ลักษณะสมบัติของยีสต์ดำ *Aureobasidium* spp. ซึ่งคัดแยกจากบริเวณชายฝั่งทะเลไทย

นางสาวเบญจวรรณ ยันต์วิเศษภักดี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาพฤกษศาสตร์ ภาควิชาพฤกษศาสตร์
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2557

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

Miss Benjawan Yanwisetpakdee

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

Thesis Title	CHARACTERIZATION OF BLACK YEAST *Aureobasidium* spp. ISOLATED FROM THAI COASTAL AREA
By	Miss Benjawan Yanwisetpakdee
Field of Study	Botany
Thesis Advisor	Associate Professor Hunsa Punnapayak, Ph.D.
Thesis Co-Advisor	Assistant Professor Pongtharin Lotrakul, 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.)

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

Aureobasidium spp. เป็นจุลินทรีย์ที่มีคุณสมบัติทนทานต่อภาวะรุนแรง มีความสำคัญที่ใช้ในเชิงเทคโนโลยีชีวภาพ ราในกลุ่มนี้ผลิตผลิตภัณฑ์ที่มีมูลค่า ได้แก่ พอลิแซ็กคาไรด์; EPS (พูลลูแลนและเบต้ากลูแคน) เอนไซม์ไซแลนเนส และสารต้านเชื้อรา ราในกลุ่มนี้มีความหลากหลายในสายพันธุ์ และถูกจัดจำแนกเพิ่มเติมออกมาอีก 4 สปีชีส์ ได้แก่ *A. pullulans, A. melanogenum, A. namibiae* และ *A. subglaciale* ในการศึกษานี้ได้คัดแยกรา *Aureobasidium* spp.จากบริเวณชายฝั่งทะเล จากจำนวน 66 สายพันธุ์ที่ใช้ในการศึกษา ประกอบด้วยสายพันธุ์ใหม่ 54 สายพันธุ์ และ 12 สายพันธุ์เปรียบเทียบ สายพันธุ์ใหม่ถูกจัดแยกประเภทโดยอาศัยการวิเคราะห์ลำดับเบสหลายตำแหน่ง จำนวนทั้งสิ้น 3 ตำแหน่ง ได้แก่ ITS *TUB* และ *ELO* บนพื้นฐานของการวิเคราะห์ระบบพันธุ์ สายพันธุ์เหล่านี้ถูกจำแนกออกเป็น 12 กลุ่ม แสดงให้ถึงความหลากหลายของราสายพันธุ์ที่คัดแยกจากบริเวณชายฝั่ง อย่างไรก็ตาม พบราเพียง 2 ชนิด โดยพบ *A. melanogenum* มากที่สุดและพบ *A. thailandense* เพียงเล็กน้อย และพบสายพันธุ์ color-variant ที่มีความจำเพาะกับแหล่งอาศัยในเขตร้อนหรือกึ่งเขตร้อน โดยราสายพันธุ์นี้ถูกจำแนกอยู่ในกลุ่มเดียวกับ *A. melanogenum* ดังนั้นลักษณะทางสัณฐานวิทยา การผลิต EPS และแอคติวิตีของเอนไซม์ไซแลนเนสจึงถูกนำมาประเมินเพื่อบ่งชี้ลักษณะพิเศษของสายพันธุ์ในแต่ละกลุ่มเพื่อเปรียบเทียบกับสายพันธุ์ที่คัดแยกได้จากบนบก ผลการศึกษาแสดงความแตกต่างของสีอาหารเลี้ยงเชื้อ ชนิด EPS และราบางกลุ่มมีการผลิต EPS และ/หรือแอคติวิตีของเอนไซม์ไซแลนเนสในระดับสูง นอกจากนี้ ฤทธิ์ต้านเชื้อรา และความเครียดจากสิ่งเร้า ได้แก่ การทนเค็ม การทนแรงดันออสโมติก การทนความร้อน และการทนความเป็นกรดด่าง ถูกนำมาใช้ทดสอบเพื่อค้นหาสายพันธุ์ที่เป็นประโยชน์ หรือ ทนทานต่อภาวะรุนแรงเพื่อนำไปประยุกต์ใช้ในเชิงเทคโนโลยีชีวภาพ ผลการศึกษาแสดงให้เห็นว่าความสามารถดังกล่าวขึ้นกับราแต่ละสายพันธุ์ เพื่อศึกษาความสัมพันธ์ระหว่างการทนเค็ม การทนแรงดันออสโมติก กับการผลิต EPS จึงคัดเลือกราจำนวน 3 สายพันธุ์มาศึกษา พบว่ารา *A. melanogenum* ที่ทนเค็มจะทนแรงดันออสโมติกได้ อย่างมีนัยสำคัญ แต่ความสัมพันธ์ดังกล่าวไม่เกี่ยวข้องกับการผลิต EPS นอกจากนี้ เพื่อศึกษาศักยภาพของเอนไซม์ไซแลนเนสในเชิงเทคโนโลยีชีวภาพ จึงคัดเลือกราเพื่อผลิตเอนไซม์ไซแลนเนสและนำไปผลิตไซโลโอลิโกแซ็กคาไรด์ (XOS) โดยสกัดไซแลนจากธูปฤาษี (*Typha angustifolia* L.) เพื่อใช้เป็นแหล่งคาร์บอน พบว่าผลิตภัณฑ์หลักที่ได้จากการย่อยด้วยเอนไซม์ไซแลนเนสเป็นไซโลไบโอสที่มีไซโลสปะปน โดย XOS ที่ผลิตได้มีฤทธิ์ต้านอนุมูลอิสระเมื่อทดสอบด้วยวิธี 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 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used for evaluation.

Department:	Botany	Student's Signature	.....
Field of Study:	Botany	Advisor's Signature	.....
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

CHAPTER I
INTRODUCTION

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* was redefined and four varieties were recognized including var. *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

Aureobasidium spp., coastal, exopolysaccharide, xylanase

1.4 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 37ºC while the other three species can only grow to 35ºC. *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 glacial, subglacial environments, and sea water. It showed unique psychrotolerance that actively 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).

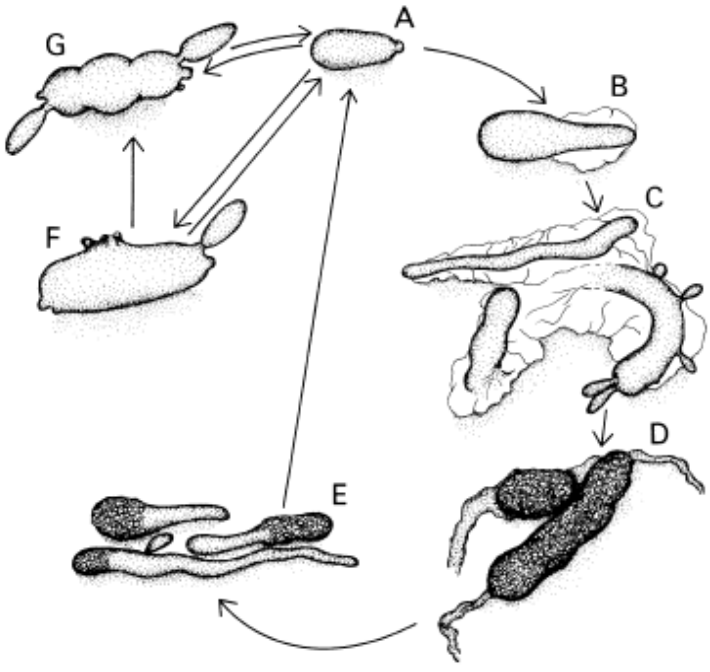


Figure 2.1 Diagram of polymorphic forms of *A. pullulans*. The development stages are as follow (A) blastospore, (B) germinating blastospore, (C) initial hyphae, (D) chlamydospores, (E) germinating chlamydospores, (F) swollen cell, (G) septate swollen cell (Pechak and Crang, 1977).

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-D- and 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-1α, 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 cell-surface 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).

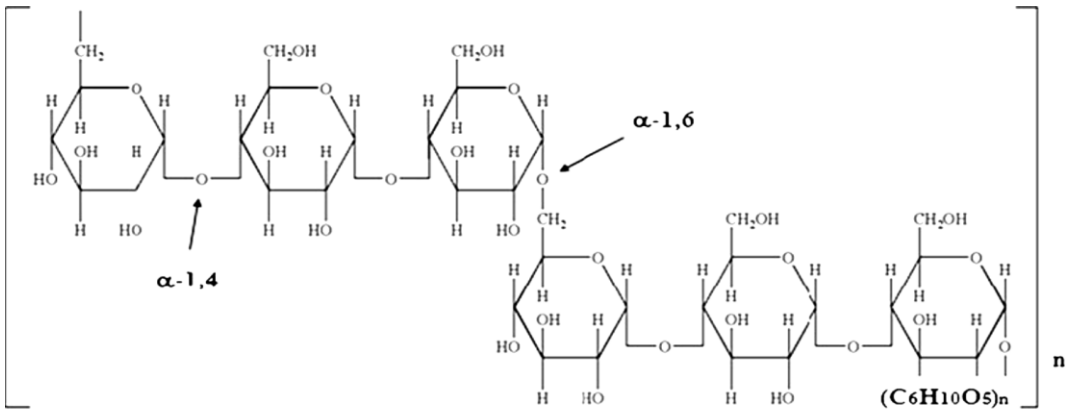


Figure 2.2 Structure of pullulan (Cheng *et al*., 2011).

The mechanism of pullulan biosynthesis is still little understood. As a result, present studies of pullulan concerning the basically findings of a pullulan-producing 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).

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).

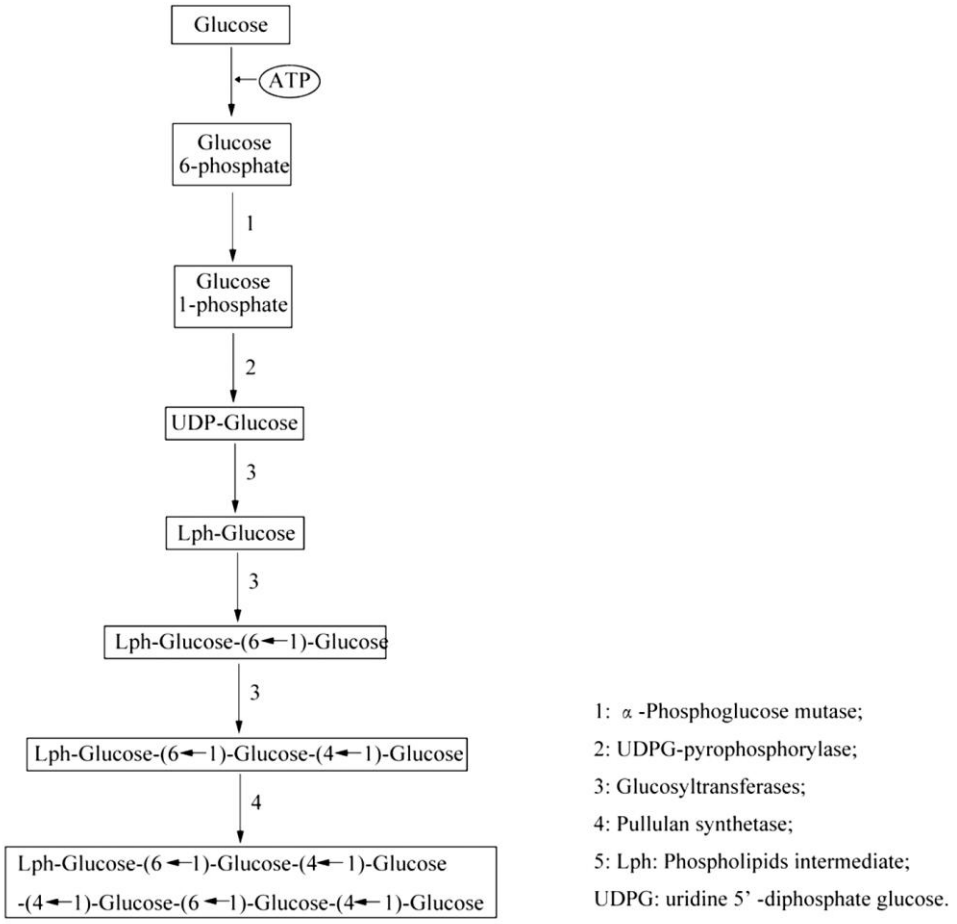


Figure 2.3 The proposed pathway of pullulan biosynthesis from glucose (Li *et al.*, 2015).

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* (Zalar *et al*., 2008).

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.

Xylanase

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-1,4-glycosidic 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 Fe^{3+} 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).

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 4ºC. 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±2°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 region	Primer [a]	Sequence 5′- 3′	Cycling reaction	Approximately PCR product (bp)	Source
ITS	ITS5 (F) ITS4 (R)	GGAAGTAAAAGTCGTAACAAGG TCCTCCGCTTATTGATATGC	95 ° C, 20 s 56 ° C, 30 s 72 ° C, 1 min	550	White *et al.* (1990) White *et al.* (1990)
TUB	Bt2a (F) Bt2b (R)	GGTAACCAAATCGGTGCTGCTTTC ACCCTCAGTGTAGTGACCCTTGGC	95 ° C, 30 s 58 ° C, 1 min 72 ° C, 1 min	450	Glass and Donaldson (1995) Glass and Donaldson (1995)
ELO	ELO2-F (F) ELO2-R (R)	CACTCTTGACCGTCCCTTCGG GCGGTGATGTACTTCTTCCACCAG	94 ° C, 15 s 58 ° C, 15 s 72 ° C, 45 s 94 ° C, 15 s 56 ° C, 15 s 72 ° C, 45 s	700	Zalar *et al.* (2008) Zalar *et al.* (2008)

[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±2°C with 150-rpm agitation. Cell density was adjusted to $2.5x10^7$ cells/ml before being transferred at 1 % (v/v) to 100 ml of production medium (PM) containing (all w/v) sucrose (5%), $(NH_4)_2SO_4$ (0.06%) , peptone (0.06%), K_2HPO_4 (0.5%), $MgSO_4 \cdot 7H_2O$ (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±2°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±2°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°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) $KMnO_4$ 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.

