

ISOLATION AND CHARACTERIZATION OF 4-CHLOROANILINE-  
DEGRADING BACTERIA

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การแยกแยะที่เรียและการศึกษาลักษณะสมบัติในการย่อยสลาย4-คลอโรอะนีน

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สารในกลุ่มคลอโรอะนิลีนมีการใช้กันอย่างแพร่หลายในอุตสาหกรรมหลายประเภท เช่น ยาฆ่าแมลงและยากำจัดศัตรูพืช เป็นต้น ซึ่งแบคทีเรียมีความสามารถในการย่อยสลายนีลีนทำให้เกิดการสะสมของสารในกลุ่มฟีนอลยูเรีย เอซิดแอนิไลด์ และ ฟีนิลคาร์บาเมทซึ่งมีความเป็นพิษต่อสิ่งแวดล้อม จึงทำการคัดเลือกแบคทีเรียในดินจากพื้นที่เกษตรกรรมของประเทศไทยที่สามารถย่อยสลาย 4-คลอโรอะนิลีนได้ 3 สายพันธุ์ และจากการศึกษาลักษณะทางสัณฐานวิทยา คุณสมบัติทางชีวเคมี และการเปรียบเทียบลำดับ 16S rDNA จำแนกได้เป็น *Acinetobacter baumannii*, *Pseudomonas putida* และ *Klebsiella pneumoniae* โดยผลของ dechlorination เป็นตัวบ่งบอกถึงอัตราการย่อยสลายของ 4-คลอโรอะนิลีน จากการศึกษาความเข้มข้นที่เหมาะสมของ 4-คลอโรอะนิลีนพบว่า ที่ 25 พีพีเอ็ม (0.2 มิลลิโมลาร์) เป็นความเข้มข้นที่เชื้อสามารถเจริญและย่อยสลาย 4-คลอโรอะนิลีนได้ดีที่สุดในเวลา 12 วันที่ 30 องศาเซลเซียส แต่ที่ความเข้มข้น 200 พีพีเอ็มเชื้อไม่สามารถเจริญและย่อยสลาย 4-คลอโรอะนิลีนได้ เมื่อเลี้ยง *Acinetobacter baumannii*, *Pseudomonas putida* และ *Klebsiella pneumoniae* ในสภาพที่มีความเข้มข้นของ 4-คลอโรอะนิลีน 25 พีพีเอ็ม ที่อุณหภูมิ 30 องศาเซลเซียส เป็นเวลา 12 วัน พบว่ามีอัตราการย่อยสลายเท่ากับ  $61.00\% \pm 1.68$   $59.82\% \pm 0.71$  และ  $62.82\% \pm 3.87$  ตามลำดับ ในแบคทีเรียทั้ง 3 ชนิดสามารถตรวจพบแอกติวิตีสูงของเอนไซม์ chlorocatechol 1,2 dioxygenase ในขณะที่มีแอกติวิตีของ catechol 1,2 dioxygenase บ้างดังนั้นพบว่าเชื้อ *Acinetobacter baumannii*, *Pseudomonas putida* และ *Klebsiella pneumoniae* มีการย่อยสลายสาร 4-คลอโรอะนิลีนผ่านวิถี modified ortho-pathway นอกจากนี้แหล่งคาร์บอนหรือและไนโตรเจนที่เหมาะสมสำหรับการย่อยสลาย 4-คลอโรอะนิลีนสำหรับเชื้อ *Acinetobacter baumannii* คือที่ซีเตรทความเข้มข้นที่ 4 มิลลิโมลาร์, แอมโมเนียมคลอไรด์ ความเข้มข้นที่ 4 มิลลิโมลาร์ และ ซีเตรทความเข้มข้นที่ 4 มิลลิโมลาร์ผสม แอมโมเนียมคลอไรด์ความเข้มข้นที่ 4 มิลลิโมลาร์, สำหรับเชื้อ *Pseudomonas putida* คือที่ซีเตรทความเข้มข้นที่ 4 มิลลิโมลาร์และ ซักซิเนทความเข้มข้นที่ 4 มิลลิโมลาร์, สำหรับเชื้อ *Klebsiella pneumoniae* คือที่ความเข้มข้นที่ 1 มิลลิโมลาร์อะนิลีน, ความเข้มข้นที่ 2 มิลลิโมลาร์อะนิลีน, นอกจากนี้ แบคทีเรียทั้ง 3 ชนิด สามารถย่อยสลาย อะนิลีน, 2-คลอโรอะนิลีน, 3-คลอโรอะนิลีน, แต่ไม่สามารถย่อยสลาย 3,4-ไดคลอโรอะนิลีนได้

