)
PBUAP5	++	++	L (8.1)
PBUAP5.1	++	+++	RL (11.5)
PBUAP9	++	++	RL (11.1)
PBUAP14	++ /	(****)	L (2.1)
PBUAP20	++	++	RL (13.9)
PBUAP23	++	++	RH (30.1)
PBUAP24	++	+++	L (7.1)
PBUAP25	++	+	RH (35.3)
PBUAP26	++ จุฬาลงกรถ	นมหุาวทยาลย	H (40.4)
PBUAP27	+€HULALONGK	ORN++UNIVERSITY	RL (18.7)
PBUAP29	++	++	H (44.7)
PBUAP30	++	++	RH (35.3)
PBUAP31	++	++	H (41.4)
PBUAP32	++	++	EH (54.6)

Halotolerance: ++++ = relative growth \geq 60 %, +++ = relative growth <60-40 %, ++ = relative growth <40-20%, + = relative growth <20%

Osmotolerance: ++++ = relative growth \geq 60 %, +++ = relative growth <60-40 %, ++ = relative growth <40-20%, + = relative growth <20%

Conversion efficiency of EPS production: EH = extremely high (\geq 50 %), H = high (<50-40 %), RH = relatively high (<40-30 %), M = moderate (<30-20 %), RL = relatively low (<20-10 %), L = low (<10 %)

* Number in parentheses indicates averaged % conversion, ND = not detectable

Table 4.6 (continued)

Strain	Halotolerance	Osmotolerance	Conversion efficiency (%)
PBUAP33	++	++	EH (59.1)
PBUAP34	++	++	EH (63.7)
PBUAP36	++	++	H (45.9)
PBUAP38	++	++	H (45.0)
PBUAP42	++	+++	L (2.7)
PBUAP44	++	+++	RL (11.4)
PBUAP46	++	++	RH (32.0)
PBUAP47	++	++	RH (31.6)
PBUAP49	++	++	L (6.9)
PBUAP50	++	+++	M (29.1)
PBUAP51	++	++	M (26.3)
PBUAP53	++	+++	L (0.8)
PBUAP61	++	++++	RL (14.9)
PBUAP67	++	++++	L (3.2)
PBUAP70	++	++++	L (5.9)
PBUAP71	++	++	RH (31.3)
PBUAP72	++	+++	L (1.4)
PBUAP73	++	++	L (ND)
PBUAP7.1	+	++	L (8.1)
PBUAP22	+	++	L (1.6)
PBUAP35	+	++	H (45.6)
PBUAP37	+	++	RH (33.1)
PBUAP45	+	+++	L (1.6)
PBUAP55	+ จหาองกรถ	<i>แ</i> ้นห่าวทยาลัย	M (24.7)
PBUAP58	+	+++	RL (11.9)
PBUAP59	+CHULALONGK	ORN++JNIVERSITY	M (25.5)
PBUAP62	+	+++	RL (18.4)
PBUAP65	+	++	M (24.4)
PBUAP75	+	+++	L (3.3)

Halotolerance: ++++ = relative growth \geq 60 %, +++ = relative growth <60-40 %, ++ = relative growth <40-20%, + = relative growth <20%

Osmotolerance: ++++ = relative growth \geq 60 %, +++ = relative growth <60-40 %, ++ = relative growth <40-20%, + = relative growth <20%

Conversion efficiency of EPS production: EH = extremely high (\geq 50 %), H = high (<50-40 %), RH = relatively high (<40-30 %), M = moderate (<30-20 %), RL = relatively low (<20-10 %), L = low (<10 %)

* Number in parentheses indicates averaged % conversion, ND = not detectable

4.2.4 Screening of antifungal activity

The potential strains of *Aureobasidium* for antifungal agent production were screened. The zone of inhibition (red color) to ward *Aspergillus* spp. on co-culture plate ranged from 3–10 mm (Figure 4.13). Among 54 strains, only strain PBUAP47 showed a powerful antifungal activity that inhibited both strains of *A. niger* and *A. fumigatus*. However, the strains PBUAP5, 7.1, 48, and 76 exhibited antifungal activity against *A. niger*, the strains PBUAP55, 58, 72, and 73 exhibited antifungal activity against *A. fumigatus* (Table 4.7).

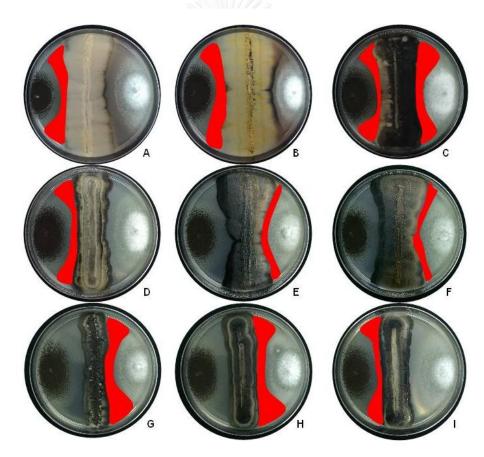


Figure 4.13 Visual agar plate assay showed screen identification of antifungal activity of PBUAP5(A), PBUAP7.1(B), PBUAP47(C), PBUAP48(D), PBUAP55(E), PBUAP58(F), PBUAP72(H), PBUAP73(I), and PBUAP76(J) against *A. niger* (left) and *A. fumigatus* (right).

Strain	Inhibition of	fungal growth	Strain	Inhibition of	fungal growth
Strain	A. niger	A. fumigatus	Juan	A. niger	A. fumigatus
PBUAP4	-	-	PBUAP40	-	-
PBUAP5	+	-	PBUAP41	-	-
PBUAP5.1	-	-	PBUAP42	-	-
PBUAP7.1	+	-	PBUAP43	-	-
PBUAP9	-	-	PBUAP44	-	-
PBUAP13	-	-	PBUAP45	-	-
PBUAP14	-	-	PBUAP46	-	-
PBUAP16	-	Alline -	PBUAP47	+	+
PBUAP17	-		PBUAP48	+	-
PBUAP20	-	111	PBUAP49	-	-
PBUAP22	-		PBUAP50	-	-
PBUAP23	-		PBUAP51	-	-
PBUAP24	-		PBUAP53	-	-
PBUAP25	-		PBUAP55	-	+
PBUAP26	-	Allecces of a	PBUAP58	-	+
PBUAP27	-	Q-	PBUAP59	-	-
PBUAP29	-	-	PBUAP61	-	-
PBUAP30	-	หาองอิรอโบ	PBUAP62	-	-
PBUAP31	- 0.		PBUAP65	-	-
PBUAP32	-	ULALUNGKURI	PBUAP67	-	-
PBUAP33	-	-	PBUAP70	-	-
PBUAP34	-	-	PBUAP71	-	-
PBUAP35	-	-	PBUAP72	-	+
PBUAP36	-	-	PBUAP73	-	+
PBUAP37	-	-	PBUAP75	-	-
PBUAP38	-	-	PBUAP76	+	-
PBUAP39	-	-	PBUAP77	-	-

 Table 4.7 Antifungal phenotypes among 54 new strains of Aureobasidium spp. on PDA.

+ / - = positive / negative for inhibitory activity against fungal growth

4.2.5 Xylanase production and characterization

All strains were grown with xylan as the sole carbon source. All strains of *Aureobasidium* spp. showed xylanase activity on the agar plate containing beechwood xylan with various diameters when congo-red assay was applied. The strains with positive result were selected and cultured in PM medium. The enzyme activity was assay under standard conditions at pH5.0, 30° C. Xylanase activity from 54 strains was shown in table 4.8 and variable xylanase activity was found. The color variant strains including PBUAP5, PBUAP5.1, PBUAP7.1, PBUAP55 and PBUAP58 exhibited high activity of xylanase when compared with the color variant straindard strain NRRLY12974. Color variant strain PBUAP58 gave the highest activity at 7.28 ± 0.07 Uml⁻¹. This strain was chosen for the next study.

จุฬาลงกรณมหาวทยาลย Chulalongkorn University

Table 4.8 Extracellular xylanase from 54 new strains of *Aureobasidium* and 3 standard strains, cultured in xylan production medium at 30°C with agitation at 200 rpm for 3 days.

Strain	Xylanase	Strain	Xylanase	Strain	Xylanase
	activity (Uml ⁻¹)		activity (Uml ⁻¹)		activity (Uml ⁻¹)
PBUAP4	7.09 <u>+</u> 0.09	PBUAP32	5.11 <u>+</u> 0.28	PBUAP51	5.95 <u>+</u> 0.09
PBUAP5	6.94 <u>+</u> 0.07	PBUAP33	5.01 <u>+</u> 0.10	PBUAP53	3.01 <u>+</u> 0.05
PBUAP5.1	6.49 <u>+</u> 0.44	PBUAP34	4.91 <u>+</u> 0.01	PBUAP55	7.12 <u>+</u> 0.34
PBUAP7.1	6.85 <u>+</u> 0.02	PBUAP35	5.14 <u>+</u> 0.01	PBUAP58	7.28 <u>+</u> 0.07
PBUAP9	6.50 <u>+</u> 0.04	PBUAP36	5.12 <u>+</u> 0.06	PBUAP59	5.80 <u>+</u> 0.16
PBUAP13	6.21 <u>+</u> 0.14	PBUAP37	6.25 <u>+</u> 0.15	PBUAP61	6.33 <u>+</u> 0.11
PBUAP14	6.64 <u>+</u> 0.12	PBUAP38	4.96 <u>+</u> 0.07	PBUAP62	6.19 <u>+</u> 0.16
PBUAP16	2.36 <u>+</u> 0.21	PBUAP39	5.48 <u>+</u> 0.09	PBUAP65	5.57 <u>+</u> 0.23
PBUAP17	3.53 <u>+</u> 0.19	PBUAP40	0.72 <u>+</u> 0.04	PBUAP67	6.65 <u>+</u> 0.07
PBUAP20	4.18 <u>+</u> 0.07	PBUAP41	1.58 <u>+</u> 0.04	PBUAP70	4.22 <u>+</u> 0.17
PBUAP22	5.23 <u>+</u> 0.07	PBUAP42	6.17 <u>+</u> 0.07	PBUAP71	6.41 <u>+</u> 0.03
PBUAP23	6.25 <u>+</u> 0.14	PBUAP43	4.38 <u>+</u> 0.15	PBUAP72	4.93 <u>+</u> 0.12
PBUAP24	6.84 <u>+</u> 0.30	PBUAP44	6.96 <u>+</u> 0.27	PBUAP73	6.92 <u>+</u> 0.29
PBUAP25	5.60 <u>+</u> 0.04	PBUAP45	2.30 <u>+</u> 0.04	PBUAP75	7.02 <u>+</u> 0.17
PBUAP26	4.54 <u>+</u> 0.21	PBUAP46	2.03 <u>+</u> 0.01	PBUAP76	2.03 <u>+</u> 0.15
PBUAP27	3.23 <u>+</u> 0.06	PBUAP47	4.99 <u>+</u> 0.28	PBUAP77	3.15 <u>+</u> 0.22
PBUAP29	4.81 <u>+</u> 0.07	PBUAP48	1.81 <u>+</u> 0.07	NRRL58560	0.64 <u>+</u> 0.05
PBUAP30	5.37 <u>+</u> 0.20	PBUAP49	6.78 <u>+</u> 0.13	NRRL58561	0.62 <u>+</u> 0.16
PBUAP31	5.38 <u>+</u> 0.01	PBUAP50	2.26 <u>+</u> 0.09	NRRLY12974	1.95 <u>+</u> 0.17

 \pm = Standard error from mean values of three replicates

4.3 Potential of xylanase for xylooligosaccharide production

Xylan was extracted from cattail by dilute alkali treatment. The crude xylanase from *A. melanogenum* PBUAP58 exhibits high activity so it was used for xylan hydrolysis. The production of XOS from cattail xylan (1 % w/v) at 50°C using 25 U of crude xylanase was shown in Table 4.9.

 Table 4.9 XOS yield produced from hydrolysis of extracellular xylanase at 1 -24 hours,

 xylan extracted from cattail was used as sole carbon source and report as reducing

 sugar measurement (mg/ g xylan). The symbol * indicates significant different between

 XOS yield.

Time (h)	XOS yield (mg/g xylan)
1	20.06 <u>+</u> 0.34
2	21.46 <u>+</u> 0.16
4	21.60 <u>+</u> 0.20
6	23.40 <u>+</u> 0.13
8	23.99 <u>+</u> 0.12
12	27.28 <u>+</u> 0.27
16	28.16 <u>+</u> 0.02 [*]
20	27.17 <u>+</u> 0.02
24	25.51 <u>+</u> 0.23

XOS yield from cattail xylan was 28.16 ± 0.02 mg/ g xylan. The result of hydrolysis period at 16h were enough for XOS production, whereas the rate of XOS production declined after 16h. The XOS obtained were mainly composed of xylobiose and xylose was also obtained (Figure 4.14).

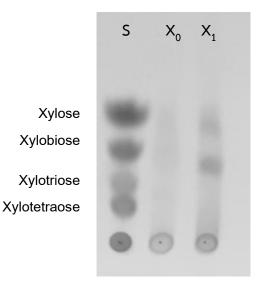


Figure 4.14 Thin-layer chromatogram of the hydrolysis products of cattail xylan treated with crude xylanase of *A. melanogenum* PBUAP58. The hydrolysis reaction using was carried out at 50° C for 24 h in 50 mM sodium acetate buffer (pH 5.0) containing 1% (w/v) cattail lxylan. S represents the oligomer markers, X₀ represents untreated xylan and X₁ represents the treatment xylan. Xylose, xylobiose, xylotriose, and xylotetraose were used for standard comparison.

HULALONGKORN UNIVERSITY

FT-IR spectroscopy was applied for XOS analysis with specific band maximum in the 1200-800 cm⁻¹ region. The result from FT-IR analysis of XOS was shown in Figure 4.15. XOS obtained from cattail showed the signal at 894 cm⁻¹ that is characteristic of β -glycosidic linkages between the sugars units. The spectral results exhibited typical of arabinoxylan type oligomers and polymers with a low degree of branched backbone as indicated by the presence of the signal at 995 cm⁻¹. The maximum absorption at 1040 cm⁻¹ is assigned to the C-O-C stretching of glycosidic linkages contributions which is characteristic for the distinction of typical xylans. The signal at 1251 and weak signal at around 1342 cm⁻¹ were related to C-H stretching and OH or C-O bending vibration. Asymmetric and symmetric (C=O) stretching vibrations of carboxylate group were found at 1566 and 1407 cm⁻¹, respectively. These bands

represented the uronic acid residues in the ionized form. The absence of absorbance around 1730 cm⁻¹ for carbonyl stretching groups implied that acetyl groups of hemicellulose substrates were cleaved during alkali extraction.

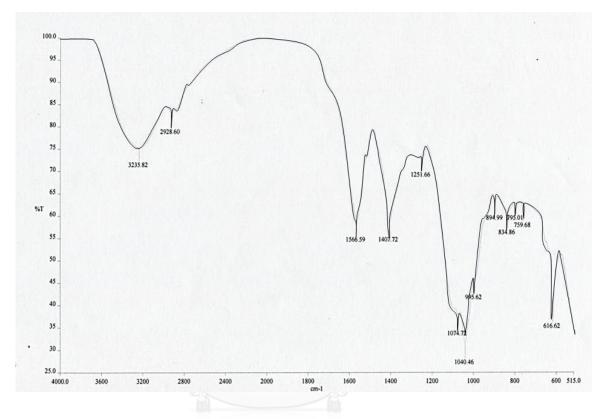
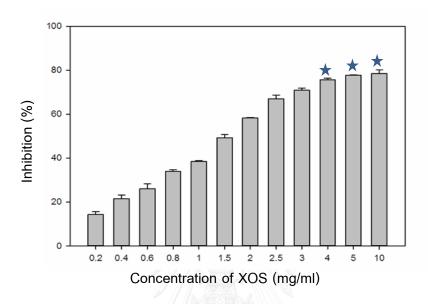
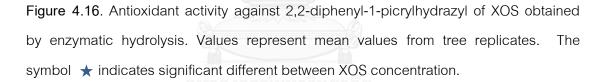


Figure 4.15 FT-IR spectrum of XOS powder obtained from cattail.

The antioxidant activity derived from XOS obtained from cattail was shown in Figure 4.16. The higher antioxidant activity (% inhibition) was found when XOS concentration was increased. The inhibition activity (%) gradually increased, at 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0, 2.5, 3, 4, 5, and 10 mgmL⁻¹. The scavenging effect of XOS were 14.39 ± 1.30 , 21.53 ± 1.59 , 26.12 ± 2.18 , 33.88 ± 0.88 , 38.47 ± 0.47 , 49.29 ± 1.53 , 58.37 ± 0.18 , 67.04 ± 1.65 , 70.92 ± 0.88 , 75.75 ± 0.68 , 77.67 ± 0.31 , and 78.45 ± 1.63 %. The maximum antioxidant activity (78.45 ± 1.63) was achieved at 10 mg of XOS. This suggested XOS produced from xylanase hydrolysis could be used as nutrient substance in food and apply for biotechnology.





Chulalongkorn University

CHAPTER V

DISCUSSIONS AND CONCLUSIONS

5.1 Identification of Aureobasidium spp. from coastal area

5.1.1 Aureobasidium spp.

Among 54 *A. pullulans*-like strains obtained from various habitats under salt stress along Thai coasts, *A. melanogenum* was apparently the dominant species showing relatively low genetic diversity compared to their terrestrial counterparts (Manitchotpisit *et al.*, 2009). Failure to isolate *A. pullulans* from the same samples was unexpected as the species has been frequently obtained from terrestrial phyllosphere and moist surfaces (Lotrakul *et al.*, 2009; Prasongsuk *et al.*, 2005; Punnapayak *et al.*, 2003; Manitchotpisit *et al.*, 2009) and it has been reported to be the most halotolerant among the four related species (Gostincar *et al.*, 2014).

Most of the strains obtained in this study were from plant leaves including perennial, annual and shrub whereas only four strains were from rock surfaces. There were no relations between host plant species and phylogenetic groupings in each clade (Manitchotpisit *et al.*, 2009). Moreover, there were no apparent relations between the geography of different sampling sites and phylogeny of individual clades.

5.1.2 Morphological identification

From the past, the most common approach to identify *A. pullulans* has been used classical methods including morphology and physiology (Cooke 1959; de Hoog and Yurlova, 1994). Result obtained by morphology and physiology showed diversity in the analysis. The new 54 tropical strains of *Aureobasidium* spp. showed polymorphism that is specific characters of the species same as *A. pullulans* standard strain from temperate zone. The only significant morphological feature for the recognition of *A. pullulans* microscopically is therefore the synchronous conidium production on young hyphal cells (de Hoog and Yurlova, 1994). Concerning to the redefinition of the species by Gostincar *et al.* (2014), morphological characters were not enough to identify 54 new *Aureobasidium* spp. into species level.

5.1.3 Physiological identification

Variation in carbon or nitrogen assimilation pattern was found in this study. In general, the carbon and nitrogen assimilation patterns of the strains correlated with the assimilation patterns of the control strains. Although a diverse range of utilized nutrients sources were found. Intra-specific variation of A. pullulans was reported so far (Prasongsuk et al., 2005; Urz'ı et al, 1999). A. pullulans utilized cellobiose but not cellulose, same as A. melanogenum was found to lack of cellulase activity as described by de Hoog and Yurlova (1994). However the results from this study showed a different ability in A. thailandense. This species absented in urease activity that distinguished from A. pullulans, the standard strain and A. melanogenum obtained in this study. All strains utilized lactose and methyl-a-D-glucoside, in agreement with A. pullulans profile (Yurlova and de Hoog, 1997). Physiological test was useful to assign the strains to Aureobasidium group, and to show some phenotypic differences among the strains. However, the physiological test did not contribute to a better knowledge of their ecological behavior. Moreover, characteristics like osmo- and halo tolerance seemed to be not so important for their settlement and colonization of coastal habitats. This ability should be state in the strain dependent (Urz`ı *et al*, 1999)

5.1.4 DNA amplification, sequencing and phylogenetic analysis

Intra-specific diversity of *Aureobasidium* strain isolated from coastal habitats was studied by assessment of morphological, physiological characters as well as multilocus sequence analysis. Recently reports proposed molecular taxonomy that would be more precise to classify *Aureobasidium* species using multilocus sequence analysis than other methods (Gostincar *et al.*, 2014; Manitchotpisit *et al.*, 2009; Zalar *et al.*, 2008).

Regarding to the phylogenetic analysis, the most desirable out group would be a member of the sister group to the in-group. S. mahoniae was used in this study based on the data of ITS region, TUB and ELO sequences were available (Zalar et From the analysis, the ITS region seems to be useful to distinguish al., 2008). Aureobasidium from other species (de Hoog et al., 1999) and it was previously used to distinguish species in the order Dothideales (Nilsson et al., 2008). However it is useless when it comes to subspecies differentiation. Other loci were found to be more informative for classification of Aureobasidium strains into distinct clades. The TUB and ELO were suggested to differentiate Aureobasidium into the species level (Manitchotpisit et al., 2009; Zalar et al., 2008). In this study, the concordance analysis of DNA sequence data classified 12 genetically isolated groups among Aureobasidium strains. Morphological and phenotypic characters are included and used for phylogenetic tree analysis. The clades obtained in this study could be formalized as species but reclassification is beyond the scope of this study. Moreover, the strain PBUAP47 and PBUAP53 are occurred in different clade with different tree from data analysis. It suggested either that these two clades were not genetically isolated or that lineage sorting between the clades was incomplete. Furthermore, the data of 12 clades from 54 new Aureobasidium strains was identified into 2 species including A. melanogenum and A. thailandense. The tree based on TUB sequences in this study showed that the standard strains A. pullulans, A. namibiae, and A. subglaciale were located out of all clades. Besides, both A. melanogenum standard strains and Aureobasidium strains in this study were separated from A. pullulans. However, A. thailandense strains were located under the same clade with A. melanogenum. It had been reported for multilocus analysis from worldwide selection of A. pullulans-like strains that A. melanogenum is distinct from A. pullulans (Gostincar et al., 2014; Zalar et al., 2008).

In conclusion, *A. melanogenum* was the dominant *Aureobasidium* species found in coastal area of Thailand whereas *A. thailandense* was also obtained. This might be due to the genetically and physiology of *A. melanogenum* that was mainly

isolated from aqueous environments and it grows at high temperature (37[°]C) (Gostincar *et al.*, 2014).

5.2 Characterization by phenotypic analysis

5.2.1 EPS production and analysis

A. pullulans is known as pullulan-producing strain. It was reported for different type production of EPS including pullular or β -glucan (Yurlova and de Hoog, 1997). All strains in this study produce EPS but two strains (PBUAP73 and PBUAP77) produced EPS in very low amount that could not recovery. EPS yield was determined using the optimal condition of strain NRRL58560, the best pullulan producing strain described by Prasongsuk et al. (2005). This strain gave 25 gl⁻¹ of pullulan when cultured in pullulan production medium containing 5.0 % (w/v) sucrose medium and 0.1 % (w/v) N-source. In this study the best strain, PBUAP34 gave the highest pullulan yield at 31.86 \pm 0.77 gl⁻¹ at standard condition with initial pH 6.5 and cultures were grown at 30 \pm 2°C, 150 rpm, for 7 days. However, β -glucan has been found in some strains. Pullulan is presently defined as **a**-D-glucan comprising **a**-maltotriosyl residues linked by 1,6-**a**-Dglucosidic bonds (Leathers et al. 1988) whereas the β -1,4-D-, β -1,6-D and β -1,3-Dglucosidic bonds was represented. Some strains produced only pullulan or β -glucan, while both EPS produced from one strain was observed. FT-IR spectra of EPS from all strains grown in PM medium were similar to the spectrum of pullulan or β -glucan, depending on type produced by each strain. However, there were EPS that absent both of α - and β -configuration. Consequently, we named this EPS as unidentified EPS. A further measure of pullulan authenticity and purity is the sensitivity of EPS to pullulanase and β -glucanase. EPS in this study exhibited both pullulanase and β -glucanase sensitivity, depends on type of EPS. However, some EPS exhibited both of enzyme sensitivities. Most of unidentified EPS responded to β -glucanase sensitivity rather than pullulanase. This indicated that its structurte natured as β -glucan type. This result should be confirmed by adding more purification steps to get rid of the contaminant substances (Yurlova and de Hoog, 1997).

5.2.2 Multiple stress tests

Aureobasidium pullulans was proposed to be polyextreamotolerance important yeast that has exceptional stress tolerance (Gostincar *et al.*, 2014). Aureobasidium from coastal habitats had been reported for their interactive effect of temperature and salinity that salt concentration can enhance their stability in high temperature (Torzilli, 1997; Torzilli *et al.*, 1985). It might be due to the specific interaction between the genetic and physiology of their adaptation ability (Gunde-Cimerman *et al.*, 2009; Gunde-Cimerman *et al.*, 2000) that could be used in biotechnological applications. From this hypothesis, all 54 new strains isolated from coastal habitats were tested for their ability to grow in different abiotic stress including halotolerance, osmotolerance, thermotolerance, and tolerance against different pH value.

According to growth determination on solid media in the presence of NaCl, it inhibited growth of all strains even the lowest concentration (5% w/v). Four strains of *A. thailandense* were moderate halotolerance whereas *A. melanogenum* were varied. However, it had been reported for *A. melanogenum* can tolerate only 10% NaCl (Peterson *et al.*, 2013; Zalar *et al.*, 2008). In this study, some strains showed that they slowly grew in the beginning then faster after day 7. The relative growth (%) decreased in all strains when the higher concentration of NaCl (10 % and 15 %, w/v) was added. Although the high ability of halotolerance was found, no growth was observed in few strains when grown in 15 % (w/v) of NaCl. This confirmed the halotoleance of *Aureobasidium* strains in this study.

Osmotolerance of *Aureobasidium* spp. were studied due to developing of pullulan production was concerned (Choudhury *et al.*, 2012; Choudhury *et al.*, 2011). The strains in this study were defined their osmotolerance on YM agar containing different glucose concentration. Almost strains seemed to be slightly osmophilic due to the result on 5 % (w/v) glucose that resulting in the highest relative growth (%). However, the low growth rate was found in higher concentration of glucose (30 %, 50 % w/v). Consequently, *Aureobasidium* is only an osmotolerant species (Hernandez-Saavedra *et al.*, 1995).

Thermotolerant strains of *A. pullulans* were interested since their capability to produce many useful of hydrolyzed enzyme that could be applied in biotechnology (Chi *et al.*, 2009a; 2009b). In addition, Torzilli *et al.* (1985) and Torzilli (1997) had been reported that thermotolerance of *Aureobasidium* might be related with halotolerance ability. In this study, *Aureobasidium* strains were isolated from coastal habitats that effected by solar radiation. The result showed that *A. melanogenum* was found to be the dominant species in aqueous habitats, especially marine environment (Gunde-Cimerman *et al.*, 2000; 2009; Wu *et al.*, 2010). Moreover, *A. melanogenum* is only one strain that grows in high temperature (37^oC) while the others four species including *A. pullulans*, *A. namibiae* and *A. subglaciale* cannot, same as *A. thailandense* (Peterson *et al.*, 2013; Zalar *et al.*, 2008).

The biomass of cell grown in the presence of different pH was considered since it might be adapted for diverse applications (Alvarez-Perez *et al.*, 2011). To discover the special ability of fungus in different pH, pH affect to cell growth and differentiate, and to find out the extremotolerance ability, each fungus was grown in 2% MEB with a range of pH (3.0 - 9.0). The results showed that pH not only effected to fungal growth but also cell differentiated. The biomass dry weight was varied depending on strains. Aerial hyphae were found at pH 9.0 with melanized hyphae whereas yeast cells were found in pH 3.0 with pink or cream color. This presents a wide potential to adapt to different environment conditions.

In conclusion a different behavior was observed in reference to the growth response with multiple stress tests. Although data obtained from halo-, osmo-, thermotolerance and effect of pH are only initially reported; it is basic data that can be useful for a possible application with selected strain in the future (Gostinčar *et al.*, 2010; Gostincar *et al.*, 2011).

5.2.3 Associations among halotolerance, osmotolerance, and EPS production

Significant associations were found among halotolerance, osmotolerance and EPS production of the 50 *A. melanogenum* strains. Tolerant strains toward ionic stress (salt) were also tolerant to non-ionic stress (sugar). However, strains tolerant to non-ionic stress might or might not be tolerant to ionic stress. It has been suggested that highly osmotolerant food yeasts would be highly halotolerant and *vice versa* because there are common mechanisms for adaptation to environments with either ionic or nonionic osmotic stress (Bubnova *et al.*, 2014). However, this association was not found among these *A. melanogenum* strains.