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±2°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±2°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 μmol 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 Xylan preparation

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 HCl. 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.

3.3.5 Statistical analysis

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.

Table 4.1 Geographic coordinates of the sample collection sites.

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

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.

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

Isolate	Accession 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.) Dryand.	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	*Avicenna officinalis* L.	Bangkok (August 2010)

Table 4.2 (continued)

Isolate	Accession number		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	*Tamarindus indica* L.	Chonburi (December 2010)
AP34	PBUAP34	BCU032	*Tamarindus indica* L.	Chonburi (December 2010)
AP35	PBUAP35	BCU033	*Tamarindus 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		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)

Isolate	Accession 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 × 15–35 μm). 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 μm, two cells slightly constricted at septum, 25–35 × 20–25 μm (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 (Figure4.1f). 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).

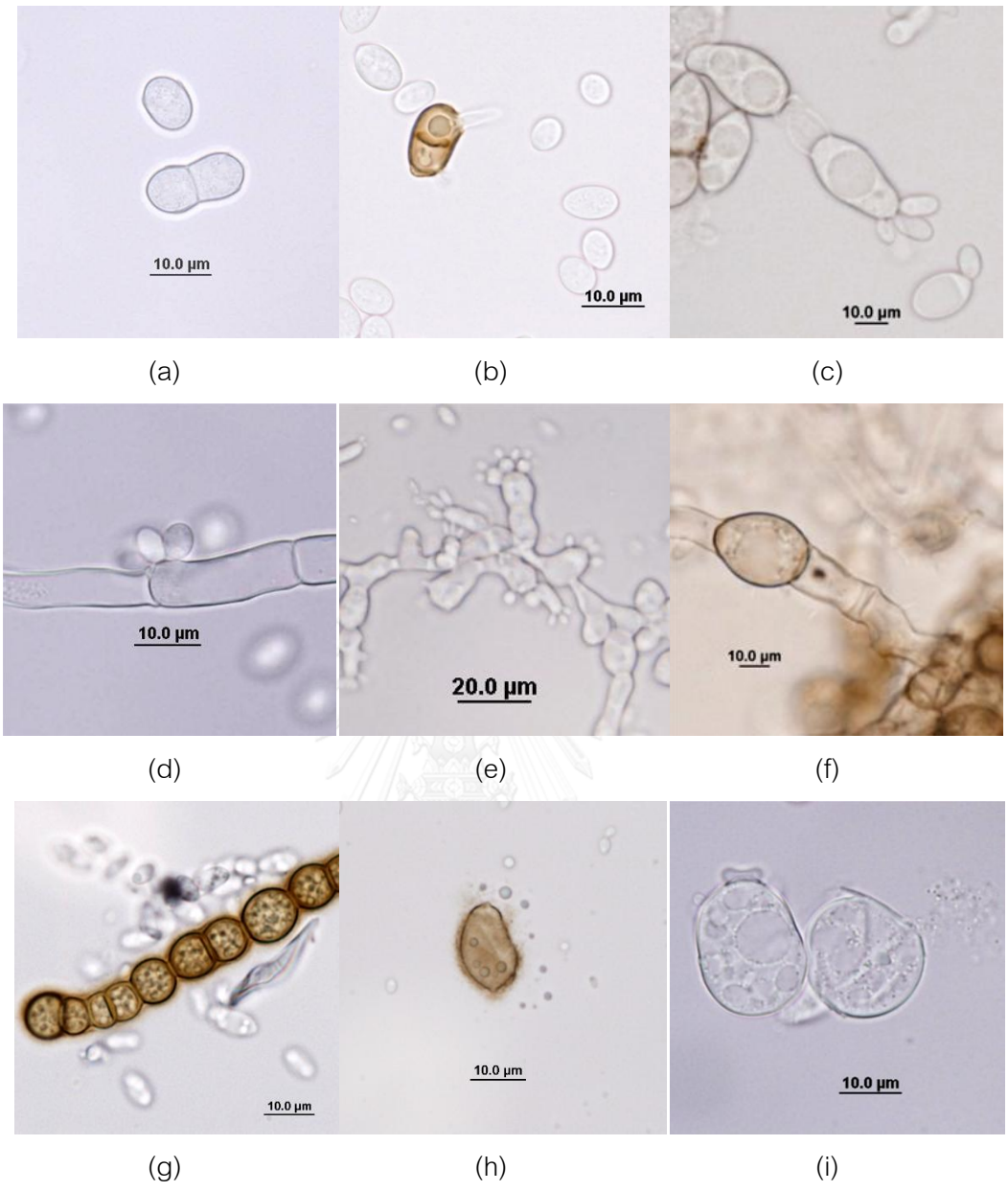


Figure 4.1 Polymorphic forms of *Aureobasidium* spp. grown in YM broth at 30 °C with agitation at 150 rpm. (a) blastospores (b) germinating chlamydospore with endoconidia (c) polar budding and budding conidia (d) intercalarychlamydospore (e) pseudohyphae with adhearing conidia (f) true hyphae with intercalary 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	PBUAP5	PBUAP5.1	PBUAP7.1	PBUAP9	PBUAP13	PBUAP14	PBUAP16	PBUAP17
L-Arabinose	+	+	+	+	+	+	+	+	+	+
D-Cellobiose	+	+	+	+	+	+	+	+	+	+
α-Cellulose	-	-	-	-	-	-	-	-	w	w
D-Fructose	+	+	+	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	+	+	+	w	w
D-Glucose	+	+	+	+	+	+	+	+	+	+
D-Glucosamine	+	+	-	-	-	+	+	+	+	+
Glycerol	+	+	+	w	w	w	+	+	+	+
β-Lactose	+	+	+	+	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+	+	+
Methyl-α-D-glucoside	+	+	+	+	+	+	+	w	w	w
L-Sorbose	w	+	w	w	w	+	+	+	-	-
D-Sucrose	+	+	+	+	+	+	+	+	+	+
D(+)Trehalose-2hydrate	+	+	+	+	+	+	+	+	w	-
D-Xylose	+	+	+	+	+	+	+	+	w	w
Xylitol	+	+	+	+	+	+	+	+	+	+

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

Table 4.3 (continued)

Carbon source	PBUAP20	PBUAP22	PBUAP23	PBUAP24	PBUAP25	PBUAP26	PBUAP27	PBUAP29	PBUAP30	PBUAP31	PBUAP32
L-Arabinose	+	+	+	+	+	+	+	+	+	+	+
D-Cellobiose	+	+	+	+	+	+	+	+	+	+	+
α-Cellulose	-	-	-	-	-	-	-	-	-	-	-
D-Fructose	+	+	+	+	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	+	+	+	+	+	+
D-Glucose	+	+	+	+	+	+	+	+	+	+	+
D-Glucosamine	+	+	+	+	+	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+	+	+	+	+	+
β-Lactose	+	+	+	+	+	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+	+	+	+
Methyl-α-D-glucoside	+	+	+	+	+	+	+	+	+	+	+
L-Sorbose	+	+	+	+	+	+	+	+	+	+	+
D-Sucrose	+	+	+	+	+	+	+	+	+	+	+
D(+)Trehalose-2hydrate	+	+	+	+	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+	+	+	+	+	+
Xylitol	+	+	+	+	+	+	+	+	+	+	+

+ = assimilation, w = weak, - = non assimilation

Table 4.3 (continued)

Carbon source	PBUAP33	PBUAP34	PBUAP35	PBUAP36	PBUAP37	PBUAP38	PBUAP39	PBUAP40	PBUAP41	PBUAP42	PBUAP43
L-Arabinose	+	+	+	+	+	+	+	+	+	+	+
D-Cellobiose	+	+	+	+	+	+	+	+	+	+	+
α-Cellulose	-	-	-	-	-	-	-	-	-	-	-
D-Fructose	+	+	+	+	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	+	+	+	+	+	+
D-Glucose	+	+	+	+	+	+	+	+	+	+	+
D-Glucosamine	+	+	+	+	+	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+	w	+	+	w	+
β-Lactose	+	+	+	+	+	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+	+	+	+
Methyl-α-D-glucoside	+	+	+	+	+	+	w	+	+	w	+
L-Sorbose	+	+	+	+	+	+	+	-	+	+	-
D-Sucrose	+	+	+	+	+	+	+	+	+	+	+
D(+)Trehalose-2hydrate	+	+	+	+	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+	+	+	+	+	+
Xylitol	+	+	+	+	+	+	+	+	+	+	+

+ = assimilation, w = weak, - = non assimilation

Table 4.3 (continued)

Carbon source	PBUAP44	PBUAP45	PBUAP46	PBUAP47	PBUAP48	PBUAP49	PBUAP50	PBUAP51	PBUAP53	PBUAP55	PBUAP58
L-Arabinose	+	+	+	+	+	+	+	+	+	+	+
D-Cellobiose	+	+	+	+	+	+	+	+	+	+	+
α-Cellulose	-	-	-	-	-	-	-	-	-	-	-
D-Fructose	+	+	+	+	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	+	+	+	+	+	+
D-Glucose	+	+	+	+	+	+	+	+	+	+	+
D-Glucosamine	+	+	+	+	+	+	+	+	+	-	-
Glycerol	w	+	+	+	+	+	+	+	+	w	w
β-Lactose	+	+	+	+	+	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+	+	+	+
Methyl-α-D-glucoside	+	+	+	+	+	+	+	+	+	+	+
L-Sorbose	+	+	+	+	+	+	+	+	+	+	+
D-Sucrose	+	+	+	+	+	+	+	+	+	+	+
D(+)Trehalose-2hydrate	+	+	+	+	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+	+	+	+	+	+
Xylitol	+	+	+	+	+	+	+	+	+	+	+

+ = assimilation, w = weak, - = non assimilation

Table 4.3 (continued)

Carbon source	PBUAP59	PBUAP61	PBUAP62	PBUAP65	PBUAP67	PBUAP70	PBUAP71	PBUAP72	PBUAP75	PBUAP76	PBUAP77
L-Arabinose	+	+	+	+	+	+	+	+	+	+	+
D-Cellobiose	+	+	+	+	+	+	+	+	+	+	+
α-Cellulose	-	-	-	-	-	w	-	w	-	-	w
D-Fructose	+	+	+	+	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	w	+	w	+	+	w
D-Glucose	+	+	+	+	+	+	+	+	+	+	+
D-Glucosamine	+	+	+	+	+	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+	+	+	+	+	+
β-Lactose	+	+	+	+	+	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+	+	+	+
Methyl-α-D-glucoside	+	+	+	+	+	w	+	w	+	+	w
L-Sorbose	+	+	+	+	+	w	+	w	+	+	w
D-Sucrose	+	+	+	+	+	+	+	+	+	+	+
D(+)Trehalose-2hydrate	+	+	+	+	+	-	+	-	+	+	-
D-Xylose	+	+	+	+	+	+	+	+	+	+	+
Xylitol	+	+	+	+	+	+	+	+	+	+	+

+ = assimilation, w = weak, - = non assimilation

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 days.

Nitrogen source	NRRL 58560	PBUAP4	PBUAP5	PBUAP5.1	PBUAP7.1	PBUAP9	PBUAP13	PBUAP14	PBUAP16	PBUAP17
Ammonium acetate	+	+	+	+	+	+	+	+	+	+
Ammonium oxalate	+	+	+	+	+	+	+	+	+	+
Ammonium sulphate	+	+	+	+	+	+	+	+	+	+
Ammonium tartrate	+	+	+	+	+	+	+	+	+	+
L-Asparagine	+	+	+	+	+	+	+	+	+	+
L-Glutamic acid	+	+	+	+	+	+	+	+	+	W
Glycine	+	+	+	-	-	w	+	+	w	W
L-Leucine	+	+	+	+	+	+	+	+	+	+
L-Lysine	+	+	+	+	+	+	+	+	+	+
Peptone	+	+	+	+	+	+	+	+	+	+
Potassium nitrate	+	+	+	+	+	+	+	+	+	+
Sodium nitrite	+	+	+	+	+	+	+	+	+	+
Sodium nitrate	+	+	+	+	+	+	+	+	+	+
Urea	+	+	+	+	+	+	+	+	w	-

+ = assimilation, w = weak, - = non assimilation

Table 4.4 (continued)

Nitrogen source	PBUAP20	PBUAP22	PBUAP23	PBUAP24	PBUAP25	PBUAP26	PBUAP27	PBUAP29	PBUAP30	PBUAP31	PBUAP32
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	+	+	+	+	+	+	+	+	+	+	+

+ = assimilation, w = weak, - = non assimilation

Table 4.4 (continued)

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

+ = assimilation, w = weak, - = non assimilation

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	w	w	+	+	w	+	+	+	w	+	+
L-Leucine	+	+	+	+	+	+	+	+	+	+	+
L-Lysine	+	+	+	+	+	+	+	+	+	+	+
Peptone	+	+	+	+	+	+	+	+	+	+	+
Potassium nitrate	+	+	+	+	+	+	+	+	+	+	+
Sodium nitrite	+	+	+	+	+	+	+	+	+	+	+
Sodium nitrate	+	+	+	+	+	+	+	+	+	+	+
Urea	+	+	+	+	+	+	+	+	+	+	+

+ = assimilation, w = weak, - = non assimilation

Table 4.4 (continued)

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	+	w	+	+	w
Glycine	+	+	+	+	+	+	+	+	+	+	+
L-Leucine	+	+	+	+	+	+	+	+	+	+	+
L-Lysine	+	+	+	+	+	+	+	+	+	+	+
Peptone	+	+	+	+	+	+	+	+	+	+	+
Potassium nitrate	+	+	+	+	+	+	+	+	+	+	+
Sodium nitrite	+	+	+	+	+	+	+	+	+	+	+
Sodium nitrate	+	+	+	+	+	+	+	+	+	+	+
Urea	+	+	+	+	+	-	+	-	+	+	-

+ = assimilation, w = weak, - = non assimilation

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 (Figure4.4). 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%.

