

การพัฒนาวิธีการตรวจจุลินทรีย์ก่อโรค *VIBRIO PARAHAEMOLYTICUS*

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DEVELOPMENT OF DETERMINATION METHODS FOR  
FOOD PATHOGEN *VIBRIO PARAHAEMOLYTICUS* BY DETECTING  
QUORUM SENSING SIGNALS

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งานวิจัยนี้มีวัตถุประสงค์เพื่อพัฒนาวิธีการตรวจสอบ *V.parahaemolyticus* โดยอาศัยคุณสมบัติในการสร้างสัญญาณ AHL การทดลองเริ่มจากการพัฒนาวิธีการตรวจสอบสัญญาณ AHL ด้วยวิธี colorimetry และประเมินประสิทธิภาพของวิธีตามหลักการพิสูจน์ความถูกต้อง (method validation) ของ USFDA guideline เมื่อประเมินวิธีที่พัฒนาขึ้น ได้แก่ Procedure A ซึ่งเป็นวิธีการตรวจสอบที่ประกอบด้วยขั้นตอนการสกัด AHL และการวัดค่าการดูดกลืนแสงของสารประกอบสีของ AHL ด้วย spectrophotometer ( $\lambda = 520$  nm) และ Procedure B คือวิธีการตรวจสอบ AHL โดยไม่ผ่านขั้นตอนการสกัด ผลการประเมินพบว่าประสิทธิภาพของทั้งสองวิธีไม่มีความแตกต่างกันอย่างมีนัยสำคัญ และจากผลการประมวลผลทางสถิติของทุกพารามิเตอร์จากการพิสูจน์ความถูกต้อง พบว่า ทั้งสองวิธีสามารถใช้ในการตรวจสอบ AHL ได้อย่างแม่นยำ เทียบตรง และมีความจำเพาะต่อ AHL และจากการประเมินความไว พบว่าทั้งสองวิธีสามารถตรวจสอบสัญญาณ AHL ในอาหารเลี้ยงเชื้อ NB ความเข้มข้นต่ำสุด  $30 \mu\text{M}$  และสามารถใช้ตรวจวัดสัญญาณ AHL ที่สร้างจาก *V.parahaemolyticus* ใน NB ที่มีปริมาณเซลล์ต่ำสุดประมาณ  $6 \log\text{CFU/ml}$  อย่างไรก็ตามในการศึกษาขั้นต่อไปได้เลือกใช้ Procedure B ในการตรวจสอบสัญญาณ AHL เนื่องจากเป็นวิธีที่สะดวกและรวดเร็วมากกว่า

เมื่อศึกษาอิทธิพลของปัจจัยภายในและปัจจัยภายนอกต่อคุณสมบัติการสร้างสัญญาณ AHL ของ *V.parahaemolyticus* ทั้งในเชิงปริมาณและเชิงคุณภาพด้วยวิธี colorimetry (Procedure B) และ HPLC ตามลำดับ ผลการทดลองชี้บ่งว่าสายพันธุ์และช่วงการเจริญของแบคทีเรีย รวมถึงภาวะการเลี้ยงได้แก่ชนิดอาหารเลี้ยงเชื้อและอุณหภูมิไม่มีผลต่อการสร้างสัญญาณ AHL ทั้งในเชิงคุณภาพและปริมาณ แต่พบว่ามีปริมาณเกลือร้อยละ 8 ในอาหารเลี้ยงเชื้อมีผลในการเหนี่ยวนำการสร้างสัญญาณ AHL ของ *V.parahaemolyticus* ให้เพิ่มขึ้นในปริมาณที่มากกว่าภาวะการเลี้ยงอื่นๆ ถึงร้อยละ 28 แต่ปัจจัยนี้ไม่ส่งผลต่อการสร้างสัญญาณ AHL ในเชิงคุณภาพ ทำให้สามารถใช้ AHL ชนิด 3-hydroxy-C4-HSL เป็นตัวชี้บ่งลักษณะเฉพาะของ *V.parahaemolyticus* ได้

การทดลองขั้นตอนสุดท้าย คือการประยุกต์ใช้ Procedure B ร่วมกับการใช้ peptone water ที่มีเกลือร้อยละ 8 เป็น selective enrichment media สำหรับใช้ในการเลี้ยงและเหนี่ยวนำให้สร้าง AHL ในการตรวจสอบ *V.parahaemolyticus* และพบว่าวิธีนี้สามารถใช้ในการตรวจสอบ *V.parahaemolyticus* ที่มีปริมาณเซลล์เริ่มต้น  $2 \log\text{CFU/ml}$  ทั้งในภาวะที่มี *V.parahaemolyticus* เพียงชนิดเดียวและอยู่ร่วมกับแบคทีเรียชนิดอื่นได้ภายใน 20 ชั่วโมง ซึ่งถือเป็นวิธีทางเลือกแนวทางใหม่สำหรับการตรวจสอบ *V.parahaemolyticus* อย่างง่ายและรวดเร็ว

ภาควิชา เทคโนโลยีทางอาหาร

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This study aimed to develop the methods for *V.parahaemolyticus* determination based on its AHL production property. The first two experiments were the development of methods for AHL determination by colorimetry method, and evaluation of the method efficiency by validation following USFDA guideline. The methods including AHL extraction and determination of AHL coloring complex by spectrophotometry ( $\lambda_{max} = 520$ ), called Procedure A, and determination of AHL existing in broth media without performing extraction step called Procedure B, were developed for AHL determination. The efficiency of both methods were not significantly different. Based on statistical analysis of validation parameters, both methods were accurate, precise and selective for AHL determination. Method sensitivity evaluation showed that, minimal AHL concentration could be detected by either procedure was 30  $\mu$ M. These methods could detect AHL produced from *V.parahaemolyticus* cultured in NB containing minimal population approximately 6 logCFU/ml. However, the Procedure B was selected for the further works because it was more rapid and less laborious.

The influences of intrinsic and extrinsic factors on AHL production properties were investigated using colorimetry (Procedure B) and HPLC analysis. It was found that strains, cultivation times and cultivation conditions could not influence on both quality and quantity of AHL produced from *V.parahaemolyticus*. Interestingly, 8% NaCl in cultural media could induce *V.parahaemolyticus* to produce 28 folds larger amount of AHL than the other factors, whereas it had not influence on AHL quality. Therefore, AHL which was 3-hydroxy-C4-HSL could be one of the *V.parahaemolyticus* identity.

Finally, Procedure B and 8% NaCl peptone water as an enrichment selective media for growing *V.parahaemolyticus* and inducing its AHL production were investigated. It was found that this methodology could determine *V.parahaemolyticus* starting at 2 logCFU/ml within 20 hours both in single and co-cultured with other bacteria. Therefore, this new strategy could be an alternative for a simple and rapid identification of *V.parahaemolyticus*.

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