The severe growth inhibition found in most *A. melanogenum* strains when grown in a medium containing 5 % (w/v) NaCl might explain why the direct isolation from marine water was unsuccessful in this study. However, a number of *A. pullulans* and *A. melanogenum* have been isolated from sea mud, hypersaline water and solar salterns (Gostincar *et al.*, 2014; Gunde-Cimerman *et al.*, 2000; Wang *et al.*, 2014; Wu *et al.*, 2012). Growth inhibition in 30 % (w/v) glucose was likely caused by the combination of osmotic stress (Gunde-Cimerman *et al.*, 2009) and oxygen deprivation due to the high medium viscosity (Kumar *et al.*, 2012). There have been extensive studies on EPS, especially pullulan, production by *A. pullulans* (Cheng *et al.*, 2013). However, most studies focused mainly on the EPS yield on a dry weight basis. Industrially a strict parameter is efficiency of conversion of the substrate. Though the majority of *A. melanogenum* strains in this study exhibited relatively low EPS production efficiency (less than 20 % conversion), eight were 30%, six 40 %, two 50 % and one over 60 %

efficient. This range of conversion efficiency is similar to previous reports (Cheng *et al.*, 2011; Choudhury *et al.*, 2011; Manitchotpisit *et al.*, 2009; Prasongsuk *et al.*, 2007), yet noteworthy as PBUAP 34 showed conversion rate above 60 %. However, high EPS conversion efficiency was found to be associated with intolerance against either salt or sugar.

There have been only a few reports focusing on EPS production by osmotolerant strains of A. pullulans at a sugar concentration above 10 % (w/v) (Cheng et al., 2011; Choudhury et al., 2011; 2012; Wu et al., 2009). At first glance it seemed that these strains produced EPS in higher amounts when the sugar concentration was increased. However, when % conversion was considered, all reported strains lost their production efficiency drastically at sugar concentrations higher than 15 % (w/v) (Cheng et al., 2011; Choudhury et al., 2011; 2012) which was similar to the results obtained in this study. According to Wu et al. (2009), A. pullulans AP329 was apparently osmotolerant because its growth was not inhibited in a medium containing 15 % (w/v) sweet potato hydrolysate [comprised 1 % (w/v) glucose, 8.19 % (w/v) maltose and 4.9 % (w/v) maltotriose]. However, at this concentration, the conversion efficiency was less than 25 % compared to 60 % with sweet ptotato hydrolysate 5 % (w/v) (Wu et al., 2009). The osmotolerant of *A. pullulans* RBF-4A3 optimally produced 70.4 gl⁻¹ pullulan in a batch medium with 16.7 % (w/v) glucose (Choudhury et al., 2012), only 42 % efficiency. In glucose concentrations of 20 and 25 % (w/v), the conversion efficiency of A. pullulans RBF-4A3 decreased to less than 30 and 20 %, respectively (Choudhury et al., 2011).

One of the common mechanisms that yeasts usually use to survive osmotic stress is the accumulation of intracellular osmolytes to lower their cellular water potential. Glycerol and mannitol were among the most common fungal osmolytes (Hohmann, 2002; Kogej *et al.*, 2005; Managbanag and Torzilli, 2002). *A. pullulans* accumulated mannitol when it was exposed to heat and/or salt stresses whereas glycerol was accumulated only under salt stress (Managbanag and Torzilli, 2002). Similarly, *A. melanogenum* used mannitol, but not glycerol, accumulation when exposed

to osmotic stress caused by high sugar concentration. Therefore, mannitol is a likely universal osmolyte for all stresses involving water activity in *A. pullulans* and related species whereas glycerol is possibly a specific osmolyte for salt stress only.

In conclusion, *A. melanogenum* was the dominant *Aureobasidium* species in habitats exposed to salt stress along coasts of Thailand. No association was found between the direct exposure to salt water and halotolerance. Halotolerance in *A. melanogenum* was significantly associated with osmotolerance, but not *vice versa*. Halo- and/or osmotolerant strains produced low to moderate EPS yield. This property might be one of their adaptation mechanisms for tolerance against osmotic stress as released EPS may lower the water potential of their surrounding water. The results may lead to development of a better understanding of the physiological mechanisms of tolerance against osmotic stress in the genus *Aureobasidium*.

5.2.4 Determination of antifungal activity

Among 54 strains, it was observed that nine strains exhibited antifungal activity against *Aspergill*. Only one strain, PBUAP47 against both *A. niger* and *A. fumigatus* that showed its potential for production of antifungal agents. In addition, eight strains showed activity against only *A. niger* or *A. fumigatus* alone. The antifungal activity of biocontrol agents in *Aureobasidium* spp. against fungal pathogens was reported that it results from the combination of different mechanisms including antibiotic, parasitism by production of lytic enzymes, and competition for limiting nutrients and space (Bencheqroun *et al.*, 2007; Mounir *et al.*, 2007). Aureobasidin production was reported from tropical *A. pullulans* that isolated from bathroom surfaces (Lotrakul *et al.*, 2009) and the production of this antifungal agent showed variation in amino acid and its activity against *Aspergilli* species (Prasongsuk *et al.*, 2013). However, no relation between habitats and antifungal activity in this study was found. Moreover, the most studies of aureobasidin A appeared in only one strain, R106 (Takesako *et al.*, 1991). The expansion to discover of new strains that might have new forms of antifungal agents with different activities should be concerned.

5.2.5 Xylanase production and characterization

According to *A. pullulans* was reported for xylanase producer (Leather, 1986; Manitchotpisit *et al.*, 2009). This enzyme has potential for commercials of biofuels, biobleaching in paper industry, food and chemicals. The xylanase production was determined using the standard condition as previously described by Manitchotpisit *et al.* (2009). All strains produced xylanase in xylan PM. Xylanase activity was varied even in the same clade. Color variant strain has been reported to overproduce xylanase (Leathers *et al.*, 1986). In this study, all color variant strains also produced high xylanase activity. This study will be beneficial for taxonomic revision of this fungus and could be used as a guideline for the identification and selection of new potential strains for biotechnological applications (Chi *et al.*, 2009b).

5.3 Potential of xylanase for xylooligosaccharide production

Xylan was extracted from cattail by dilute alkali treatment based on this technique can cause swelling of substrate and lead to the increase in internal surface areas, decreasing the degree of polymerization and crystallization, and breaking of linkages between lignin and hydrolyzed, resulting in easy xylan recovery (Chapla *et al.*, 2012; Yoon *et al.*, 2006).

Due to the XOS yield found to be limited even after the prolonged incubation period (Christov and Prior, 1993), the result of hydrolysis period at 16h were enough for XOS production, was same as previously report (Chapla *et al.*, 2012; Kallel *et al.*, 2014). The rate of XOS production declined after 16h since the reduction of accessible hydrolytic sites in xylan, the degradation of XOS, and/or reduction of enzyme activity due to end product inhibition (Mandelli *et al.*, 2014). The Xylanases from *Aspergillus oryzae* MTCC 5154 and *Geobacillus thermoleovorans* have been reported to hydrolyze xylan to xylose, and XOS with degrees of polymerization of three or higher. Similarly, the hydrolysis of xylan by the xylanase from *Streptomyces olivaceoviridis* E-86 produced

xylobiose as the main product together with a minor amount of xylose and xylotriose (Bian *et al.*, 2013). In general, the product varies in degree of polymerization ranging from xylose, xylobiose, xylotriose, to higher xylo-oligosaccharides (Veenashri and Muralikrishna, 2011). In the present study, the crude enzyme of *A. melanogenum* PBUAP58 is effective for xylan hydrolysis. The XOS obtained were mainly composed of xylobiose and xylose that the reaction was both time and substrate dependent (Christov *et al.*, 1997).

FT-IR spectroscopy was applied for the study of cell wall polysaccharides because each particular polysaccharide had a specific band maximum in the 1200-800 cm⁻¹ region (Robert et al., 2005). FT-IR technique proved cell wall monosaccharide composition and monitored their changes during the isolation process. The spectral exhibited typical of arabinoxylan type oligomers and polymers with a low degree of branched backbone as indicated by the presence of the signal at 995 cm⁻¹. The absorbances between 1170 and 1000 cm⁻¹ are typical of arabinoxylans (Peng et al., 2009). Asymmetric and symmetric (C=O) stretching vibrations of carboxylate group were found at 1566 and 1407 cm⁻¹, respectively. These bands represented the uronic acid residues in the ionized form that was found to have role in antioxidant activity (Rao and Muralikrishna, 2006; Rivas et al., 2013). The absence of absorbance around 1730 cm⁻¹ for carbonyl stretching groups implied that acetyl groups of hemicellulose substrates were cleaved during alkali extraction (Bian et al., 2013). In conclusion, the potential and beneficial property of antioxidant has made it very important in biological systems as well as in industrial processes. It is known to possess anti-inflammatory, anti-cardiovascular disease, anti-neurogenerative and anticancer properties (Veenashri and Muralikrishna, 2011). XOS from cattail indicated itself for natural antioxidant substance that could be used as potential resource for food industry.

Alvarez-Perez, S., J. L. Blanco, P. Alba and M. E. Garcia. 2011. Fungal growth in culture media simulating an extreme environment. <u>Revista Iberoamericana De</u> <u>Micologia</u> 28(4): 159-165.

Bencheqroun, S. K., M. Bajji, S. Massart, M. Labhilili, S. E. Jaafari and M. H. Jijakli. 2007. In vitro and in situ study of postharvest apple blue mold biocontrol by Aureobasidium pullulans: Evidence for the involvement of competition for nutrients. <u>Postharvest Biology and Technology</u> 46(2): 128-135.

Bian, J., F. Peng, X. P. Peng, P. Peng, F. Xu and R. C. Sun. 2013. Structural features and antioxidant activity of xylooligosaccharides enzymatically produced from sugarcane bagasse. <u>Bioresour Technol</u> 127: 236-241.

Bubnova, M., J. Zemancikova and H. Sychrova. 2014. Osmotolerant yeast species differ in basic physiological parameters and in tolerance of non-osmotic stresses. <u>Yeast</u> 31(8): 309-321.

Chapla, D., P. Pandit and A. Shah. 2012. Production of xylooligosaccharides from corncob xylan by fungal xylanase and their utilization by probiotics. <u>Bioresour Technol</u> 115: 215-221.

Cheng, K. C., A. Demirci and J. M. Catchmark. 2011. Pullulan: biosynthesis, production, and applications. <u>Appl Microbiol Biotechnol</u> 92(1): 29-44.

Chi, Z., Z. Chi, T. Zhang, G. Liu, J. Li and X. Wang. 2009a. Production, characterization and gene cloning of the extracellular enzymes from the marine-derived yeasts and their potential applications. <u>Biotechnol Adv</u> 27(3): 236-255.

Chi, Z., F. Wang, Z. Chi, L. Yue, G. Liu and T. Zhang. 2009b. Bioproducts from Aureobasidium pullulans, a biotechnologically important yeast. <u>Appl Microbiol</u> <u>Biotechnol</u> 82(5): 793-804.

Choudhury, A. R., M. S. Bhattacharyya and G. S. Prasad. 2012. Application of response surface methodology to understand the interaction of media components during pullulan production by Aureobasidium pullulans RBF-4A3. <u>Biocatalysis and Agricultural Biotechnology</u> 1(3): 232-237.

Choudhury, A. R., P. Saluja and G. S. Prasad. 2011. Pullulan production by an osmotolerant Aureobasidium pullulans RBF-4A3 isolated from flowers of Caesulia axillaris. <u>Carbohydrate Polymers</u> 83(4): 1547-1552.

Christov, L. and B. Prior. 1993. Xylan removal from dissolving pulp using enzymes of Aureobasidium pullulans. <u>Biotechnology letters</u> 15(12): 1269-1274.

Christov, L. and B. Prior. 1996. Repeated treatments with Aureobasidium pullulans hemicellulases and alkali enhance biobleaching of sulphite pulps. <u>Enzyme and Microbial technology</u> 18(4): 244-250.

Cooke, W. B. 1959. An ecological life history of Aureobasidium pullulans (De Bary) Arnaud. <u>Mycopathologia</u> 12: 1-45.

de Hoog, G. S. and N. A. Yurlova. 1994. Conidiogenesis, nutritional physiology and taxonomy of Aureobasidium and Hormonema. <u>Antonie Van Leeuwenhoek</u> 65(1): 41-54.

Deshpande, M. S., V. B. Rale and J. M. Lynch. 1992. Aureobasidium pullulans in applied microbiology: a status report. <u>Enzyme and Microbial Technology</u> 14(7): 514-527.

Dhiman, S. S., J. Sharma and B. Battan. 2008. Industrial applications and future prospects of microbial xylanases: a review. <u>BioResources</u> 3(4): 1377-1402.

Glass, N. L. and G. C. Donaldson. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. <u>Applied and Environmental Microbiology</u> 61(4): 1323-1330.

Gostin**Č**ar, C., M. Grube, S. De Hoog, P. Zalar and N. Gunde-Cimerman. 2010. Extremotolerance in fungi: evolution on the edge. <u>FEMS microbiology ecology</u> 71(1): 2-11.

Gostincar, C., M. Grube and N. Gunde-Cimerman. 2011. Evolution of fungal pathogens in domestic environments? <u>Fungal Biol</u> 115(10): 1008-1018.

Gostincar, C., R. A. Ohm, T. Kogej, S. Sonjak, M. Turk, J. Zajc, *et al.* 2014. Genome sequencing of four Aureobasidium pullulans varieties: biotechnological potential, stress tolerance, and description of new species. <u>BMC Genomics</u> 15: 549.

Guimarães, J. B., P. Pereira, L. Chambel and R. Tenreiro. 2011. Assessment of filamentous fungal diversity using classic and molecular approaches: case study – Mediterranean ecosystem. <u>Fungal Ecology</u> 4(5): 309-321.

Gunde-Cimerman, N., J. Ramos and A. Plemenitas. 2009. Halotolerant and halophilic fungi. <u>Mycol Res</u> 113(Pt 11): 1231-1241.

Gunde-Cimerman, N., P. Zalar, S. de Hoog and A. Plemenitaš. 2000. Hypersaline waters in salterns–natural ecological niches for halophilic black yeasts. <u>FEMS</u> <u>Microbiology Ecology</u> 32(3): 235-240.

Hernandez-Saavedra, N. Y., J. L. Ochoa and R. Vazquez-Dulhalt. 1995. Osmotic adjustment in marine yeast. Journal of plankton research 17(1): 59-69.

Hohmann, S. 2002. Osmotic Stress Signaling and Osmoadaptation in Yeasts. <u>Microbiology and Molecular Biology Reviews</u> 66(2): 300-372.

Hua, S.-S. T., J. L. Baker and M. Flores-Espiritu. 1999. Interactions of Saprophytic Yeasts with anor Mutant of Aspergillus flavus. <u>Applied and environmental microbiology</u> 65(6): 2738-2740.

Ikai, K., K. Shiomi, K. Takesako, S. Mizutani, J. Yamamoto, Y. Ogawa, *et al.* 1991. Structures of aureobasidins B to R. <u>J Antibiot (Tokyo)</u> 44(11): 1187-1198.

Kallel, F., D. Driss, F. Bouaziz, M. Neifer, R. Ghorbel and S. Ellouz Chaabouni. 2015. Production of xylooligosaccharides from garlic straw xylan by purified xylanase from Bacillus mojavensis UEB-FK and their in vitro evaluation as prebiotics. <u>Food and</u> <u>Bioproducts Processing</u> 94: 536-546.

Kane, J. and R. C. Summerbell. 1987. Sodium chloride as aid in identification of Phaeoannellomyces werneckii and other medically important dematiaceous fungi. <u>J Clin</u> <u>Microbiol</u> 25(5): 944-946.

Kang, B. K., H. J. Yang, N. S. Choi, K. H. Ahn, C. S. Park, B. D. Yoon, *et al.* 2010. Production of pure beta-glucan by Aureobasidium pullulans after pullulan synthetase gene disruption. <u>Biotechnol Lett</u> 32(1): 137-142.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. <u>J Mol Evol</u> 16(2): 111-120.

หาลงกรณ์มหาวิทยาลัย

Kogej, T., J. Ramos, A. Plemenitas and N. Gunde-Cimerman. 2005. The halophilic fungus Hortaea werneckii and the halotolerant fungus Aureobasidium pullulans maintain low intracellular cation concentrations in hypersaline environments. <u>Appl Environ Microbiol</u> 71(11): 6600-6605.

Kumar, V., V. Sahai and V. Bisaria. 2012. Production of amylase and chlamydospores by Piriformospora indica, a root endophytic fungus. <u>Biocatalysis and Agricultural</u> <u>Biotechnology</u> 1(2): 124-128.

Kurtzman, C. P., J. W. Fell, T. Boekhout and V. Robert. 2011. Methods for isolation, phenotypic characterization and maintenance of yeasts. <u>The yeasts, a taxonomic study,</u> <u>5th edn. Elsevier, Amsterdam</u>: 87-110.

Larkin, M. A., G. Blackshields, N. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, *et al.* 2007. Clustal W and Clustal X version 2.0. <u>Bioinformatics</u> 23(21): 2947-2948.

Leathers, T., G. Nofsinger, C. Kurtzman and R. Bothast. 1988. Pullulan production by color variant strains of Aureobasidium pullulans. <u>Journal of industrial microbiology</u> 3(4): 231-239.

Leathers, T. D. 1986. Color Variants of Aureobasidium pullulans Overproduce Xylanase with Extremely High Specific Activity. <u>Appl Environ Microbiol</u> 52(5): 1026-1030.

Leathers, T. D. 2003. Biotechnological production and applications of pullulan. <u>Appl</u> <u>Microbiol Biotechnol</u> 62(5-6): 468-473.

Leathers, T. D. and P. Manitchotpisit. 2013. Production of poly(beta-L-malic acid) (PMA) from agricultural biomass substrates by Aureobasidium pullulans. <u>Biotechnol Lett</u> 35(1): 83-89.

Li, Y., Z. Chi, G. Y. Wang, Z. P. Wang, G. L. Liu, C. F. Lee, *et al.* 2015. Taxonomy of Aureobasidium spp. and biosynthesis and regulation of their extracellular polymers. <u>Crit</u> <u>Rev Microbiol</u> 41(2): 228-237.

Liu, S. and A. Steinbüchel. 1996. Investigation of poly (β -L-malic acid) production by strains of Aureobasidium pullulans. <u>Applied Microbiology and Biotechnology</u> 46(3): 273-278.

Lotrakul, P., P. Deenarn, S. Prasongsuk and H. Punnapayak. 2009. Isolation of Aureobasidium pullulans from bathroom surfaces and their antifungal activity against some Aspergilli. <u>African Journal of Microbiology Research</u> 3(5): 253-257.

Lotrakul, P., P. Unhapattaratitikul, T. Seelanan, S. Prasongsuk and H. Punnapayak. 2013. An aubasidan-like beta-glucan produced by Aureobasidium pullulans in Thailand. <u>Scienceasia</u> 39(4): 363-368.

Managbanag, J. R. and A. P. Torzilli. 2002. An analysis of trehalose, glycerol, and mannitol accumulation during heat and salt stress in a salt marsh isolate of Aureobasidium pullulans. <u>Mycologia</u> 94(3): 384-391.

Mandelli, F., L. B. Brenelli, R. F. Almeida, R. Goldbeck, L. D. Wolf, Z. B. Hoffmam, *et al.* 2014. Simultaneous production of xylooligosaccharides and antioxidant compounds from sugarcane bagasse via enzymatic hydrolysis. <u>Industrial Crops and Products</u> 52: 770-775.

Manitchotpisit, P., T. D. Leathers, S. W. Peterson, C. P. Kurtzman, X. L. Li, D. E. Eveleigh, *et al.* 2009. Multilocus phylogenetic analyses, pullulan production and xylanase activity of tropical isolates of Aureobasidium pullulans. <u>Mycol Res</u> 113(Pt 10): 1107-1120.

Manitchotpisit, P., N. P. Price, T. D. Leathers and H. Punnapayak. 2011. Heavy oils produced by Aureobasidium pullulans. <u>Biotechnol Lett</u> 33(6): 1151-1157.

Mounir, R., A. Durieux, E. Bodo, C. Allard, J. P. Simon, E. H. Achbani, *et al.* 2007. Production, formulation and antagonistic activity of the biocontrol like-yeast Aureobasidium pullulans against Penicillium expansum. <u>Biotechnol Lett</u> 29(4): 553-559.

Muramatsu, D., A. Iwai, S. Aoki, H. Uchiyama, K. Kawata, Y. Nakayama, *et al.* 2012. beta-Glucan derived from Aureobasidium pullulans is effective for the prevention of influenza in mice. <u>PLoS One</u> 7(7): e41399.

Nilsson, R. H., E. Kristiansson, M. Ryberg, N. Hallenberg and K.-H. Larsson. 2008. Intraspecific ITS variability in the kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. <u>Evolutionary bioinformatics online</u> 4: 193. Ohta, K., H. Fujimoto, S. Fujii and M. Wakiyama. 2010. Cell-associated beta-xylosidase from Aureobasidium pullulans ATCC 20524: Purification, properties, and characterization of the encoding gene. <u>J Biosci Bioeng</u> 110(2): 152-157.

Ohta, K., S. Moriyama, H. Tanaka, T. Shige and H. Akimoto. 2001. Purification and characterization of an acidophilic xylanase from Aureobasidium pullulans var. melanigenum and sequence analysis of the encoding gene. <u>J Biosci Bioeng</u> 92(3): 262-270.

Pechak, D. G. and R. E. Crang. 1977. An analysis of Aureobasidium pullulans developmental stages by means of scanning electron microscopy. Mycologia: 783-792.

Peng, F., J. L. Ren, F. Xu, J. Bian, P. Peng and R. C. Sun. 2009. Comparative study of hemicelluloses obtained by graded ethanol precipitation from sugarcane bagasse. <u>J</u> <u>Agric Food Chem</u> 57(14): 6305-6317.

Peterson, S. W., P. Manitchotpisit and T. D. Leathers. 2013. Aureobasidium thailandense sp. nov. isolated from leaves and wooden surfaces. <u>Int J Syst Evol</u> <u>Microbiol</u> 63(Pt 2): 790-795.

าลงกรณ์มหาวิทยาลัย

Prajapati, V. D., G. K. Jani and S. M. Khanda. 2013. Pullulan: an exopolysaccharide and its various applications. <u>Carbohydrate polymers</u> 95(1): 540-549.

Prasongsuk, S., S. Ployngam, S. Wacharasindhu, P. Lotrakul and H. Punnapayak. 2013. Effects of sugar and amino acid supplementation on Aureobasidium pullulans NRRL 58536 antifungal activity against four Aspergillus species. <u>Appl Microbiol Biotechnol</u> 97(17): 7821-7830.

Prasongsuk, S., R. F. Sullivan, M. Kuhirun, D. E. Eveleigh and H. Punnapayak. 2005. Thailand habitats as sources of pullulan-producing strains of Aureobasidium pullulans. <u>World Journal of Microbiology and Biotechnology</u> 21(4): 393-398. Price, N. P., P. Manitchotpisit, K. E. Vermillion, M. J. Bowman and T. D. Leathers. 2013. Structural characterization of novel extracellular liamocins (mannitol oils) produced by Aureobasidium pullulans strain NRRL 50380. <u>Carbohydrate research</u> 370: 24-32.

Punnapayak, H., M. Sudhadham, S. Prasongsuk and S. Pichayangkura. 2003. Characterization of Aureobasidium pullulans isolated from airborne spores in Thailand. J Ind Microbiol Biotechnol 30(2): 89-94.

Rao, R. S. and G. Muralikrishna. 2006. Water soluble feruloyl arabinoxylans from rice and ragi: changes upon malting and their consequence on antioxidant activity. <u>Phytochemistry</u> 67(1): 91-99.

Rivas, S., E. Conde, A. Moure, H. Dominguez and J. C. Parajo. 2013. Characterization, refining and antioxidant activity of saccharides derived from hemicelluloses of wood and rice husks. <u>Food Chem</u> 141(1): 495-502.

Robert, P., M. Marquis, C. Barron, F. Guillon and L. Saulnier. 2005. FT-IR investigation of cell wall polysaccharides from cereal grains. Arabinoxylan infrared assignment. J Agric Food Chem 53(18): 7014-7018.

หาลงกรณ์มหาวิทยาลัย

Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. <u>Molecular cloning</u>. Cold spring harbor laboratory press New York.

Selbmann, L., G. S. de Hoog, L. Zucconi, D. Isola, S. Ruisi, A. H. van den Ende, *et al.* 2008. Drought meets acid: three new genera in a dothidealean clade of extremotolerant fungi. <u>Stud Mycol</u> 61: 1-20.

Singh, R. S. and G. K. Saini. 2008. Pullulan-hyperproducing color variant strain of Aureobasidium pullulans FB-1 newly isolated from phylloplane of Ficus sp. <u>Bioresour</u> <u>Technol</u> 99(9): 3896-3899.

Takesako, K., K. Ikai, F. Haruna, M. Endo, K. Shimanaka, E. Sono, *et al.* 1991. Aureobasidins, new antifungal antibiotics. Taxonomy, fermentation, isolation, and properties. <u>The Journal of antibiotics</u> 44(9): 919-924.

Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. <u>Mol Biol Evol</u> 30(12): 2725-2729.

Thambugala, K. M., H. A. Ariyawansa, Y.-M. Li, S. Boonmee, S. Hongsanan, Q. Tian, *et al.* 2014. Dothideales. <u>Fungal Diversity</u> 68(1): 105-158.

Torzilli, A., S. Vinroot and C. West. 1985. Interactive effect of temperature and salinity on growth and activity of a salt marsh isolate of Aureobasidium pullulans. <u>Mycologia</u>: 278-284.

Torzilli, A. P. 1997. Tolerance to high temperature and salt stress by a salt marsh isolate of Aureobasidium pullulans. <u>Mycologia</u>: 786-792.

Urzì, C., F. De Leo, C. L. Passo and G. Criseo. 1999. Intra-specific diversity of Aureobasidium pullulans strains isolated from rocks and other habitats assessed by physiological methods and by random amplified polymorphic DNA (RAPD). Journal of microbiological methods 36(1): 95-105.

Veenashri, B. R. and G. Muralikrishna. 2011. In vitro anti-oxidant activity of xylooligosaccharides derived from cereal and millet brans – A comparative study. <u>Food</u> <u>Chemistry</u> 126(3): 1475-1481.

Wang, C. L., Y. Li, F. H. Xin, Y. Y. Liu and Z. M. Chi. 2014. Evaluation of single cell oil from Aureobasidium pullulans var. melanogenum P10 isolated from mangrove ecosystems for biodiesel production. <u>Process Biochemistry</u> 49(5): 725-731.

Wang, D., X. Yu and W. Gongyuan. 2013. Pullulan production and physiological characteristics of Aureobasidium pullulans under acid stress. <u>Appl Microbiol Biotechnol</u> 97(18): 8069-8077.

White, T. J., T. Bruns, S. Lee and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. <u>PCR protocols: a guide to methods</u> <u>and applications</u> 18: 315-322.

Wickerham, L. J. and C. P. Kurtzman. 1975. Synergistic color variants of Aureobasidium pullulans. <u>Mycologia</u> 67(2): 342-361.

Wu, S., J. Chen and S. Pan. 2012. Optimization of fermentation conditions for the production of pullulan by a new strain of Aureobasidium pullulans isolated from sea mud and its characterization. <u>Carbohydrate Polymers</u> 87(2): 1696-1700.

Wu, S., Z. Jin, Q. Tong and H. Chen. 2009. Sweet potato: A novel substrate for pullulan production by Aureobasidium pullulans. <u>Carbohydrate Polymers</u> 76(4): 645-649.

Wu, Y. R., Z. H. Luo and L. L. Vrijmoed. 2010. Biodegradation of anthracene and benz[a]anthracene by two Fusarium solani strains isolated from mangrove sediments. <u>Bioresour Technol</u> 101(24): 9666-9672.

Yoon, K. Y., E. E. Woodams and Y. D. Hang. 2006. Enzymatic production of pentoses from the hemicellulose fraction of corn residues. <u>LWT - Food Science and Technology</u> 39(4): 388-392.

Youssef, F., T. Roukas and C. G. Biliaderis. 1999. Pullulan production by a nonpigmented strain of Aureobasidium pullulans using batch and fed-batch culture. <u>Process Biochemistry</u> 34(4): 355-366. Yurlova, N. A. and G. S. de Hoog. 1997. A new variety of Aureobasidium pullulans characterized by exopolysaccharide structure, nutritional physiology and molecular features. <u>Antonie Van Leeuwenhoek</u> 72(2): 141-147.