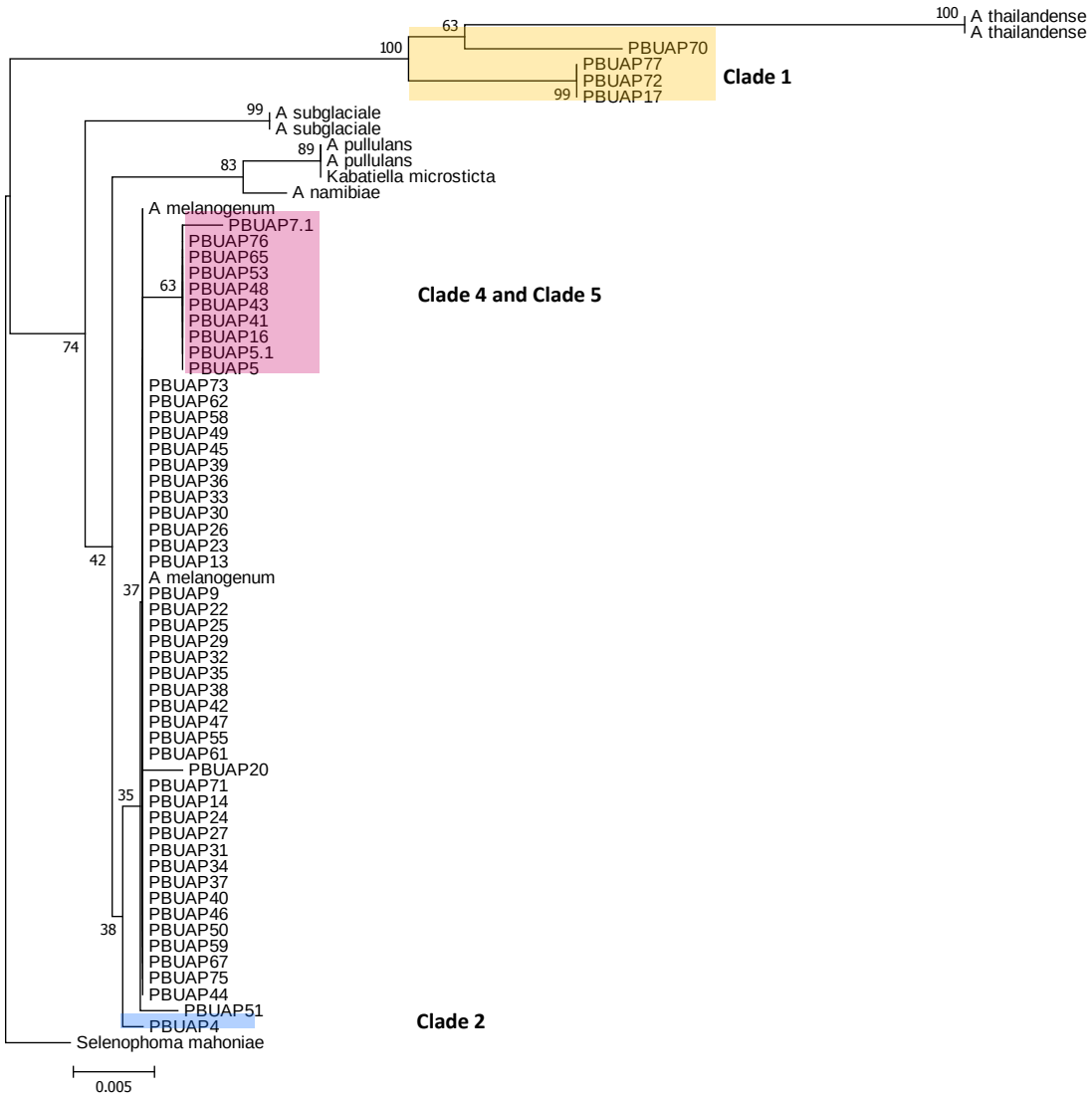


Figure 4.2 Neighbor-joining tree depicting the relationships based on the partial ITS sequences between the 54 *Aureobasidium* new strains and five standard *Aureobasidium* species. Only the branch leading to PBUAP17, PBUAP72 and PBUAP77 is strongly supported clade outside of the ingroup. Numbers on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

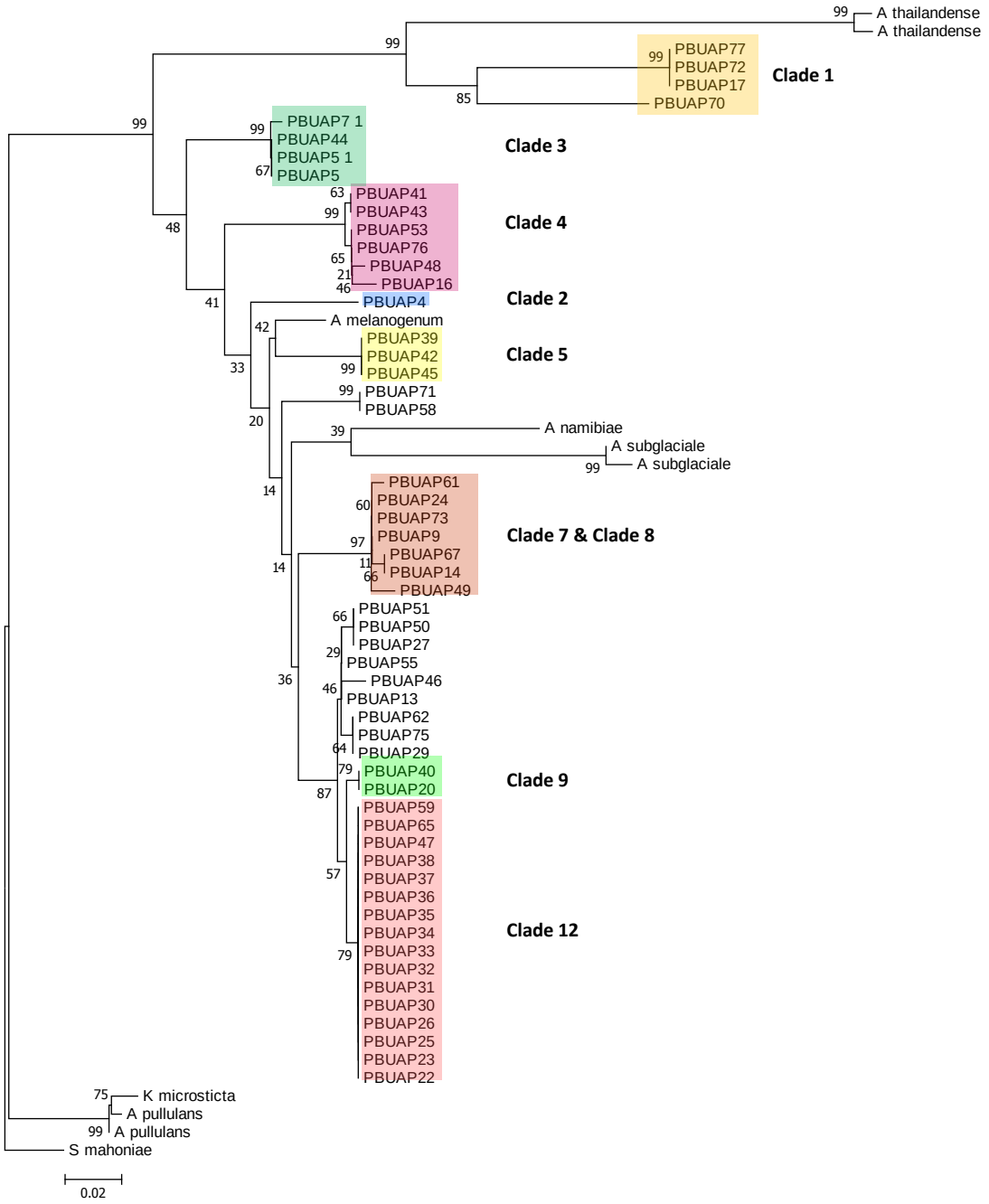


Figure 4.3 Neighbor-joining tree of *TUB* sequences between the 54 *Aureobasidium* new strains and five standard *Aureobasidium* species. Some of the terminal groups are strongly supported by statistic, deeper branches in the tree are mostly not statistically significant. Numbers on the nodes indicate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

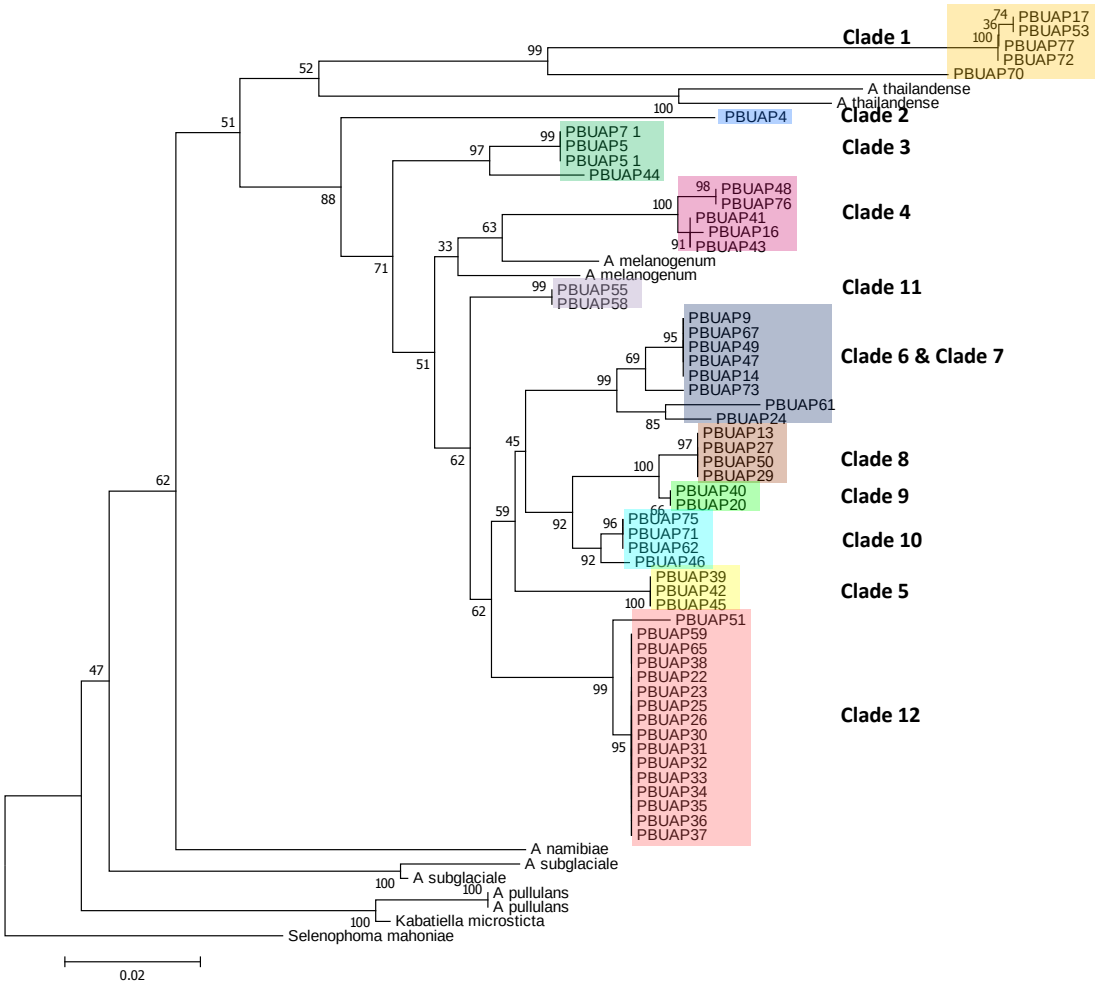


Figure 4.4 Neighbor-joining tree of *ELO* sequences between the 54 *Aureobasidium* new strains and five standard *Aureobasidium* species. All strains are formed ingroup, but related only 2 clades including *A. melanogenum* and *A. thailandense*. Most of the terminal groups are strongly supported by statistic,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

