

# Transfer of Degradation Capacity Between Microorganisms Treating a Persistent of Organic

陳盈盈、張玉明

E-mail: 9511339@mail.dyu.edu.tw

## ABSTRACT

The ability of an indigenous microbial population to degrade a persistent xenobiotic organic compound is generally acquired after the microbes are acclimated to the target compound. The degradation pathway is believed to be mediated by an extra chromosomal agent plasmids. Plasmid is free to transfer from the original host to neighboring cells, thus a xenobiotic degradation capacity can be transfer to population that has not previously been acclimated to the target. The purpose of this research was to investigate the extent to which an activated sludge acclimated to 2,4-D was to transfer its degradation ability horizontally to one not acclimated. We sifted out the single bacteria from the sludge that have a ability to degrade the 2,4-D, and identification of this pure bacteria. We were trying to transfer the degrade capacity to *Escherichia coli* and *Bacillus subtilis*, and investigate these can get capacity of degradation or not. The results showed that: 1) we got the pure bacteria that can degrade the 2,4-D. It ' s called *Bacillus cereus* after identification, and it can degrade 2,4-D very well. 2) After transferring, the degrade velocity of mixed bacterium are faster than single *Bacillus cereus*. The part of increase velocity is means acceptor of transfer already got the capacity of degradation.

Keywords : plasmid ; 2,4-D ; conjugation ; capacity of degradation ; horizontal transfer

## Table of Contents

封面內頁 簽名頁 授權書.....	iii 中文摘要.....
.....iv 英文摘要.....	v 誌謝.....
.....vi 目錄.....	vii 圖目錄.....
.....x 表目錄.....	xiv 第一章
前言 1.1 研究緣起.....	1 1.2 實驗內容.....
1.3 實驗目的.....	2 第二章 文獻回顧 2.1 分解能力之水平傳遞形
式.....	4 2.1.1 接合作用(conjugation).....
.....8 2.1.2 轉導作用(transformation).....	5 2.1.2 轉導作用(transformation).....
.....11 2.2 2,4-D之特性及背景資料.....	13 2.2 2,4-D之特性及背景資料.....
2.2.1 2,4-D之生物分解路徑.....	15 2.3 菌株之間分解污染物的交互作用 .
.....27 2.4 分解能力之來源.....	25 2.4 分解能力之來源.....
.....27 2.4.1 plasmid之簡介.....	27 2.4.2 具2,4-D分解能力之plasmid.....
.....28 第三章 研究方法 3.1 菌株來源.....	30 3.1.1 研究材料及儀器設備.....
.....31 3.1.2 使用藥品.....	31 3.1.2 使用藥品.....
.....31 3.1.3 研究使用材料.....	31 3.1.3 研究使用材料.....
.....31 3.1.4 研究使用儀器設備.....	34 3.2 研究架構.....
.....34 3.2 研究架構.....	36 3.3 菌株分解2,4-D
實驗.....	37 3.3.1 2,4-D之配製.....
.....37 3.3.2 營養鹽成分與配比.....	37 3.3.2 營養鹽成分與配比.....
.....39 3.4 2,4-D之評估項與方法.....	39 3.4.1 2,4-D含量之測量方式.....
.....39 3.4.2 2,4-D濃度量測.....	40 3.4.2 2,4-D濃度量測.....
.....40 3.4.3 菌體濃度測量方法.....	40 3.4.3 菌體濃度測量方法.....
.....41 3.5 分解2,4-D能力之水平傳遞接觸實驗.....	41 3.5.1 分解能力水平傳遞.....
.....41 3.5.2 分解能力水平	41 3.5.2 分解能力水平
傳遞之探討.....	44 3.6 <i>Escherichia coli</i> 菌接受分解能力傳遞後之獨立分解實 驗.....
.....45 3.6.1 <i>Escherichia coli</i> 菌獨立分解2,4-D之實驗.....	45 3.6.1 <i>Escherichia coli</i> 菌獨立進行分解實驗之結果分
.....45 3.6.2 <i>Escherichia coli</i> 菌獨立進行分解實驗之結果分	析.....
.....45 第四章 結果與討論 4.1 活性污泥之篩選.....	47 4.1 活性污泥之篩選.....
4.1.1 C1純菌之鑑定.....	49 4.1.1.1 菌種鑑定方法.....
.....49 4.1.1.2 菌種鑑定結果...	.....49 4.1.1.2 菌種鑑定結果...
.....50 4.2 2,4-D分解能力之水平傳遞.....	51 4.2.1 不同初始2,4-D濃度實驗組之分解能力
.....51 4.2.2 不同菌量實驗組之分解能力傳遞.....	55 4.3 分解能力傳遞接觸實驗後之純菌獨立分離.....
.....58 4.3.1 延長接觸時間後進行分離獨立分解實驗.....	60 第五章 結論與建議 5.1 結論.....
.....65 5.2 建議.....	66 參考文獻.....
.....68 圖目錄 圖2-1 接合作用示意圖.....	7 圖2-2 轉型合作用示意圖.....
.....10 圖2-3 轉導作用示意圖.....	12 圖2-4 2,4-D分子結構.....
.....13 圖2-5 2,4-D之生化分解途徑Pathway ( 1 ) .....	16 圖2-6 2,4-D之生化分解途徑 (
2,4-Dichlorophenoxyacetic acid to 2,4-Dichlorophenol and Glyoxylate ) .....	17 圖2-7
2,4-D之生化分解途徑 ( From 2,4-Dichlorophenol to 3,5-Dichlorocatechol ) .....	.....