Yurlova, N. A., J. M. Uijthof and G. S. de Hoog. 1996. Distinction of species in Aureobasidium and related genera by PCR-ribotyping. <u>Antonie Van Leeuwenhoek</u> 69(4): 323-329.

Zalar, P., C. Gostincar, G. S. de Hoog, V. Ursic, M. Sudhadham and N. Gunde-Cimerman. 2008. Redefinition of Aureobasidium pullulans and its varieties. <u>Stud Mycol</u> 61: 21-38.



จุฬาลงกรณมหาวทยาลย Chulalongkorn University

REFERENCES



จุฬาลงกรณ์มหาวิทยาลัย Chulalongkorn University



Appendix A

Culture media

1. Malt Extract Agar (MEA)

Malt extract	20.0	g
Peptone	1.0	g
Dextrose	20.0	g
Agar	25.0	g

Dissolved in distilled water to final volume 1 liter.

Note that: sterile dextrose should be prepared separately and added after autoclaving to prevent caramelization.

2. Yeast Malt Agar (YMA)

Yeast extract	3.0	g
Malt extract	3.0	g
Bacto-Peptone	5.0	g
Dextrose	10.0	g
Agar	20.0	g

Dissolved in distilled water to final volume 1 liter.

Chulalongkorn University

3. Pullulan Production (PM) Medium

Sucrose	50.0	g
Bacto-Peptone	0.6	g
K ₂ HPO ₄	5.0	g
MgSO ₄ .7H ₂ O	0.4	g
NCI	1.0	g
Yeast extract	0.4	g

Dissolved in 800 ml of distilled water and adjusted to pH to 6.5 with HCL.

Added distilled water to final volume 1 liter.

4. Xyalanase production medium.

4.1

Basal medium		
Yeast nitrogen base	6.7	g
L-asparagine	2.0	g
K ₂ HPO ₄	5.0	g
Glucose	10.0	g

Dissolved in distilled water to final volume 1 liter.

4.2 Xylan production medium

6.7	g
2.0	g
5.0	g
10.0	g
	2.0

จุฬาลงกรณ์มหาวิทยาลัย Chulalongkorn University

Appendix B Morphological studies of each isolates

PDA MEA YMA PBUAP4 PBUAP5 PBUAP5.1 PBUAP7.1 PBUAP9 PBUAP13 .0 0 -PBUAP14 -.

	PDA	MEA	YMA
PBUAP16			
PBUAP17			
PBUAP20		•	
PBUAP22			
PBUAP23			
PBUAP24			
PBUAP25			
PBUAP26			

	PDA	MEA	YMA
PBUAP27			
PBUAP29			
PBUAP30	P		
PBUAP31			
PBUAP32			
PBUAP33	0.0		
PBUAP34			
PBUAP35			

	PDA	MEA	YMA
PBUAP36			
PBUAP37			
PBUAP38			
PBUAP39	0		
PBUAP40			
PBUAP41			
PBUAP42			
PBUAP43			

	PDA	MEA	YMA
PBUAP44			
PBUAP45			
PBUAP46			
PBUAP47			
PBUAP48			
PBUAP49			
PBUAP50			
PBUAP51			

	PDA	MEA	YMA
PBUAP53			
PBUAP55			
PBUAP58			
PBUAP59			
PBUAP61			
PBUAP62			
PBUAP65			
PBUAP67			

	PDA	MEA	YMA
PBUAP70			
PBUAP71			
PBUAP72			
PBUAP73	\bigcirc		
PBUAP75			
PBUAP76			
PBUAP77			

Appendix C

GenBank accession number and sequences of organisms used in this study.

Strain*	ITS	TUB	ELO
EXF-2481 ^T	FJ150895	FJ157878	FJ039845
EXF-2479	FJ150893	FJ157877	FJ039846
CBS 147.97 ^T	FJ150875	FJ157863	FJ039822
CBS 105.22 ^T	FJ150886	FJ157858	FJ039812
CBS 123.37	FJ150881	FJ157852	FJ039818
CBS 109810	FJ150901	FJ157868	FJ039838
CBS 100524 ^T	FJ150905	FJ157867	FJ039839
CBS 100524 CBS 342.66	FJ150903	FJ157872	FJ039823
CBS 388.92 CBS 133856 ^T	FJ150872	FJ157874 EU719407	FJ039847 GCCTTTACCTCGCTCAAGGGCTACAGACCCCAAG
		จุษาลงกรณ์มหาวิทย	ACTTCCGTTTCGTGCCTGGAAAGACCCCCGATGGC TACCTTCAGGGAGACTGCCACCATGCTCATCGCC TACTACATCATCATCTTTGGTGGCAGAGAGTTCAT GCGCGGTCGCGAGCCTTTCAAGCTCAGCTTTTCT TCAAGCTCCACAACTTCTACTTGACCGTCATCAGC GGTGTCCTCCTCGCGCTCTTCGTTGAGCAGCTTCT GCCCGAGATTGTCAGAAACGGCGTCTTCCACGCT GTCTGCGCCTACGAGGGTGGCTGGACTGACAAG CTTGTTGTTCTTTACTACGTATGTTGATTGCGATTC GCGACTGAATGCGCTTACTGACGAGGTTGCAGCTC AACTACCTCACCAAGTACCTCGAGCTGATTGACAC CTGCTTCCTGTTCCTCAAGAAGAAGCCCCTGAGTA AGTTCAATCCATCTTCGGCGCATCTCATCGCACT ACCTGACCAACCTCACAGCTTTCCACCCT ACCTGACCAACCTCACAGCTTTCCCACACTTAC CACCACGGTGCTACCGCCCTTCTCTGCTTCACCC AGCTCCTCGGTCACACCGCCGTCTCCTGGGTTCC CATCACCTCAACCTGACCGCCGTCTCCTGGGTTCC CATCACCTCAACCTGACCGCCTCCTGGGTTCC
CBS 133857	JX462675	EU719412	CTACAGACCCCAAGACTTCCGTTTCGTGCCCGGA AAGACCCCAATGGCTACCTTCAAGGAGACTGCCA CCATGCTCATCGCCTACTACATCATCATTTTTGGTG GCAGAGAGTTCATGCGCGGTCGCGAGCCCTTCAA GCTCAGCTTCTTCTTCAAGCTCCACAACTTCTACTT GACCGTCATCAGCGGTGTTCTCCTCGCGCTCTTC GTTGAGCAGCTTCTGCCCGAGATTGTCAGAAACG GCGTCTTCCACGCTGTCTGCGCCTACGAGGGTGG CTGGACTGACAAGCTTGTTGTTCTTTACTACGTAC GTGGAATGCGATCAGCGACTGAATGCGCTTACTG ACGAGTTGCAGCTCAACTACCTGACCAAGTACCT CGAGCTGATTGACACCTGCTTCCTGTTTCTCAAGA AGAAGCCCCTGAGTGAGTTCAATCCATTTTCGGC GCAATTCACTCGCACCAACTGATCACCTCCTAGC TTTCCTCCACACTTACCACCTGATCAACCTCCTAGC TTTCCTCCACACTTACCACCTGATCAACCTCCTAGC CTTCTCTGGTTCACCCAGCTCCTGGTCACACCTC CGTCTCCTGGGTTCCCATCACCTCAACCTCCTG GTCCACGTCGTCATGTACTGGTACTACTTCCAGGC CGCACTGGCATCCGCATCTGGTACTACTTCCAGGC

	KDOGE426		
PBUAP4	KP965436	GGTGCTGCTTTCTGGCAGACCA	GTAGTACCAGTACATGACAACGTGGACGGTCAGG
		TCTCTGGCGAGCACGGCCTCG	TTCAGGGTGATGGGGGACCCATGAGACTGCAGTGT
		ACGGTGCTGGTGTGTACGTCG	GGCCGAGAAGCTGGGTGAAGCAGAGGAGGGCA
		ACTCCCTGCGCATCCTCTCATG	GTGGCACCGTGGTGGTAGGTGTGGAGGAAAGCT
		TAGTGACAGCTCGCTGACTGCC	AGGAAGCCAGTCAGCTAGTGGGTCGATAATGGAG
		TAACAGCTACAACGGTACCTCA	ATGGAGCGCACTTACTCAAAGGCTTCTTCTTGAGG
		GATCTCCAGCTGGAGCGCATG	AAAAGGAAGCAAGTGTCAATCAGCTCGAGGTACT
		AACGTCTACTTCAACGAGGTCA	TGGTGAGGTAGTTGAGCTGCCACTTGTCAGTAAAC
		GTCACCCATCTTTGGCCCTTGA	GAATTCTGGTGCAGACGTGGCAGAACTCACGTAG
		TTCGCAACTTCAATGCTGATGC	TAAAGGACGACAAGCTTGTCAGTCCAGCCACCCT
		GCACCGTAGGCCTCTGGTAAC	CGTAGGCGCAGACAGCGTGGAAAATGCCGTTTCT
		AAGTATGTCCCCCGTGCCGTCC	GACAATCTCGGGCAGGAGCTGCTCAACGAACAG
		TCGTCGACTTGGAGCCTGGTAC	AACCAAGAGAAGACCACTGATGAGGGTCAGGTAG
		CATGGACGCCGTCCGTGCCGG	AAGTTGTGAACCTTGAAGAAGAAGTTGAGCTTGAA
		TCCTTTCGGTCAGCTCTTCCGT	CGGCTCGCGACCACGCATGAGCTCTCTGCCACC
		CCCGACAACTTCGTCTTCGGTC	GAAGATGATGATGTAGTAGGCAATGAGCATGGTG
		AGTCCGGTGCTGGCAACAACT	GCAGTCTCCTTGAAGGTAGCCATAGGGGTCTTTC
		GGGCCAAGGGTCACTACACTG	CAGGAACGAAGCGGAAGTCCTGGGGCTTGTAGC
		AGGGTA	CCTTGATCGAGGTGAAAGCCT
PBUAP5	KP965437	GTGCTGCTTTCTGGCAGACCAT	GTAGTACCAGTACATGACAACGTGGACGGTCAGGTTC
		CTCCGGCGAGCACGGCCTTGA	AGGGTGATGGGAACCCATGAGACTGCGGTGTGGCCG
		CGGTGCTGGTGTGTACGTCGA	AGAAGCTGGGTGAAGCAGAGAAGGGCGGTGGCACCG
		CTCCCTGCGCACCCCACCCG	TGGTGGTAGGTGTGGAGGAAAGCTAGGAGACTGGTCA
		CCGTGATAGCTCGCTGACTGC	GCTAAAGCATTGGTTGTGGAGATATTGCGCGCTTACTC
		CTAACAGCTACAACGGTACCTC	AAAGGCTTCTTCTTGAGGAAAAGGAAGCAGGTGTCAAT
		AGATCTCCAGCTGGAGCGCAT	CAGCTCGAGGTACTTGGTGAGGTAGTTGAGCTGCCAG
		GAACGTCTACTTCAACGAGGTC	CTGTTAGTAAGGGCATTCTGGCCCGATTATGGCAGAAC
		AGCCATCCAACGTCGACCCTTG	ATACGTAGTAAAGAACAACAAGCTTGTCGGTCCAGCCA
		CTTCACTACTTGAATGCTAATGC	CCCTCGTAGGCGCAGACGGCGTGGAAAATGCCGTTTC
		GCACCATAGGCCTCCGGTAAC	TGACAATCTCGGGCAGGAGCTGCTCAACGAAGAGAAC
		AAGTATGTCCCCCGTGCCGTCC	CAAGAGAAGACCACTGATGACGGTCAGGTAGAAGTTG
		TCGTCGACTTGGAGCCCGGTA	TGGACCTTGAAGAAGAAGTTGAGCTTGAAAGGCTCGC
		CCATGGACGCCGTCCGTGCCG	GACCACGCATGAACTCTCTGCCACCAAAGATGATGAT
		GTCCCTTCGGTCAGCTCTTCCG	GTAGTAGGCAATGAGCATGGTGGCCGTCTCCTTGAAG
		TCCCGACAACTTCGTCTTTGGT	GTAGCCATAGGGGTCTTTCCAGGCACAAAGCGGAAGT
		CAGTCCGGTGCTGGCAACAAC	CCTGGGGCTTGTAGCCCTTGATCGAGGTGAAAGCCTT
		TGGGCCAAGGGTCACTA	CTCGAAT
PBUAP5.1	KP965438	GACGAAGTTGTCGGGACGGAA	CGATCAAGGGCTACAAGCCCCAGGACTTCCGCTTTGT
		GAGCTGACCGAAGGGACCGG	GCCTGGAAAGACCCCTATGGCTACCTTCAAGGAGACG
		CACGGACGGCGTCCATGGTAC	GCCACCATGCTCATTGCCTACTACATCATCATCTTTGGT
		CGGGCTCCAAGTCGACGAGGA	GGCAGAGAGTTCATGCGTGGTCGCGAGCCTTTCAAGC
		CGGCACGGGGGGACATACTTGT	TCAACTTCTTCTTCAAGGTCCACAACTTCTACCTGACCG
		TACCGGAGGCCTATGGTGCGC	TCATCAGTGGTCTTCTCTTGGTTCTCTTCGTTGAGCAGC
		ATTAGCATTCAAGTAGTGAAGC	TCCTGCCCGAGATTGTCAGAAACGGCATTTTCCACGCC
		AAGGGTCGACGTTGGATGGCT	GTCTGCGCCTACGAGGGTGGCTGGACCGACAAGCTT
		GACCTCGTTGAAGTAGACGTTC	GTTGTTCTTTACTACGTATGTTCTGCCATAATCGGGCCA
		ATGCGCTCCAGCTGGAGATCT	GAATGCCCTTACTAACAGCTGGCAGCTCAACTACCTCA
		GAGGTACCGTTGTAGCTGTTAG	CCAAGTACCTCGAGCTGATTGACACCTGCTTCCTTTTC
		GCAGTCAGCGAGCTATCACGG	CTCAAGAAGAAGCCTTTGAGTAAGCGCGCAATATCTCC
		CGGGTGGGGGTGCGCAGGGA	ACAACCAATGCTTTAGCTGACCAGTCTCCTAGCTTTCCT
		GTCGACGTACACACCAGCACC	CCACACCTACCACCGCGCCCCCCCCCCCCCCCCCCCCCC
1			
		GTCAAGGCCGTGCTCGCCGGA	TTCACCCAGCTTCTCGGCCACACCGCAGTCTCATGGG
		GTCAAGGCCGTGCTCGCCGGA GATGGTCTGCCAGAAAGCAGC	TTCACCCAGCTTCTCGGCCACACCGCAGTCTCATGGG TTCCCATCACCCTGAACCTGACCGTCCACGTTGTCATG

		TACCOTOACTOTACTOACCOTT	
PBUAP7.1	KP965439	TACCCTCAGTGTAGTGACCCTT	TATTCGAGAAGGCTTTCACCTCGATCAAGGGCTAC
		GGCCCAGTTGTTGCCAGCACC	AAGCCCCAGGACTTCCGCTTTGTGCCTGGAAAGA
		GGACTGACCAAAGACGAAGTT	CCCCTATGGCTACCTTCAAGGAGACGGCCACCAT
		GTCGGGACGGAAGAGCTGACC	GCTCATTGCCTACTACATCATCATCTTTGGTGGCA
		GAAGGGACCGGCACGGACGG	GAGAGTTCATGCGTGGTCGCGAGCCTTTCAAGCT
		CGTCCATGGTACCGGGCTCCA	CAACTTCTTCTTCAAGGTCCACAACTTCTACCTGA
		AGTCGACGAGGACGGCACGG	CCGTCATCAGTGGTCTTCTCTTGGTTCTCTTCGTTG
		GGGACATACTTGTTACCGGAG	AGCAGCTCCTGCCCGAGATTGTCAGAAACGGCAT
		GCCTATGGTGCGCATTAGCATT	TTTCCACGCCGTCTGCGCCTACGAGGGTGGCTGG
		CAAGCAGTGAAGCAAGGGTCG	ACCGACAAGCTTGTTGTTCTTTACTACGTATGTTCT
		ACGTTGGATGgCTGACCTCGTT	GCCATAATCGGGCCAGAATGCCCTTACTAACAGC
		GAAGTAGACGTTCATGCGCTCC	TGGCAGCTCAACTACCTCACCAAGTACCTCGAGC
		AGCTGGAGATCTGAGGTACCG	TGATTGACACCTGCTTCCTTTCCTCAAGAAGAAG
		TTGTAGCTGTTAGGCAGTCAGC	CCTTTGAGTAAGCGCGCAATATCTCCACAACCAAT
		GAGCTATCACGGCGGGTGGGG	GCTTTAGCTGACCAGTCTCCTAGCTTTCCTCCACA
		GTGCGCAGGGAGTCGACGTAC	CCTACCACCACGGTGCCACCGCCCTTCTCTGCTT
		ACACCAGCACCGTCAAGGCCG	CACCCAGCTTCTCGGCCACACCGCAGTCTCATGG
		TGCTCGCCGGAGATGGTCTGC	GTTCCCATCACCCTGAACCTGACCGTCCACGTTGT
		CAGAAAGCAGCAC	CATGTACTGGTACTACTtCCAG
PBUAP9	KP965440	GGTGCTGCTTTCTGGCAGACCATC	GGGCTACAAGCCCCAGGACTTCCGCTTCGTTCCTGGA
I BOAI 3	11 903440	TCTGGCGAGCACGGCCTTGACGG	AAGACCCCCATGGCTACCTTCAAGGAGACGGCCACCA
			TGCTCATTGCCTACTACATCATCATCTTTGGTGGTAGAG
		CGCTGGTGTGTGTACGTCGACCCCC	
		TGCGCATCCTCACCCGCAATGACA	AGCTCATGCGTGGTCGCGAGCCTTTCAAGCTCAACTTC
		GCTCGCTGACTGCCTAACAGCTAC	TTCTTCAAGGTCCACAACTTCTACCTGACCGTCATCAG
		AACGGTACCTCAGATCTCCAGCTG	CGGTCTTCTCTTGGTTCTCTTCGTTGAGCAGCTCCTGC
		GAGCGCATGAACGTCTACTTCAAC	CCGAGATTGTCAGAAACGGCATTTTCCACGCTGTCTGC
		GAGGTCGGTCACCAACTGTCGGC	GCCTACGAGGGCGGCTGGACTGACAAGCTCGTTGTTC
		CTTTCACTCACTCCTTCAATGCTAA	TTTACTACGTACGTTTATCCAATTCCGCGATAGAATGCG
		TGCGCACCATAGGCCTCTGGTAAC	CTTACTGACAGCTGGCAGCTCAACTACCTCACCAAGTA
		AAGTATGTCCCCCGTGCCGTCCTC	CCTCGAGCTGATTGACACCTGCTTCCTTTCCTCAAGA
		GTCGATTTGGAGtCCTGGTACCAT	AGAAGCCTTTGAGTAAGCGCGCATTACCTCCACAATCA
		GGACGCCGTCCGTGCTGGTCCCT	ATGCTTTAGCTGACTGATCTCCCAGCTTTCCTCCACAC
		TCGGTCAGCTCTTCCGTCCCGACA	CTACCACCACGGTGCCACTGCTCTTCTCTGCTTCACCC
		ACTTCGTCTTCGGTCAGTCCGGTG	AGCTTCTCGGCCACACCGCAGTTTCATGGGTCCCCAT
		CTGGCAACAACTGGGCCAAGGGT	CACCCTGAACTTGACCGTCCACGTCGTCATGTACTGGT
		CA	ACTAC
PBUAP13	KP965441	GGTGCTGCTTTCTGGCAGACCA	TTTCACCTCGCTCAAGGGCTACAAGCCCCAGGACTTC
		TCTCTGGCGAGCACGGCCTTG	CGCTTCGTCCCTGGAAAGACCCCCATGGCTACCTTCA
		ACGGTGCTGGTGTGTACGTTGA	AGGAGACGGCCACCATGCTCATCGCCTACTACATCAT
		CTCCCTGCGCATCCTCACCCG	CATCTTTGGTGGCAGAGAGCTCATGCGTGGTCGCGAG
		CAATGACAGCTCGCTGACTGC	CCTTTCAAGCTCAACTTCTTCTTCAAGGTCCACAACTTC
		CTAACAGCTACAACGGTACCTC	TACTTGACCGTCATCAGCGGTCTTCTCTTGGTTCTCTTC
		AGATCTCCAGCTGGAGCGCAT	GTCGAGCAGCTCCTGCCCGAGATTGTCAGAAACGGCA
		GAACGTCTACTTCAACGAGGTC	TTTTCCACGCTGTCTGCGCCTACGAGGGCGGTTGGAC
		AGTCACCAACTGTCCGCCCTTC	TGACAAGCTCGTTGTTCTTTACTACGTACGTTTTTCTAAT
		AGTGACCAACTGTCCGCCCTTC	TCCGCGCCAGAACGCGCCTACTGACAGCTGGCAGCT
		GCGCACCATAGGCCTCTGGTA	
		ACGAAGTATGTCCCCCGTGCC	GCTTCCTTTTCCTCAAGAAGAAGCCTCTGAGTAAGCGC
		GTCCtTCGTCGACTTGGAGCCT	GCAATATCTCCACCATCCATGCTCTAGCTGATTGATCTA
		GGTACCATGGACGCCGTCCGT	TTAGCTTTCCTCCACACCTACCACCACGGTGCCACTGC
		GCTGGTCCCTTCGGTCAGCTTC	CCTCCTCTGCTTTACCCAGCTTCTCGGCCACACCGCA
		TTCCGTCCCGACAACTTCGTCT	GTCTCATGGGTTCCCATCACCCTGAACCTGACCGTCCA
		TCGGTCAGTCCGGTGCTGGCA	CGTTGTCATGTACTGGTACTACTYCCAGGCCGCACGTG
		ACAACTGGGCCAAGGGTC	GCATCCGCATCTGGTGNN

PBUAP14	KP965442	GTGCTGCTTTCTGGCAGACCAT	AGGCTTTCACCTCCCTCAAGGGCTACAAGCCCCA
		CTCTGGCGAGCACGGCCTTGA	GGACTTCCGCTTCGTTCCTGGAAAGACCCCCCATG
		CGGCGCTGGTGTGTACGTCGA	GCTACCTTCAAGGAGACGGCCACCATGCTCATTG
		CCCCCTGCGCATCCTCACCCG	CCTACTACATCATCATCTTTGGTGGTAGAGAGCTC
		CAATGACAGCTCGCTGACTGC	ATGCGTGGTCGCGAGCCTTTCAAGCTCAACTTCTT
		CTAACAGCTACAACGGTACCTC	CTTCAAGGTCCACAACTTCTACCTGACCGTCATCA
		AGATCTCCAGCTGGAGCGCAT	GCGGTCTTCTCTTGGTTCTCTTCGTTGAGCAGCTC
		GAACGTCTACTTCAACGAGGTC	CTGCCCGAGATTGTCAGAAACGGCATTTTCCACG
		GGTCACCAACTGTCGGCCTTTC	CTGTCTGCGCCTACGAGGGCGGCTGGACTGACA
		ACTCACTCCTTCAATGCTAATAC	AGCTCGTTGTTCTTTACTACGTACGTTTATCCAATT
		GCACCATAGGCCTCTGGTAACg	CCGCGATAGAATGCGCTTACTGACAGCTGGCAGC
		AAGTATGTCCCCCGTGCCGTCC	TCAACTACCTCACCAAGTACCTCGAGCTGATTGAC
		tTCGTCGATTTGGAGCCTGGTA	ACCTGCTTCCTTTCCTCAAGAAGAAGCCTTTGAG
		CCaATGGACGCCGTCCGTGCT	TAAGCGCGCATTACCTCCACAATCAATGCTTTAGC
		GGTCCCTTCGGTCAAGCTTACT	TGACTGATCTCCCAGCTTTCCTCCACACCTACCAC
		TCCGTCCCGACAACTTCGTCTT	CACGGTGCCACTGCTCTTCTCTGCTTCACCCAGCT
		CGGTCAGTCCGGTGCTGGCAA	TCTCGGCCACACCGCAGTTTCATGGGTCCCCATC
		CAACTGGGCCAAGGGTC	ACCCTGAACTTGACCGTCCACGTCGTCATGTACTG
			GTAC
PBUAP16	KP965443	TGCTGCTTTCTGGCAGACCATCTC	TGGAAGTAGTACCAGTACATGACAACATGAACGGTCAA
		TGGCGAGCACGGCCTTGACGGTG	ATTGAGGGTGATGGGAACCCATGAGACTGCGGTGTGG
		CTGGTGTGTACGTCGATTCCCTGC	CCGAGAAGCTGGGTGAAGCAGAGAAGGGCAGTGGCA
		GCATCCCCATCCGTCGTGATAGCT	CCGTGGTGGTAGGTGTGGAGGAAAGCTAGGAGCCCA
		CGCTGACTGCCTGACAGCTACAAT	ATTAGTTGGTGTATCGGGTGTGAAGATATTGCGCGCTT
		AGGTACCTCAGATCTCCAGCTGGA	ACTCAAAGGCTTCTTCTTGAGGAAAAGGAAGCAGGTGT
		GCGCATGAACGTCTACTTCAACGA	CAATCAGCTCGAGGTACTTGGTAAGGTAGTTGAGCTGC
		GGTCAGTGGCCAACCTTGGGCCC	CAGCTGTCAGTAAGCGCATTCTGGTGCGGATACGACA
		TTCCTTCACGACTTCATTGCTAATG	AAACGTACGTAGTAAAGAACAACAAGCTTGTCGGTCCA
		ACTATAGGCCTCTGGTAACAAGTA	GCCACCCTCGTAGGCGCAGACAGCGTGGAAGATACC
		TGTCCCCCGCGCCGTCCTCGTCG	GTTTCTGACAATCTCGGGCAGGAGCTGCTCAACGAAG
		ACTTGGAGCCTGGTACCATGGAC	AGAACCAAGAGAAGACCACTGATGACGGTCAGGTAGA
		GCCGTCCGTGCCGGCCCCTTCGG	AGTTGTGGACCTTGAAGAAGAAGTTGAGCTTGAAAGG
		TCAGCTTCTTCCGTCCCGACAACT	CTCGCGACCACGCATGAACTCTCTGCCACCAAAGATG
		TCGTCTTCGGCCAGTCCGGTGCTG	ATGATGTAGTAGGCGATGAGCATGGTGGCTGTCTCCTT
		GCAACAACTGGGCCAAGGGTC	GAAGGTAGCCATGGGAGTCTTTCCAGGGACGAAGCG
			GAAGTCCTGGGGCTTGTAGCCCTTGATCGAGGTGAAA
PBUAP17	KP965444	GGTGCTGCTTTCTGGCAGACCA	GGCCTTCACCGCCGTCAAGGGCTACAAGCCCCAGGA
1.50/ 4.11		TCTCTGGCGAGCACGGCCTTG	CTTCCGCTTCGTCCCCGGAAAGACGCCTATGGCTACTT
		ACGGTGCTGGTGTGTGTCG	TCAAGGAGACGGCCACCATGCTCATTGCCTACTATATC
		ACAGCGCTAGCGCATCCCATG	ATCATTTTTGGCGGCAGAGAGTTTATGCGTGGCCGCGA
		CCTCTCGTGACGCCTCTCTGAC	GCCCTTCAAGCTCAGCTTCTTCTTCAAGCTCCACAACTT
		ATGCTCGCAGCTACAATGGCAC	CTACCTGACTCTGATCAGCGGCATTCTCCTGGTTCTGT
		CTCGGACCTCCAGCTTGAGCG	TCGTTGAGCAGCTTCTGCCCGAAATTGTCAGAAATGGC
		CATGAACGTCTACTTCAACGAG	ATTTTCCACGCAGTCTGCGCCTACGAGGGTGGCTGGA
		GTGAGCCCTTCACACCACCTCC	CCGACAAGCTTGTTGTTCTCTACTACGTGAGTGGCTGGA
		GCTGCCCTCCCATGCATCGGC	GAGTCGCGACAAGGTGCCCTTACTACGTGACGAGAGTCGCAG
		TAACGCGCTGCAGGCCTCCGG	CTCAACTACCTGACCAAGGTGCCCTTACTGACAAGATCGCAG
		CAACAAGTATGTTCCCCGTGCC	CTCAACTACCTGACCAAGTACCTCGAGCTCATTGACAC
		GTCCTCGTCGACTTGGAGCCC	
			CCACCTGACGGAACCGTCTACCAGTCGCATTAGCTGA
		GGTACCATGGACGCCGTCCGT	
		GCCGGTCCCTTCGGCCAGCTC	CGCTACCGCTCTCCTCTGCTTCACTCAGCTCCTCGGTC
		TTCCGTCCCGACAACTTCGTCT	ACACTTCCGTCTCTTGGGTTCCCATCACCCTGAACCTG
		TCGGTCAGTCCGGTGCTGGCA	ACCGTCCACGTCGTCATGTACTGGTACTACTTCCAGGC
		ACAACTGGGCCAAGGGT	CGCACGTGGCATCCGTATCTGGTGGAANAN