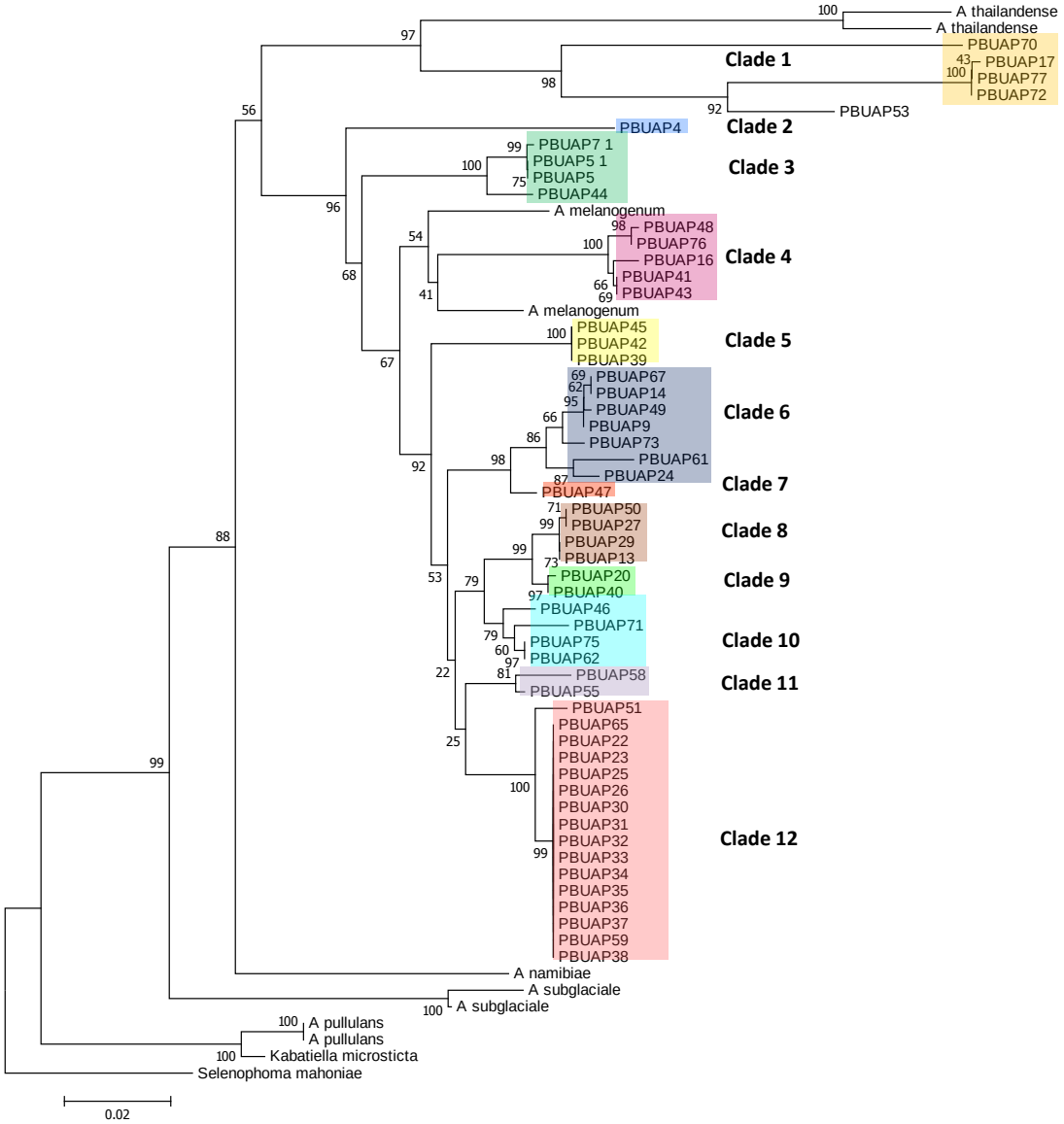


Figure 4.5 Neighbor-joining tree of the combined data from *TUB* and *ELO* sequences from the 54 *Aureobasidium* new strains and five standard *Aureobasidium* species. All strains are formed ingroup but related only 2 clades including *A. melanogenum* and *A. thailandense*. Most of the terminal groups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

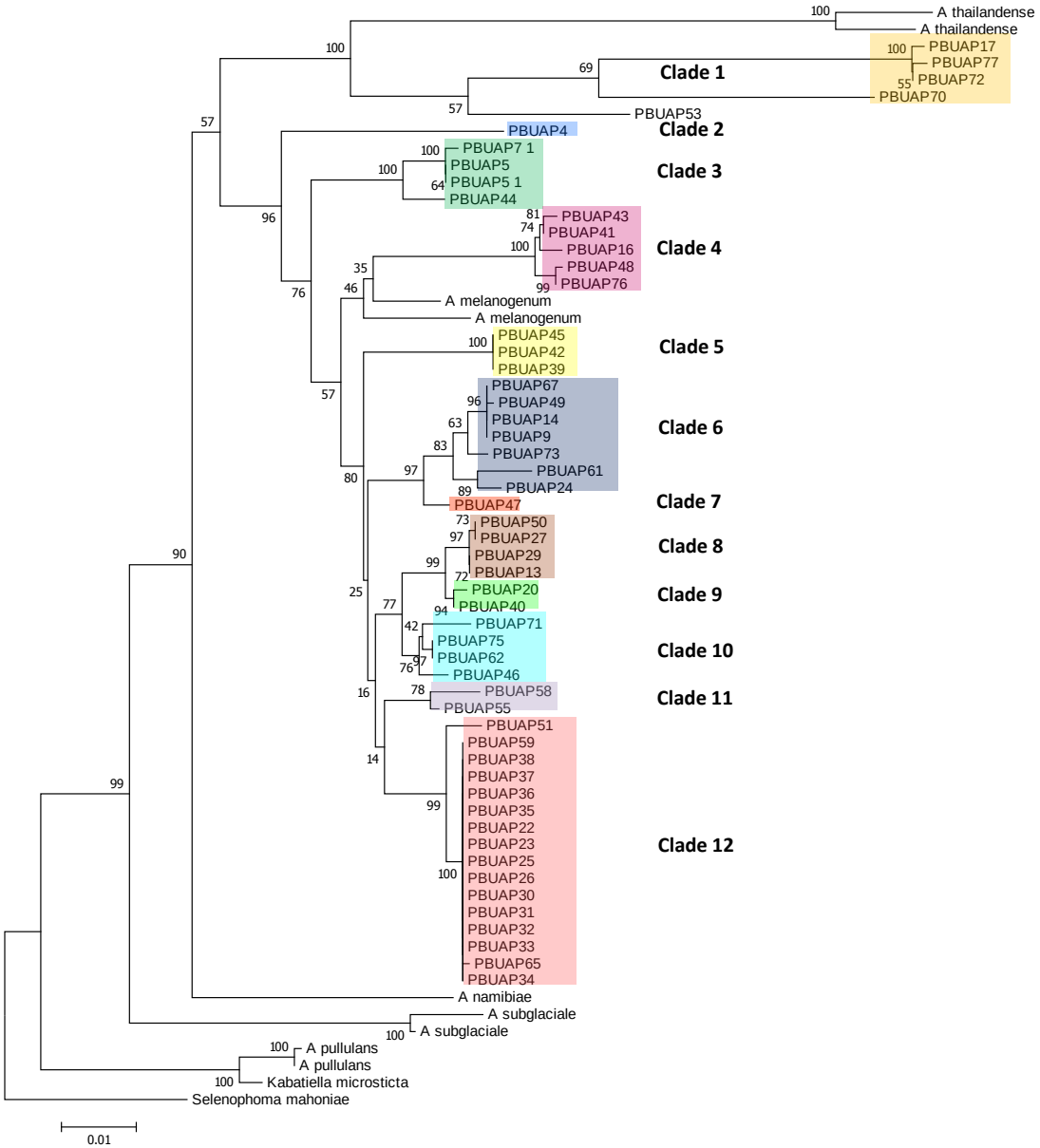


Figure 4.6 Neighbor-joining tree of the combined data from *TUB*, *ELO* and ITS region DNA from the 54 *Aureobasidium* new strains and five standard *Aureobasidium* species. All strains are formed ingroup but related only 2 clades including *A. melanogenum* and *A. thailandense*. Most of the terminal 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.

Table 4.5 EPS production and its properties from *Aureobasidium* spp.

Strain	EPS production		Sensitivity (%)		Appearance		Water solubility	NaOH solubility
	Type	gl^{-1}	Pullulanase	β-Glucanase	Color	Character	(25°C)	(0.1M)
PBUAP4	P	15.71 ± 0.52	89.86	ND	white	hard	easily soluble	easily soluble
PBUAP5	P	4.04 ± 0.46	79.75	ND	white	hard	insoluble	easily soluble
PBUAP5.1	P	5.76 ± 0.64	61.89	13.5	white	hard	easily soluble	swells
PBUAP7.1	P	4.03 ± 0.16	73.01	ND	White	hard	insoluble	insoluble
PBUAP9	P	5.64 ± 0.40	75.26	ND	brown	hard-sticky	incompletely soluble	easily soluble
PBUAP13	P	12.71 ± 0.91	87.62	ND	cream	fragile	easily soluble	easily soluble
PBUAP14	N	1.07 ± 0.05	64.03	9.6	brown	fragile	incompletely soluble	easily soluble
PBUAP16	N	1.00 ± 0.06	82.00	7.07	dark-grey	fragile	insoluble	easily soluble
PBUAP17	B	0.8 + 0.03	10.77	71.15	brown	fragile	insoluble	swells
PBUAP20	P	6.98 ± 0.08	62.90	ND	cream	fragile	insoluble	easily soluble
PBUAP22	P	0.8 ± 0.03	83.12	ND	dark-brown	fragile	incompletely soluble	easily soluble
PBUAP23	P	15.07 ± 0.31	70.77	ND	dark-brown	fragile	incompletely soluble	easily soluble
PBUAP24	P&B	3.66 ± 1.01	80.88	9.6	cream	fragile	easily soluble	easily soluble
PBUAP25	P	17.69 ± 0.60	83.12	ND	brown	fragile	easily soluble	easily soluble
PBUAP26	P	20.2 ± 0.81	78.63	ND	brown	fragile	incompletely soluble	easily soluble
PBUAP27	P	9.34 ± 0.19	77.51	ND	white	fragile	insoluble	easily soluble

*P = pullulan, B = β-glucan, P&B = pullulan and β-glucan, N= unidentified EPS, ND = non detectable

Table 4.5 (continued)

Strain	EPS production		Sensitivity (%)		Appearance		Water solubility	NaOH solubility
	Type	gl^{-1}	Pullulanase	β-Glucanase	Color	Character	($25^{o}C$)	(0.1M)
PBUAP29	P	22.37 ± 0.77	86.49	ND	white	fragile	incompletely soluble	easily soluble
PBUAP30	P	17.65 ± 0.44	80.88	ND	cream	fragile	easily soluble	easily soluble
PBUAP31	P	20.70 ± 0.58	84.25	ND	cream	fragile	easily soluble	easily soluble
PBUAP32	P	27.31 ± 0.66	78.63	ND	cream	fragile	easily soluble	easily soluble
PBUAP33	P	29.55 ± 1.81	74.14	ND	dark-brown	fragile	incompletely soluble	easily soluble
PBUAP34	P	31.66 ± 0.77	83.12	ND	cream-brown	fragile	easily soluble	easily soluble
PBUAP35	P	22.80 ± 0.10	77.51	ND	cream	fragile	easily soluble	easily soluble
PBUAP36	P	22.95 ± 0.79	79.75	ND	cream	fragile	easily soluble	easily soluble
PBUAP37	P	16.55 ± 0.52	82.00	ND	cream	fragile	easily soluble	easily soluble
PBUAP38	P & B	22.50 ± 1.12	88.74	19.2	cream	fragile	easily soluble	easily soluble
PBUAP39	N	0.97 ± 0.04	61.78	23.1	black	fragile-fine	insoluble	incompletely soluble
PBUAP40	B	3.43 ± 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	B	1.34 ± 0.12	11.78	93.8	black	fragile-fine	insoluble	insoluble
PBUAP43	P	1.40 ± 0.28	99.97	ND	cream	fragile	incompletely soluble	incompletely soluble
PBUAP44	P & B	5.72 ± 0.07	66.27	23.1	cream	fragile	insoluble	easily soluble

*P = pullulan, B = β-glucan, P&B = pullulan and β-glucan, N= unidentified EPS, ND = non detectable

Table 4.5 (continued)

Strain	EPS production		Sensitivity (%)		Appearance		Water solubility	NaOH solubility
	Type	gl^{-1}	Pullulanase	β-Glucanase	Color	Character	($25^{\circ}C$)	(0.1M)
PBUAP45	P	0.80 ± 0.15	59.53	13.8	black	fragile-fine	insoluble	insoluble
PBUAP46	P	16.01 ± 0.46	86.49	ND	orange-cream	fragile	easily soluble	easily soluble
PBUAP47	P	15.80 ± 0.98	77.51	29.2	orange-cream	fragile	incompletely soluble	easily soluble
PBUAP48	P	0.93 ± 0.03	65.15	ND	dark-brown	fragile-fine	insoluble	incompletely soluble
PBUAP49	P	3.45 ± 0.24	69.64	ND	dark-brown	fragile	insoluble	incompletely soluble
PBUAP50	P	14.55 ± 0.30	88.74	ND	cream	fragile	insoluble	easily soluble
PBUAP51	P	13.17 ± 1.12	79.75	ND	grey	fragile	incompletely soluble	easily soluble
PBUAP53	B	0.40 ± 0.06	12.09	77.77	cream	fragile	easily soluble	incompletely soluble
PBUAP55	N	12.37 ± 0.36	68.52	29.92	cream-brown	fragile	insoluble	swells
PBUAP58	N	5.99 ± 0.23	65.15	12.5	grey-black	fragile	insoluble	swells
PBUAP59	P	12.77 ± 0.68	77.51	ND	brown	hard	incompletely soluble	easily soluble
PBUAP61	P	7.45 ± 0.82	91.10	ND	brown-grey	hard	incompletely soluble	easily soluble
PBUAP62	P	9.20 ± 0.54	80.88	ND	cream	hard	incompletely soluble	easily soluble
PBUAP65	P	12.23 ± 1.22	67.40	ND	dark-brown	fragile-fine	insoluble	incompletely soluble
PBUAP67	N	1.62 ± 0.06	61.78	26.9	cream	hard-fine	incompletely soluble	easily soluble