.....17 圖2-8 2,4-D之生化分解途徑 (From 3,5-Dichlorocatechol to 2,4-Dichloro-cis, cis- muconate) .....	17
.....17 圖2-9 2,4-D之生化分解途徑 (From 2,4-Dichloro-cis,cis-muconate to trans-2-Chlorodiene -lactone) .....	17
.....18 圖2-10 2,4-D之生化分解途徑 (From trans-2-Chlorodienelactone to cis-2-Chlorodiene -lactone).....	18
.....18 圖2-11 2,4-D之生化分解途徑 (From cis-2-Chlorodienelactone to 2-Chloromaleylacetate) .....	18
.....18 圖2-12 2,4-D之生化分解途徑 (From 2-Chloromaleylacetate to Maleylacetate).....	19
.....19 圖2-13 2,4-D之生化分解途徑 (From Maleylacetate to 3-Oxadipate).....	19
.....19 圖2-14 2,4-D之生化分解途徑Pathway ( 2 ) .....	20
.....20 圖2-15 2,4-D之生化分解途徑 (From 2,4-Dichlorophenoxyacetic acid to 4-Chloro phenoxy -acetate).....	21
.....21 圖2-16 2,4-D之生化分解途徑 (From 4-Chlorophenoxyacetate to 4-Chlorophenol and Glyoxylate).....	21
.....21 圖2-17 2,4-D之生化分解途徑 (From 4-Chlorophenol to 4-Chlorocatechol).....	21
.....21 圖2-18 2,4-D之生化分解途徑 (From 4-Chlorocatechol to 3-Chloro-cis,cis-muconate).....	22
.....22 圖2-19 2,4-D之生化分解途徑 (From 3-Chloro-cis,cis-muconate to cis-4-Carboxymethyl -ebut-2-en-4-olide) .....	22
.....22 圖2-20 2,4-D之生化分解途徑 (From cis-4-Carboxymethylenebut-2-en-4-olide to Maleylacetate).....	22
.....22 圖2-21 2,4-D之生化分解途徑 (From Maleylacetate to 3-Oxadipate).....	22
.....22 圖2-22 2,4-D之生化分解途徑 (From 3-Chloro-cis,cis-muconate to 4-Methylenebut -2-en-4-olide).....	23
.....23 圖2-23 2,4-D之生化分解途徑 (From 4-Methylenebut-2-en-4-olide to cis-Acetylacrylate ).....	23
.....23 圖2-24 2,4-D之生化分解途徑 (From 4-Chlorocatechol to 5-Chloro-2-hydroxymuconic semialdehyde).....	24
.....24 圖2-25 2,4-D之生化分解途徑 (From 5-Chloro-2-hydroxymuconic semialdehyde to 2-Hydroxymuconic semialdehyde).....	24
.....24 圖2-26 2,4-D之生化分解途徑 (From 2-Hydroxymuconic semialdehyde to cis-2-Hydroxypenta-2,4-dienoate and Formate).....	24
.....24 圖2-27 2,4-D之生化分解途徑 (From cis-2-Hydroxypenta-2,4-dienoate to 4-Hydroxy- 2-oxovalerate).....	25
.....25 圖2-28 2,4-D之生化分解途徑 (From 4-Hydroxy-2-oxovalerate to Pyruvate and Acetaldehyde).....	25
.....25 圖3-1 實驗架構與流程.....	36
圖4.1 C1、C2及C3之2,4-D分解曲線.....	48
圖4.2 C1、C2及C3不同排列組合之2,4-D分解曲線.....	48
圖4.3 相同2,4-D濃度為50 mg/L之不同菌量比例(B:E).....	52
圖4.4 相同2,4-D濃度為50 mg/L之不同菌量比例(B:K).....	53
圖4.5 相同2,4-D濃度為200 mg/L之不同菌量比例(B:E).....	54
圖4.6 相同2,4-D濃度為200 mg/L之不同菌量比例(B:K).....	54
圖4.7 不同菌量濃度之分解能力傳遞 (1B:XE).....	55
圖4.8 不同菌量濃度之分解能力傳遞 (1B:XK).....	56
圖4.9 不同菌量濃度之分解能力傳遞 (XB:1E).....	57
圖4.10 不同菌量濃度之分解能力傳遞 (XB:1K).....	57
圖4.11 經過2,4-D分解能力傳遞之Escherichia coli菌.....	59
圖4.12 經過2,4-D分解能力傳遞之Escherichia coli菌.....	60
圖4.13 延長接觸時間之實驗 (B和E).....	61
圖4.14 延長接觸時間之實驗 (B和K).....	61
圖4.15 經過2,4-D分解能力傳遞之Escherichia coli菌.....	62
圖4.16 延長接觸時間之實驗 (B和E).....	63
圖4.17 延長接觸時間之實驗 (B和K).....	63
圖4.18 經過2,4-D分解能力再傳遞之各單一純菌.....	64
表目錄 表3-1 研究使用藥品清單.....	32
表3-2 研究使用材料清單.....	33
表3-3 研究使用儀器設備清單.....	35
表3-4 營養鹽配比.....	39
表3-5 不同初始2,4-D濃度對分解能力水平傳遞之影響實驗組合 .....	42
表3-6 不同具分解能力菌株量對分解能力水平傳遞實驗組合 .....	43
表3-7 不同不具分解能力菌株量對分解能力水平傳遞實驗組合 .....	44