PBUAP20	KP965445	GGTGCTGCTTTCTGGCAGACCA	GTAGTACCAGTACATGACAACGTGGACGGTCAGG
F BUAF 20	KF 905445	TCTCTGGCGAGCACGGCCTTG	TTCAGGGTGATGGGAACCCATGAGACTGCGGTGT
		ACGGTGCTGGTGTGTGTGCGTTGA	GGCCGAGAAGCTGGGTAAAGCAGAGGAGGGCA
		CTCCCTGCGCATCCTCACCCG	GTGGCACCGTGGTGGTGGGGGGGGGGGGGGGGGGGGGGG
		CAATGACAGCTCGCTGACTGC	AATAGATCAATCAGCTAGAGCATTGATGGTGGAGA
		CTAACAGCTACAACGGTACCTC	TATTGCGCGCTTACTCAGCAGGCTTCTTCTTGAGGA
		AGATCTCCAGCTGGAGCGCAT	AAAGGAAGCAGGTGTCAATCAGCTCGAGGTACTT
			GGTGAGGTAGTTGAGCTGCCAGCTGTCAGTAGCT
		GAACGTCTACTTCAACGAGGTC	
		AGTCACCAACTAGTCCGCCCTT	GCGTTCTGGCGCAGAATTAGAAAAACGTACGTAG
		CACTGACTACTCCGAATGCTAA	TAAAGAACAACGAGCTTGTCAGTCCAGCCGCCCT
		TGCGCACCATAGGCCTCTGGTA	CGTAGGCGCAGACAGCGTGGAAAATGCCGTTTCT
		ACNAAGTATGTCCCCCGTGCC	GACAATCTCGGGCAGGAGCTGCTCGACGAAGAG
		GTCCTCGTCGACTTGGAGCCTG	AACCAAGAGAAGACCGCTGATGACGGTCAAGTAG
		GTACCATGGACGCCGTCCGTG	AAGTTGTGGACCTTGAAGAAGAAGTTGAGCTTGAA
		CCGGTCCCTTCGGTCAGCTTCT	AGGCTCGCGACCACGCATGAGCTCTCTGCCACCA
		TCCGTCCCGACAACTTCGTCTT	AAGATGATGATGATGTAGTAGGCGATGAGCATGGTGG
		CGGTCAGTCCGGTGCTGGCAA	CCGTCTCCTTGAAGGTAGCCATGGGGGTCTTTCC
		CAACTGGGCCAAGGGTC	AGGGACGAAGCGGAAGTCCTGGGGCTTGTAGC
PBUAP22	KP965446	GACCCTTGGCCCAGTTGTTGCC	TTTCACCTCCCTCAAGGGCTACAAGCCCCAGGACTTC
		AGCACCGGACTGACCGAAGAC	CGCTTCGTCCCTGGAAAGACCCCTATGGCTACCTTCAA
		GAAGTTGTCAGGACGGAAGAA	GGAGACGGCCACCATGCTCATTGCCTACTACATCATCA
		GCTGACCGAAGGGACCGGCAC	TCTTTGGTGGCAGAGAGCTCATGCGTGGTCGCGAGCC
		GGACGGCGTCCATGGTACCAG	TTTCAAGCTCAACTTCTTCTTCAAGGTCCACAACTTCTA
		GCTCCAAGTCGACGAGGACGG	CCTGACCGTCATCAGCGGTCTCCTCTTGGTTCTGTTCG
		CACGGGGGGACATACTTGTTACC	TCGAGCAGCTCTTGCCCGAGATTGTCAGAAACGGCAT
		AGAGGCCTATGGTGCGCATTA	TTTCCACGCTGTCTGCGCCTACGAGGGCGGCTGGACC
		GCATTGGAGTAGTCAGTGAAG	GACAAGCTCGTTGTTCTTTACTACGTACGTTTTTCCAGC
		GGCGGACAGTTGGTGACTGAC	TTCTCGCCAGAATGCGCTTACTGACAGCTGGCAGCTC
		CTCGTTGAAGTAGACGTTCATG	AACTACCTCACCAAGTACCTTGAGCTGATTGACACCTG
		CGCTCCAGCTGGAGATCTGAG	CTTCCTTTTCCTCAAGAAGAAGCCTTTGAGTAAGCGCG
		GTACCGTTGTAGCTGTTAGGCA	CAATATTTTCACAATCAATGCTTTAGCTGACTGGTCTCC
		GTCAGCGAGCTGTCATTGAGG	TAGCTTTCCTCCACACCTACCACCACGGTGCCACTGC
		ATGCGCAGAGAGTCAACGTAC	CCTTCTCTGCTTTACCCAGCTTCTTGGCCACACCGCAG
		ACACCAGCACCGTCAAGGCCG	TCTCATGGGTTCCCATCACCCTGAACTTGACCGTCCAC
		TGCTCGCCAGAGATGGTCTGC	GTTGTCATGTACTGGTACTAC
		CAGAAAGCAGCACC	
PBUAP23	KP965447	GGTGCTGCTTTCTGGCAGACCA	TCACCTCCCTCAAGGGCTACAAGCCCCAGGACTTCCG
		TCTCTGGCGAGCACGGCCTTG	CTTCGTCCCTGGAAAGACCCCTATGGCTACCTTCAAGG
		ACGGTGCTGGTGTGTACGTTGA	AGACGGCCACCATGCTCATTGCCTACTACATCATCATC
		CTCTCTGCGCATCCTCAATGAC	TTTGGTGGCAGAGAGCTCATGCGTGGTCGCGAGCCTT
		AGCTCGCTGACTGCCTAACAG	TCAAGCTCAACTTCTTCTTCAAGGTCCACAACTTCTACC
		CTACAACGGTACCTCAGATCTC	TGACCGTCATCAGCGGTCTCCTCTTGGTTCTGTTCGTC
		CAGCTGGAGCGCATGAACGTC	GAGCAGCTCTTGCCCGAGATTGTCAGAAACGGCATTTT
		TACTTCAACGAGGTCAGTCACC	CCACGCTGTCTGCGCCTACGAGGGCGGCTGGACCGA
		AACTGTCCGCCCTTCACTGACT	CAAGCTCGTTGTTCTTTACTACGTACGTTTTTCCAGCTT
		ACTCCAATGCTAATGCGCACCA	CTCGCCAGAATGCGCTTACTGACAGCTGGCAGCTCAA
		TAGGCCTCTGGTAACAAGTATG	CTACCTCACCAAGTACCTTGAGCTGATTGACACCTGCT
		TCCCCCGTGCCGTCCTCGTCG	TCCTTTTCCTCAAGAAGAAGCCTTTGAGTAAGCGCGCA
		ACTTGGAGCCTGGTACCATGGA	ATATTTTCACAATCAATGCTTTAGCTGACTGGTCTCCTA
		CGCCGTCCGTGCCGGTCCCTT	GCTTTCCTCCACACCTACCACCACGGTGCCACTGCCC
		CGCCGTCCGTGCCGGTCCCTT CGGTCAGCTCTTCCGTCCTGAC	GCTTTCCTCCACACCTACCACCACGGTGCCACTGCCC TTCTCTGCTTTACCCAGCTTCTTGGCCACACCGCAGTC
		CGGTCAGCTCTTCCGTCCTGAC	TTCTCTGCTTTACCCAGCTTCTTGGCCACACCGCAGTC

PBUAP24	KP965448	GTGCTGCTTTCTGGCAGACCAT	GGCTTTCACTTCCCTCAAGGGCTACAAGCCCCCAG
		CTCTGGCGAGCACGGCCTTGA	GACTTCCGCTTCGTTCCTGGAAAGACCCCCATGG
		CGGCGCTGGTGTGTACGTCGA	CCACCTTCAAGGAGACGGCCACCATGCTCATTGC
		CCCCCTGCGCATCCTCACCCG	CTACTACATCATCATCTTTGGTGGTAGAGAGCTCA
		CAATGACAGCTCGCTGACTGC	TGCGTGGTCGCGAGCCCTTCAAGCTCAACTTCTTC
		CTAACAGCTACAACGGTACCTC	TTCAAGGTCCACAACTTCTACCTGACCGTCATCAG
		AGATCTCCAGCTGGAGCGCAT	CGGTCTTCTCTTGGTTCTCTTCGTTGAGCAGCTCC
		GAACGTCTACTTCAACGAGGTC	TGCCCGAGATTGTCAGAAACGGCATTTTCCACGCT
		GGTCACCAACTGTCGGCCTTTC	GTCTGCGCCTACGAGGGCGGCTGGACTGACAAG
		ACTCACTCCTTCAATGCTAATG	CTCGTTGTTCTTTACTACGTACGTTTATCCAATTCC
		CGCACCATAGGCCTCTGGTAA	GCGACAGAATGCGCTTACTGACAGCTGGCAGCTC
		CAAGTATGTCCCCCGTGCCGTC	AACTACCTCACCAAGTACCTCGAGCTGATTGACAC
		CTCGTCGATTTGGAGCCTGGTA	CTGCTTCCTTTTCCTCAAGAAGAAGCCTTTGAGTA
		CCATGGACGCCGTCCGTGCTG	AGCGCGCACTACCTCCACAATCAATGCTTTAGCTA
		GTCCCTTCGGTCAGCTTCTTCC	ATTGGGCTCCTAGCTTTCCTCCACACCTACCACCA
		GTCCCGACAACTTCGTCTTCGG	CGGTGCCACTGCTCTTCTCTGCTTCACCCAGCTTC
		TCAGTCCGGTGCTGGCAACAA	TCGGCCACACCGCAGTTTCATGGGTCCCCATCAC
		CTGGGCCAAGGGTC	CCTGAACTTGACCGTCCACGTCGTCATGTACTGGT
			ACTA
PBUAP25	KP965449	GTGCTGCTTTCTGGCAGACCATCT	AGGCTTTCACCTCCCTCAAGGGCTACAAGCCCCAGGA
		CTGGCGAGCACGGCCTTGACGGT	CTTCCGCTTCGTCCCTGGAAAGACCCCTATGGCTACCT
		GCTGGTGTGTACGTTGACTCTCTG	TCAAGGAGACGGCCACCATGCTCATTGCCTACTACATC
		CGCATCCTCAATGACAGCTCGCTG	ATCATCTTTGGTGGCAGAGAGCTCATGCGTGGTCGCG
		ACTGCCTAACAGCTACAACGGTAC	AGCCTTTCAAGCTCAACTTCTTCTTCAAGGTCCACAACT
		CTCAGATCTCCAGCTGGAGCGCAT	TCTACCTGACCGTCATCAGCGGTCTCCTCTTGGTTCTG
		GAACGTCTACTTCAACGAGGTCAG	TTCGTCGAGCAGCTCTTGCCCGAGATTGTCAGAAACG
		TCACCAACTGTCCGCCCTTCACTG	GCATTTTCCACGCTGTCTGCGCCTACGAGGGCGGCTG
		ACTACTCCAATGCTAATGCGCACC	GACCGACAAGCTCGTTGTTCTTTACTACGTACGTTTTTC
		ATAGGCCTCTGGTAACAAGTATGT	CAGCTTCTCGCCAGAATGCGCTTACTGACAGCTGGCA
		CCCCCGTGCCGTCCTCGTCGACTT	GCTCAACTACCTCACCAAGTACCTTGAGCTGATTGACA
		GGAGCCTGGTACCATGGACGCCG	CCTGCTTCCTTTCCTCAAGAAGAAGCCTTTGAGTAAG
		TCCGTGCCGGTCCCTTCGGTCAG	CGCGCAATATTTTCACAATCAATGCTTTAGCTGACTGGT
		CTCTTCCGTCCTGACAACTTCGTCT	CTCCTAGCTTTCCTCCACACCTACCACCACGGTGCCAC
		TCGGTCAGTCCGGTGCTGGCAAC	TGCCCTTCTCTGCTTTACCCAGCTTCTTGGCCACACCG
		AACTGGGCCAAGGGTC	CAGTCTCATGGGTTCCCATCACCCTGAACTTGACCGTC
			CACG
PBUAP26	KP965450	GGTGCTGCTTTCTGGCAGACCATC	TAGTACCAGTACATGACAACGTGGACGGTCAAGTTCA
1 00/11 20	11 000-00	TCTGGCGAGCACGGCCTTGACGG	GGGTGATGGGAACCCATGAGACTGCGGTGTGGCCAA
		TGCTGGTGTGTGTACGTTGACTCTCT	GAAGCTGGGTAAAGCAGAGAGAGGGCAGTGGGCCAA
		GCGCATCCTCAATGACAGCTCGCT	GGTGGTAGGTGTGGAGGAGAGGGCAGTGGCACCGT
		GACTGCCTAACAGCTACAACGGTA	GCTAAAGCATTGATTGTGAAAATATTGCGCGCTTACTCA
		CCTCAGATCTCCAGCTGGAGCGC	
		ATGAACGTCTACTTCAACGAGGGC	AAGGCTTCTTCTTGAGGAAAAGGAAGCAGGTGTCAATC
			AGCTCAAGGTACTTGGTGAGGTAGTTGAGCTGCCAGC
		AGTCACCAACTGTCCGCCCTTCAC	TGTCAGTAAGCGCATTCTGGCGAGAAGCTGGAAAAAC
		TGACTACTCCAATGCTAATGCGCA	GTACGTAGTAAAGAACAACGAGCTTGTCGGTCCAGCC
		CCATAGGCCTCTGGTAACAAGTAT	GCCCTCGTAGGCGCAGACAGCGTGGAAAATGCCGTTT
		GTCCCCCGTGCCGTCCTCGTCGA	CTGACAATCTCGGGCAAGAGCTGCTCGACGAACAGAA
		CTTGGAGCCTGGTACCATGGACG	CCAAGAGGAGACCGCTGATGACGGTCAGGTAGAAGTT
		CCGTCCGTGCCGGTCCCTTCGGT	GTGGACCTTGAAGAAGAAGATGAGCTTGAAAGGCTCG
		CAGCTCTTCCGTCCTGACAACTTC	CGACCACGCATGAGCTCTCTGCCACCAAAGATGATGA
		GTCTTCGGTCAGTCCGGTGCTGG	TGTAGTAGGCAATGAGCATGGTGGCCGTCTCCTTGAA
		CAACAACTGGGCCAAGGGTCA	GGTAGCCATAGGGGTCTTTCCAGGGACGAAGCGGAA
			GTCCTGGGG

DDU 4 D07		010011101000000000000000000000000000000	
PBUAP27	KP965451	CTGCTTTCTGGCAGACCATCTC	GGCTTTCACCTCGCTCAAGGGCTACAAGCCCCAG
		TGGCGAGCACGGCCTTGACGG	GACTTCCGCTTCGTCCCTGGAAAGACCCCCATGG
		TGCTGGTGTGTACGTTGACTCC	CTACCTTCAAGGAGACGGCCACCATGCTCATCGC
		CTGCGCATCCTCACCCGCAAT	CTACTACATCATCATCTTTGGTGGCAGAGAGCTCA
		GACAGCTCGCTGACTGCCTAA	TGCGTGGTCGCGAGCCTTTCAAGCTCAACTTCTTC
		CAGCTACAACGGTACCTCAGAT	TTCAAGGTCCACAACTTCTACTTGACCGTCATCAG
		CTCCAGCTGGAGCGCATGAAC	CGGTCTTCTCTTGGTTCTCTTCGTCGAGCAGCTCC
		GTCTACTTCAACGAGGTCAGTC	TGCCCGAGATTGTCAGAAACGGCATTTTCCACGCT
		ACCAACTGTCCGCCCTTCACTG	GTCTGCGCCTACGAGGGCGGTTGGACTGACAAG
		ACTACTCCAATGCTAATGCGCA	CTCGTTGTTCTTTACTACGTACGTTTTTCTAATTCCG
		CCATAGGCCTCTGGTAACAAGT	CGCCAGAACGCGCCTACTGACAGCTGGCAGCTC
		ATGTCCCCCGTGCCGTCCTCGT	AACTACCTCACCAAGTACCTCGAGCTGATTGACAC
		CGACTIGGAGCCTGGTACCAT	CTGCTTCCTTTTCCTCAAGAAGAAGCCTCTGAGTA
		GGACGCCGTCCGTGCTGGTCC	AGCGCGCAATATCTCCACCATCCATGCTCTAGCT
		TTTCGGTCAGCTACTTCCGTCC	GATTGATCTATTAGCTTTCCTCCACACCTACCACC
		CGACAACTTCGTCTTCGGTCAG	ACGGTGCCACTGCCCTCCTCTGCTTTACCCAGCTT
		TCCGGTGCTGGCAACAACTGG	CTCGGCCACACCGCAGTCTCATGGGTTCCCATCA
		GCCAAGGGTCAC	CCCTGAACCTGACCGTCCACGTTGTCATGTACTG
			GTACTA
PBUAP29	KP965452	GGTGCTGCTTTCTGGCAGACCATC	AGGCTTTCACCTCGCTCAAGGGCTACAAGCCCCAGGA
		TCTGGCGAGCACGGCCTTGACGG	CTTCCGCTTCGTCCCTGGAAAGACCCCCATGGCTACC
		TGCTGGTGTGTACGTTGACTCCCT	TTCAAGGAGACGGCCACCATGCTCATCGCCTACTACAT
		GCGCATTCTCACCCGCAATGACA	CATCATCTTTGGTGGCAGAGAGCTCATGCGTGGTCGC
		GCTCGCTGACTGCCTAACAGCTAC	GAGCCTTTCAAGCTCAACTTCTTCTTCAAGGTCCACAA
		AACGGTACCTCAGATCTCCAGCTG	CTTCTACTTGACCGTCATCAGCGGTCTTCTCTTGGTTCT
		GAGCGCATGAACGTCTACTTCAAC	CTTCGTCGAGCAGCTCCTGCCCGAGATTGTCAGAAAC
		GAGGTCAGTCACCAACTGTCCGC	GGCATTTTCCACGCTGTCTGCGCCTACGAGGGCGGTT
		CCTTCACTGACTACTCCAATGCTA	GGACTGACAAGCTCGTTGTTCTTTACTACGTACGTTTTT
		ATGCGCACCATAGGCCTCTGGTAA	CTAATTCCGCGCCAGAACGCGCCTACTGACAGCTGGC
		CAAGTATGTCCCCCGTGCCGTCCT	AGCTCAACTACCTCACCAAGTACCTCGAGCTGATTGAC
		CGTCGACTTGGAGCCTGGTACCAT	ACCTGCTTCCTTTTCCTCAAGAAGAAGCCTCTGAGTAA
		GGACGCCGTCCGTGCTGGTCCCT	GCGCGCAATATCTCCACCATCCATGCTCTAGCTGATTG
		TCGGTCAGCTCTTCCGTCCCGACA	ATCTATTAGCTTTCCTCCACACCTACCACCACGGTGCC
		ACTTCGTCTTCGGTCAGTCCGGTG	ACTGCCCTCCTCTGCTTTACCCAGCTTCTCGGCCACAC
		CTGGCAACAACTGGGCCAAGGGT	CGCAGTCTCATGGGTTCCCATCACCCTGAACCTGACC
		С	GTCCACGTTGTCATGTACTGGTACTACT
PBUAP30	KP965453	GGTGCTGCTTTCTGGCAGACCA	AGGCTTTCACCTCCCTCAAGGGCTACAAGCCCCAGGA
		TCTCTGGCGAGCACGGCCTTG	CTTCCGCTTCGTCCCTGGAAAGACCCCTATGGCTACCT
		ACGGTGCTGGTGTGTACGTTGA	TCAAGGAGACGGCCACCATGCTCATTGCCTACTACATC
		CTCTCTGCGCATCCTCAATGAC	ATCATCTTTGGTGGCAGAGAGCTCATGCGTGGTCGCG
		AGCTCGCTGACTGCCTAACAG	AGCCTTTCAAGCTCAACTTCTTCTTCAAGGTCCACAACT
		CTACAACGGTACCTCAGATCTC	TCTACCTGACCGTCATCAGCGGTCTCCTCTTGGTTCTG
		CAGCTGGAGCGCATGAACGTC	TTCGTCGAGCAGCTCTTGCCCGAGATTGTCAGAAACG
		TACTTCAACGAGGTCAGTCACC	GCATTTTCCACGCTGTCTGCGCCTACGAGGGCGGCTG
		AACTGTCCGCCCTTCACTGACT	GACCGACAAGCTCGTTGTTCTTTACTACGTACGTTTTTC
		ACTCCAATGCTAATGCGCACCA	CAGCTTCTCGCCAGAATGCGCTTACTGACAGCTGGCA
		TAGGCCTCTGGTAACAAGTATG	GCTCAACTACCTCACCAAGTACCTTGAGCTGATTGACA
		TCCCCCGTGCCGTCCTCGTCG	CCTGCTTCCTTTTCCTCAAGAAGAAGCCTTTGAGTAAG
		ACTTGGAGCCTGGTACCATGGA	CGCGCAATATTTTCACAATCAATGCTTTAGCTGACTGGT
		CGCCGTCCGTGCCGGTCCCTT	
		CGGTCAGCTCTTCCGTCCTGAC	TGCCCTTCTGCTTTACCCAGCTTCTTGGCCACACCG
		AACTTCGTCTTCGGTCAGTCCG	CAGTCTCATGGGTTCCCATCACCCTGAACTTGACCGTC
		GTGCTGCAACAACTGGGCCAA	CACGTTGTCATGTACTGGT

	T		
PBUAP31	KP965454	GACCCTTGGCCCAGTTGTTGCC	TCACCTCCCTCAAGGGCTACAAGCCCCAGGACTT
		AGCACCGGACTGACCGAAGAC	CCGCTTCGTCCCTGGAAAGACCCCTATGGCTACC
		GAAGTTGTCAGGACGGAAGAG	TTCAAGGAGACGGCCACCATGCTCATTGCCTACT
		CTGACCGAAGGGACCGGCACG	ACATCATCATCTTTGGTGGCAGAGAGCTCATGCGT
		GACGGCGTCCATGGTACCAGG	GGTCGCGAGCCTTTCAAGCTCAACTTCTTCTTCAA
		CTCCAAGTCGACGAGGACGGC	GGTCCACAACTTCTACCTGACCGTCATCAGCGGT
		ACGGGGGACATACTTGTTACCA	CTCCTCTTGGTTCTGTTCGTCGAGCAGCTCTTGCC
		GAGGCCTATGGTGCGCATTAG	CGAGATTGTCAGAAACGGCATTTTCCACGCTGTCT
		CATTGGAGTAGTCAGTGAAGG	GCGCCTACGAGGGCGGCTGGACCGACAAGCTCG
		GCGGACAGTTGGTGACTGACC	TTGTTCTTTACTACGTACGTTTTTCCAGCTTCTCGC
		TCGTTGAAGTAGACGTTCATGC	CAGAATGCGCTTACTGACAGCTGGCAGCTCAACT
		GCTCCAGCTGGAGATCTGAGG	ACCTCACCAAGTACCTTGAGCTGATTGACACCTGC
		TACCGTTGTAGCTGTTAGGCAG	TTCCTTTTCCTCAAGAAGAAGCCTTTGAGTAAGCG
		TCAGCGAGCTGTCATTGAGGAT	CGCAATATTTTCACAATCAATGCTTTAGCTGACTGG
		GCGCAGAGGAGTCAACGTACA	TCTCCTAGCTTTCCTCCACACCTACCACCACGGTG
		CACCAGCACCGTCAAGGCCGT	CCACTGCCCTTCTCTGCTTTACCCAGCTTCTTGGC
		GCTCGCCAGAGATGGTCTGCC	CACACCGCAGTCTCATGGGTTCCCATCACCCTGA
		AGAAAGCAGCACC	ACTTGACCGTCCACGTTGTCATGTACTGG
PBUAP32	KP965455	TAACCAAATCGGTGCTGCTTTC	GTAGTACCAGTACATGACAACGTGGACGGTCAAG
		TGGCAGACCATCTCTGGCGAG	TTCAGGGTGATGGGAACCCATGAGACTGCGGTGT
		CACGGCCTTGACGGTGCTGGT	GGCCAAGAAGCTGGGTAAAGCAGAGAAGGGCAG
		GTGTACGTTGACTCTCTGCGCA	TGGCACCGTGGTGGTAGGTGTGGAGGAAAGCTA
		TCCTCAATGACAGCTCGCTGAC	GGAGACCAGTCAGCTAAAGCATTGATTGTGAAAAT
		TGCCTAACAGCTACAACGGTAC	ATTGCGCGCTTACTCAAAGGCTTCTTCTTGAGGAA
		CTCAGATCTCCAGCTGGAGCG	AAGGAAGCAGGTGTCAATCAGCTCAAGGTACTTG
		CATGAACGTCTACTTCAACGAG	GTGAGGTAGTTGAGCTGCCAGCTGTCAGTAAGCG
		GTCAGTCACCAACTGTCCGCC	CATTCTGGCGAGAAGCTGGAAAAACGTACGTAGT
		CTTCACTGACTACTCCAATGCT	AAAGAACAACGAGCTTGTCGGTCCAGCCGCCCTC
		AATGCGCACCATAGGCCTCTG	GTAGGCGCAGACAGCGTGGAAAATGCCGTTTCTG
		GTAACAAGTATGTCCCCCGTGC	ACAATCTCGGGCAAGAGCTGCTCGACGAACAGAA
		CGTCCTCGTCGACTTGGAGCCT	CCAAGAGGAGACCGCTGATGACGGTCAGGTAGA
		GGTACCATGGACGCCGTCCGT	AGTTGTGGACCTTGAAGAAGAAGTTGAGCTTGAAA
		GCCGGTCCCTTCGGTCAGCTCT	GGCTCGCGACCACGCATGAGCTCTCTGCCACCAA
		TCCGTCCTGACAACTTCGTCTC	AGATGATGATGTAGTAGGCAATGAGCATGGTGGC
		GGTCAGTCCGGGTGCTGGCAA	CGTCTCCTTGAAGGTAGCCATAGGGGTCTTTCCA
		CAACTGGGCCAAGGGTCACTA	GGGACGAAGCGGAAGTCCTGGGGCTTGTAGCCC
		CACTGGGCCAAGGGTCACTA	GGGACGAAGCGGAAGTCCTGGGGCTTGTAGCCC
			TTO A COTO O TO A A COCO TA CA A COCO O A CO A O TTO O
PBUAP33	KP965456	GGTAACCAAATCGGTGCTGCTTTC	TTCACCTCCCTCAAGGGCTACAAGCCCCAGGACTTCC
		TGGCAGACCATCTCTGGCGAGCA	GCTTCGTCCCTGGAAAGACCCCCTATGGCTACCTTCAAG
		CGGCCTTGACGGTGCTGGTGTGT	GAGACGGCCACCATGCTCATTGCCTACTACATCATCAT
		ACGTTGACtTCTCTGCGCATCCTCA	CTTTGGTGGCAGAGAGCTCATGCGTGGTCGCGAGCCT
		ATGACAGCTCGCTGACTGCCTAAC	TTCAAGCTCAACTTCTTCTTCAAGGTCCACAACTTCTAC
		AGCTACAACGGTACCTCAGATCTC	CTGACCGTCATCAGCGGTCTCCTCTTGGTTCTGTTCGT
		CAGCTGGAGCGCATGAACGTCTA	CGAGCAGCTCTTGCCCGAGATTGTCAGAAACGGCATT
		CTTCAACGAGGTCAGTCACCAACT	TTCCACGCTGTCTGCGCCTACGAGGGCGGCTGGACC
		GTCCGCCCTTCACTGACTACTCCA	GACAAGCTCGTTGTTCTTTACTACGTACGTTTTTCCAGC
		ATGCTAATGCGCACCATAGGCCTC	TTCTCGCCAGAATGCGCTTACTGACAGCTGGCAGCTC
		TGGTAACAAGTATGTCCCCCGTGC	AACTACCTCACCAAGTACCTTGAGCTGATTGACACCTG
		CGTCCTCGTCGACTTGGAGCCTG	CTTCCTTTTCCTCAAGAAGAAGCCTTTGAGTAAGCGCG
		GTACCATGGACGCCGTCCGTGCC	CAATATTTTCACAATCAATGCTTTAGCTGACTGGTCTCC
		GGTCCCTTCGGTCAGCTCTTCCGT	TAGCTTTCCTCCACACCTACCACCACGGTGCCACTGC
		CCTGACAACTTCGTCTTCGGTCAG	CCTTCTCTGCTTTACCCAGCTTCTTGGCCACACCGCAG
		TCCGGTGCTGGCAACAACTGGGC	TCTCATGGGTTCCCATCACCCTGAACTTGACCGTCCAC
		CAAGGGT	GTTGTCATGTACTGGTACTA