*P = pullulan, B = β-glucan, P&B = pullulan and β-glucan, N= unidentified EPS, ND = non detectable

Table 4.5 (continued)

Strain	EPS production		Sensitivity (%)		Appearance		Water solubility	NaOH solubility
	Type	gl^{-1}	Pullulanase	β-Glucanase	Color	Character	(25°C)	(0.1M)
PBUAP70	B	2.95 ± 0.64	11.78	82.88	cream-brown	fragile-fine	insoluble	insoluble
PBUAP71	P	15.67 ±	79.75	ND	brown	fragile	insoluble	easily soluble
PBUAP72	B	0.70 ± 0.05	18.41	84.23	black	fragile-fine	insoluble	insoluble
PBUAP73	ND	-	0.00	0.00	-	-	-	-
PBUAP75	B	1.64 ± 0.12	16.27	80.00	black	fragile	insoluble	insoluble
PBUAP76	P	3.45 ± 0.21	66.27	ND	brown	hard-sticky	insoluble	insoluble
PBUAP77	ND	-	0.00	0.00	-	-	-	-
NRRLY12974	P	33.76 ± 0.64	100.00	ND	light-grey	hard	easily soluble	easily soluble
NRRL58561	P	2.08 ± 0.23	66.27	ND	brown-grey	fragile	incompletely soluble	incompletely soluble
NRRL58560	P	25.00 ± 0.10	100.00	ND	light-cream	fragile	easily soluble	easily soluble
NRRL58013	B	3.60 ± 0.67	13.01	100.00	black	fragile	incompletely soluble	incompletely soluble

*P = pullulan, B = β-glucan, P&B = pullulan and β-glucan, N= unidentified EPS, ND = non detectable

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).

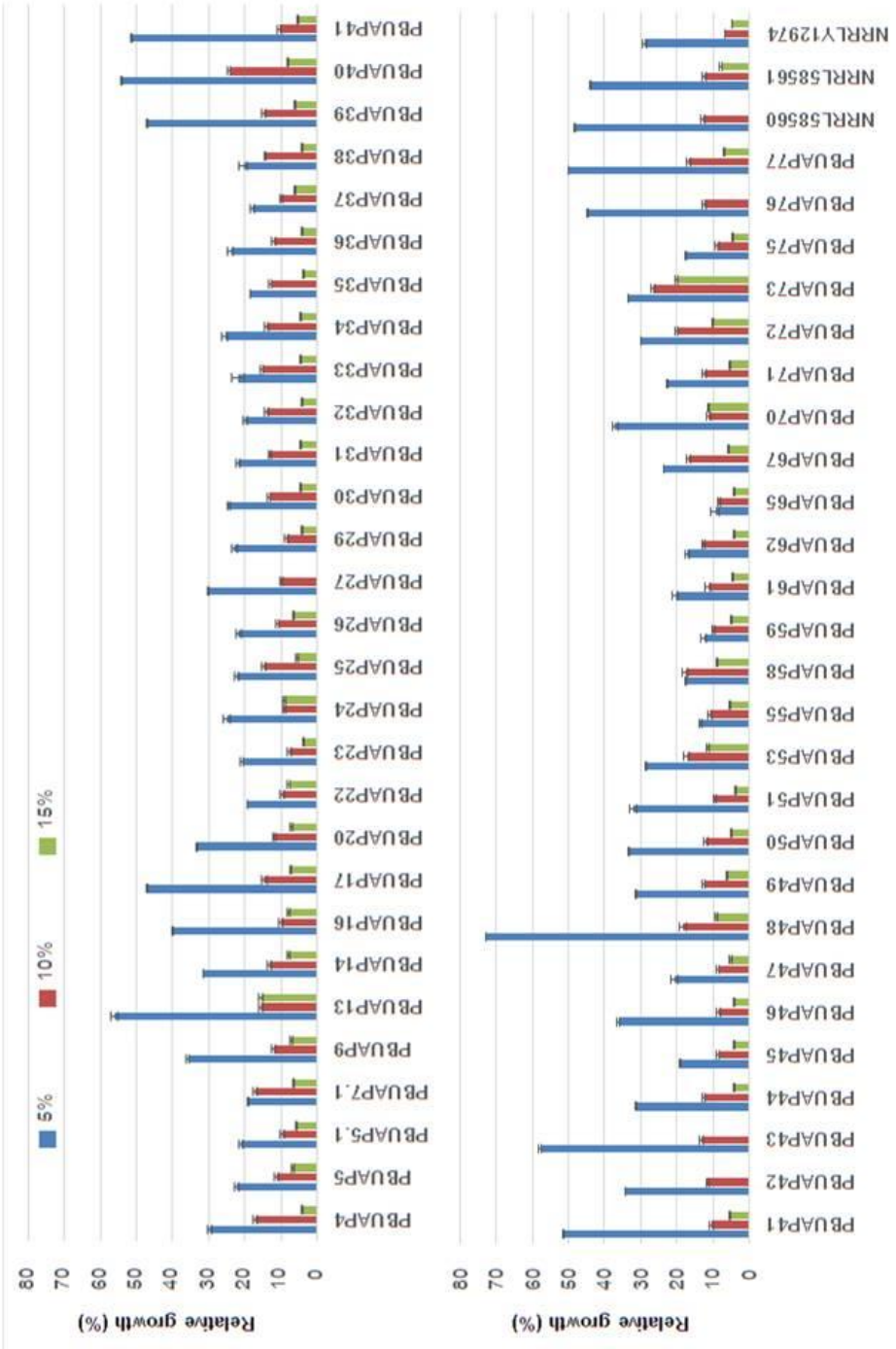


Figure 4.7 Relative growth (%) of 54 new strains of *Aureobasidium* spp. from coastal and three standard *Aureobasidium* species grown on PDA supplemented with different concentrations of NaCl (5 %, 10 %, 15 % w/v) at 30°C for 7 day, compared with the growth in PDA without addition of NaCl.

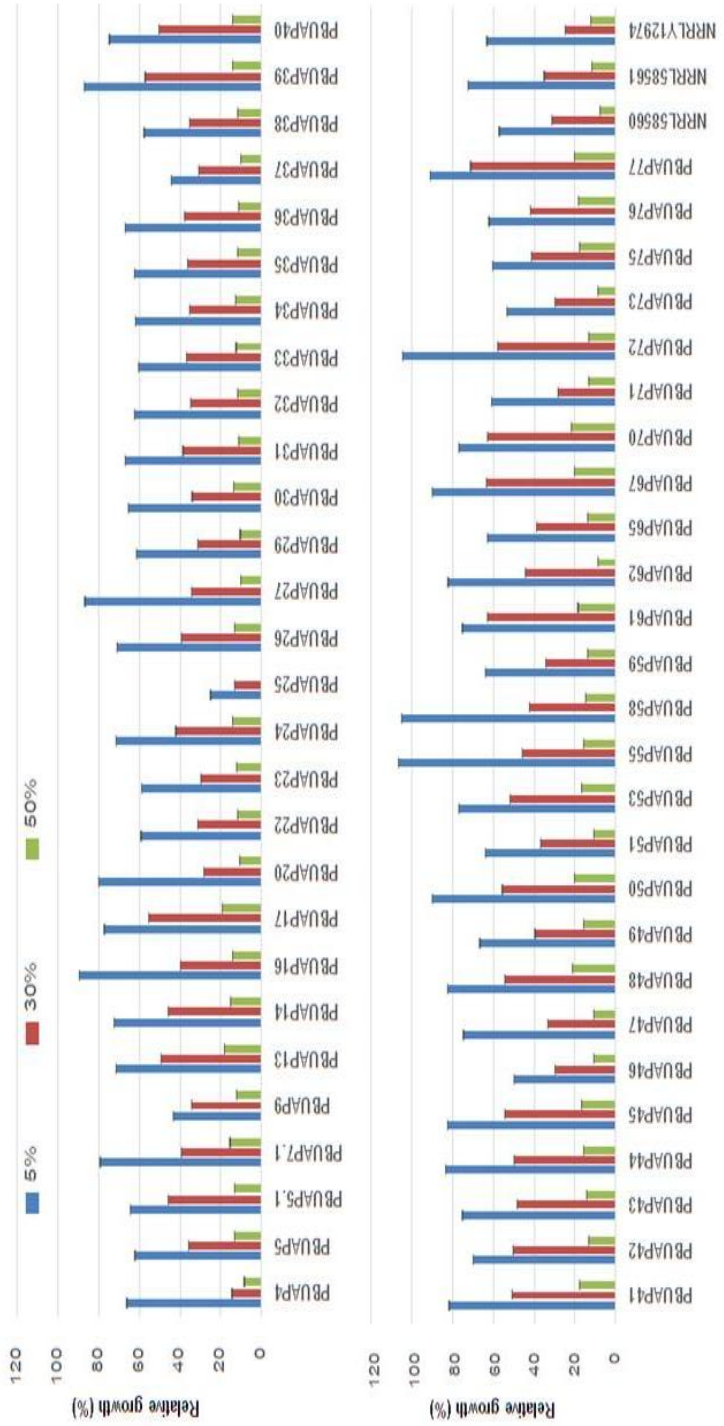


Figure 4.8 Relative growth (%) of 54 new strains of *Aureobasidium* spp. from coastal and three standard *Aureobasidium* species grown on YMA supplemented with different concentrations of glucose (5 %, 30 %, 50 % w/v) at 30°C for 7 day, compared with the growth on YMA with addition of 1% glucose.

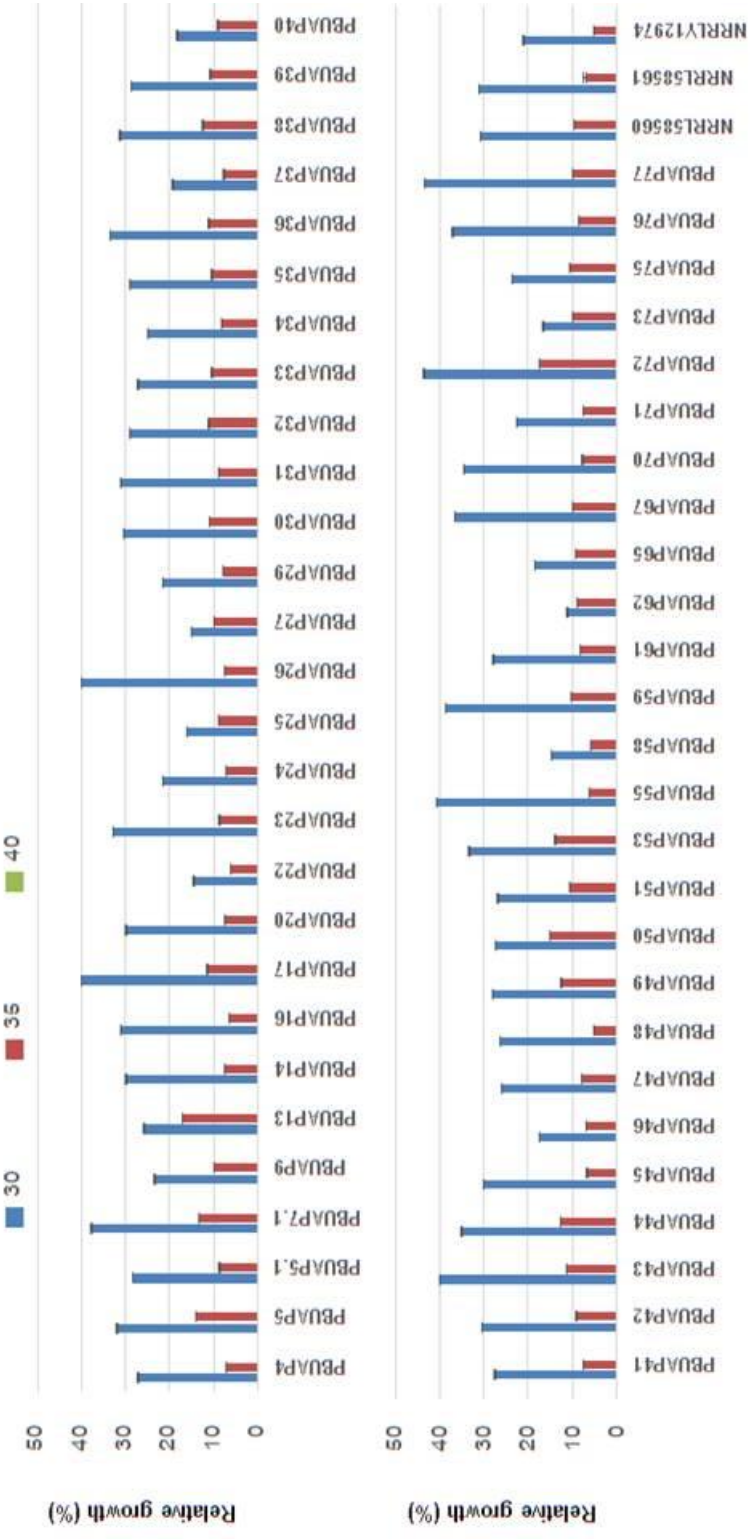


Figure 4.9 Relative growth (%) of 54 new strains of *Aureobasidium* spp. from coastal and three standard *Aureobasidium* species grown on 2% MEA incubated at various temperatures (30^0C, 35^0C, 40^0C) for 7 day.