สาขาวิชา การจัดการสิ่งแวดล้อม  
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ลายมือชื่อนิสิต.....  
ลายมือชื่ออาจารย์ที่ปรึกษา.....

# # 4689482220: MAJOR ENVIRONMENTAL MANAGEMENT  
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WANSIRI PETCHKROH: ISOLATION AND CHARACTERIZATION OF 4-CHLOROANILINE-DEGRADING BACTERIA THESIS ADVISOR: ASSIST. PROF. ALISA VANGNAI, Ph.D., 208 pp. ISBN 974-14-3280-1.

Chloroanilines have been widely used in several industries such as insecticides and herbicides etc. It is also accumulated in the environment owing to the microbial degradation of various types of chemical substances, namely, herbicides, phenyl urea, acylanilide, and phenylcarbamate. Three bacterial strains enabled to degrade 4CA were isolated from Thailand agricultural soil. They were identified based on the basis of morphology, biochemical characteristics and comparison of 16S rDNA sequence. These strains were *Acinetobacter baumannii*, *Pseudomonas putida*, and *Klebsiella pneumoniae*. These bacteria showed the highest degradation rate and exhibited the percentage of 4-chloroaniline biodegradation were  $61.00\% \pm 1.68$ ,  $59.82\% \pm 0.71$ , and  $62.82\% \pm 3.87$ , respectively at 25 ppm (0.2 mM) of 4-chloroaniline which incubated for 12 days at 30°C. Furthermore, the dechlorination was monitored for the 4-chloroaniline degradation. *Acinetobacter baumannii*, *Pseudomonas putida*, and *Klebsiella pneumoniae* showed high chlorocatechol 1,2 dioxygenase activity and fair activity of catechol 1,2 dioxygenase. Therefore, the biodegradation of 4-chloroaniline by these three isolates was occurred via the modified ortho-pathway. Moreover, the additional carbon or/and nitrogen source to enhance 4-chloroaniline degradation are, for *Acinetobacter baumannii*: 4mM citrate, 4mM NH<sub>4</sub>Cl and 4mM citrate + 4mM NH<sub>4</sub>Cl; for *Pseudomonas putida*: 4 mM citrate and 4mM succinate, for *Klebsiella pneumoniae*: 1mM aniline and 2mM aniline. Furthermore, all isolates could degrade aniline, 2-chloroaniline, 3-chloroaniline but not degrade 3,4-dichloroaniline.

Field of Study: Environmental management

Academic Year: 2006

Student's signature.....

Advisor's signature.....

Wansiri Petchkroh

Alisa Vangnai



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## LIST OF ABBREVIATIONS

4CA	=	4-Chloroaniline
2CA	=	2-Chloroaniline
3CA	=	3-Chloroaniline
3,4DCA	=	3,4-Dichloroaniline
EPA	=	Environmental Protection Agency
ml	=	Milliliter
mg	=	Miligram
ppm	=	Part per million
OD	=	Optical density
rpm	=	Revolution per minute
RT	=	Room temperature
mm	=	Mineral medium
LB	=	Luria bertani
bp	=	base pairs
HPLC	=	High performance liquid chromatography
ISE	=	Ion selective electrode
nm	=	Nanometer
min	=	Minutes
h	=	Hour