PBUAP34	KP965457	TGGTAACCAAATCGGTGCTGCT	GCTTTCACCTCCCTCAAGGGCTACAAGCCCCAGG
		TTCTGGCAGACCATCTCTGGCG	ACTTCCGCTTCGTCCCTGGAAAGACCCCTATGGC
		AGCACGGCCTTGACGGTGCTG	TACCTTCAAGGAGACGGCCACCATGCTCATTGCC
		GTGTGTACGTTGACTCTCTGCG	TACTACATCATCATCTTTGGTGGCAGAGAGCTCAT
		CATCCTCAATGACAGCTCGCTG	GCGTGGTCGCGAGCCTTTCAAGCTCAACTTCTTCT
		ACTGCCTAACAGCTACAACGGT	TCAAGGTCCACAACTTCTACCTGACCGTCATCAGC
		ACCTCAGATCTCCAGCTGGAG	GGTCTCCTCTTGGTTCTGTTCGTCGAGCAGCTCTT
		CGCATGAACGTCTACTTCAACG	GCCCGAGATTGTCAGAAACGGCATTTTCCACGCT
		AGGTCAGTCACCAACTGTCCG	GTCTGCGCCTACGAGGGCGGCTGGACCGACAAG
		CCCTTCACTGACTACTCCCAATG	CTCGTTGTTCTTTACTACGTACGTTGTACCGACCAG
		CTAATGCGCACCATAGGCCTCT	CGCCAGAATGCGCTTACTGACAGCTGGCAGCTCA
		GGTAACAAGTATGTCCCCCGTG	ACTACCTCACCAAGTACCTTGAGCTGATTGACACC
		CCGTCCTCGTCGACTTGGAGC	TGCTTCCTTTTCCTCAAGAAGAAGCCTTTGAGTAA
		CTGGTACCATGGACGCCGTCC	GCGCGCAATATTTTCACAATCAATGCTTTAGCTGA
		GTGCCGGTCCCTTCGGTCAGC	CTGGTCTCCTAGCTTTCCTCCACACCTACCACCAC
		TCTTCCGTCCTGACAACTTCGT	GGTGCCACTGCCCTTCTCTGCTTTACCCAGCTTCT
		CTTCGGTCAGTCCGGTGCTGG	TGGCCACACCGCAGTCTCATGGGTTCCCATCACC
		CAACAACTGGGCCAAGGGTCA	CTGAACTTGACCGTCCACGTTGTCATGTACT
		С	2
PBUAP35	KP965458	GGTAACCAAATCGGTGCTGCTT	TTTCACCTCCCTCAAGGGCTACAAGCCCCAGGAC
		TCTGGCAGACCATCTCTGGCGA	TTCCGCTTCGTCCCTGGAAAGACCCCTATGGCTA
		GCACGGCCTTGACGGTGCTGG	CCTTCAAGGAGACGGCCACCATGCTCATTGCCTA
		TGTGTACGTTGACTCTCTGCGC	CTACATCATCATCTTTGGTGGCAGAGAGCTCATGC
		ATCCTCAATGACAGCTCGCTGA	GTGGTCGCGAGCCTTTCAAGCTCAACTTCTTCTTC
		CTGCCTAACAGCTACAACGGTA	AAGGTCCACAACTTCTACCTGACCGTCATCAGCG
		CCTCAGATCTCCAGCTGGAGC	GTCTCCTCTTGGTTCTGTTCGTCGAGCAGCTCTTG
		GCATGAACGTCTACTTCAACGA	CCCGAGATTGTCAGAAACGGCATTTTCCACGCTGT
		GGTCAGTCACCAACTGTCCGC	CTGCGCCTACGAGGGCGGCTGGACCGACAAGCT
		CCTTCACTGACTACTCCAATGC	CGTTGTTCTTTACTACGTACGTTTTTCCAGCTTCTC
		TAATGCGCACCATAGGCCTCTG	GCCAGAATGCGCTTACTGACAGCTGGCAGCTCAA
		GTAACAAGTATGTCCCCCGTGC	CTACCTCACCAAGTACCTTGAGCTGATTGACACCT
		CGTCCTCGTCGACTTGGAGCCT	GCTTCCTTTTCCTCAAGAAGAAGCCTTTGAGTAAG
		GGTACCATGGACGCCGTCCGT	CGCGCAATATTTTCACAATCAATGCTTTAGCTGACT
		GCCGGTCCCTTCGGTCAGCTCT	GGTCTCCTAGCTTTCCTCCACACCTACCACCACG
		TCCGTCCTGACAACTTCGTCTT	GTGCCACTGCCCTTCTCTGCTTTTACCCAGCTTCTT
		CGGTCAGTCCGGTGCTGGCAA	GGCCACACCGCAGTCTCATGGGTTCCCATCACCC
		CAACTGGGCCAAGGGT	TGAACTTGACCGTCCACGTTGTCATGTACTGGT
PBUAP36	KP965459	CCAAATCGGTGCTGCTTTCTGGCA	AGTAGTACCAGTACATGACAACGTGGACGGTCAAGTT
		GACCATCTCTGGCGAGCACGGCC	CAGGGTGATGGGAACCCATGAGACTGCGGTGTGGCC
		TTGACGGTGCTGGTGTGTACGTTG	AAGAAGCTGGGTAAAGCAGAGAAGGGCAGTGGCACC
		ACTCTCTGCGCATCCTCAATGACA	GTGGTGGTAGGTGTGGAGGAAAGCTAGGAGACCAGT
		GCTCGCTGACTGCCTAACAGCTAC	CAGCTAAAGCATTGATTGTGAAAATATTGCGCGCTTACT
		AACGGTACCTCAGATCTCCAGCTG	CAAAGGCTTCTTCTTGAGGAAAAGGAAGCAGGTGTCA
		GAGCGCATGAACGTCTACTTCAAC	ATCAGCTCAAGGTACTTGGTGAGGTAGTTGAGCTGCCA
		GAGGTCAGTCACCAACTGTCCGC	GCTGTCAGTAAGCGCATTCTGGCGAGAAGCTGGAAAA
		CCTTCACTGACTACTCCAATGCTA	ACGTACGTAGTAAAGAACAACGAGCTTGTCGGTCCAG
		ATGCGCACCATAGGCCTCTGGTAA	CCGCCCTCGTAGGCGCAGACAGCGTGGAAAATGCCG
		CAAGTATGTCCCCCGTGCCGTCCT	TTTCTGACAATCTCGGGCAAGAGCTGCTCGACGAACA
		CGTCGACTTGGAGCCTGGTACCAT	GAACCAAGAGGAGACCGCTGATGACGGTCAGGTAGA
		GGACGCCGTCCGTGCCGGTCCCT	AGTTGTGGACCTTGAAGAAGAAGTTGAGCTTGAAAGG
		TCGGTCAGCTCTTCCGTCCTGACA	CTCGCGACCACGCATGAGCTCTCTGCCACCAAGATG
		ACTTCGTCTTCGGTCAGTCCGGTG	ATGATGTAGTAGGCAATGAGCATGGTGGCCGTCTCCTT
		CTGGCAACAACTGGGCCAAGGGT	GAAGGTAGCCATAGGGGTCTTTCCAGGGACGAAGCG
		CAC	GAAGTCCTGGGGCTTG

PBUAP37	KP965460	GCTGCTTTCTGGCAGACCATCT	TTCGAGAAGGCTTTCACCTCCCTCAAGGGCTACA
1 20/ 1 0/	14 000 100	CTGGCGAGCACGGCCTTGACG	AGCCCCAGGACTTCCGCTTCGTCCCTGGAAAGAC
		GTGCTGGTGTGTGTGCGTTGACTC	CCCTATGGCTACCTTCAAGGAGACGGCCACCATG
		TCTGCGCATCCTCAATGACAGC	CTCATTGCCTACTACATCATCATCTTTGGTGGCAG
		TCGCTGACTGCCTAACAGCTAC	AGAGCTCATGCGTGGTCGCGAGCCTTTCAAGCTC
		AACGGTACCTCAGATCTCCAGC	AACTTCTTCTTCAAGGTCCACAACTTCTACCTGAC
		TGGAGCGCATGAACGTCTACTT	CGTCATCAGCGGTCTCCTCTTGGTTCTGTTCGTCG
		CAACGAGGTCAGTCACCAACT	AGCAGCTCTTGCCCGAGATTGTCAGAAACGGCAT
		GTCCGCCCTTCACTGACTACTC	TTTCCACGCTGTCTGCGCCTACGAGGGCGGCTGG
		CAATGCTAATGCGCACCATAGG	
		CCTCTGGTAACAAGTATGTCCC	TCCAGCTTCTCGCCAGAATGCGCTTACTGACAGCT
		CCGTGCCGTCCTCGTCGACTTG	GGCAGCTCAACTACCTCACCAAGTACCTTGAGCT
		GAGCCTGGTACCATGGACGCC	GATTGACACCTGCTTCCTTTTCCTCAAGAAGAAGC
		GTCCGTGCCGGTCCCTTCGGT	CTTTGAGTAAGCGCGCGCAATATTTTCACAATCAATG
		CAGCTCTTCCGTCCTGACAACT	CTTTAGCTGACTGGTCTCCTAGCTTTCCTCCACAC
		TCGTCTTCGGTCAGTCCGGTGC	CTACCACCACGGTGCCACTGCCCTTCTCTGCTTTA
		TGGCAACAACTGGGCCAAGGG	CCCAAGCTTCTTGGCCACACCGCAGTCTCATGGG
		TCACTACACTGGGCCAAGGG	TTCCCATCACCCTGAACTTGACCG
		CTGGCAGACCATCTCTGGCGA	
PBUAP38	KP965461		AGGCTTTCACCTCCCTCAAGGGCTACAAGCCCCA
		GCACGGCCTTGACGGTGCTGG	GGACTTCCGCTTCGTCCCTGGAAAGACCCCCTATG
		TGTGTACGTTGACTCTCTGCGC	GCTACCTTCAAGGAGACGGCCACCATGCTCATTG
		ATCCTCAATGACAGCTCGCTGA	CCTACTACATCATCATCTTTGGTGGCAGAGAGCTC
		CTGCCTAACAGCTACAACGGTA	ATGCGTGGTCGCGAGCCTTTCAAGCTCAACTTCTT
		CCTCAGATCTCCAGCTGGAGC	CTTCAAGGTCCACAACTTCTACCTGACCGTCATCA
		GCATGAACGTCTACTTCAACGA	GCGGTCTCCTCTTGGTTCTGTTCGTCGAGCAGCTC
		GGTCAGTCACCAACTGTCCGC	TTGCCCGAGATTGTCAGAAACGGCATTTTCCACGC
		CCTTCACTGACTACTCCAATGC	TGTCTGCGCCTACGAGGGCGGCTGGACCGACAA
		TAATGCGCACCATAGGCCTCTG	GCTCGTTGTTCTTTACTACGTACGTTTTTCCAGCTT
		GTAACAAGTATGTCCCCCGTGC	CTCGCCAGAATGCGCTTACTGACAGCTGGCAGCT
		CGTCCTCGTCGACTTGGAGCCT	CAACTACCTCACCAAGTACCTTGAGCTGATTGACA
		GGTACCATGGACGCCGTCCGT	CCTGCTTCCTTTTCCTCAAGAAGAAGCCTTTGAGT
		GCCGGTCCCTTCGGTCAGCTCT	AAGCGCGCAATATTTTCACAATCAATGCTTTAGCT
		TCCGTCCTGACAACTTCGTCTT	GACTGGTCTCCTAGCTTTCCTCCACACCTACCACC
		CGGTCAGTCCGGTGCTGGCAA	ACGGTGCCACTGCCCTTCTCTGCTTTACCCAGCTT
		CAACTGGGCCAAGGGTCACTA	CTTGGCCACACCGCAGTCTCATGGGTTCCCATCA
		CACTGAGGGTA	CCCTGAACTTGACCGTCCACGTTGTCATGTACTGG
			TACTAC
PBUAP39	KP965462	CCCTCAGTGTAGTGACCCTTGGCC	AGTAGTACCAGTACATGACGACGTGGACGGTCAGGTT
		CAGTTGTTGCCAGCACCGGACTG	CAGGGTGATGGGGACCCATGAGACTGCGGTGTGGCC
		ACCGAAGACGAAGTTGTCGGGAC	GAGAAGCTGGGTGAAGCAGAGAAGGGCAGTGGCACC
		GGAAGAGCTGACCGAAAGGACCA	GTGGTGGTAGGTGTGGAGGAAAGCTAGGAGATCGGTC
		GCACGGACGGCGTCCATGGTACC	AGCTGATGCATTGATTGTAGAGATAATGCGCGCTTACT
		AGGCTCCAAGTCGACGAGGACGG	CAAAGGCTTCTTCTTGAGGAAAAGGAAGCAGGTGTCA
		CACGGGGAACATATTTGTTACCAG	ATCAGCTCGAGGTACTTGGTGAGGTAGTTGAGCTGCC
		AGGCCTATGGTGCGCATTAGCATT	AGCTGTCAGTAAGCGCGTTCTGGCGTGAGATTGGAAA
		GAAGTAGTGAGCGAAGGGGCGAC	AACGTACGTAGTAAAGAACAACGAGCTTGTCGGTCCA
		GGTCGATGACTGACCTCGTTGAAG	GCCGCCCTCGTAGGCGCAGACAGCGTGGAAAATGCC
		TAGACGTTCATGCGCTCCAGCTGG	GTTTCTGACAATCTCGGGCAGGAGCTGCTCGACGAAG
		AGATCTGAGGTACCGTTGTAGCTG	AGAACCAAGAGAAGACCGCTGATGACGGTCAGGTAGA
		TTAGGCAGTCAGCGAGCTGTGATT	AGTTGTGGACCTTGAAGAAGAAGTTGAGCTTGAAAGG
		GCGGGTGAGGATGCGCAGGGAG	CTCGCGACCACGCATGAGCTCTCTGCCACCAAAGATG
		TCGACGTACACACCAGCACCGTC	ACGATGTAGTAGACAATGAGCATGGTGGCCGTCTCCTT
		AAGGCCGTGCTCGCCAGAGATGG	GAAGGTAGCCATGGGGGTCTTTCCAGGGACAAAGCG
		TCTGCCA	GAAGTCCTGGGGCTTGTAGCCCTTGATGGA

PBUAP40	KP965463	GGTGCTGCTTTCTGGCAGACCA	CACCTCGCTCAAGGGCTACAAGCCCCAGGACTTC
PBUAP40	KP905403	TCTCTGGCGAGCACGGCCTTG	CACCTCGCTCAAGGGCTACAAGCCCCAGGACTTC
		ACGGTGCTGGTGTGTGTGCGTTGA	TCAAGGAGACGGCCACCATGCTCATCGCCTACTA
		CTCCCTGCGCATCCTCACCCG	CATCATCATCTTTGGTGGCAGAGAGCTCATCGCCTACTA
		CAATGACAGCTCGCTGACTGC	GTCGCGAGCCTTTCAAGCTCAACTTCTTCTTCAAG
		CTAACAGCTACAACGGTACCTC	GTCCACAACTTCTACTTGACCGTCATCAGCGGTCT
			AGATTGTCAGAAACGGCATTTTCCACGCTGTCTGC GCCTACGAGGGCGGCTGGACTGACAAGCTCGTT
		AGTCACCAACAGTCCGCCCTTC	
		ACTGACTACTCCAATGCTAATG	GTTCTTTACTACGTACGTTTTTCTAATTCTGCGCCA GAACGCGCTTACTGACAGCTGGCAGCTCAACTAC
		CGCACCATAGGCCTCTGGTAA	
		CAAGTATGTCCCCCGTGCCGTC	CTCACCAAGTACCTCGAGCTGATTGACACCTGCTT
		CTCGTCGACTTGGAGCCTGGTA	CCTTTTCCTCAAGAAGAAGCCTCTGAGTAAGCGC
		CCATGGACGCCGTCCGTGCCG	GCAATATCTCCACCATCAATGCTCTAGCTGATTGA
		GTCCCTTCGGTCAGCTCTTCCG	TCTATTAGCTTTCCTCCACACCTACCACCACGGTG
		TCCCGACAACTTCGTCTTCGGT	CCACTGCCCTCCTCTGCTTTACCCAGCTTCTCGGC
		CAGTCCGGTGCTGGCAACAAC	CACACCGCAGTCTCATGGGTTCCCATCACCCTGA
		TGGGCCAAGGGTCACTACACT	ACCTGACCGTCCACGTTGTCATGTACTGGTACTAC
		GAGGGTA	
PBUAP41	KP965464	TGTTGCCAGCACCGGACTGGC	GGGCTACAAGCCCCAGGACTTCCGCTTCGTCCCT
		CGAAGACGAAGTTGTCGGGAC	GGAAAGACTCCCATGGCTACCTTCAAGGAGACGG
		GGAAGAGCTGACCGAAGGGG	CCACCATGCTCATCGCCTACTACATCATCATCTTT
		CCGGCACGGACGGCGTCCATG	GGTGGCAGAGAGTTCATGCGTGGTCGCGAGCCTT
		GTACCAGGCTCCAAGTCGACG	TCAAGCTCAACTTCTTCTTCAAGGTCCACAACTTCT
		AGGACGGCGCGGGGGACATA	ACCTGACCGTCATCAGTGGTCTTCTCTTGGTTCTC
		CTTGTTACCAGAGGCCTATAGT	TTCGTTGAGCAGCTCCTGCCCGAGATTGTCAGAA
		CATTAGCAATGAAGTCGTGAAG	ACGGTATCTTCCACGCTGTCTGCGCCTACGAGGG
		GAAGGGCCTACGGTTGGCCAC	TGGCTGGACCGACAAGCTTGTTGTTCTTTACTACG
		TGACCTCGTTGAAGTAGACGTT	TACGTTTTGTCGTATCCGCACCAGAATGCGCTTAC
		CATGCGCTCCAGCTGGAGATCT	TGACAGCTGGCAGCTCAACTACCTTACCAAGTAC
		GAGGTACCGTTGTAGCTGTCAG	CTCGAGCTGATTGACACCTGCTTCCTTTTCCTCAA
		GCAGTCAGCGAGCTATCACGA	GAAGAAGCCTTTGAGTAAGCGCGCAATATCTTCAC
		CGGATGGGGATGCGCAGGGAA	ACCCGATACACCAACTAATTGGGCTCCTAGCTTTC
		TCGACGTACACACCAGCACCG	CTCCACACCTACCACCACGGTGCCACTGCCCTTC
		TCAAGGCCGTGCTCGCCAGAG	TCTGCTTCACCCAGCTTCTCGGCCACACCGCAGT
		ATGGTCTGCCAGAAAGCAGCA	CTCATGGGTTCCCATCACCCTCAATTTGACCGTCC
		CCGATTTGGTT	ACGTTGTCATGTACTGGTACTAC
PBUAP42	KP965465	ACCCTTGGCCCAGTTGTTGCCAGC	GTAGTACCAGTACATGACGACGTGGACGGTCAGGTTC
		ACCGGACTGACCGAAGACGAAGT	AGGGTGATGGGGACCCATGAGACTGCGGTGTGGCCG
		TGTCGGGACGGAAGAGCTGACCG	AGAAGCTGGGTGAAGCAGAGAAGGGCAGTGGCACCG
		AAAGGACCAGCACGGACGGCGTC	TGGTGGTAGGTGTGGAGGAAAGCTAGGAGATCGGTCA
		CATGGTACCAGGCTCCAAGTCGA	GCTGATGCATTGATTGTAGAGATAATGCGCGCTTACTC
		CGAGGACGGCACGGGGAACATAT	AAAGGCTTCTTCTTGAGGAAAAGGAAGCAGGTGTCAAT
		TTGTTACCAGAGGCCTATGGTGCG	CAGCTCGAGGTACTTGGTGAGGTAGTTGAGCTGCCAG
		CATTAGCATTGAAGTAGTGAGCGA	CTGTCAGTAAGCGCGTTCTGGCGTGAGATTGGAAAAA
		AGGGGCGACGGTCGATGACTGAC	CGTACGTAGTAAAGAACAACGAGCTTGTCGGTCCAGC
		CTCGTTGAAGTAGACGTTCATGCG	CGCCCTCGTAGGCGCAGACAGCGTGGAAAATGCCGTT
		CTCCAGCTGGAGATCTGAGGTAC	TCTGACAATCTCGGGCAGGAGCTGCTCGACGAAGAGA
		CGTTGTAGCTGTTAGGCAGTCAGC	ACCAAGAGAAGACCGCTGATGACGGTCAGGTAGAAGT
		GAGCTGTGATTGCGGGTGAGGAT	TGTGGACCTTGAAGAAGAAGTTGAGCTTGAAAGGCTC
		GCGCAGGGAGTCGACGTACACAC	GCGACCACGCATGAGCTCTCTGCCACCAAAGATGACG
		CAGCACCGTCAAGGCCGTGCTCG	ATGTAGTAGACAATGAGCATGGTGGCCGTCTCCTTGAA
		CCAGAGATGGTCTGCCAGAAAGC	GGTAGCCATGGGGGTCTTTCCAGGGACAAAGCGGAA
		AGCAC	GTCCTGGGGCTTGTAGCCCTTGATGGA

		TTOOT & A OO & A TOO OTOOTOO	000000000000000000000000000000000000000
PBUAP43	KP965466	TTGGTAACCAAATCGGTGCTGC	GGCTTTCACCTCGATCAAGGGCTACAAGCCCCAG
		TTTCTGGCAGACCATCTCTGGC	GACTTCCGCTTCGTCCCTGGAAAGACTCCCATGG
		GAGCACGGCCTTGACGGTGCT	CTACCTTCAAGGAGACGGCCACCATGCTCATCGC
		GGTGTGTACGTCGATTCCCTGC	CTACTACATCATCATCTTTGGTGGCAGAGAGTTCA
		GCATCCCCATCCGTCGTGATAG	TGCGTGGTCGCGAGCCTTTCAAGCTCAACTTCTTC
		CTCGCTGACTGCCTGACAGCTA	TTCAAGGTCCACAACTTCTACCTGACCGTCATCAG
		CAACGGTACCTCAGATCTCCAG	TGGTCTTCTCTTGGTTCTCTTCGTTGAGCAGCTCCT
		CTGGAGCGCATGAACGTCTACT	GCCCGAGATTGTCAGAAACGGTATCTTCCACGCT
		TCAACGAGGTCAGTGGCCAAC	GTCTGCGCCTACGAGGGTGGCTGGACCGACAAG
		CGTAGGCCCTTCCTTCACGACT	CTTGTTGTTCTTTACTACGTACGTTTTGTCGTATCC
		TCATTGCTAATGACTATAGGCCT	GCACCAGAATGCGCTTACTGACAGCTGGCAGCTC
		CTGGTAACAAGTATGTCCCCCG	AACTACCTTACCAAGTACCTCGAGCTGATTGACAC
		CGCCGTCCTCGTCGACTTGGA	CTGCTTCCTTTTCCTCAAGAAGAAGCCTTTGAGTA
		GCCTGGTACCATGGACGCCGT	AGCGCGCAATATCTTCACACCCGATACACCAACT
		CCGTGCCGGCCCCTTCGGTCA	AATTGGGCTCCTAGCTTTCCTCCACACCTACCACC
		GCTCTTCCGTC	ACGGTGCCACTGCCCTTCTCTGCTTCACCCAGCTT
		5. 16 M 16 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	CTCGGCCACACCGCAGTCTCATGGGTTCCCATCA
			CCCTCAATTTGACCGTCCACGTTGTCATGTACTGG
			TACTACT
PBUAP44	KP965467	CTGCTTTCTGGCAGACCATCTC	GTAGTACCAGTACATGACAACGTGGACGGTCAGGTTA
		CGGCGAGCACGGCCTTGACGG	AGGGTGATGGGAACCCATGAGACTGCGGTGTGGCCG
		TGCTGGTGTGTACGTCGACTCC	AGAAGCTGGGTGAAGCAGAGAAGGGCGGTGGCACCG
		CTGCGCACCCCACCCGCCGT	TGGTGGTAGGTGTGGAGGAAAGCTAGGAGACTGGTCA
		GATAGCTCGCTGACTGCCTAAC	GCTAAAGCCTTGGTCATGGAGATATTGCGCGCTTACTC
		AGCTACAACGGTACCTCAGATC	AAAGGCTTCTTCTTGAGGAAAAGGAAGCAAGTGTCAAT
		TCCAGCTGGAGCGCATGAACG	CAGCTCGAGGTACTTGGTGAGATAGTTGAGCTGCCAC
		TCTACTTCAACGAGGTCAGCCA	CTGTCAGTAAGCGCATTCTGGCGCGGATATAGCAGAA
		TCCAACGTCGACCCTTGCTTCA	CGTACGTAGTAAAGAACAACAAGCTTGTCGGTCCAGC
		CTACTTGAATGCTAATGCGCAC	CACCCTCGTAGGCGCAGACGGCGTGGAAAATGCCGTT
		CATAGGCCTCCGGTAACAAGTA	TCTGACAATCTCGGGCAGGAGCTGCTCAACGAAGAGA
		TGTCCCCCGTGCCGTCCTCGTC	ACCAAGAGAAGACCACTGATGACGGTCAGGTAGAAGT
		GACTTGGAGCCCGGTACCATG	TGTGGACCTTGAAGAAGAAGTTGAGCTTGAAAGGCTC
		GACGCCGTCCGTGCCGGTCCC	GCGACCACGCATGAACTCTCTGCCACCAAAGATGATG
		TTCGGTCAGCTCTTCCGTCCCG	ATGTAGTAAGCAATGAGCATGGTGGCCGTCTCCTTGAA
		ACAACTTCGTC	GGTAGCCATAGGGGTCTTTCCAGGCACAAAGCGGAAG
			TCCTGGGGCTTGTAGCCCTTGATCGAGGT
PBUAP45	KP965468	CATCTCTGGCGANCACGGCNTT	GCTTTCACCTCCATCAAGGGCTACAAGCCCCAGGACT
		GACGGTGCTGGTGTGTACGTTC	TCCGCTTTGTCCCTGGAAAGACCCCCATGGCTACCTTC
		GACTCCCTGCGCATCCTCACC	AAGGAGACGGCCACCATGCTCATTGTCTACTACATCGT
		CGCAATCACAGCTCGCTGACT	CATCTTTGGTGGCAGAGAGCTCATGCGTGGTCGCGAG
		GCCTAACAGCTACAACGGTAC	CCTTTCAAGCTCAACTTCTTCTTCAAGGTCCACAACTTC
		CTCAGATCTCCAGCTGGAGCG	TACCTGACCGTCATCAGCGGTCTTCTCTTGGTTCTCTTC
		CATGAACGTCTACTTCAACGAG	GTCGAGCAGCTCCTGCCCGAGATTGTCAGAAACGGCA
		GTCAGTCATCGACCGTCGCCC	TTTTCCACGCTGTCTGCGCCTACGAGGGCGGCTGGAC
		CTTCGCTCACTACTTCAATGCTA	CGACAAGCTCGTTGTTCTTTACTACGTACGTTTTTCCAA
		ATGCGCACCATAGGCCTCTGGT	TCTCACGCCAGAACGCGCTTACTGACAGCTGGCAGCT
		AACAAATATGTTCCCCGTGCCG	
		TCCTCGTCGACTTGGAGCCTGG	GCTTCCTTTTCCTCAAGAAGAAGCCTTTGAGTAAGCGC
		TACCATGGACGCCGTCCGTGC	GCATTATCTCTACAATCAATGCATCAGCTGACCGATCT
		TGGTCCTTTCGGTCAGCTCTTA	CCTAGCTTTCCTCCACACCTACCACCACGGTGCCCACT
		CCGTCCCGACAACTTCGTCTTC	GCCCTTCTCTGCTTCACCCACCACCGCTGCCACCG
		GGTCAGTCCGGTGCTGGCAAC	CAGTCTCATGGGTCCCCATCACCCTGAACCTGACCGT
		AACTGGGCCAAGGGTCACTG	CCACGTCGTCATGTACTGGTA