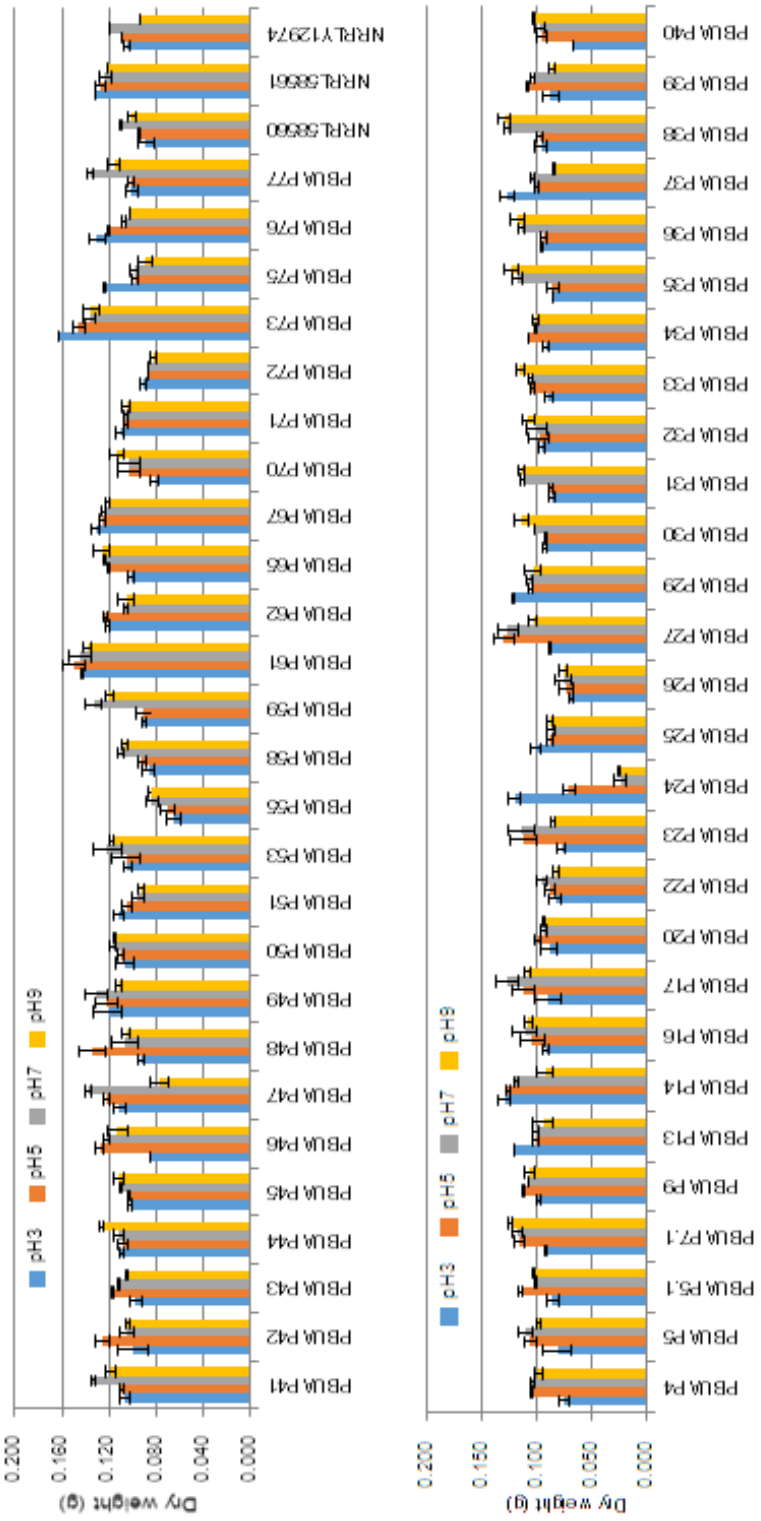


Figure 4.10 Relative growth (%) of 54 new strains of *Aureobasidium* from coastal and three standard *Aureobasidium* species grown in 2% MEB with different pH values (pH3, pH5, pH7, pH9) at 30^0C, 70 rpm, for 1 month.

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) sucrose. On the contrary, significant growth inhibition occurred in the relatively 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 % (w/v). In the relatively halotolerant and osmointolerant PBUAP34, accumulation of 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.

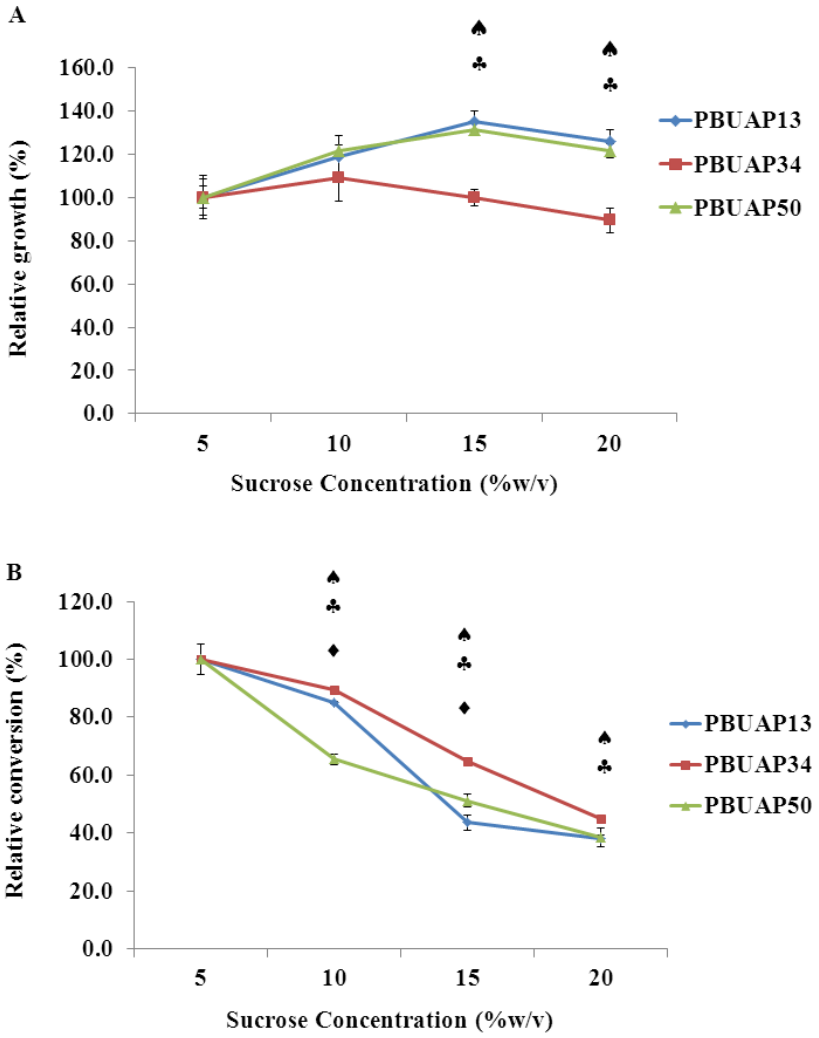


Figure 4.11 Effect of sucrose concentration on growth and comparison to its efficiency of conversion. (A) Relative growth (compared with growth in the medium containing 5% (w/v) of sucrose) and (B) relative conversion (compared with conversion in the medium containing 5% (w/v) of sucrose) of *A. melanogenum* strains PBUAP13, 34 and 50. All strains were grown in production medium containing sucrose at concentrations of 5 – 20 % (w/v) at 30±2°C with 150-rpm agitation for five days. The symbols: ♠ indicates significant difference between PBUAP13 and 34, ♣ indicates significant difference between PBUAP 34 and 50 and ♦ indicates significant difference between PBUAP13 and 50.

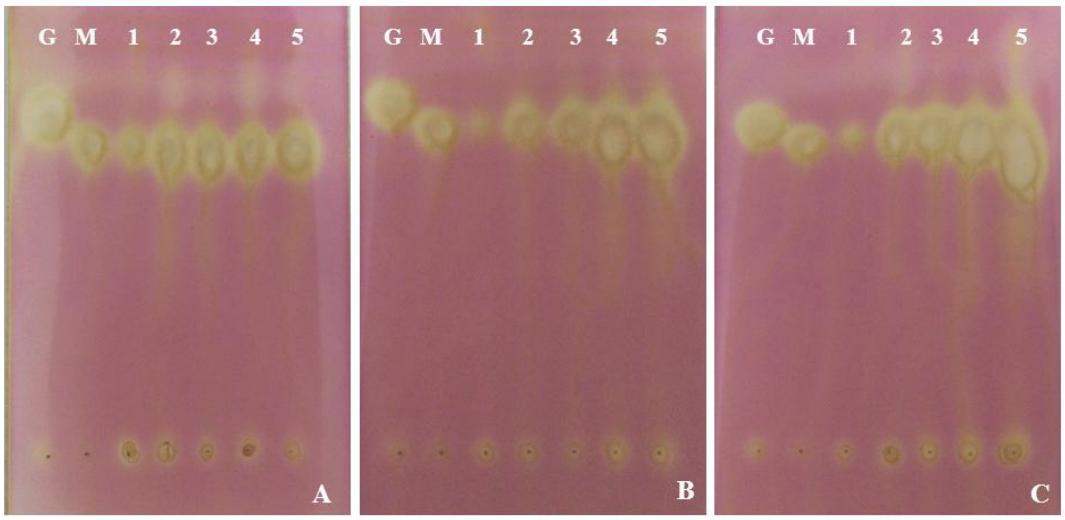


Figure 4.12 Cellular extracts of *A. melanogenum* analyzed by thin layer chromatography. *A. melanogenum* strains were grown in production medium containing various concentration of sucrose at 30±2°C with 150-rpm agitation for five days. (A) PBUAP13, (B) PBUAP34 and (C) PBUAP50. Lane G: glycerol (0.01 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 << 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	++	++	H (40.4)
PBUAP27	++	++	RL (18.7)
PBUAP29	++	++	H (44.7)
PBUAP30	++	++	RH (35.3)
PBUAP31	++	++	H (41.4)
PBUAP32	++	++	EH (54.6)

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

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

Conversion efficiency of EPS production: EH = extremely high (≥ 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	+	+++	M (24.7)
PBUAP58	+	+++	RL (11.9)
PBUAP59	+	++	M (25.5)
PBUAP62	+	+++	RL (18.4)
PBUAP65	+	++	M (24.4)
PBUAP75	+	+++	L (3.3)

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

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

Conversion efficiency of EPS production: EH = extremely high (≥ 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).

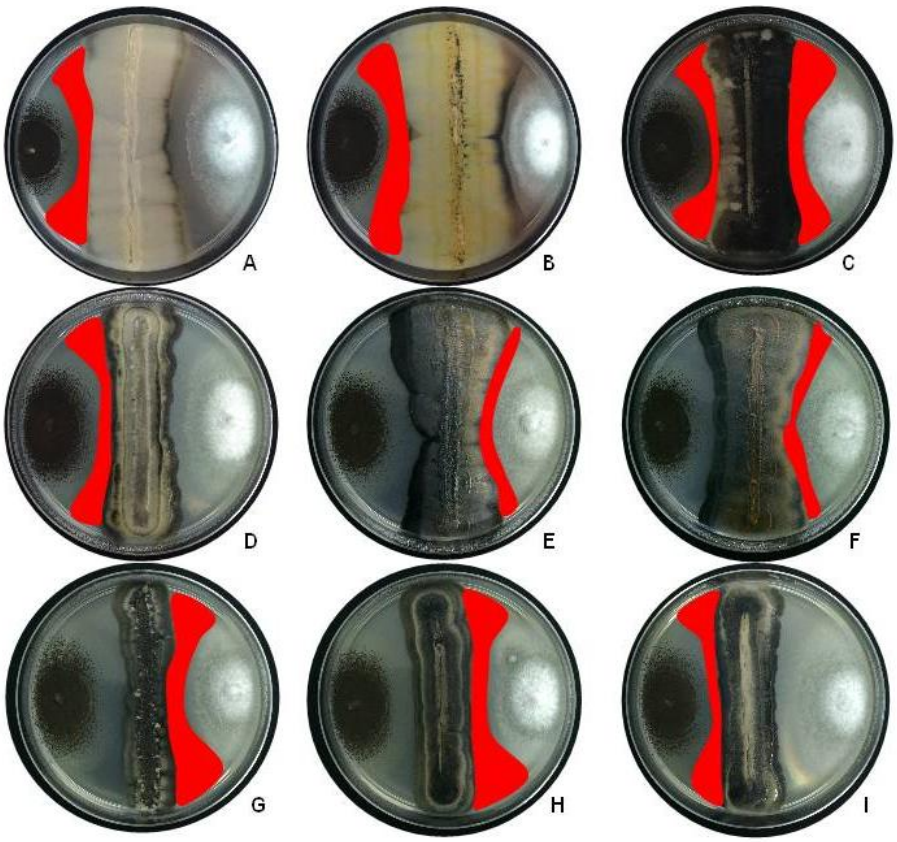


Figure 4.13 Visual agar plate assay showed screen identification of antifungal activity of PBUAP5(A), PBUAP7.1(B), PBUAP47(C), PBUAP48(D), PBUAP55(E), PBUAP58(F), PBUAP72(H), PBUAP73(I), and PBUAP76(J) against *A. niger* (left) and *A. fumigatus* (right).

