

Effect of Soil Bacterial Communities by Paclobutrazol

王彥文、劉淑瑛；林重宏

E-mail: 9806546@mail.dyu.edu.tw

ABSTRACT

Paclobutrazol (PP333) is a plant growth retardant, due to its known function on the inhibition of Gibberellins acid (GA) biosynthesis. In agricultural practices paclobutrazol applied to arrest the vegetative growth so as to increase the reproductive growth of many orchard as well as grain crops. However, paclobutrazol is very stable in soil, overdose or dose accumulation would cause the accumulation of paclobutrazol residue in soil resulting in the extensive inhibition of plant growth and the inhibition even lasting to the following plant cultivation. This study focus on the comparison of field soil bacterial communities treated with paclobutrazol. Denaturing gradient gel electrophoresis (DGGE) was applied to the analysis the soils were collected from paclobutrazol contaminated mango and waxapple orchards (Southern Taiwan) and paclobutrazol applied peanut fields (Central Taiwan), followed by the DNA extraction, PCR amplification of 16S rRNA gene fragments and PCR products purification. Analysis on DGGE patterns of 16S rRNA gene fragments suggests that two separated groups can be identified, soil bacteria from paclobutrazol contaminated orchard of southern Taiwan and soil bacteria from paclobutrazol applied peanut field of central Taiwan. Further purification and sequence analysis of DGGE bands followed by phylogenetic tree assay showed similar results. Mung bean plants were treated with paclobutrazol for three consecutive seasons and each time the soil samples were collected and analyzed. With paclobutrazol treatment, bacteria Proteobacteria would account for the majority. After paclobutrazol treatment, the soil bacterial diversity reduced 33%.

Keywords : Paclobutrazol、 Denaturing gradient gel electrophoresis (DGGE)、 bacterial diversity、 agricultural soil

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.....	xi
1. 緒論.....	1
1.1 前言.....	1
1.2 Paclobutrazol 簡介.....	2
1.3 土壤微生物.....	4
1.4 變性梯度凝膠電泳介紹.....	5
1.5 研究目的與動機.....	6
2. 材料與方法.....	7
2.1 培養基配置.....	7
2.2 土壤樣品來源.....	8
2.3 土壤 pH 值.....	9
2.4 篩選菌株.....	9
2.5 培養篩選菌株.....	10
2.6 生物活性分析.....	10
2.7 土壤細菌 DNA 萃取.....	11
2.8 聚合?連鎖反應.....	11
2.9 純化 PCR 產物.....	13

2.10變性梯度凝膠電泳分析.....	14
2.10.1 變性梯度凝膠電泳操作步驟.....	14
2.10.2 DGGE 膠體圖分析.....	16
2.11 轉殖作用 (Cloning).....	17
2.11.1 載體 (Vector).....	17
2.11.2 接合作用 (ligation).....	17
2.11.3 轉型作用 (transformation).....	17
2.11.4 抽取質體.....	17
2.11.5 親緣關係樹分析.....	18
3. 結果.....	20
3.1 Paclobutrazol 標準品.....	20
3.2 Paclobutrazol 濃度對生物活性測試.....	20
3.3 土壤微生物培養.....	21
3.4 菌株的生物活性分析.....	22
3.5 土壤細菌菌相.....	23
3.5.1 DGGE bands 分析.....	24
3.5.2 親源關係樹分析.....	24
3.6 土壤微生物菌相變化.....	26
3.6.1 DGGE bands 分析.....	26
3.6.2 親源關係樹分析.....	27
4. 討論.....	29
4.1 篩選 Paclobutrazol 菌株培養.....	29
4.2 污染土壤中微生物活動之探討.....	29
4.3 Paclobutrazol 對土壤中微生物活動之探討.....	30
5. 結論.....	33
參考文獻.....	73
附錄.....	79

圖目錄

圖1. Paclobutrazol 結構式.....	34
圖2. 土壤以 0.1% Paclobutrazol 處理之生長情形.....	35
圖3. Paclobutrazol 標準曲線.....	36
圖4. 不同濃度的 Paclobutrazol 之綠豆生物活性分析..	37
圖5. 細菌生長情形 (控制組).....	38
圖6. 細菌生長情形 (蓮霧土壤).....	39
圖7. 細菌生長情形 (芒果土壤).....	40

圖8. 細菌生長情形 (花生土壤).....	41
圖9. 檢測菌株之綠豆生物活性分析.....	42
圖10. 以 DGGE分析控制組、污染土和正處理中之土壤細菌菌相.....	43
圖11. 以 Jaccard ' s 係數分析土壤 DNA bands.....	44
圖12. 土壤微生物序列之親緣關係樹.....	45
圖13. 以 DGGE 分析 Paclobutrazol 處理之土壤細菌菌相變化.....	49
圖14. 以 Jaccard ' s 係數分析土壤 DNA bands.....	50
圖15. 土壤微生物序列之親緣關係樹.....	51
圖16. 經 Paclobutrazol 處理的土壤菌相比例.....	56