	KD065460	CTCCTTTCTCCCACACCATCTC	
PBUAP46	KP965469	CTGCTTTCTGGCAGACCATCTC	GTAGTACCAGTACATGACGACGTGAACGGTCAGG
		TGGCGAGCACGGCCTTGACGG	TTCAGGGTGATGGGAACCCATGAGACTGCGGTGT
		TGCTGGTGTGTGTCGTCGCCCCA	GGCCGAGAAGCTGGGTAAAGCAGAGGAGGGCA GTGGCACCGTGGTGGTAGGTGTGGAGGAAAGCT
		CTGCGCATCCTCACCCGCAAT	
		GACAGCTCGCTGACTGCCTAA	AGGAGATCAGTCAGCTAAAGTATTGATTGTGGAAA
		CAGCTACAACGGTACCTCAGAT	TATTGCGCGCTTACTCAAAGGCTTCTTCTTGAGGA
		CTCCAGCTGGAGCGCATGAAC	ACAAGAAGCAGGTGTCAATCAGCTCGAGGTACTT
		GTCTACTTCAACGAGGTCAGTC	GGTGAGGTAGTTGAGCTGCCAGCTGTCAGTAAGC
		ACCAGCTGTCCGCCCTTCACTG	GCGTTCTGGCGCGGAATTAGAAAAACGTACGTAG
		ACCACTCCAATGCTAATGCGCA	TAAAGAACAACGAGCTTGTCAGTCCAGCCGCCCT
		CCATAGGCCTCTGGTAACAAGT	CGTAGGCGCAGACAGCGTGGAAAATGCCGTTTCT
		ATGTCCCCCGTGCCGTCCTCGT	GACAATCTCGGGCAGGAGCTGCTCGACGAAGAG
		CGACTTGGAGCCTGGTACCAT	AACCAAGAGAAGACCGCTGATGACGGTCAAGTAG
		GGACGCCGTCCGTGCTGGTCC	AAGTTGTGGACCTTGAAGAAGAAGTTGAGCTTGAA
		CTTCGGTCAGCTCTTCCGTCCC	AGGCTCGCGACCACGCATGAGCTCTCTGCCACCA
		GACAACTTCGTCTTCGGTCAGT	AAGATGATGATGTAGTAGGCGATGAGCATGGTGG
		CCGGTGCTGGCAACAACTGGG	CCGTCTCCTTGAAGGTAGCCATGGGAGTCTTTCCA
		CCAAGGGTCACTACACTGAGG	GGGACGAAGCGGAAGTCCTGGGGCTTGTAGCCC
		GTA	TTGAGCGAGGTGAAA
PBUAP47	KP965470	CCAGCACCGGACTGNCCGAAG	TTCGAGAAGGCTTTCACCTCCCTCAAGGGCTACAAGC
		ANGAAGTTGTCAGGGACGGAA	CCCAGGACTTCCGCTTCGTTCCTGGAAAGACCCCCAT
		GAGCTGACCGAAGGGACCGG	GGCTACCTTCAAGGAGACGGCCACCATGCTCATTGCC
		CACGGACGGCGTCCATGGTAC	TACTACATCATCATCTTTGGTGGTAGAGAGCTCATGCG
		CAGGCTCCAAGTCGACGAGGA	TGGTCGCGAGCCTTTCAAGCTCAACTTCTTCTTCAAGG
		CGGCACGGGGGGACATACTTGT	TCCACAACTTCTACCTGACCGTCATCAGCGGTCTTCTC
		TACCAGAGGCCTATGGTGCGC	TTGGTTCTCTTCGTTGAGCAGCTCCTGCCCGAGATTGT
		ATTAGCATTGGAGTAGTCAGTG	CAGAAACGGCATTTTCCACGCTGTCTGCGCCTACGAG
		AAGGGCGGACAGTTGGTGANT	GGCGGCTGGACTGACAAGCTCGTTGTTCTTTACTACGT
		GACCTCGTTGAAGTAGACGTTC	ACGTTTATCCAATTCCGCGATAGAATGCGCTTACTGAC
		ATGCGCTCCAGCTGGAGATCT	AGCTGGCAGCTCAACTACCTCACCAAGTACCTCGAGC
		GAGGTACCGTTGTAGCTNTTAG	TGATTGACACCTGCTTCCTTTCCTCAAGAAGAAGCCTT
		GCAGTCAGCGAGCTGTCATTGA	TGAGTAAGCGCGCATTACCTCCACAATCAATGCTTTAG
		GGATGCGCAGAGAGTCAACGT	CTGACTGATCTCCCAGCTTTCCTCCACACCTACCACCA
		ACACACCAGCACCGTCAAGGC	CGGTGCCACTGCTCTTCTCTGCTTCACCCAGCTTCTCG
		CGTGCTCGCCAGAGATGGTCT	GCCACACCGCAGTTTCATGGGTCCCCATCACCCTGAA
		GCCGCAAANCAGCACCGAGTT	CTTGACCGTCCACGTCGTCATGTACT
		GTTTACCAC	
PBUAP48	KP965471	TGCTGCTTTCTGGCAGACCATC	CACCTCGATCAAGGGCTACAAGCCCCAGGACTTCCGC
I DOAI 40	11 903471	TCTGGCGAGCACGGCCTTGAC	TTCGTCCCTGGAAAGACTCCCATGGCTACCTTCAAGGA
		GGTGCTGGTGTGTGTACGTCGATT	
		CCCTGCGCATCCCCATCCGTC	GACGGCCACCATGCTCATTGCCTACTACATCATCATCT
			TTGGTGGCAGAGAGTTCATGCGTGGTCGCGAGCCTTT
		GTGATAGCTCGCTGACTGCCTG	
		ACAGCTACAACGGTACCTCAGA	GACCGTCATCAGTGGTCTTCTCTTGGTTCTCTTCGTTGA
		TCTCCAGCTGGAGCGCATGAA	GCAGCTCCTGCCCGAGATTGTCAGAAACGGTATCTTC
		CGTCTACTTCAACGAGGTCAGT	CACGCTGTCTGCGCCTACGAGGGTGGCTGGACCGAC
		GGCCAACCGTGGGCCCTTCCT	AAGCTTGTTGTTCTTTACTACGTACGTTTTGTCGTATGG
		TCACGATTTCATTGCTAATGACT	GCACCAGAATGCGCTTACTGACAGCTGGCAGCTCAAC
		ATAGGCCTCTGGTAACAAGTAT	TACCTTACCAAGTACCTCGAGCTGATTGACACCTGCTT
		GTCCCCCGCGCCGTCCTCGTC	CCTTTTCCTCAAGAAGAAGCCTTTGAGTAAGCGCGCAA
		GACTTGGAGCCTGGTACCATG	TATCTTCACACCCGATACACCAACTAATTGGGCTCCTA
		GACGCCGTCCGTGCCGGCCCC	GCTTTCCTCCACACCTACCACCACGGTGCCACTGCCC
		TTCGGTCAGCTCTTCCGTCCCG	TTCTCTGCTTCACCCAGCTTCTCGGCCACACCGCAGTC
		ACAACTTC	TCATGGGTTCCCATCACCCTCAATTTGACCGTTCACGTT
			GTCATGTACTGGTACTAC

PBUAP49	KP965472	GCTGCTTTCTGGCAGACCATCT	GGGCTACAAGCCCCAGGACTTCCGCTTCGTTCCT
		CTGGCGAGCACGGCCTTGACG	GGAAAGACCCCCATGGCTACCTTCAAGGAGACG
		GCGCTGGTGTGTGTACGTCGACC	GCCACCATGCTCATTGCCTACTACATCATCATT
		CCCTGCGCATTCTCACCCGCAA	GGTGGTAGAGAGCTCATGCGTGGTCGCGAGCCTT
		CGACAGCTCGCTGACTGCCTA	TCAAGCTCAACTTCTTCTTCAAGGTCCACAACTTCT
		ACAGCTACAACGGTACCTCAGA	ACCTGACCGTCATCAGCGGTCTTCTCTTGGTTCTC
		TCTCCAGCTGGAGCGCATGAA	TTCGTTGAGCAGCTCCTGCCCGAGATTGTCAGAA
		CGTCTACTTCAACGAGGTCGGT	ACGGCATTTTCCACGCTGTCTGCGCCTACGAGGG
		CACCAACTGTCGGCCTTTCACT	CGGCTGGACTGACAAGCTCGTTGTTCTTTACTACG
		CACTCCTTCAATGCTAATGCGC	TACGTITATCCAATTCCGCGATAGAATGCGCTTAC
		ACCATAGGCCTCTGGTAACAAG	TGACAGCTGGCAGCTCAACTACCTCACCAAGTAC
		TATGTCCCCCGTGCCGTCCTCG	CTCGAGCTGATTGACACCTGCTTCCTTTTCCTCAA
		TCGATTTGGAGCCTGGTACCAT	GAAGAAGCCTTTGAGTAAGCGCGCATTACCTCCA
		GGACGCCGTCCGTGCTGGTCC	CAATCAATGCTTTAGCTGACTGATCTCCCAGCTTT
		CTTCGGTCAGCTCTTCCGTCCC	CCTCCACACCTACCACCACGGTGCCACTGCTCTT
		GACAACTTCGTCTTCGGTCAGT	CTCTGCTTCACCCAGCTTCTCGGCCACACCGCAG
		CCGGTGCTGGCAACAACTGGG	TTTCATGGGTCCCCATCACCCTGAACTTGACCGTC
		CCAAGGGTCACTACACTGA	CACGTCGTCATGTACTGGTACTACTCC
PBUAP50	KP965473	CAAATCGGTGCTGCTTTCTGGC	ACCAGTACATGACAACGTGGACGGTCAGGTTCAG
		AGACCATCTCTGGCGAGCACG	GGTGATGGGAACCCATGAGACTGCGGTGTGGCC
		GCCTTGACGGTGCTGGTGTGTA	GAGAAGCTGGGTAAAGCAGAGGAGGGCAGTGGC
		CGTTGACTCCCTGCGCATCCTC	ACCGTGGTGGTAGGTGTGGAGGAAAGCTAATAGA
		ACCCGCAATGACAGCTCGCTG	TCAATCAGCTAGAGCATGGATGGTGGAGATATTG
		ACTGCCTAACAGCTACAACGGT	CGCGCTTACTCAGAGGCTTCTTCTTGAGGAAAAG
		ACCTCAGATCTCCAGCTGGAG	GAAGCAGGTGTCAATCAGCTCGAGGTACTTGGTG
		CGCATGAACGTCTACTTCAACG	AGGTAGTTGAGCTGCCAGCTGTCAGTAGGCGCGT
		AGGTCAGTCACCAACTGTCCG	TCTGGCGCGGAATTAGAAAAACGTACGTAGTAAA
		CCCTTCACTGACTACTCCAATG	GAACAACGAGCTTGTCAGTCCAACCGCCCTCGTA
		CTAATGCGCACCATAGGCCTCT	GGCGCAGACAGCGTGGAAAATGCCGTTTCTGACA
		GGTAACAAGTATGTCCCCCGTG	ATCTCGGGCAGGAGCTGCTCGACGAAGAGAACC
		CCGTCCTCGTCGACTTGGAGC	AAGAGAAGACCGCTGATGACGGTCAAGTAGAAGT
		CTGGTACCATGGACGCCGTCC	TGTGGACCTTGAAGAAGAAGTTGAGCTTGAAAGG
		GTGCTGGTCCTTTCGGTCAGCT	CTCGCGACCACGCATGAGCTCTCTGCCACCAAAG
		CTTCCGTCCCGACAACTTCGTC	ATGATGATGTAGTAGGCGATGAGCATGGTGGCCG
		TTCGGTCAGTCCGGTGCTGGC	TCTCCTTGAAGGTAGCCATGGGGGTCTTTCCAGG
		AACAACT	GACGAAGCGGAAGTCCTGGGGCTTG
PBUAP51	KP965474	AATCGGTGCTGCTTTCTGGCAGAC	AGCCCCAGGACTTCCGCTTCGTCCCTGGAAAGACCCC
		CATCTCTGGCGAGCACGGCCTTG	TATGGCTACCTTCAAGGAGACGGCCACCATGCTCATC
		ACGGTGCTGGTGTGTACGTTGACT	GCCTACTACATCATCATCTTTGGTGGCAGAGAGCTCAT
		CCCTGCGCATCCTCACCCGCAAT	GCGTGGTCGCGAGCCTTTCAAGCTCAACTTCTTCTTCA
		GACAGCTCGCTGACTGCCTAACA	AGGTCCACAACTTCTACCTGACCGTCATCAGCGGTCTC
		GCTACAACGGTACCTCAGATCTCC	CTCTTGGTTCTGTTCGTCGAGCAGCTCTTGCCCGAGAT
		AGCTGGAGCGCATGAACGTCTACT	TGTCAGAAACGGCATTTTCCACGCTGTCTGCGCCTACG
		TCAACGAGGTCAGTCACCAACTGT	AGGGCGGCTGGACCGACAAGCTCGTTGTTCTTTACTA
		CCGCCCTTCACTGACTACTCCAAT	CGTACGTTTTTCCAGCTTCTCGCCAGAATGCGCTTATTG
		GCTAATGCGCACCATAGGCCTCTG	ACAGCTGGCAGCTCAACTACCTCACCAAGTACCTTGA
		GTAACAAGTATGTCCCCCGTGCCG	GCTGATTGACACCTGCTTCCTTTTCCTCAAGAAGAAGC
		TCCTCGTCGACTTGGAGCCTGGTA	CTTTGAGTAAGCGCGCAATATTTTCACAATTAATGCTTT
		i de la companya de l	
		CCATGGACGCCGTCCGTGCTGGT	AGCTGATTGGTCTCCTAGCTTTCCTCCACACCTACCAC
		CCATGGACGCCGTCCGTGCTGGT CCTTTCGGTCAGCTCTTCCGTCCC	AGCTGATTGGTCTCCTAGCTTTCCTCCACACCTACCAC CACGGTGCCACTGCCCTTCTCTGCTTTACCCAGCTTCT
		CCTTTCGGTCAGCTCTTCCGTCCC	CACGGTGCCACTGCCCTTCTCTGCTTTACCCAGCTTCT

PBUAP53	KP965475	TGCTTTCTGGCAGACCATCTCT	GGGCTACAAGCCCCAGGACTTCCGCTTCGTCCCC
		GGCGAGCACGGCCTTGACGGT	GGAAAGACGCCTATGGCTACTTTCAAGGAGACGG
		GCTGGTGTGTACGTCGATTCCC	CCACCATGCTCATTGCCTACTATATCATCATTTTTG
		TGCGCATCCCCATCCGTCGTGA	GCGGCAGAGAGTTTATGCGTGGCCGCGAGCCCT
		TAGCTCGCTGACTGCCTGACAG	TCAAGCTCAGCTTCTTCTTCAAGCTCCACAACTTCT
		CTACAACGGTACCTCAGATCTC	ACCTGACTCTGATCAGCGGCATTCTCCTGGTTCTG
		CAGCTGGAGCGCATGAACGTC	TTCGTTGAGCAGCTTCTGCCCGAAATTGTCAGAAA
		TACTTCAACGAGGTCAGTGGCC	TGGCATTTTCCACGCAGTCTGCGCCTACGAGGGT
		AACCGTGGGCCCTTCCTTCACG	GGCTGGACCGACAAGCTTGTTGTTCTCTACTACGT
		ACTTCATTGCTAATGACTATAGG	GAGTGTCTCTCGAGTCGCGACAAGGTGCCCTTAC
		CCTCTGGTAACAAGTATGTCCC	TGACAAGATCGCAGCTCAACTACCTGACCAAGTA
		CCGCGCCGTCCTCGTCGACTT	CCTCGAGCTCATTGACACCTGCTTCCTTTCCTCA
		GGAGCCTGGTACCATGGACGC	AGAAGAAGCCCTTGAGTAAGCCCACCTGACGGAA
		CGTCCGTGCCGGCCCCTTCGG	CCGTCTACCAGTCGCATTAGCTGATCGCTCCCCTA
		TCAGCTCTTCCGTCCCGACAAC	GCCTTCCTCCACACCTACCACCACGGCGCTACCG
		TTCGTCTTCGGCCAGTCCGGTG	CTCTCCTCTGCTTCACTCAGCTCCTCGGTCACACT
		CTGGCAACAACTGGGCCAAGG	TCCGTCTCTTGGGTTCCCATCACCCTGAACCTGAC
		GTCACTACACTGAGGGTA	CGTCCACGTCGT
PBUAP55	KP965476	GGTGCTGCTTTCTGGCAGACCA	CTTCGAGAAGGCCTTCACTTCCCTCAAGGGACTA
		TCTCTGGCGAGCACGGCCTTG	CAAGCCCCAGGACTTCCGCTTCGTCCCTGGAAAG
		ACGGTGCTGGTGTGTACGTTGA	ACCCCCATGGCTACCTTCAAGGAGACGGCCACCA
		CTCCCTGCGCATCCTCACCCG	TGCTCATTGCCTACTACATCATCATCTTTGGTGGCA
		CAATGACAGCTCGCTGACTGC	GAGAGCTCATGCGTGGTCGCGAGCCTTTCAAGCT
		CTAACAGCTACAACGGTACCTC	CAACTTCTTCTTCAAGGTCCACAACTTCTACCTGA
		AGATCTCCAGCTGGAGCGCAT	CCGTCATCAGTGGTCTTCTCTTGGTTCTCTTCGTC
		GAACGTCTACTTCAACGAGGTC	GAGCAGCTCCTGCCCGAGATTGTCAGAAACGGCA
		AGTCACCAACTGTCCGCCCTTC	TTTTCCACGCTGTCTGCGCCTACGAGGGCGGCTG
		ACTGACTACTCCAATGCTAATG	GACTGACAAGCTCGTTGTTCTTTACTACGTACGTTT
		CGCACCATAGGCCTCTGGTAA	TGCCATGTACGCGCCAGAATGCGCTTACTGACAG
		CAAGTATGTCCCCCGTGCCGTC	CTGGCAGCTCAACTACCTCACCAAGTACCTCGAG
		CTCGTCGACTTGGAGCCTGGTA	CTGATTGACACCTGCTTCCTTTTCCTCAAGAAGAA
		CCATGGACGCCGTCCGTGCTG	GCCTTTGAGTAAGCGCGCAATATCTTCACATTTAG
		GTCCCTTCGGTCAGCTCTTCCG	TGCTTGAGCTGACTGGTCTCCTAGCTTTCCTCCAC
		TCCCGACAACTTCGTCTTCGGT	ACCTACCACCACGGTGCCACTGCCCTTCTCTGCTT
		CAGTCCGGTGCTGGCAACAAC	CACCCAGCTTCTCGGCCACACCGCAGTCTCATGG
		TGGGCCA	GTCCCCATCACCCTGAACCTGACCGTCCACGT
PBUAP58	KP965477	GCTGCTTTCTGGCAGACCATCT	CCCAGGACTTCCGCTCGTCCCTGGAAAGACCCC
1 2 6 / 4 6 6		CTGGCGAGCACGGCCTTGACG	CATGGCTACCTTCAAGGAGACGGCCACCATGCTC
		GTGCTGGTGTGTACGTCGACTC	ATTGCCTACTACATCATCATCATCTTTGGTGGCAGAGA
		CCTGCGCATCCTCACCGCAAT	GCTCATGCGTGGTCGCGAGCCTTTCAAGCTCAAC
		GACAGCTCGCTGACTGCCTAA	TTCTTCTTCAAGGTCCACAACTTCTACCTGACCGT
		CAGCTACAACGGTACCTCAGAT	
		CTCCAGCTGGAGCGCATGAAC	AGCTCCTGCCCGAGATTGTCAGAAACGGCATTTTC
		GTCTACTTCAACGAGGTCAGTC	CACGCTGTCTGCGCCTACGAGGGCGGCTGGACT
		ACTAGTTATCGGCCCTTCGCTC	GACAAGCTCGTTGTTCTTTACTACGTACGTTTTGCC
		ACTACTICAATACTAATGCGCA	ATGTACGCGCCAGAATGCGCTTACTGACAGCTGG
		CCATAGGCCTCTGGTAACAAGT	CAGCTCAACTACCTCACCAGCTGC
		ATGTCCCCCGTGCTGTCCTCGT	TTGACACTACTACCTCACCAAGTACCTCGAGCTGA
		CGACTTGGAGCCTGGTACCAT	TTGAGTAAGCGCGCGCAATATCTTCACATTTAGTGCTT
		GGACGCCGTCCGTGCCGGTCC	GAGCTGACTGGTCTCCTAGCTTTCCTCCACACCTA
		CTTCGGTCAGCTCTTCCGTCCC	
		GACAACTTCGTCTTCGGCCAGT	
		CCGGTGCTGGCAACAACTGGG	CCATCACCCTGAACCTGACCGTCCACGTTGTCAT
		CCAAGGG	GTACTGGTACTACTCC