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

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

+ / - = 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^{0}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 standard 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.

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

± = 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 **x**ylanase 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±0.34
2	21.46±0.16
4	21.60±0.20
6	23.40±0.13
8	23.99±0.12
12	27.28±0.27
16	28.16±0.02*
20	27.17±0.02
24	25.51±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).

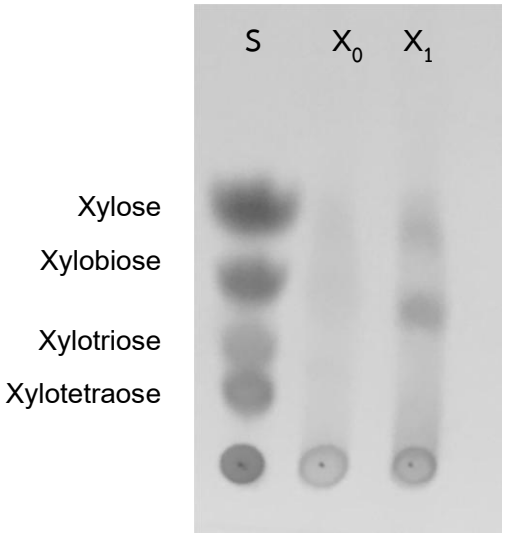


Figure 4.14 Thin-layer chromatogram of the hydrolysis products of cattail xylan treated with crude xylanase of *A. melanogenum* PBUAP58. The hydrolysis reaction using was carried out at 50°C for 24 h in 50 mM sodium acetate buffer (pH 5.0) containing 1% (w/v) cattail lxylan. S represents the oligomer markers, X_0 represents untreated xylan and X_1 represents the treatment xylan. Xylose, xylobiose, xylotriose, and xylotetraose were used for standard comparison.

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.

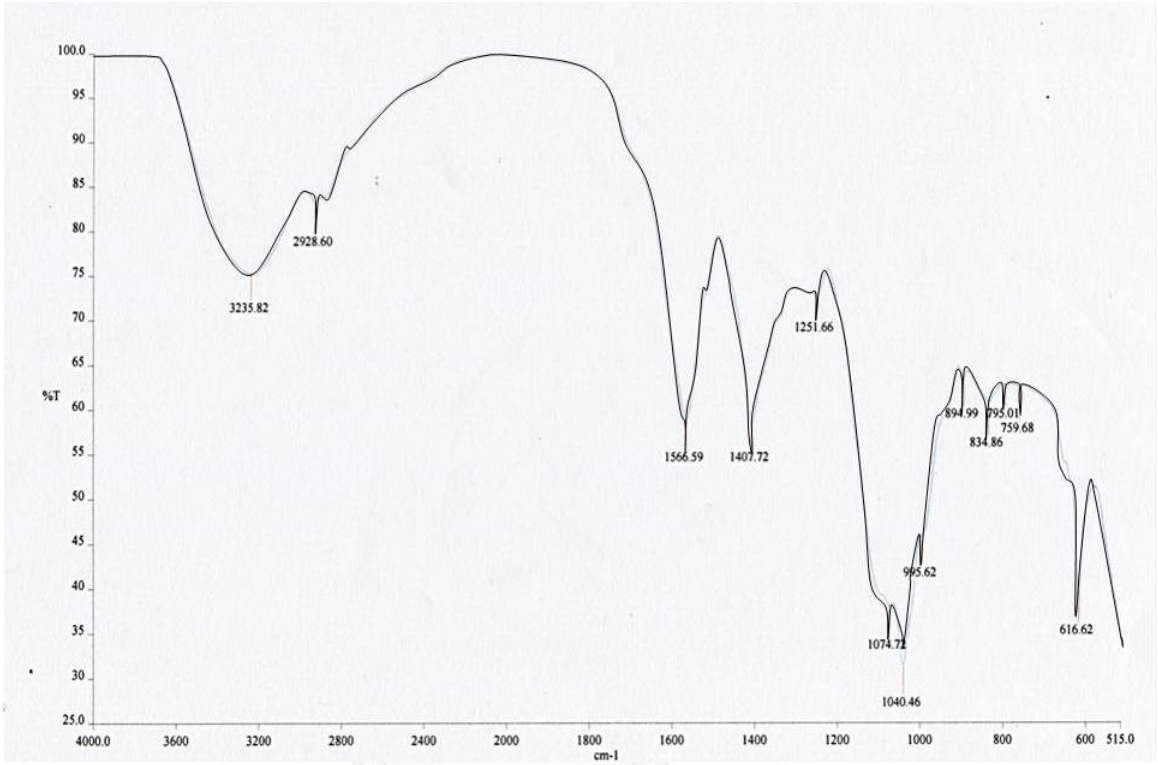


Figure 4.15 FT-IR spectrum of XOS powder obtained from cattail.

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.

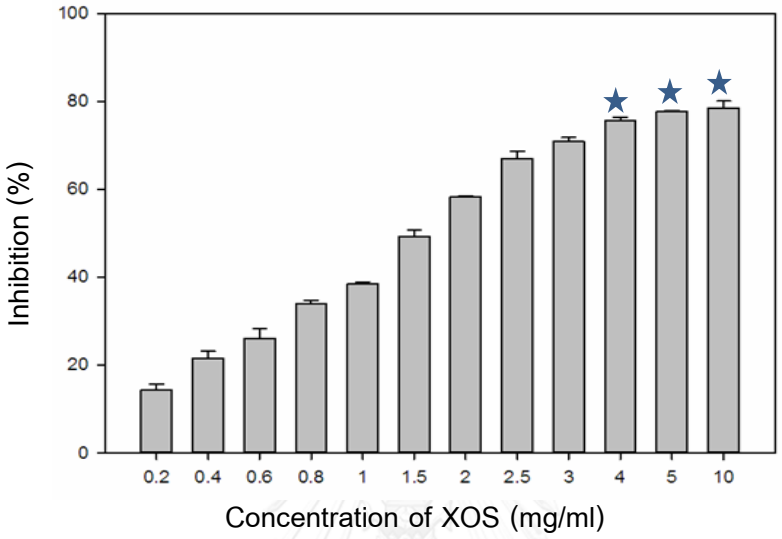


Figure 4.16. Antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl of XOS obtained by enzymatic hydrolysis. Values represent mean values from tree replicates. The symbol ★ indicates significant different between XOS concentration.

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-**α**-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 al*., 2008). From the analysis, the ITS region seems to be useful to distinguish *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 (37^0C) (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 pullulan 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 α-D-glucan comprising α-maltotriosyl residues linked by 1,6-α–D-glucosidic bonds (Leathers *et al*. 1988) whereas the β-1,4-D-, β -1,6-D and β-1,3-D-glucosidic 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 (37^0C) 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 non-ionic 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.* (2011); Choudhury *et al*., 2012; Prajapati *et al*., 2013; Singh and Saini, 2008; Wang *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. Revista Iberoamericana De Micologia 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. Postharvest Biology and Technology 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. Bioresour Technol 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. Yeast 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. Bioresour Technol 115: 215-221.

Cheng, K. C., A. Demirci and J. M. Catchmark. 2011. Pullulan: biosynthesis, production, and applications. Appl Microbiol Biotechnol 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. Biotechnol Adv 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. Appl Microbiol Biotechnol 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. Biocatalysis and Agricultural Biotechnology 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. Carbohydrate Polymers 83(4): 1547-1552.

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

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

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

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

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

Dhiman, S. S., J. Sharma and B. Battan. 2008. Industrial applications and future prospects of microbial xylanases: a review. BioResources 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. Applied and Environmental Microbiology 61(4): 1323-1330.

Gostinčar, C., M. Grube, S. De Hoog, P. Zalar and N. Gunde-Cimerman. 2010. Extremotolerance in fungi: evolution on the edge. FEMS microbiology ecology 71(1): 2-11.

Gostincar, C., M. Grube and N. Gunde-Cimerman. 2011. Evolution of fungal pathogens in domestic environments? Fungal Biol 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. BMC Genomics 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. Fungal Ecology 4(5): 309-321.

Gunde-Cimerman, N., J. Ramos and A. Plemenitas. 2009. Halotolerant and halophilic fungi. Mycol Res 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. FEMS Microbiology Ecology 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. Microbiology and Molecular Biology Reviews 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. Applied and environmental microbiology 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. J Antibiot (Tokyo) 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. Food and Bioproducts Processing 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. J Clin Microbiol 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. Biotechnol Lett 32(1): 137-142.

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

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. Appl Environ Microbiol 71(11): 6600-6605.

Kumar, V., V. Sahai and V. Bisaria. 2012. Production of amylase and chlamydospores by Piriformospora indica, a root endophytic fungus. Biocatalysis and Agricultural Biotechnology 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. The yeasts, a taxonomic study, 5th edn. Elsevier, Amsterdam: 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. Bioinformatics 23(21): 2947-2948.

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

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

Leathers, T. D. 2003. Biotechnological production and applications of pullulan. Appl Microbiol Biotechnol 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. Biotechnol Lett 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. Crit Rev Microbiol 41(2): 228-237.

Liu, S. and A. Steinbüchel. 1996. Investigation of poly (β-L-malic acid) production by strains of Aureobasidium pullulans. Applied Microbiology and Biotechnology 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. African Journal of Microbiology Research 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. Scienceasia 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. Mycologia 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. Industrial Crops and Products 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. Mycol Res 113(Pt 10): 1107-1120.

Manitchotpisit, P., N. P. Price, T. D. Leathers and H. Punnapayak. 2011. Heavy oils produced by Aureobasidium pullulans. Biotechnol Lett 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. Biotechnol Lett 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. PLoS One 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. Evolutionary bioinformatics online 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. J Biosci Bioeng 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. J Biosci Bioeng 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. J Agric Food Chem 57(14): 6305-6317.

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

Prajapati, V. D., G. K. Jani and S. M. Khanda. 2013. Pullulan: an exopolysaccharide and its various applications. Carbohydrate polymers 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. Appl Microbiol Biotechnol 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. World Journal of Microbiology and Biotechnology 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. Carbohydrate research 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. Phytochemistry 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. Food Chem 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.

Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. Molecular cloning. 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. Stud Mycol 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. Bioresour Technol 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. The Journal of antibiotics 44(9): 919-924.

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

Thambugala, K. M., H. A. Ariyawansa, Y.-M. Li, S. Boonmee, S. Hongsanan, Q. Tian*, et al.* 2014. Dothideales. Fungal Diversity 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. Mycologia: 278-284.

Torzilli, A. P. 1997. Tolerance to high temperature and salt stress by a salt marsh isolate of Aureobasidium pullulans. Mycologia: 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 xylo-oligosaccharides derived from cereal and millet brans – A comparative study. Food Chemistry 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. Process Biochemistry 49(5): 725-731.

Wang, D., X. Yu and W. Gongyuan. 2013. Pullulan production and physiological characteristics of Aureobasidium pullulans under acid stress. Appl Microbiol Biotechnol 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. PCR protocols: a guide to methods and applications 18: 315-322.

Wickerham, L. J. and C. P. Kurtzman. 1975. Synergistic color variants of Aureobasidium pullulans. Mycologia 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. Carbohydrate Polymers 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. Carbohydrate Polymers 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. Bioresour Technol 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. LWT - Food Science and Technology 39(4): 388-392.

Youssef, F., T. Roukas and C. G. Biliaderis. 1999. Pullulan production by a non-pigmented strain of Aureobasidium pullulans using batch and fed-batch culture. Process Biochemistry 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. Antonie Van Leeuwenhoek 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. Antonie Van Leeuwenhoek 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. Stud Mycol 61: 21-38.

REFERENCES

APPENDIX

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.

3. **Pullulan Production (PM) Medium**

Sucrose	50.0	g
Bacto-Peptone	0.6	g
K_2HPO_4	5.0	g
$MgSO_4.7H_2O$	0.4	g
NCl	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_2HPO_4	5.0	g
Glucose	10.0	g

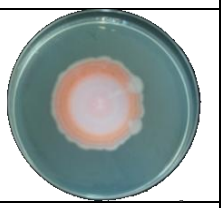
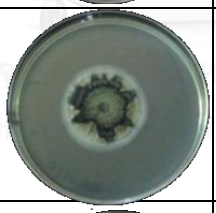
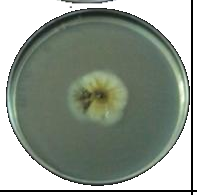
Dissolved in distilled water to final volume 1 liter.

