

氯酚分解的質體核酸(plasmid DNA)量化分析

張紘偉、張玉明

E-mail: 9221179@mail.dyu.edu.tw

摘要

本研究是質體核酸(plasmid DNA)來量化微生物對難分解有機物分解的能力。結果顯示:plasmid的產生,與微生物分解目標有機物二氯酚氧基乙酸(2,4-D)有一定的關係。微生物分解2,4-D過後,菌體中偵測出相對應的plasmid,這在許多的文獻中都已記載。在相關的文獻討論中,僅將特定的plasmid用來代表可否分解2,4-D,並沒有對分解能力的強弱作進一步的探討。本研究的結果顯示,在微生物分解2,4-D過後,會產生90kb的plasmid,與諸多文獻中的plasmid pJP4相似。本研究的後段實驗中,便是以分解2,4-D後所產生的90kb plasmid,來量化微生物分解2,4-D的能力;量化成功在處理2,4-D的應用上,有很深的意義。在微生物分解與處理2,4-D的過程,會遭遇不同的階段,分別是:馴化分解階段、分解能力退化階段,及再次分解2,4-D階段。研究結果顯示,單位菌體中所具有分解plasmid量越多,分解2,4-D的能力越強。在馴化分解階段結束時,菌體中具有分解plasmid最多,但如果長時間沒有接觸2,4-D,菌體中的分解plasmid會減少,代表微生物產生分解2,4-D的能力是會消退(消失)的。Plasmid與分解能力的正比關係,當可用來作為量化的工具;本研究最後將量化的結果作實用性的討論,希望能改善2,4-D所造成的嚴重污染。

關鍵詞: plasmid; 馴化; 退化; 分解能力

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參考文獻

王三郎 (1997),應用微生物學 林志勇 (2002),生物分解能力之化學計量,大葉大學環境工程學系碩士論文 Amy PS, Schulke JW, Frazier LM, and Seidler RJ (1985). Characterization of aquatic bacteria and cloning of genes specifying partial degradation of 2,4-dichlorophenoxyacetic acid. *Appl Environ Microbiol.* 49,1237-1245 Brunner W, Staub D, and Leisinger T (1980). Bacterial Degradation of Dichloromethane. *Appl. Environ. Microbiol.* 40(5), 950-958 Cookson J T (1995). Bioremediation Engineering: Design and Application, McGraw-Hill, Inc., New York Chang HL, and Alvarez CL (1995). Transformation Capacities of Chlorinated Organics by Mixed Culture Enriched on Methane, Propane, Toluene, or Phenol. *Biotechnol. Bioeng.* 45(5), 440-449 Don RH, Pemberton JM (1981). Properties of six pesticide degradation plasmids isolated from *Alcaligenes paradoxus* and *Alcaligenes eutrophus*. *J Bacteriol.* 145,681-686 Don RH, Pemberton, JM (1985). Genetic and physical map of the 2,4-dichlorophenoxyacetic acid-degradative plasmid pJP4. 161(1),466-468 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* JMP134(pJP4). 161(1),85-90 Dejonghe W, and Top EM (2000). Effect of Dissemination of 2,4-Dichlorophenoxyacetic Acid (2,4-D) Degradation Plasmids on 2,4-D Degradation and on Bacterial Community Structure in Two Different Soil Horizons. *Journal of Envir. Microbio.* 66(8),3297-3304 Fisher PR, Appleton J, and Pemberton JM (1978). Isolation and characterization of the pesticide-degrading plasmid pJP1 from *Alcaligenes paradoxus*. *J Bacteriol* 135,798-804 Furukawa K, and Chakrabarty AM (1982). Involvement of plasmids in total degradation of chlorinated biphenyls. *Appl Environ Microbiol.* 44,619-626 Filer K, and Harker AR (1997). Identification of the Inducing Agent of the 2,4-Dichlorophenoxyacetic Acid Pathway Encoded by Plasmid pJP4. *Journal of Envir. Microbio.* 63(1),317-320 Ghosal D, You IS, Chatterjee DK, and Chakrabarty AM (1985). Microbial degradation of halogenated compounds. *Science* 228,135-142 Hartmann J, Reinke W, and Knackmuss HJ (1979). Metabolism of 3-chloro, 4-chloro, and 3,5-dichlorobenzoate by a *Pseudomonad*. *Appl Environ Microbiol.* 37,421-428 Heiger DN (1992). High Performance Capillary Electrophoresis — An Introduction., 2th ed. Hewlett-Packard Company. Hawkins AC, and Harwood CS (2002). Chemotaxis of *Ralstonia eutropha* JMP134(pJP4) to the Herbicide 2,4-Dichlorophenoxyacetate. *Journal of Envir. Microbiol.* 68(2),968-972 Hu HY, Nozawa M, Fujie K, Makabe T, and Urano K (1996). Studies of microbial acclimation to hard chemicals on the basis of respiratory quinone profiles and kinetic analyses. *Wat.Sci.Tech.* 34,249~256 Hu HY, Nozawa M, Fujie K, Makabe T, and Urano K (1998). Analysis of microbial acclimation to refractory chemicals in wastewater using respiratory quinone profiles. *Wat.Sci.Tech.* 37, 407~411 Hu HY, Nozawa M, Fujie K, Makabe T, and Urano, K (1998). Effects of biodegradable substrates and microbial concentration on the acclimation of microbes to acrylonitrile in aerobic submerged biofilter. *Wat.Sci.Tech.* 38, 81~89 Innis MA, and Gelfand H (1990). Optimization of PCRs. In *PCR Protocols: A Guide to Methods and Applications*, ed. Janssen DB, Schepers A, Dijkhuizen L, and Witholt B (1985). Degradation of Halogenated Aliphatic Compounds by *Xanthobacter autotrophicus* GJ10. *Appl. Environ. Microbiol.* 49(3), . 673-677 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 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 Latorre J, Reineke W, and Knackmuss H (1984). Microbial metabolism of chloranilines: enhanced evolution by natural genetic exchange. *Arch Microbiol.* 140,159-165 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. Microbiol.* 58(4),1271-1275 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 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 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

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

Pierce G.JB, and Colaruotole JR (1983). Substrate diversity of *Pseudomonas* spp. Containing chlorotoluene degradative plasmids. Dev. Ind. Microbiol. 24,499-507

Verhagen C, Smit E, Janssen DB, and Van EJD (1995). Bacterial Dichloroproene degradation in soil, screening of soils and involvement of plasmids carrying dhla gene. Soil BW Biochem. 27(12),1547-1557