## REFERENCES

- 參考文獻 1.Aly, O.M., and S.D Faust, (1964). Studies on the fate of 2,4-D and ester derivatives in natural surface waters. *Agric. Food Chem.* 12 (6) : 541-546. 2.Andrew CH, Harwood CS (2002). Chemotaxis of *Ralstonia eutropha* JMP134 (pJP4) to the Herbicide 2,4-Dichlorophenoxyacetate. *AEM.*68.968-972.2002. 3.Crosby, D. G., and H. O. Tutass. (1996). Photodecomposition of 2,4-Dichlorophenoxyacetic acid. *J. Agr. Food Chem.* 14(6) : 596-599. 4.Digiovanni G.D, Neilson J.W,Pepper,I.L,Sinclair N.A(1996). Gene Transfer of *Alcaligenes eutrophus* JMP134 Plasmid pJP4 to Indigenous Soil Recipients.*AEM.*62.7.2521-2526. 5.Don RH, Pemberton, JM(1985).Genetic and physical map of the 2,4-dichlorophenoxyacetic acid-degradative plasmid pJP4 161(1),466-468 6.Don RH, Weightman AJ, Knackmuss HJ, and Timmis KN (1985). Transposon mutagenesis and cloning analysis of the pathways for degradation of 2,4-dichlorophenoxyacetic acid and 3-chlorobenzoate in *Alcaligenes eutrophus* JMP 134(pJP4).161(1),85-90. 7.Don Rh, Pemberton JM(1981). Properties of six pesticide degradation plasmids isolated from *Alcaligenes paradoxus* and *Alcaligenes eutrophus*.*J Bacteriol.* 145,681-686. 8.Filer K, and Harker AR(1997). Identification of the Inducing Agent of the 2,4-Dichlorophenoxyacetic acid pathway Encoded by plasmid pJP4. *Journal of Envi. Microbio.*63(1),317-320. 9.Fulthorpe RR, McGowan C, Maltseva OV, Holben WE, Tiedje JM(1995). 2,4-dichlorophenoxyacetic acid-degrading Bacteria Contain Mosaics of Catabolic Genes.*AEM.*61.9.p.3274-3281. 10.Geerdink, M.J., van Loosdrecht M.C., and Luyben, K.Ch.A.(1996) Biodegradability of Diesel Oil, *Biodegradation*,7,73-81. 11.Halter, M,(1980).2,4-D in the aquatic environment. Section II in *Literature Reviews of Four Selected Herbicides:2,4-D,*