PBUAPE1 KP96547 CCGGACTGACCGAAGACGTAG TTCAGGGTGATGGGAACCCATGAGAAGCTGGC PBUAPE1 KP96547 GCCACGCCCCCAGGACGACGCCAG GGCACGCACTGGTACGAGGAAGCTGGC PBUAPE1 KP965478 GACTGACCGGACGACGCCCAGGACGTCCGTGTGGTACGAGGAAGTGTGGCCACGGGGCATTGCAGGAAGCTGGGGCATTGCCGTGCAGGAGGTGCCAGGCGGACGTCCGGAGGACGTCCGGGGGCATTGCGGTGCCGCCAGGCGGGGAGGTCCTGGCGAGGAGGTCCGGCGCCGCGCGCG		10005470	011000000001101100000000	
PBUAP61 KP965479 GCCAGGGCGCGCGCGCGCGCCGCCCCCCCCCCCCCCCC	PBUAP59	KP965478	CTTGGCCCAGTTGTTGCCAGCA	GTAGTACCAGTACATGACAACGTGGACGGTCAAG
PBUAP61 KP965400 GGCACCAGGCAC GGCACCAGGCAGGCAC PBUAP61 KP965400 GGCACCAGGCAGGACGGCACG AGGAACCAGGCAGGCACG ACAGTICAGCAGGCAGGACGGCACG AGGAAGCAGGGCAGGCACGACGACGGCAGGAAAAGCATACGACGAAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG				
PBUAP61 CCCGTCCATCGTACCAGGCCC GGAGACCAGTCAGCTAAGCATTGTTGTAGAGA CAAGTCGACCAGAGGACGGCCC CCCTATGCTCCACCAGGCCC ATGCGCCGCTATCACCATT CGAGATAGTCAGTCAAGCGCCG ATGCGCCGCAATCAGCCTGCCAAGCCCCCCCCCCCCCCC				
PBUAP61 KP965479 CAAGTCGACGAGGACGGACGG ATTGCGCGCTTACTCAAAGGCTTCTTCTTGAGGA PBUAP61 KP965479 CAAGTCGTCACCTCGTTGACCTCGACGTCGCAGGAGGCTCTCGAGGAGAAGCTGCGCGCAGGAGGCTCG CAAGTCGCGCAAGAGCTGCTGGAGGAGCG PBUAP62 KP965480 CGCGCACCCCTCGCGTGAGGAGGCGCG CAAGTCGCGCGTGAGGAGGCGCG CACAGTCGTCGGCGGAGAGCGCGCGTCGGAGAAAGCGGCGTGCGCGAGAAGCGGCGGCGAGAGGCGCGTGCCGGAGGAGGCGGCG PBUAP62 KP965480 CGCGCACCCCCTCGGCGGCGGAGGCGCG CGCGCCGCGCGCGCGCGGCGGCGGCGGCGGCGGGGGGGG				
PBUAP61 KP965479 GAGGACATACTIGTTACCAGAG AAGGAAGCAGGTGTAATCAGCTCAAGGTACTT GAGGTAGTCAGCATTAGCCTCG CAAGTAGACGTTGAGCAGCG AACAGTGGAAGTCGAGCAGCG CAACAGTGGAGATCGAGCTCG CAAGTAGACGTTCAGCGCCC AGGCTGGAGATCGAGCTCGCC AGGCTGGTATGAGCGTCCC CAGGAGAGTCGACGGTCCCC AGAGGAGCGGCCAGCGCAGAGAGCGCCCCCAGAGACGGCCCCCGCCCC CCAGAGATGGCCTCCCC CCAGAGATGGCCT CCAGGAGCAGCGCCCCCCCC CCAGAGATGGCCT CCAGGAGCGCGCCCCCCCCCC				
PBUAP61 KP965479 GACGTACGCACCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGC				
PBUAP61 KP965479 GAGTGGTCAGCCGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGC				
PBUAP61 ACAGTIGGTGACTGACCTGGTT MAGAACAGGAGCTIGTCGGGTCAAGCGCGCCT GIAGCGCGCAAGAAGCTGCGACAAGAGCTGCTGAAGAAGCCGTGAGAAAGCCGTGAGAAAGCCGTGACGAAGA GGACTGACTGACGAGCACCACGA GGACGTGATTAGGCAGTCACCC ACAATCTCGCGCAAGAAGCTGCACGACGACGCGTGAGGAAG GCACGCGTGAACGACGACGCA GGACGCGTCAAGCGCAGCACCAC GGACGCACCAACGGCGCGCG CCAGAGAGTGGTCT ACAATCTCGCGCACCACGAAGAGCTGCACGACGCACGACGCGCGCG				
PBUAP61 CAAGTAGACGTTCATGCGCTCC ACCTGAGACACGTCAAGCACCGCGACAACAGCGTGCAAAAAGCG TTGTAGCTGTTAGGCAGTCAGC CACAGTGTCATGAGGACGCAGC GACCGTCATGAGCAGCCCA GCACCGTCAAGGCCGTGCTCG CCAGAGATGCCAACGGCGTGCTCG CCAGAGATGGTCA CCAGAGATGGTCA CCAGAGATGGTCA CCAGAGATGGTCA CCAGAGATGGTCA CCAGAGATGGTCA CCAGAGATGGTCA CCAGAGAGCCAACGCCAAGGCGCAAGTCCTGCGGGC CCAGAGATGGCCA CCAGAGATGGTCA CCAGAGAGCCAACGCCAAGTCGT CCGGACCGAAGACCGAAGTCGT CCGGACCGAAGACCGAAGTCCTGGGGC CCAGAGAGCGAAGCCAAGTCCTGGGGC CCAGAGAGCCAAGCGCAAGTCCTGCGGGC ACATGCTGACGGAAGCCAAGGCCGA AGGGACCCATGAACCTGGGGCC ACATGCTGCCGACGGAGGCG ACATACTGTTCCAGGGCCCAAGCCGAAGCCCAAGGACGAAGCAAGACGAGCAGAAGCCGGAAGCCGCAAGTCCTGGGGC ACATACTGTTACCAGGCCCCAAG AGGGGCCCAGGACGGCGC ACATACTGTGTCCCAGGGCGC ACATACTGTTCCAGGGCCCCAAGCCGCGCG ACATACTGTGTCCCAGGCGCCAAGCCGCGCG ACATACTGTGTCCCAGGCCCCACGCG GGCGGCCACGCCGCGCCG				
PBUAP61 KP965479 CACTGGAGGACCGTGAGG ACAATCTCGGGCAAGAGCGTCGACGGACGGAGAGGTGAGCGTGAGAGAGA				
PBUAP61 KP965479 GACGACCACCAGAGACCGACGACAGACAGAGAGAGAGAG				
BUAP61 GAGCTGTCATTGAGGATGCGC AGTTGTGGACCTTGAAGAAGAGTTGAGCTTGACAGCTAG PBUAP61 KP965479 GACTGACGGAAGAGAGTTGT AGTGATGGACGCAGGAGGAAGGAGTTGT PBUAP61 KP965479 GACTGACGGAAGGAGCGACGAG GATGGTGGTAGGAGGCGAAGGAGTGTGT CGGGACGAGAGGAGGGGACGAGGGG AGTGACGGAAGGAGGTGACGA AGTGGGGACGACGAGGAGGAGTGGACGGTCAAGTGCGGACGGTCAGGAGGACGAAGAGACAGAGAGGAGGACGAGGGGC CCATGGTACCAGGGCGC AGCTGGCGGACGAGGGG AGTGGGGACGAGGAGGGACGAGGGG AGCTGGGTGGAGGTGGAGGAAGGACGAGGCG CCACGAGGACGGGCGC CCACGAGGACGGACGGGG ATTAACTAAACGATTGGTGGACGGTAGGCGC AGCTGGGTGGAGGAAGGACGAAGGACAAAGGACAAAGGACAAAGGACAAAGGACCAATACTGTGTGAGCGTCAATACGTGAGGAGACGAAGGACCAAC TGGTGCGCATTAGCGGCCACGA AGTGGAGGCGACGGGGG ATTAACTAACGATGGGGAGGAAGGACAAAA TGGTGCGCATTAGCGGGCCAGGAGG CGGGGAGATGGCGCACGGCGCC CGCGCAGATGGCGTCAGTAAGGAAGAAAAGGACAAA TGTGTACGGGGGGGGGGGGGGGGGGGGGGAGGAGCCACCTCTGCGCCAAGAGAGCAAA CGCGCGCGCAGAGGCCGCC CGGACACGCCGCCCCCCCGCAAGAGGCCCCCCCGGCAAGAGGCCCCCTCGTGAAGAAAGGACCAAA CCAGGCGCCTGAAGGCCGGCC CGAGGAGCACCCCTCTGGCGAGG CGAGCAGCCCCTCGACGAAGGCGCAGGGCCCCCCCAAGAGGGGCCTGAAGGCCCCCC CGGGGCGCGCAAGGCCGCC CGGGGCGCGAAGGCCGCCCGGCCCCCCGAGGGGCTCGAAGGCCGCCCCCCCC				
AGAGAGTCAACGTACACGACA GGCTCGCCACGCATGAGCCTCTGCCACC GCACCCGTCAAGGCCGTGCTCG CGTGATGATGATGAGCATGAGCATGGCGC CCAGAGATGGTCT CGGGACGCAAGCCGAGCGCACGAGCGCCAAGGAGGCGTCATCCGGGCC PBUAP61 KP965479 GACTGACCGAGAGCGACGCGCA CAGTGGGGACGGACGGCGGCAAGGCGGCAGGCGGCAGGCCAGGAGG				
BUAP61 KP965479 GACTGACCGACGAAGGCCGACGAAGTGATGAGGAAGTGAGGCAAGGGGAAGGAA				
PBUAP61 KP965479 GACTGACCGAGAGCGAAGTGTT CGTCTCCTTGAAGGTAGCCGTGACGTCAAGTTCAGG PBUAP61 KP965479 GACTGACCGAGACGCAAGTTGT AGTGACTGACGGACGTGACGGCAAGTTCAGGG CGGGACGGAAGACGCACGGACGGCG AAGCTGGGTGAACCGAGACAGCAGCAGCAGCGCCAAGTTCAGGG AAGCTGGGTGAAGCAGAAGAGCAGGAGCGCCAGGGCG AGGGACCACGCACGGACGGCGGG AACTCTGGTGGTGGAGGAAAGCCAGAGAGCAGGGCCC GGGTGGTGAGGGTGGAGGAAAGCCAGGAGAGGAC AGGGTGCCATTGATGAGG CGGCCGCGAGGGGGG AATTACTAAGCATTGAAG CAGGGTGTCAATCAGCTGGAGGAAAGCCAGGAGAGGA ATGGTGCCCATTGATGAGG GCGGGAGTGAGCGCCAGGCGG AGTTGAGCGACCACGGCGCAGGGG AGTGGTGCATCAGCGCAGCAG AGTGGTGCATCAGCGCCAGCT TAGACGTTCAAGGGTCACGACGTCGTGAGAGCGCGGCCAGGGGGGAGCGCGCCGGGCAGGAGCTGCCCAGCGGCGCAGCGGCGTGACGAGCG CGGGGAGAGCGCGTGC CGGGCAGGAGGCGTGCCAGCGGCGAGAGGCGTCCAACGTGGGCGCTCTGACGAAGAGAGAACCAAC GGCAGGGGGGTGAAGGTGGC GGCAGGGCGTGCACGGCGGCG CGGCCAGGAGGCGTGC CGGCAGGGGGTCGACGGCGGCG CGGCAGGCGCTGCCCAGCGGGGCGTGAGGGCCTTCTCACCACCAAGAAGGAGCACCCAC CGGCCCCCAGAGGAGGCCGTGC CGGGCAGGCCATCGGGGGCGTGCC CGGGCAGGCCGTGCC CGGGCAGGCCGTGC CGGGCAGGCCGTGCC CGGGCAGGCCGTGCCCCCGCGCACCAGGGGGGCGGCGCGGCGCGCCCCCAGGGGGG			AGAGAGTCAACGTACACACCA	GGCTCGCGACCACGCATGAGCTCTCTGCCACCAA
PBUAP61 KP965479 GACTGACCGAGACGAAGTTGT AGTACATGACCACGGGACGACGACGACGACGACGACGACGACGACGA			GCACCGTCAAGGCCGTGCTCG	AGATGATGATGTAGTAGGCAATGAGCATGGTGGC
PBUAP61 KP965479 GACTGACCGAGACGAAGTTGT AGTACATGACCAGCGCGCGAAGCGACGAGTTGCAGCTCAAGTTCAGGC AGGGACCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGC			CCAGAGATGGTCT	CGTCTCCTTGAAGGTAGCCATAGGGGTCTTTCCA
PBUAP62 KP965480 GGCAGCAGCACGACCGACC GATGGGGACCCATGAAACTGCGGTGTGGCCCAAAGAAACTGCGGTGTGGCCCATGAAACTGCGGAAAACGACGAGGACGCCAAGAAAGCAAGAAGACAAGGAAGAA				GGGACGAAGCGGAAGTCCTGGGGC
PBUAP62 KP965480 GGCAGGACCACCACGACGGCG AAGCTGGGTGAAGCAGAGAGAGAGAGCAGTGGCGACGGAGAGAGA	PBUAP61	KP965479	GACTGACCGAGACGAAGTTGT	AGTACATGACGACGTGGACGGTCAAGTTCAGGGT
PBUAP62 KP965480 GCCAGGCACCACCGCACCACCACCACCACCACCACCACCA			CGGGACGGAAGAGCTGACCGA	GATGGGGACCCATGAAACTGCGGTGTGGCCGAG
PBUAP62 KP965480 GCAGAGCACCACTCACTCACGAGGCCG ACAGCCTCACTCACACGCCCCCCCCCC			AGGGACCAGCACGGACGGCG	AAGCTGGGTGAAGCAGAGAAGAGCAGTGGCGCC
ACATACTTGTTACCAGAGGCCT GCTTACTCAAAGGCTTCTTTTGAGGAAAAGGAA ATGGTGCGCATTAGCATTGAAG CAGGTGTCAATCAGCTCGAGGTACTTGGTGAGG GAGTGAGTGAAAGGCCGACAG AGTTGAGCTGCCAGCTGTCAGTAAGCGCATTCT TTGTTGACCGACCTCGTTGAAG CGCGGCAGTGAATGGACGTTCCAGCCAGCT GGAGATTGAGGTACCGTTGTA CGCGGCAGGAATGGCAGGAAGC GGTGTTAGCGGTCAGCGACGT CGCGGCAGGAAGGAGCCCCCCCTCGTAGGAGAAACCAAC GCTGTTAGCGGTGAGGAGGC CGGGCAGGAGCTGCCCGCCCGCGCAGGAGAGAAACCAAC GCTGTTAGCGGCGCGC CGGGCAGGAAGGTCCGGCGCAGGAAGGACCAAC GCAGGCGCGTCAAGGCCGTGC CGACGCCGCGCAGGAGCCCCCCGGAGGGGCCTCTCACCACCAAAGGACCCC PBUAP62 KP965480 GGCAGACCATCCTCGGCGAGC PBUAP62 KP965480 GGCAGGACCATCCTGGCGAGC GAGTGACGTACCAGGGGAGCTGCCCGGGGGGAGCCGCCCGGGGGAGCAGGGGCCCCCGGAGAGGGCAGGGGCAGGGGAAGCCAAGGAGG			TCCATGGTACCAGGCTCCAAAT	GTGGTGGTAGGTGTGGAGGAAAGCTAGGAGCCC
ATGGTGCCCATTAGCATTGAAG CAGGTGTCAATCAGCTCGAGGTACTTGGTGAGG GAGTGAGTGAAAGGCCGACAG AGTTGAGCTGCCAGCTGCAGTAAGCGCATTCT TTGTTGACCGACCTCGTTGAAG CGCGGAATTGGATAGACCATACGTAGTAAGCGCATTCT GGAGATCTGAGGTACCGTTGTA CGCGGCAGCAGCTGCCAGCGTGCAAGAAATGCCGTTTCTGACAATC GCTGTTAGGCGCAGCAGCAGCAGCAGCAGCAGCAGCGTGCCAAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGC			CGACGAGGACGGCACGGGGG	AATTAACTAAAGCATTGATTTTGGAGGTAGTGCGC
PBUAP62 KP965480 GGCAGACCACCTCGTCGCGACAGC AGTTGAGCGACCACGACAGC CGCGCATTGAGGGACCACCGTTGTA CGCGCCACGCGCCCCCGTGAGGACACACACGACGACACACGACGACACACGACGACGA			ACATACTTGTTACCAGAGGCCT	GCTTACTCAAAGGCTTCTTCTTGAGGAAAAGGAAG
PBUAP62 KP965480 GGCAGACCATCTTCAGCGCGCCAGCT CAGCAGCATCAGAGAAAGAACAGAGAAGAAGAAGAAGAAGAAGAAGAAG			ATGGTGCGCATTAGCATTGAAG	CAGGTGTCAATCAGCTCGAGGTACTTGGTGAGGT
PBUAP62 KP965480 GGCAGACCATCTCTGGCGTCCAGCTCG ACGGACTGTGGGAAAATGCCGTGGAAAAAGCAAGGAACCAACC			GAGTGAGTGAAAGGCCGACAG	AGTTGAGCTGCCAGCTGTCAGTAAGCGCATTCTGT
PBUAP62 KP965480 GGCAGACCATCTCTGGCGAGC CAGACAGCGTGGAAAATGCCGTTGTAAGCAGCGTCAAGTAGAAGGAACCAAC PBUAP62 KP965480 GGCAGGCCAGTCAAGCCGCGTGC GAAGACCAGCAGTAGCAGCAGTGGTGAAGGAAGCAAGCGAAC AGGCGCATGACCAAGGACCCATCTCTGGCGAAGACCAACCA			TTGTTGACCGACCTCGTTGAAG	CGCGGAATTGGATAGACATACGTAGTAAAGAACA
PBUAP62 KP965480 GGCAGAGCCAGCCAGCGAGC CGAGAGGAGGAGCAGCAGCAGAGAAGAGAAGCAAGAGAGAGACCAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA			TAGACGTTCATGCGCTCCAGCT	ACGAGCTTGTCGGTCCAGCCGCCCTCGTAGGCG
TGTCATTGCGGGTGAGGATGC GCAGGGGGGTCGACGACGACGACGACACA GCAGGGGGGTCGACGTCACACA CCAGCGCCGTCAAGGCCGTGC TCGCCAGAGAGTGGTCTGCCAG AAAGCAGCACCGAAGACCGCATGAGCGTCAAGGACGTCAAAGAGGCGTG GACCTTGAAGAGACGCCATGGGGGTCTTTCCAGGAAG CGACCATGGGGGCTGTGAGCCGTGAGGCCGTCT TGATGTAGTGGCGAGC AAGCGGCATGACGTCCCGCGCAG TGTACGTTGACTCCCTGCGCAT CGGCACCCCCGCATGACGCCAGACGGCGAGGAGGACGACGGCGGTG TGTACGTCACGACGTCCCAGCATGAGGCGTGGGGGGAGGACGAGGGCAGTGGC CTGACTGCCCAGAACGGCCAGCACGCCAGACGGCGGGTGGAGGAAGGCAGGGGAGGAGGCAGTGGC CGGCCCTCAGACGCCAGAACGGCCAGCAGGGCGGTGGAGGAAGGCAGGGGAGGAGGAGGCAGTGGC CGGCCCTCAGGTGCCCCGCGCGGTGCGGCGGGTGAGGCAGGGAGGACAAGAAGAAGAAGAAGAAGAAGAAGAA			GGAGATCTGAGGTACCGTTGTA	CAGACAGCGTGGAAAATGCCGTTTCTGACAATCT
GCAGGGGGGGTCGACGTACACA GACCTTGAAGAAGAAGTTGAGCTTGAAAGGGTCT CCAGCGCCGTCAAGGCCGTGC CGACCACGCATGAGCTCTCTACCACCAAAGATG TCGCCAGAGATGGTCTGCCAG TGATGTAGTAGGCATGAGCATGGGGGGTCTTTCCAGGAAC AAAGCAGCACC CTTGAAAGTAGCCATGGGGGGTCTTTCCAGGAAC PBUAP62 KP965480 GGCAGACCATCTCTGGCGAGC GAAGTAGTACCAGTACATGACGACGTGAACGGTCAC PBUAP62 KP965480 GGCAGACCATCTCTGGCGAGC GAAGTAGTACCAGTACATGACGACGTGAACGGTCAC CGGCCTTGACGGTGCTGGTG TTCAGGGTGATGGGAACCCATGAGAGAGGGGCAGTGGC TCTCACCCGCAATGACAGCTCG CGAGGAAGCTAGGAGAGGAGGGCAGTGGC CGACGTCGCCTAACAGCTACAC CGAGGTCAGCTACAACG CGGGGTGGAGGAAAGCAAGGAGGGCAGTGGC CGAGGTCAGCTACACAC CGACGCCTTGACGCAATGACAGCTCC CGGGGTGGAGAAAGCAAGAAGAAGCAAGAAGCAAGGAGGTGT CGAGGTCAGTCACCAACTGCG CGGGGTGGTGAGGAACAAGAAGAAGCAAGGAGCTGCT CGACGCCTTCACTGAACAGCTCTACTTCAA AGCGCCCTTCGACGACCAACTGTC ACGGCCCTTCGGCGGGAAACAAGAAGAAGCAAGGAGCTGCTGAGCAGCAGCGTGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGA			GCTGTTAGGCAGTCAGCGAGC	CGGGCAGGAGCTGCTCGACGAAGAGAACCAAGA
PBUAP62 KP965480 GGCAGACCATCTCTGCCAGG CGACCACGCATGAGGCTCTCTACCACCAAAGATCA PBUAP62 KP965480 GGCAGACCATCTCTGGCGAGC GAAGTAGTACCAGTACAGGACGGTGAACGGCGGTGAACGGCGGGGCAGGGGAAGCCATGACGGTGCTGGTG TCTCACCCGCAATGACGGTGCTGGTG TCACGGTGCTGGGGGAGCCAGGGGAAGCCATGAGGAGGGGAAGGCAGGGGCAGTGGCG CGAGGACCATCTCCGGCGAGC GGTACCTCACGGACCATGACGGTGCTCG CGAGGACCATCGCGGGGAAGCAGGAGGGAAGGCAGGGGCAGTGGC CGAGGAAGCTGGGGGAAGCAGAGGAGGGGCAGTGGC GGTACCTCAGATCTCCAGCTGG TCTCACCCGCAATGACGCTCACAAC CAGGCCATGAACGTCACAAC CAGCGCATGAACGTCACAAC GGTACCTCAGATCTCCAGCTGG ACGGCCATGAACGTCACAAC CAGCGCATGAACGTCACAAC CAGCGCATGAAGAAGAAGAAGAAGAAGAAGCAGGTGTA ACGACCAGGTCAGCAACTGTC CGACGTCTCACTGACACTGTC ACCGCCCTTCGCGCGAATCAACAACAAGAAGAACAAGAAGCAAGGAGGTGTGAAGAACAACGAAGAACAAGAAGCAAGGAAGAACAAGAAG			TGTCATTGCGGGTGAGGATGC	GAAGACCGCTGATGACGGTCAAGTAGAAGTTGTG
TCGCCAGAGATGGTCTGCCAG TGATGTAGTAGGCAATGAGCATGGTGGCCGTCT PBUAP62 KP965480 GGCAGACCATCTCTGGCGAGC CAAGCGGAAGTCCTGGGGGCTTGTAGCCC PBUAP62 KP965480 GGCAGACCATCTCTGGCGAGC GAAGTAGTACCAGTACATGACGACGTGAACGGTGAC CGGCCTTGACGTGGTG TGTACGTTGACTCCCTGCGCAT CGAGGAGGCAGGGGGAAGCGAGGGGGAGGGGCAGTGGC TCTCACCCGCAATGACAGCTCC CGAGGTGGGGGGAAGCAGAGGAGGGGCAGTGGC CGGTACTCCCTGCGCAT CGAGGTAGGGGGGAAGCAGAGGAGGGGCAGTGGC CGGGTACCTCAGATCTCCAGCTACAAC CGGGGTGGTGGAGGAAAAGCTAGGAGAGCAGGAGGAGGCAGTGGC CGAGGTCAGCCAACGACTACAAC CGAGCATGAACGTCTACTACAA CGAGGTCAGTCAGTCACCAACTGTC CGAGGTCAGTCACCAACTGTC AGCGCCTTCACTGACTACCAACTGTC AGCGCCCTTCGCGCGGAATCAGA AGCGCCTTCACTGACTACCCAACTGTC AGCGCCTTCGGCGCGGAATCAGA CGAGGTCAGTCACCAACTGTC AGCGCGCCTCGTGGAGGCGGCGGAACAAGAAGCAGCGGCGGAATCAGA AGCGCCCTTCACTGACTACTCCAA AACGTACGTAGTAAGAACAACGAGCTTGTCAGTCC CGCCCCTTCACTGACTACTCCAA AACGTACGTAGTAAGAACAACGAGCTTGTCAGTCC CGCCCCTTCACTGACAACTAGCCC GCCGCCCTCGTAGGCGAAAAGAACAACGAGCTGCTCAACGAA CGAGGTCAGTCACCATAGGCCT GCCGCCCTCGTAGGCAAAAGAACAACGAGCTGCTCGACGAAAAGACCAGCGGGAGACTGCTCGACGAAAAGAACAAGAAGACCAGGGGAGAGCGCTGACAAGAAA CTGCCTCCTCGTCGTCGACCATTGGAG AGAACCAAAGAAGAACACGCGGTGAAAAAAAAAAAAAAA			GCAGGGGGGTCGACGTACACA	GACCTTGAAGAAGAAGTTGAGCTTGAAAGGCTCG
AAAGCAGCACC KORN UNIN CTTGAAAGTAGCCATGGGGGTCTTTCCAGGAAC AAAGCAGCACC KORN UNIN CTTGAAAGTAGCCATGGGGGGTCTTTCCAGGAAC PBUAP62 KP965480 GGCAGACCATCTCTGGCGAGC GAAGTAGTACCAGTACATGACGACGTGAACGGTCAC PBUAP62 KP965480 GGCAGACCATCTCTGGCGAGC GAAGTAGTACCAGTACATGACGACGGTGAACGGTGAG TGTACGTTGACGGTGCTGGTG TTCAGGGTGATGGGAACCAGAGAGAGGGCAGTGGC TTCAGGGTGGTGGAGGTAGGGGAAGCTAGGAGAGCGGCAGTGGC TGTCACCCGCAATGACAGCTCG CGAGGAGGTGTGGAGGAAGCAGGGGAAGCTAGGAGAATCA CGAGGTACTCCAGGCTGG CGTGGTGGTGGGGAGGAAGCAAGAAGAAGCAGGGGTGT GGTACCTCAGATCTCCAGCTGG ACGCGCATGAACGTCTACTTCAA CAGCTGAAGGAGACAAGAAGAAGCAAGGAGGTGT ATCAGCTCGAGGTACTTGGTGAGGTAGTTGAGCTTG GGCAGGTCAGTCACCAACTGTC AGCGTGCTGAGGAGACAACGAGCTGTCGCGGAAACAAGAACAACGAGCTTGTCAGTCC ACGTGTCAGTAAGAACAACGAGCTTGTCAGTCC CGCCCTTCACTGACACACACTACTCCAA AACGTACGTAGTAAGAACAACGAGCTTGTCAGTCC ACGTGTCAGTAAGAACAACGAGCTTGTCAGTCC CGCCCTTCACTGACTACTCCAA AACGTACGTAGTAAGAACAACGAGCTTGTCAGTCC ACGTGCCCCGGAGAACAACGAGCGTGGAAAATGC CGCGCCTTCAATGCCCCG GCCGCCCTCGTAGGCGAGAACAACGAGCTGCTCGACGAA GCCGCCCTCGTAGGCGAGAAGCCGCTGATGACGGTCAAGTA			CCAGCGCCGTCAAGGCCGTGC	CGACCACGCATGAGCTCTCTACCACCAAAGATGA
PBUAP62 KP965480 GGCAGACCATCTCTGGCGAGC GAAGCGGAAGTCCAGTACATGACGACGTGAACGGTCACA ACGGCCTTGACGGTGCTGGTG TGTACGTTGACGTGCCGCAT CGAGAAGCTGGGGTGAAGCAGAGAGAGGAGGGCAGTGGC TGTACGTTGACTCCCTGCGCAT CGAGAAGCTGGGTGAAGCAGAGGAGGGCAGTGGC CGAGGAGGTGGTGGAGGGAAGCAGAGGAGGGCAGTGGC CTGACTGCCTAACAGCTACAAC CGTGGTGGTGGAGGAAGCAGAGGAGGGCAGTGGC CGTGACTGCCTAACAGCTACAAC GGTACCTCAGATCTCCAGCTGG CCAAAGCATTGATTGTGGAAAAAGAAGAAGCAGGGTGT AGCGCATGAACGTCTACTTCAA CAGCTCAGGGGAACAAGAAGCAGGGGGA ATCAGCTCGAGGTACTTGGTGAGGAGATGAGCAGCGTGT AGCGCCTTCACTGACTACTCCAA CGACGTCAGTCACCAACTGTC AGCTGTCAGTAAGAACAACGAGCTTGTCAGTCC CGCCCTTCACTGACTACTCCAA AACGTACGTAGTAAAGAACAACGAGCTTGTCAGTCC GCCGCCTTCACTGACTACTCCAA TGCTAATGCGCACCATAGGCCT GCCGCCCTCGTAGGCGAGAACAACGAGCTGCTCGACGAA TGCTAATGCGCACCATAGGCCT GCCGCCCTCGTAGGCGCAGACAGCGTGGAAAATGC GTTCTGACAATCTCGGGCAGGAGCTGCTCGACGAA ACGTACCTAGGAACAACGACGCTGCTCGACGACAGCGTGAAAATGC CTGGTAACAAGTATGTCCCCCG GTTTCTGACAATCTCGGGCAGGAGCTGCTCGACGAA ACGAACCAAGAGAAAGACCGCTGATGACGGTCAAGTAA			TCGCCAGAGATGGTCTGCCAG	TGATGTAGTAGGCAATGAGCATGGTGGCCGTCTC
PBUAP62 KP965480 GGCAGACCATCTCTGGCGAGC GAAGTAGTACCAGTACATGACGACGTGAACGGTGAACGGTGAACGGTGAACGGTGAACGGTGAACGGTGAACGGTGAACGGTGAACGGTGAGGAACCCATGAGAACGGGTGAAGCAGAGGAGGGCAGTGGC TGTACGTTGACGCCCCGCCAT TCAGGGTGATGGGAACCCATGAGAGCGGGGAAGCGAGGGCAGTGGC CGAGAACGTCGCCCACGGCGAAGCAGAGGAGGGCAGTGGC TCTCACCCGCAATGACAGCTCG CGTGGTGGTGGGAGGAAGCAGGGGAAAGCTAGGAGAATATTGCGCGCGTTA CGTGACTGCCTAACAGCTACAAC CAGCTAAAGGCTTCTTCTTGAGGAAACAAGAAGCAGGAGGTGT GGTACCTCAGATCTCCAGACTGC CGAGGTCAGTCACCAACTGTC CGACGTACTGGCGCGGGAATCAGA ATCAGCTCGAGGTACTTGGTGAGGTAGTTGAGCTGC CGACGCTCACCAACTGTC AGCTGTCAGTAAGAACAACGAGCTGCTGAGGTACTTGGCGCGGGAATCAGAA AACGTACGTAGTAAGAACAACGAGCTTGTCAGTCA CGCCCTTCACTGACTACTCCAA AACGTACGTAGTAAAGAACAACGAGCTTGTCAGTCA AACGTACGTAGTAAAGAACAACGAGCTTGTCAGTCA CGCCCTTCACTGACAACTATCCCCA AACGTACGTAGTAAAGAACAACGAGCTTGTCAGTCAGTAA AACGTACGTAGTAAAGAACAACGAGCTTGTCAGTCAGAA CGGCCCTCGTAACAAGTATGCCCCCG GCTGCCCCCGTAGGCAGAAGCAGCGGCGAGACAGCGTGGAAAATGC GCCGCCCCCGTAGGCAGAAGACCGCTGATGACGGTCAAGTAA			AAAGCAGCACC	CTTGAAAGTAGCCATGGGGGTCTTTCCAGGAACG
ACGGCCTTGACGGTGCTGGTGTTCAGGGTGATGGGAACCCATGAGACTGCGGTGGGGTGTACGTTGACTCCCTGCGCATCGAGAAGCTGGGTGAAGCAGAGGAGGGCAGTGGCTCTCACCCGCAATGACAGCTCGCGTGGTGGTGGAGGAAAGCTAGGAGAATATTGCGCGCGTTACTGACTGCCTAACAGCTACAACCAGCTAAAGCATTGATTGTGGAAATATTGCGCGCGTTAGGTACCTCAGATCTCCAGCTGGTCAAAGGCTTCTTCTTGAGGAACAAGAAGCAGGAGGTGTAGCGCATGAACGTCTACTTCAAATCAGCTCGAGGTACTTGGTGAGGTAGTTGAGCTGCCGACGTCAGTCACCAACTGTCAGCTGTCAGTAAGCAGCGTTCTGGCGCGGAATCAGAACGCCCTTCACTGACTACTCCAAAACGTACGTAGTAAGAACAACGAGCTTGTCAGTCAGTCATGCTAATGCGCACCATAGGCCTGCCGCCCTCGTAGGCGCAGACAGCGTGGAAAATGCCTGGTAACAAGTATGTCCCCCGGTTTCTGACAATCTCGGGCAGGAGCTGCTCGACGAATGCCGTCCTCGTCGACTTGGAGAGAACCAAGAGAAGAACCCCTGATGACGGTCAAGTAA				AAGCGGAAGTCCTGGGGCTTGTAGCCC
TGTACGTTGACTCCCTGCGCATCGAGAAGCTGGGTGAAGCAGAGGAGGGCAGTGGCTCTCACCCGCAATGACAGCTCGCGTGGTGGTAGGTGGAGGAAAGCTAGGAGAATCACTGACTGCCTAACAGCTACAACCAGCTAAAGCATTGATTGTGGAAAATATTGCGCGCTTAGGTACCTCAGATCTCCAGCTGGTCAAAGGCTTCTTCTTGAGGAACAAGAAGCAGGGGTGTAGCGCATGAACGTCTACTTCAAATCAGCTCGAGGTACTTGGTGAGGTAGTTGAGCTGCCGACGTCAGTCACCAACTGTCAGCTGTCAGTAAGCGCGTTCTGGCGCGGAATCAAGACGACCTCTACTGACTACTCCAAAACGTACGTAGTAAGCACCACGAGCTTGTCAGTCCCGCCCTTCACTGACTACTCCAAAACGTACGTAGTAAAGAACAACGAGCTTGTCAGTCCTGCTAATGCGCACCATAGGCCTGCCGCCCTCGTAGGCGCAGAACAGCGTGGAAAATGCCTGGTAACAAGTATGTCCCCCGGTTTCTGACAATCTCGGGCAGGAGCTGCTCGACGAATGCCGTCCTCGTCGACGTCGACGACTGGCGAGAAGACCGCTGATGACGGTCAAGTAAAGAACCAAGAGAAGAACCGCTGATGACGGTCAAGTAA	PBUAP62	KP965480	GGCAGACCATCTCTGGCGAGC	GAAGTAGTACCAGTACATGACGACGTGAACGGTCAGG
TCTCACCCGCAATGACAGCTCGCGTGGTGGTAGGTGTGGAGGAAAGCTAGGAGATCACTGACTGCCTAACAGCTACAACCAGCTAAAGCATTGATTGTGGAAATATTGCGCGCGTTAGGTACCTCAGATCTCCAGCTGGTCAAAGGCTTCTTCTTGAGGAACAAGAAGCAGGTGTTAGCGCATGAACGTCTACTTCAAATCAGCTCGAGGTACTTGGTGAGGTAGTTGAGCTGCCGAGGTCAGTCACCAACTGTCAGCTGTCAGTAAGCACCAGCGGCGAATCAGAACGCCCTTCACTGACTACTCCAAAACGTACGTAGTAAGCACCAGCGGCGAATCAGAATGCTAATGCGCACCAATAGGCCTGCCGCCCTCGTAGGCGCGAGACAGCGTGGCAAATGCCTGGTAACAAGTATGTCCCCCGGTTTCTGACAATCTCGGGCAGGAGCTGCTCGACGACATGCCGTCCTCGTCGTCGACCTTGGAGAGAACCAAGAGAAGACCGCTGATGACGGTCAAGTAA			ACGGCCTTGACGGTGCTGGTG	TTCAGGGTGATGGGAACCCATGAGACTGCGGTGTGGC
CTGACTGCCTAACAGCTACAAC CAGCTAAAGCATTGATTGTGGAAATATTGCGCGCGTTA GGTACCTCAGATCTCCAGCTGG TCAAAGGCTTCTTCTTGAGGAACAAGAAGCAGGTGT AGCGCATGAACGTCTACTTCAA ATCAGCTCGAGGTACTTGGTGAGGTAGTTGAGCTGC CGAGGTCAGTCACCAACTGTC AGCTGTCAGTAAGCAGCGCGTTCTGGCGCGGAATCAGAA CGCCCTTCACTGACTACTCCAA AACGTACGTAGTAAGCACCAGCGGCGCGCGGAATCAGAA CGCCCTTCACTGACTACTCCAA AACGTACGTAGTAAAGAACAACGAGCTTGTCAGTCC TGCTAATGCGCACCATAGGCCT GCCGCCCTCGTAGGCGCAGACAGCGTGGAAAATGC CTGGTAACAAGTATGTCCCCCG GTTTCTGACAATCTCGGGCAGGAGCTGCTCGACGAA TGCCGTCCTCGTCGACTTGGAG AGAACCAAGAGAAGAACCGCTGATGACGGTCAAGTAA			TGTACGTTGACTCCCTGCGCAT	CGAGAAGCTGGGTGAAGCAGAGGAGGGCAGTGGCAC
GGTACCTCAGATCTCCAGCTGG TCAAAGGCTTCTTCTTGAGGAACAAGAAGCAGGTGT AGCGCATGAACGTCTACTTCAA ATCAGCTCGAGGTACTTGGTGAGGTAGTTGAGCTGC CGAGGTCAGTCACCAACTGTC AGCTGTCAGTAAGCGCGTTCTGGCGCGGGAATCAGAA CGCCCTTCACTGACTACTCCAA AACGTACGTAGTAAGCAACAACGAGCTTGTCAGTCAGTCA			TCTCACCCGCAATGACAGCTCG	CGTGGTGGTAGGTGTGGAGGAAAGCTAGGAGATCAGT
AGCGCATGAACGTCTACTTCAA ATCAGCTCGAGGTACTTGGTGAGGTAGTTGAGCTGC CGAGGTCAGTCACCAACTGTC AGCTGTCAGTAAGCGCGTTCTGGCGCGGAATCAGAA CGCCCTTCACTGACTACTCCAA AACGTACGTAGTAAAGAACAACGAGCTTGTCAGTCAGTCA			CTGACTGCCTAACAGCTACAAC	CAGCTAAAGCATTGATTGTGGAAATATTGCGCGCTTAC
CGAGGTCAGTCACCAACTGTC AGCTGTCAGTAAGCGCGTTCTGGCGCGGAATCAGA CGCCCTTCACTGACTACTCCAA AACGTACGTAGTAAAGAACAACGAGCTTGTCAGTCC TGCTAATGCGCACCATAGGCCT GCCGCCCTCGTAGGCGCAGACAGCGTGGAAAATGC CTGGTAACAAGTATGTCCCCCG GTTTCTGACAATCTCGGGCAGGAGCTGCTCGACGACA TGCCGTCCTCGTCGACGTCGACGACTTGGCG AGAACCAAGAGAAGACCGCTGATGACGGTCAAGTAA			GGTACCTCAGATCTCCAGCTGG	TCAAAGGCTTCTTCTTGAGGAACAAGAAGCAGGTGTCA
CGCCCTTCACTGACTACTCCAA AACGTACGTAGTAAAGAACAACGAGCTTGTCAGTCC TGCTAATGCGCACCATAGGCCT GCCGCCCTCGTAGGCGCAGACAGCGTGGAAAATGC CTGGTAACAAGTATGTCCCCCG GTTTCTGACAATCTCGGGCAGGAGCTGCTCGACGAC TGCCGTCCTCGTCGACTTGGAG AGAACCAAGAGAAGACCGCTGATGACGGTCAAGTA			AGCGCATGAACGTCTACTTCAA	ATCAGCTCGAGGTACTTGGTGAGGTAGTTGAGCTGCC
TGCTAATGCGCACCATAGGCCT GCCGCCCTCGTAGGCGCAGACAGCGTGGAAAATGC CTGGTAACAAGTATGTCCCCCG GTTTCTGACAATCTCGGGCAGGAGCTGCTCGACGAC TGCCGTCCTCGTCGACTTGGAG AGAACCAAGAGAAGACCGCTGATGACGGTCAAGTAA			CGAGGTCAGTCACCAACTGTC	AGCTGTCAGTAAGCGCGTTCTGGCGCGGAATCAGAAA
TGCTAATGCGCACCATAGGCCT GCCGCCCTCGTAGGCGCAGACAGCGTGGAAAATGC CTGGTAACAAGTATGTCCCCCG GTTTCTGACAATCTCGGGCAGGAGCTGCTCGACGAC TGCCGTCCTCGTCGACTTGGAG AGAACCAAGAGAAGACCGCTGATGACGGTCAAGTAA			CGCCCTTCACTGACTACTCCAA	AACGTACGTAGTAAAGAACAACGAGCTTGTCAGTCCA
CTGGTAACAAGTATGTCCCCCG GTTTCTGACAATCTCGGGCAGGAGCTGCTCGACGAA TGCCGTCCTCGTCGACTTGGAG AGAACCAAGAGAAGACCGCTGATGACGGTCAAGTA			TGCTAATGCGCACCATAGGCCT	GCCGCCCTCGTAGGCGCAGACAGCGTGGAAAATGCC
			CTGGTAACAAGTATGTCCCCCG	GTTTCTGACAATCTCGGGCAGGAGCTGCTCGACGAAG
			TGCCGTCCTCGTCGACTTGGAG	AGAACCAAGAGAAGACCGCTGATGACGGTCAAGTAGA
			CCTGGTACCATGGACGCCGTC	AGTTGTGGACCTTGAAGAAGAAGTTGAGCTTGAAAGG
				CTCGCGACCACGCATGAGCTCTCTGCCACCAAAGATG
				ACGATGTAGTAGGCGATGAGCATGGTGGCCGTCTCCT
				TGAAGGTAGCCATGGGGGGTCTTTCCAGGGACGAAGCG
GAAGTCCTGGGGCTTGTAGCCCTTGAG				

