CHAPTER I

INTRODUCTION

Aureobasidium is a ubiquitous yeast-like fungus in the class Ascomycetes, order Dothideales (Deshpande et al., 1992). This black yeast that produces a black melanin pigment, also make a range of color variants (Leathers et al., 1988). This mold colonizes a range of habitats, especially surfaces: paints and plastics, soil particles and the phyllosphere. Several reports showed widespread incidence of this fungus (Cooke, 1959; Deshpande et al., 1992, and Punnapayak et al., 2003). It occurs around the world, many reports being from temperate zones in the Americas and Europe. In contrast reports from tropical spheres, such as Thailand, are sparse, though the isolation of distinctive color variants has promoted further study of tropical strains (Punnapayak et al., 2003).

The genus *Aureobasidium* is comprised of fourteen (14) species greatest focus being on *A. pullulans* due to its usefulness in many biotechnological applications including industrial enzymes and production of a biopolymer, pullulan (Deshpande et al., 1992). Pullulan, an exopolysaccharide (EPS), is a linear homopolymer composed of maltotriose subunits interconnected with α-1,6 glucosidic linkage (Leathers, 2002, 2003). This polymer is exploited in the food, cosmetic, manufacturing, and plywood industries. *A. pullulans* produces a further polysaccharide, aubasidan, a β-1,3 linked glucan. Aubasidan is produced from *A. pullulans var. aubasidani* while pullulan is produced from *A. pullulans var. pullulans*.

Pullulan based on maltotriose subunits interconnected with α-1,6 glucosidic linkage, somewhat resembles amylose, and not surprisingly can be degraded by

amylolytic enzymes (Leathers, 2003). Interestingly under certain fermentation conditions *A. pullulans* produces amylase. These amylases have been believed to reduce the molecular weight of pullulan (Leathers, 1993).

As *A. pullulans* exopolysaccharides are of potential application and also chemically interesting, further isolates were collected. Focus was on Thai tropical isolates, ecological niches including plant leaves, and bathroom walls and painted surfaces, using selective culture media. These yeasts were characterized based on classical morphological characteristics, nutritional and physiological criteria, molecular biological techniques such as ribosomal DNA Internal Transcribed Spacer (ITS) sequences, and EPS structure. EPS production was optimized in liquid culture. The EPSs were chemically characterized: carbohydrate content (anthrone), sensitivity to pullulanase, infrared and ^{13}C-Nuclear Magnetic Resonance (NMR) spectroscopy, plus molecular weight and viscosity assessments. The detection of amylase and pullulanase activities during the EPS production was monitored.

From this background, my thesis is that a range of distinctive strains of *A. pullulans* exist especially in sites that have not received major study (such as Thailand), which could yield novel and useful pullulans, and that the quality of these polysaccharides can be optimized by optimizing fermentation conditions. In this latter regard, high-quality and high molecular weight EPS could perhaps be obtained through use of amylase inhibitors during fermentation, or through selection of high yielding mutants.

Objectives of this study

1. To obtain the isolates of *A. pullulans* from different habitats in Thailand
2. To characterize exopolysaccharides of these isolates, and to optimize EPS yields and quality.