dichlobenil, diquat&endotall. Shearer R., and M. Halter, eds. 12. Hemmett, R.B. and S.D Faust, (1969). Biodegradation Kinetics of 2,4-dichlorophenoxyacetic acid by aquatic microorganisms. Residue.Rev. 29: 191-207 13. Jensen, H.L., (1957). Can. J. Microbiol. 3, 165. Johan HJ, Leveau, Alexander JB, Zehnder, and Jan Roelof van der Mee (1998). The tfdK Gene Product Facilitates Uptake of 2,4-dichlorophenoxyacetate by *Ralstonia eutropha* JMP134(pJP4) 180, 2237-2243. 14. Johnson. W.G., T.L. Lavy, and E.E. Gbur, (1995a). Persistence of Triclopyr and 2,4-D in Flooded and Non-Flooded Soil. Journal of Environmental Quality, 24(3) pp493-497. 15. Johnson. W.G., T.L. Lavy, and E.E. Gbur, (1995b). Sorption mobility, and degradation of triclopyr and 2,4-D and four soils. Weed Sci. 43:678-684. 16. Ka JO, Holben W.E, and Tiedje JM (1994). Genetic and phenotypic diversity of 2,4-dichlorophenoxyacetic acid (2,4-D)-degrading bacteria isolated from 2,4-D-treated field soils. Journal of Envir. Microbiol. 60(4), 1106-1115. 17. Neilson JW, Josephson KL, Pillai SD, and Pepper IL (1992). Polymerase chain reaction and gene probe detection of the 2,4-dichlorophenoxyacetic acid degradation plasmid, pJP4 Journal of Envir. 18. Newby DT, Josephson KL, and Pepper IL (2000). Detection and Characterization of plasmid pJP4 Transfer to Indigenous Soil Bacteria. Journal of Envir. Microbiol. 66, 290-296. 19. Newby DT, Gentry TJ, and Pepper IL (2000). Comparison of 2,4-dichlorophenoxyacetic acid Degradation and plasmid Transfer in Soil Resulting from Bioaugmentation with Two Different pJP4 Donors. Journal of Envir. Microbiol. 66, 3399-3407. 20. Que Hee, S.S., and R.G. Sutherland, (1981). The phenoxyalkanic Herbicides, Volume 1 :Chemistry, Analysis, and Environmental Pollution Press. Inc., Boca Raton, Florida 319 pgs. 21. Top EM, Maltseva OV, and Forney LJ (1996). Capture of a catabolic plasmid that encodes only 2,4-dichlorophenoxyacetic acid:alpha-ketoglutaric acid dioxygenase (TfdA) by genetic complementation. Journal of Envir. Microbiol. 62(7), 2470-2476. 22. Turnbull GA, and Morgan JAW (2001). Degradation of Substituted Phenylurea Herbicides by *Arthrobacter globiformis* Strain D47 and Characterization of a plasmid-Associated Hydrolase Gene, puhA. Journal of Envir. Microbiol. 67(5), 2270-2275. 23. 張紘偉 (2003), 氯酚分解的質體核酸(plasmid DNA) 量化分析, 大葉大學環境工程學系研究所碩士論文 24. 廖文景 (2005), 活性污泥接受分解能力水平傳遞之探討, 大葉大學環境工程學系研究所碩士論文