DDUADOS		400400000	0000740440000040040770007700770077
PBUAP65	KP965481	AGCACGGCCTTGACGGTGCTG	GGGCTACAAGCCCCAGGACTTCCGCTTCGTCCCT
		GTGTGTACGTTGACTCTCTGCG	GGAAAGACCCCTATGGCTACCTTCAAGGAGACGG
		CATCCTCAATGACAGCTCGCTG	CCACCATGCTCATTGCCTACTACATCATCATCTTTG
		ACTGCCTAACAGCTACAACGGT	GTGGCAGAGAGCTCATGCGTGGTCGCGAGCCTTT
		ACCTCAGATCTCCAGCTGGAG	CAAGCTCAACTTCTTCTTCAAGGTCCACAACTTCTA
		CGCATGAACGTCTACTTCAACG	CCTGACCGTCATCAGCGGTCTCCTCTTGGTTCTGT
		AGGTCAGTCACCAACTGTCCG	TCGTCGAGCAGCTCTTGCCCGAGATTGTCAGAAA
		CCCTTCACTGACTACTCCAATG	CGGCATTTTCCACGCTGTCTGCGCCTACGAGGGC
		CTAATGCGCACCATAGGCCTCT	GGCTGGACCGACAAGCTCGTTGTTCTTTACTACGT
		GGTAACAAGTATGTCCCCCGTG	ACGTTTTTCCAGCTTCTCGCCAGAATGCGCTTACT
		CCGTCCTCGTCGACTTGGAGC	GACAGCTGGCAGCTCAACTACCTCACCAAGTACC
		CTGGTACCATGGACGCCGTCC	TTGAGCTGATTGACACCTGCTTCCTTTCCTCAAGA
		GTGCCGGTCCCTTCGGTCAGC	AGAAGCCTTTGAGTAAGCGCGCAATATTTTCACAA
		TCTTCCGTCCTGACAACTTCGT	TCAATGCTTTAGCTGACTGGTCTCCTAGCTTTCCTC
		CTTCGGTCAGTCCGGTGCTGG	CACACCTACCACCACGGTGCCACTGCCCTTCTCT
		CAACAACTGGGCCAAG	GCTTTACCCAGCTTCTTGGCCACACCGCAGTCTCA
			TGGGTTCCCATCACCCTG
PBUAP67	KP965482	GTACGTCGACCCCCTGCGCAT	GGGCTACAAGCCCCAGGACTTCCGCTTCGTTCCT
1 00/11 0/	11 000402	CCTCACCCGCAATGACAGCTC	GGAAAGACCCCCATGGCTACCTTCAAGGAGACG
		GCTGACTGCCTAACAGCTACAA	GCCACCATGCTCATGCCTACCATCATCATCATCTT
		CGGTACCTCAGATCTCCAGCTG	GGTGGTAGAGAGAGCTCATGCGTGGTCGCGAGCCTT
		GAGCGCATGAACGTCTACTTCA	TCAAGCTCAACTTCTTCTTCAAGGTCCACAACTTCT
		ACGAGGTCGGTCACCAACTGT	ACCTGACCGTCATCAGCGGTCTTCTCTTGGTTCTC
		CGGCCTTTCACTCACTCCTTCA	TTCGTTGAGCAGCTCCTGCCCGAGATTGTCAGAA
		ATGCTAATACGCACCATAGGCC	ACGGCATTTTCCACGCTGTCTGCGCCTACGAGGG
		TCTGGTAACAAGTATGTCCCCC	CGGCTGGACTGACAAGCTCGTTGTTCTTTACTACG
		GTGCCGTCCTCGTCGATTTGGA	TACGTTTATCCAATTCCGCGATAGAATGCGCTTAC
		GCCTGGTACCATGGACGCCGT	TGACAGCTGGCAGCTCAACTACCTCACCAAGTAC
		CCGTGCTGGTCCCTTCGGTCA	CTCGAGCTGATTGACACCTGCTTCCTTTCCTCAA
		GCTCTTCCGTCCCGACAACTTC	GAAGAAGCCTTTGAGTAAGCGCGCATTACCTCCA
		GTCTTCGGTCAGTCCGGTGCTG	CAATCAATGCTTTAGCTGACTGATCTCCCAGCTTT
		GCAACAACTGGGCCAAGGGT	CCTCCACACCTACCACCACGGTGCCACTGCTCTT
		จหาลงกรณ์มหาวิทย	CTCTGCTTCACCCAGCTTCTCGGCCACACCGCAG
			TTTCATGGGTCCCCATCACCCT
PBUAP70	KP965483	GGCAGACCATCTCTGGCGAGC	GGGCTACAAGCCTCAAGACTTCCGCTTCGTCCCC
		ACGGCCTTGACGGTGCTGGTG	GGAAAGACCCCTATGGCTACTTTCAAGGAGACGG
		TGTACGTCGATCCCGCCTGCG	CCACCATGCTCATTGCCTACTACATCATCATCTTTG
		CATCTCACACCCATTGTGACGG	GTGGCAGAGAGTTCATGCGCAGCCGCGAGCCCT
		CTCTCTGACATGCTCGCAGCTA	TCAAGCTCAGCTTCTTCTTCAAGCTCCACAACTTCT
		CAATGGCACCTCTGATCTCCAG	ACTTGACCCTGATCAGTGGTGTTCTCCTGGTTCTG
		CTTGAGCGCATGAACGTCTACT	TTTGTCGAGCAGCTTCTGCCCGAGATTGTCAGAAA
		TCAACGAGGTCAGCCTTTCACA	CGGCATTTTCCACGCCGTCTGCGCCTACGACGGC
		TAGCCTCGACCCTCACACTGTC	GGCTGGACCGACAAGCTCGTTGTTCTCTACTACGT
		GCCCGACTAACGCGCTGCAGG	GAGTGACTCCCAAGTCGCAATGAGATGCGCTTGC
		CATCCGGCAACAAGTATGTTCC	TGACGAGCTCCCAAGTCGCAATGAGATGCGCTTGC
		CCGTGCCGTCCTCGTCGACTTG	
		GAGCCCGGTACCATGGACGCC	GAAGAAGCCCTTGAGTAAGCCCATCCTGTACGCT
		GTCCGTGCCGGTCCCTTCGGC	CTCCGGCGAACCGCAGCAGCTGATTTTGTACCCC
		CAGCTCTTCCGTCCCGACAACT	AGCTTTCCTCCACACCTACCACCACGGCGCTACC
		TTGTCTTCGGTCAGTCCGGTGC	GCTCTCCTCTGCTTCACCCAGCTCCTCGGCCACA
		TGGCAACAACTGGGCCAAGGG	CCTCGGTTTCATGGGTTCCCATCACTCTGAACCTG
		TCACTACACTGAGGGTA	ACCGTCCACGTCGTCATGTACTGGTACTA
	1		

		001110100000000000000000000000000000000	000000000000000000000000000000000000000
PBUAP71	KP965484	GCTITCTGGCAGACCATCTCTG	GCCCCAGGACTTCCGCTTCGTCCCTGGAAAGACC
		GCGAGCACGGCCTTGACGGTG	CCCATGGCTACCTTCAAGGAGACGGCCACCATGC
		CTGGTGTGTACGTCGACTCCCT	TCATCGCCTACTACATCGTCATCTTTGGTGGCAGA
		GCGCATCCTCACCGCAATGAC	GAGCTCATGCGTGGTCGCGAGCCTTTCAAGCTCA
		AGCTCGCTGACTGCCTAACAG	ACTTCTTCTTCAAGGTCCACAACTTCTACTTGACCG
		CTACAACGGTACCTCAGATCTC	TCATCAGCGGTCTTCTCTTGGTTCTCTTCGTCGAG
		CAGCTGGAGCGCATGAACGTC	CAGCTCCTGCCCGAGATTGTCAGAAACGGCATTTT
		TACTTCAACGAGGTCAGTCACT	CCACGCTGTCTGCGCCTACGAGGGCGGCTGGAC
		AGTTATCGGCCCTTCGCTCACT	TGACAAGCTCGTTGTTCTTTACTACGTACGTTTTTC
		ACTTCAATACTAATGCGCACCA	TGATTCCGCGCCAGAACGCGCTTACTGACAGCTG
		TAGGCCTCTGGTAACAAGTATG	GCAGCTCAACTACCTCACCAAGTACCTCGAGCTG
		TCCCCCGTGCTGTCCTCGTCGA	ATTGACACCTGCTTCTTGTTCCTCAAGAAGAAGCC
		CTTGGAGCCTGGTACCATGGA	TTTGAGTAAGCGCGCAATATTTCCACAATCAATGC
		CGCCGTCCGTGCCGGTCCCTT	TTTAGCTGACTGATCTCCTAGCTTTCCTCCACACCT
		CGGTCAGCTCTTCCGTCCCGA	ACCACCACGGTGCCACTGCCCTCCTCTGCTTCAC
		CAACTTCGTCTTCGGCCAGTCC	CCAGCTTCTCGGCCACACCGCAGTCTCATGGGTT
		GGTGCTGGCAACAACTGGGCC	CCCATCACCCTGAACCTGACCGTTCACGTCGTCA
		AAGGGTCACTACACTGAGGGT	TGTACTGGTACTAC
		A	
PBUAP72	KP965485	GAGCACGGCCTTGACGGTGCT	GGGCTACAAGCCCCAGGACTTCCGCTTCGTCCCC
		GGTGTGTACGTCGACAGCGCT	GGAAAGACGCCTATGGCTACTTTCAAGGAGACGG
		AGCGCATCCCATGCCTCTCGTG	CCACCATGCTCATTGCCTACTACATCATCATTTTG
		ACGCCTCTCTGACATGCTCGCA	GCGGCAGAGAGTTTATGCGTGGCCGCGAGCCCT
		GCTACAATGGCACCTCGGACC	TCAAGCTCAGCTTCTTCTTCAAGCTCCACAACTTCT
		TCCAGCTTGAGCGCATGAACGT	ACCTGACTCTGATCAGCGGCATTCTCCTGGTTCTG
		CTACTTCAACGAGGTGAGCCCT	TTCGTTGAGCAGCTTCTGCCCGAAATTGTCAGAAA
		TCACACCACCTCCGCTGCCCTC	TGGCATTTTCCACGCAGTCTGCGCCTACGAGGGT
		CCATGCATCGGCTAACGCGCT	GGCTGGACCGACAAGCTTGTTGTTCTCTACTACGT
		GCAGGCCTCCGGCAACAAGTA	GAGTGTCTCGAGTCGCGACAAGGTGCCCTTACTG
		TGTTCCCCGTGCCGTCCTCGTC	ACAAGATCGCAGCTCAACTACCTGACCAAGTACC
		GACTTGGAGCCCGGTACCATG	TCGAGCTCATTGACACCTGCTTCCTTTCCTCAAG
		GACGCCGTCCGTGCCGGTCCC	AAGAAGCCCTTGAGTAAGCCCACCTGACGGAACC
		TTCGGCCAGCTCTTCCGTCCCG	GTCTACCAGTCGCATTAGCTGATCGCTCCCCTAG
		ACAACTTCGTCTTCGGTCAGTC	CCTTCCTCCACACCTACCACCACGGCGCTACCGC
		CGGTGCTGGCAACAACTGGGC	TCTCCTCTGCTTCACTCAGCTCCTCGGTCACACTT
		CAAGGGTCACTACACTGAGGG	CCGTCTCTTGGGTTCCCATCACCCTGAACCTGAC
		ТА	CGTCCACGTCGTCATGT
PBUAP73	KP965486	TCTGGCGAGCACGGCCTTGACGG	GGGCTACAAGCCCCAGGACTTCCGCTTCGTTCCTGGA
		CGCTGGTGTGTACGTCGACCCCC	AAGACCCCCATGGCTACCTTCAAGGAGACTGCCACCA
		TGCGCATCCTCACCCGCAATGACA	TGCTCATTGCCTACTACATCATCATCTTCGGTGGCAGA
		GCTCGCTGACTGCCTAACAGCTAC	GAGCTCATGCGTGGTCGCGAGCCTTTCAAGCTCAACTT
		AACGGTACCTCAGATCTCCAGCTG	CTTCTTCAAGGTCCACAACTTCTACCTGACCGTCATCA
		GAGCGCATGAACGTCTACTTCAAC	GCGGTCTTCTCTTGGTTCTCTTCGTTGAGCAGCTCCTG
		GAGGTCGGTCACCAACTGTCGGC	CCCGAGATTGTCAGAAACGGCATTTTCCACGCTGTCTG
		CTTTCACTCACTCCTTCAATGCTAA	CGCCTACGAGGGCGGCTGGACTGACAAGCTCGTTGTT
		TGCGCACCATAGGCCTCTGGTAAC	CTTTACTACGTACGTTTATCCAATTCCGCGACAGAATGC
		AAGTATGTCCCCCGTGCCGTCCTC	GCTTACTGACAGCTGGCAGCTCAACTACCTCACCAAGT
		GTCGATTTGGAGCCTGGTACCATG	ACCTCGAGCTGATTGACACCTGCTTCCTTTCCTCAAG
		GACGCCGTCCGTGCTGGTCCCTT	AAGAAGCCTTTGAGTAAGCGCGCATTACCTCCACAATC
		CGGTCAGCTCTTCCGTCCCGACAA	AATGCTTTAGCCGACTGATCTCCCAGCTTTCCTCCACA
		CTTCGTCTTCGGTCAGTCCGGTGC	CCTACCACCGGTGCCACTGCTCTTCTCTGCTTCACC
		TGGCAACAACTGGGCCAAGGGTC	CAGCTTCTCGGCCACACCGCAGTCTCATGGGTCCCTA
		ACTACACTGAGGGT	TCACCCTGAACTTGACCGTCCACGTCGTCATGTA
	1		

	KD005407		
PBUAP75	KP965487	GGACTGACCGAAGACGAAGTT	GCTCAAGGGCTACAAGCCCCAGGACTTCCGCTTC
		GTCGGGACGGAAGAGCTGACC	GTCCCTGGAAAGACCCCCCATGGCTACCTTCAAGG
		GAAGGGACCAGCACGGACGG	AGACGGCCACCATGCTCATCGCCTACTACATCGT
		CGTCCATGGTACCAGGCTCCA	CATCTITGGTGGCAGAGAGCTCATGCGTGGTCGC
		AGTCGACGAGGACGGCACGG	GAGCCTTTCAAGCTCAACTTCTTCTTCAAGGTCCA
		GGGACATACTTGTTACCAGAGG	CAACTTCTACTTGACCGTCATCAGCGGTCTTCTCTT
		CCTATGGTGCGCATTAGCATTG	GGTTCTCTTCGTCGAGCAGCTCCTGCCCGAGATT
		GAGTAGTCAGTGAAGGGCGGA	GTCAGAAACGGCATTTTCCACGCTGTCTGCGCCT
		CAGTTGGTGACTGACCTCGTTG	ACGAGGGCGGCTGGACTGACAAGCTCGTTGTTCT
		AAGTAGACGTTCATGCGCTCCA	TTACTACGTACGTTTTTCTGATTCCGCGCCAGAAC
		GCTGGAGATCTGAGGTACCGTT	GCGCTTACTGACAGCTGGCAGCTCAACTACCTCA
		GTAGCTGTTAGGCAGTCAGCG	CCAAGTACCTCGAGCTGATTGACACCTGCTTCTTG
		AGCTGTCATTGCGGGTGAGAAT	TTCCTCAAGAAGAAGCCTTTGAGTAAGCGCGCAAT
		GCGCAGGGAGTCAACGTACAC	ATTTCCACAATCAATGCTTTAGCTGACTGATCTCCT
		ACCAGCACCGTCAAGGCCGTG	AGCTTTCCTCCACACCTACCACCACGGTGCCACT
		CTCGCCAGAGATGGTCTGCCA	GCCCTCCTCTGCTTCACCCAGCTTCTCGGCCACA
		GAAAGCAGCA	CCGCAGTCTCATGGGTTCCCATCACCCTGAACCT
		S. 11 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	GACCGTTCACGTCGTCATGTACT
PBUAP76	KP965488	AGACCATCTCTGGCGAGCACG	CTCGATCAAGGGCTACAAGCCCCAGGACTTCCGC
		GCCTTGACGGTGCTGGTGTGTA	TTCGTCCCTGGAAAGACTCCCATGGCTACCTTCAA
		CGTCGATTCCCTGCGCATCCCC	GGAGACGGCCACCATGCTCATTGCCTACTACATC
		ATCCGTCGTGATAGCTCGCTGA	ATCATCTTTGGTGGCAGAGAGTTCATGCGTGGTCG
		CTGCCTGACAGCTACAACGGTA	CGAGCCTTTCAAGCTCAACTTCTTTTCAAGGTCC
		CCTCAGATCTCCAGCTGGAGC	ACAACTTCTACCTGACCGTCATCAGTGGTCTTCTC
		GCATGAACGTCTACTTCAACGA	TTGGTTCTCTTCGTTGAGCAGCTCCTGCCCGAGAT
		GGTCAGTGGCCAACCGTGGGC	TGTCAGAAACGGTATCTTCCACGCTGTCTGCGCCT
		CCTTCCTTCACGACTTCATTGCT	ACGAGGGTGGCTGGACCGACAAGCTTGTTGTTCT
		AATGACTATAGGCCTCTGGTAA	TTACTACGTACGTTTTGTCGTATGGGCACCAGAAT
		CAAGTATGTCCCCCGCGCCGT	GCGCTTACTGACAGCTGGCAGCTCAACTACCTTA
		CCTCGTCGACTTGGAGCCTGGT	CCAAGTACCTCGAGCTGATTGACACCTGCTTCCTT
		ACCATGGACGCCGTCCGTGCC	TTCCTCAAGAAGAAGCCTTTGAGTAAGCGCGCAAT
		GGCCCCTTCGGTCAGCTCTTCC	ATCTTCACACCCGATACACCAACTAATTGGGCTCC
		GTCCCGACAACTTCGTCTTCGG	TAGCTTTCCTCCACACCTACCACCACGGTGCCACT
		CCAGTCCGGTGCTGGCAACAA	GCCCTTCTCTGCTTCACCCAGCTTCTCGGCCACA
		CTGGGCCAAGGGTCACTACAC	CCGCAGTCTCATGGGTTCCCATCACCCTCAATTTG
		TGAGGGTA	ACCGTTCACGTTGTCATGTACTGGTA
PBUAP77	KP965489	CTGGCAGACCATCTCTGGCGAGC	GTACATGACGACGTGGACGGTCAGGTTCAGGGTGATG
		ACGGCCTTGACGGTGCTGGTGTG	GGAACCCAAGAGACGGAAGTGTGACCGAGGAGCTGA
		TACGTCGACAGCGCTAGCGCATC	GTGAAGCAGAGGAGAGCGGTAGCGCCGTGGTGGTAG
		CCATGCCTCTCGTGACGCCTCTCT	GTGTGGAGGAAGGCTAGGGGAGCGATCAGCTAATGC
		GACATGCTCGCAGCTACAATGGC	GACTGGTAGACGGTTCCGTCAGGTGGGCTTACTCAAG
		ACCTCGGACCTCCAGCTTGAGCG	GGCTTCTTCTTGAGGAAAAGGAAGCAGGTGTCAATGA
		CATGAACGTCTACTTCAACGAGGT	GCTCGAGGTACTTGGTCAGGTAGTTGAGCTGCGATCTT
		GAGCCCTTCACACCACCTCCGCT	GTCAGTAAGGGCACCTTGTCGCGACTCGAGACACTCA
		GCCCTCCCATGCATCGGCTAACG	CGTAGTAGGGGACCACCTIGTCGCGACTCGAGACACCACCC
		CGCTGCAGGCCTCCGGCAACAAG	CTCGTAGGCGCAGACTGCGTGGAAAATGCCATTCTG
		TATGTTCCCCGTGCCGTCCTCGTC	ACAATTTCGGGCAGAAGCTGCTCCAACGAACAGAACCA
		GACTTGGAGCCCGGTACCATGGA	GGAGAATGCCGCTGATCAGAGTCAGGTAGAAGTTGTG
		CGCCGTCCGTGCCGGTCCCTTCG	
			GAGCTTGAAGAAGAAGCTGAGCTTGAAGGGCTCGCG
		GCCAAGCTTCTTCCGTCCCGACAA	GCCACGCATAAACTCTCTGCCGCCAAAAATGATGATGT
		CTTCGTCTTCGGTCAGTCCGGTGC	AGTAGGCAATGAGCATGGTGGCCGTCTCCTTGAAAGT
		TGGCAACAACTGGGCCAA	AGCCATAGGCGTCTTTCCGGGGACGAAGCGGAAGTC
"T., ".,		<u> </u>	CTGGGGCTTGTAG

 $*^{\mathrm{T}}$ indicates type specimen.

VITA

Miss Benjawan Yanwisetpakdee was born on April 19th1976 in Hatyai, Songkhla, Thailand. She received the Batchelor of Science degree with a major in Biology from Prince of Songkhla University in March 1999. She continued to study for the Master degree of Science in Botany at Department of Botany, Faculty of Science, Chulalongkorn University and graduated in May 2003. After that, she worked as a lecturer at Program of biology and applied Biology, Faculty of Science and Technology, Songkhla Rajabhat University until 2009. Since June 2009, she has studied the degree Doctor of Philosophy program in Botany at Faculty of Science, Chulalongkorn University. While studying she received the best oral presentation award at the 19th Biological Graduate Congress in 2015, the best poster award from the 8th Korea-Asean Joint Symposium 2014, and the bronze medal award from oral presentation at the 18th Biological Graduate Congress in 2014.

> จุฬาลงกรณ์มหาวิทยาลัย Chulalongkorn University



จุฬาลงกรณ์มหาวิทยาลัย Chulalongkorn University