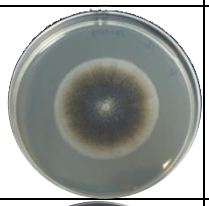
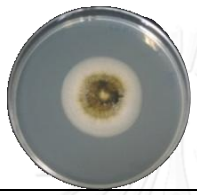
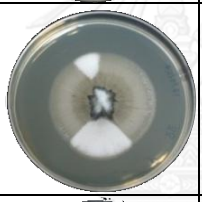
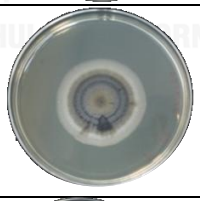
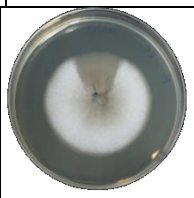
4.2 Xylan production medium

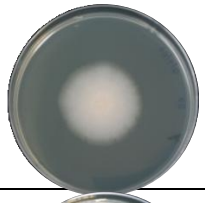
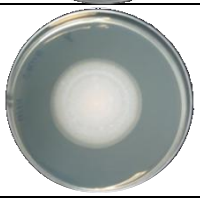
Yeast nitrogen base	6.7	g
L-asparagine	2.0	g
K_2HPO_4	5.0	g
Beechwood xylan	10.0	g

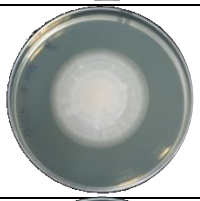
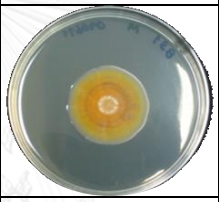
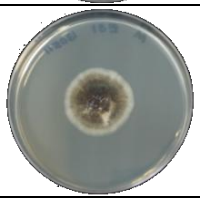
Appendix B

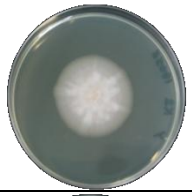
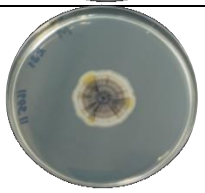
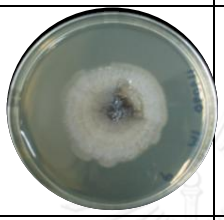
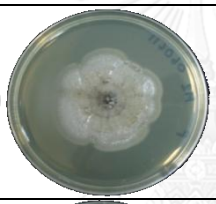
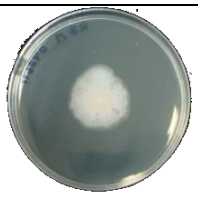
Morphological studies of each isolates

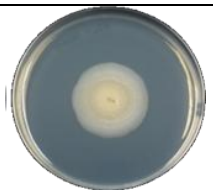
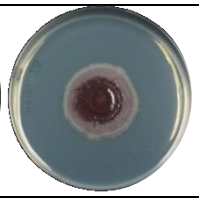
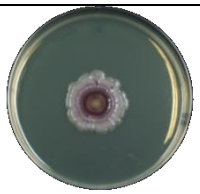
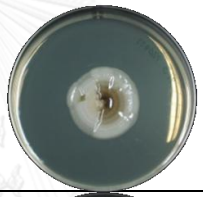
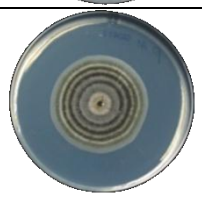
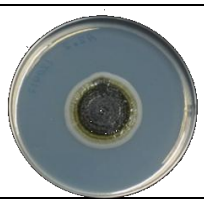
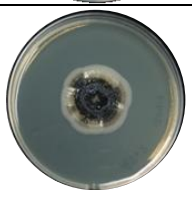
	PDA	MEA	YMA
PBUAP4			
PBUAP5			
PBUAP5.1			
PBUAP7.1			
PBUAP9			
PBUAP13			
PBUAP14			

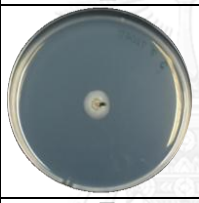
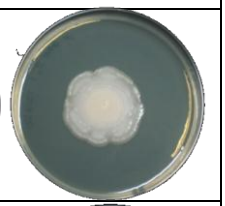
	PDA	MEA	YMA
PBUAP16			
PBUAP17			
PBUAP20			
PBUAP22			
PBUAP23			
PBUAP24			
PBUAP25			
PBUAP26			

	PDA	MEA	YMA
PBUAP27			
PBUAP29			
PBUAP30			
PBUAP31			
PBUAP32			
PBUAP33			
PBUAP34			
PBUAP35			

	PDA	MEA	YMA
PBUAP36			
PBUAP37			
PBUAP38			
PBUAP39			
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			
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 342.66	FJ150903	FJ157872	FJ039823
CBS 388.92	FJ150872	FJ157874	FJ039847
CBS 133856[T]	JX462674	EU719407	GCCTTTACCTCGCTCAAGGGCTACAGACCCCAAG ACTTCCGTTTCGTGCCTGGAAAGACCCCGATGGC TACCTTCAGGGAGACTGCCACCATGCTCATCGCC TACTACATCATCATCTTTGGTGGCAGAGAGTTCAT GCGCGGTCGCGAGCCTTTCAAGCTCAGCTTTTTCT TCAAGCTCCACAACTTCTACTTGACCGTCATCAGC GGTGTCCTCCTCGCGCTCTTCGTTGAGCAGCTTCT GCCCGAGATTGTCAGAAACGGCGTCTTCCACGCT GTCTGCGCCTACGAGGGTGGCTGGACTGACAAG CTTGTTGTTCTTTACTACGTATGTTGATTGCGATTC GCGACTGAATGCGCTTACTGACGAGTTGCAGCTC AACTACCTCACCAAGTACCTCGAGCTGATTGACAC CTGCTTCCTGTTCCTCAAGAAGAAGCCCCTGAGTA AGTTCAATCCATCTTCGGCGCATCTCATTCGCACT ACCTGACCAACCTCACAGCTTTCCTCCACACTTAC CACCACGGTGCTACCGCCCTTCTCTGCTTCACCC AGCTCCTCGGTCACACCGCCGTCTCCTGGGTTCC CATCACCCTCAACCTGACTGTCCA
CBS 133857	JX462675	EU719412	CTACAGACCCCAAGACTTCCGTTTCGTGCCCGGA AAGACCCCAATGGCTACCTTCAAGGAGACTGCCA CCATGCTCATCGCCTACTACATCATCATTTTTGGTG GCAGAGAGTTCATGCGCGGTCGCGAGCCCTTCAA GCTCAGCTTCTTCTTCAAGCTCCACAACTTCTACTT GACCGTCATCAGCGGTGTTCTCCTCGCGCTCTTC GTTGAGCAGCTTCTGCCCGAGATTGTCAGAAACG GCGTCTTCCACGCTGTCTGCGCCTACGAGGGTGG CTGGACTGACAAGCTTGTTGTTCTTTACTACGTAC GTGGAATGCGATCAGCGACTGAATGCGCTTACTG ACGAGTTGCAGCTCAACTACCTGACCAAGTACCT CGAGCTGATTGACACCTGCTTCCTGTTTCTCAAGA AGAAGCCCCTGAGTGAGTTCAATCCATTTTCGGC GCAATTCACTCGCACCAACTGATCAACCTCCTAGC TTTCCTCCACACTTACCACCACGGTGCCACCGCC CTTCTCTGCTTCACCCAGCTCCTCGGTCACACCTC CGTCTCCTGGGTTCCCATCACCCTCAACCTGACT GTCCACGTCGTCATGTACTGGTACTACTTCCAGGC CGCACGTGGCATCCGCATCTGGTGG

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

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

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

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

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

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

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

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

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

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

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

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

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

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

PBUAP59	KP965478	CTTGGCCCAGTTGTTGCCAGCA CCGGACTGACCGAAGACGAAG TTGTCAGGACGGAAGAGCTGA CCGAAGGGACCGGCACGGAC GGCGTCCATGGTACCAGGCTC CAAGTCGACGAGGACGGCACG GGGGACATACTTGTTACCAGAG GCCTATGGTGCGCATTAGCATT GGAGTAGTCAGTGAAGGGCGG ACAGTTGGTGACTGACCTCGTT GAAGTAGACGTTCATGCGCTCC AGCTGGAGATCTGAGGTACCG TTGTAGCTGTTAGGCAGTCAGC GAGCTGTCATTGAGGATGCGC AGAGAGTCAACGTACACACCA GCACCGTCAAGGCCGTGCTCG CCAGAGATGGTCT	GTAGTACCAGTACATGACAACGTGGACGGTCAAG TTCAGGGTGATGGGAACCCATGAGACTGCGGTGT GGCCAAGAAGCTGGGTAAAGCAGAGAAGGGCAG TGGCACCGTGGTGGTAGGTGTGGAGGAAAGCTA GGAGACCAGTCAGCTAAAGCATTGATTGTGAAAAT ATTGCGCGCTTACTCAAAGGCTTCTTCTTGAGGAA AAGGAAGCAGGTGTCAATCAGCTCAAGGTACTTG GTGAGGTAGTTGAGCTGCCAGCTGTCAGTAAGCG CATTCTGGCGAGAAGCTGGAAAAACGTACGTAGT AAAGAACAACGAGCTTGTCGGTCCAGCCGCCCTC GTAGGCGCAGACAGCGTGGAAAATGCCGTTTCTG ACAATCTCGGGCAAGAGCTGCTCGACGAACAGAA CCAAGAGGAGACCGCTGATGACGGTCAGGTAGA AGTTGTGGACCTTGAAGAAGAAGTTGAGCTTGAAA GGCTCGCGACCACGCATGAGCTCTCTGCCACCAA AGATGATGATGTAGTAGGCAATGAGCATGGTGGC CGTCTCCTTGAAGGTAGCCATAGGGGTCTTTCCA GGGACGAAGCGGAAGTCCTGGGGC
PBUAP61	KP965479	GACTGACCGAGACGAAGTTGT CGGGACGGAAGAGCTGACCGA AGGGACCAGCACGGACGGCG TCCATGGTACCAGGCTCCAAAT CGACGAGGACGGCACGGGGG ACATACTTGTTACCAGAGGCCT ATGGTGCGCATTAGCATTGAAG GAGTGAGTGAAAGGCCGACAG TTGTTGACCGACCTCGTTGAAG TAGACGTTCATGCGCTCCAGCT GGAGATCTGAGGTACCGTTGTA GCTGTTAGGCAGTCAGCGAGC TGTCATTGCGGGTGAGGATGC GCAGGGGGGTCGACGTACACA CCAGCGCCGTCAAGGCCGTGC TCGCCAGAGATGGTCTGCCAG AAAGCAGCACC	AGTACATGACGACGTGGACGGTCAAGTTCAGGGT GATGGGGACCCATGAAACTGCGGTGTGGCCGAG AAGCTGGGTGAAGCAGAGAAGAGCAGTGGCGCC GTGGTGGTAGGTGTGGAGGAAAGCTAGGAGCCC AATTAACTAAAGCATTGATTTTGGAGGTAGTGCGC GCTTACTCAAAGGCTTCTTCTTGAGGAAAAGGAAG CAGGTGTCAATCAGCTCGAGGTACTTGGTGAGGT AGTTGAGCTGCCAGCTGTCAGTAAGCGCATTCTGT CGCGGAATTGGATAGACATACGTAGTAAAGAACA ACGAGCTTGTCGGTCCAGCCGCCCTCGTAGGCG CAGACAGCGTGGAAAATGCCGTTTCTGACAATCT CGGGCAGGAGCTGCTCGACGAAGAGAACCAAGA GAAGACCGCTGATGACGGTCAAGTAGAAGTTGTG GACCTTGAAGAAGAAGTTGAGCTTGAAAGGCTCG CGACCACGCATGAGCTCTCTACCACCAAAGATGA TGATGTAGTAGGCAATGAGCATGGTGGCCGTCTC CTTGAAAGTAGCCATGGGGGTCTTTCCAGGAACG AAGCGGAAGTCCTGGGGCTTGTAGCCC
PBUAP62	KP965480	GGCAGACCATCTCTGGCGAGC ACGGCCTTGACGGTGCTGGTG TGTACGTTGACTCCCTGCGCAT TCTCACCCGCAATGACAGCTCG CTGACTGCCTAACAGCTACAAC GGTACCTCAGATCTCCAGCTGG AGCGCATGAACGTCTACTTCAA CGAGGTCAGTCACCAACTGTC CGCCCTTCACTGACTACTCCAA TGCTAATGCGCACCATAGGCCT CTGGTAACAAGTATGTCCCCCG TGCCGTCCTCGTCGACTTGGAG CCTGGTACCATGGACGCCGTC CGTGCTGGTCCCTTCGGTCAG CTCTTCCGTCCCGACAACTTC	GAAGTAGTACCAGTACATGACGACGTGAACGGTCAGG TTCAGGGTGATGGGAACCCATGAGACTGCGGTGTGGC CGAGAAGCTGGGTGAAGCAGAGGAGGGCAGTGGCAC CGTGGTGGTAGGTGTGGAGGAAAGCTAGGAGATCAGT CAGCTAAAGCATTGATTGTGGAAATATTGCGCGCTTAC TCAAAGGCTTCTTCTTGAGGAACAAGAAGCAGGTGTCA ATCAGCTCGAGGTACTTGGTGAGGTAGTTGAGCTGCC AGCTGTCAGTAAGCGCGTTCTGGCGCGGAATCAGAAA AACGTACGTAGTAAAGAACAACGAGCTTGTCAGTCCA GCCGCCCTCGTAGGCGCAGACAGCGTGGAAAATGCC GTTTCTGACAATCTCGGGCAGGAGCTGCTCGACGAAG AGAACCAAGAGAAGACCGCTGATGACGGTCAAGTAGA AGTTGTGGACCTTGAAGAAGAAGTTGAGCTTGAAAGG CTCGCGACCACGCATGAGCTCTCTGCCACCAAAGATG ACGATGTAGTAGGCGATGAGCATGGTGGCCGTCTCCT TGAAGGTAGCCATGGGGGTCTTTCCAGGGACGAAGCG GAAGTCCTGGGGCTTGTAGCCCTTGAG

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

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

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

*[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.