表目錄

表1. PCR 引子序列.....	57
表2. 控制組、污染土和正處理中之 16S rDNA 序列分析比對所得最相似序列與相似度.....	58
表3. 未處理土 (3) 之 16S rDNA 序列分析比對所得最相似序列與相似度.....	62
表4. 處理土 (9) 之 16S rDNA 序列分析比對所得最相似序列與相似度.....	64
表5. 未處理土 (12) 之 16S rDNA 序列分析比對所得最相似序列與相似度.....	66
表6. 處理土 (21) 之 16S rDNA 序列分析比對所得最相似序列與相似度.....	68
表7. 未處理土 (24) 之 16S rDNA 序列分析比對所得最相似序列與相似度.....	70
表8. 土壤微生物菌相分析.....	72

REFERENCES

- 莊智翔。2009。健康與疾病微孔珊瑚伴生之細菌相。國?東華大學海洋生物科技研究所碩士論文，花蓮。
- 黃敏展。1998。矮化劑在花卉上之應用。李信山、張村仁、林嘉興等主編植物生長調節劑在園藝作物之應用研討會專輯。台中區農業改良場出版，P.141-159。
- 費雯綺、王喻其編輯。2004。植物保護手冊。農委會農業藥物毒物試驗所編印，P.801-822。
- 楊秋忠。2004。土壤與肥料 第八版。農業世界叢書，P.398-399。
- 鍾旻潔。2002。溫度、季節、容器大小與矮化劑對盆菊生育及品質之影響。國立台灣大學園藝學研究所碩士論文，台北，pp.128。
- Alexander, M. 1977. Introduction to soil microbiology. 2nd ed. Wiley, New York.
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. 1990. Basic local alignment search tool. J. Mol. Biol. 215: 403-410.
- Aron, Y., Monselise, S. P., Goren, R. and Costo, J. 1985. Chemical control of vegetative growth in citrus trees by paclobutrazol. HortScience 20: 96-98.
- Atlas, R. M. and Bartha, R. 1998. Microbial ecology: Fundamentals and applications. 4th ed. Benjamin/Cummings Pub. CO., Inc., California.
- Bano, N. and Hollibaugh, J. T. 2002. Phylogenetic composition of bacterioplankton assemblages from the Arctic Ocean. Appl. Environ. Microbiol. 68: 505-511.
- Barrett, J. E. 1982. Chrysanthemum height control by ancymidol, PP333 and EL-500 dependent on medium composition. HortScience 17: 896-897.
- Borneman, J., Skroch, P. W., O ' Sullivan, K. M., Palus, J. A., Rumjanek, N.G., Jansen, J. L., Nienhuis, J. and Triplett, E. W. 1996. Molecular microbial diversity of an agricultural soil in Wisconsin. Appl. Environ. Microbiol. 62: 1935 – 1943.
- Buitron, G., Gonzalez, A. and Lopez-Marin, L. M. 1998. Biodegradation of phenolic compounds by an acclimated activated sludge and isolated bacteria. Wat. Sci. Tech. 37: 371-378.
- Cathey, H. M. 1975. Comparative plant growth retarding activities of ancymidol with ACPC, phosfon, chlormequat and SADH on ornamental plant species. HortScience 10: 204-216.
- Cole, J. R., Chai, B., Farris, R. J., Wang, Q., Kulam, S. A., McGarrell, D. M., Garrity, G.

M. and Tiedje, J. M. 2005. The Ribosomal Database Project (RDP-II): sequences and tools for high-throughput rRNA analysis. *Nucleic Acids Res.* 33: 294-296.

16. Croker, S. J., Gaskin, P., Beale, M. H. and Lenton, J. R. 1995. Ent-3-hydroxykaur-16-ene and ent-17-hydroxykaur-15-ene in paclobutrazol-treated wheat seedlings. *Phytochemistry* 39: 11-14.

17. Erez, A. 1984. Dwarfing peaches by pruning and by paclobutrazol. *Acta Hort.* 146: 235-241.

18. Fernandez, J. A., Balenzategui, L., Banon, S. and Franco, J. A. 2006. Induction of drought tolerance by paclobutrazol and irrigation deficit in *Phillyrea angustifolia* during the nursery period. *Sci. Hort.* 107: 277-283.

19. Fierer, N. and Jackson, R. B. 2006. The diversity and biogeography of soil bacterial communities. *Proc. Natl. Acad. Sci.* 103: 626-631.

20. Gopi, R., Jaleel, C. A., Sairam, R., Lakshmanan, G. M. A., Gomathinayagam, M. and Panneerselvam, R. 2007. Differential effects of hexaconazole and paclobutrazol on biomass, electrolyte leakage, lipid peroxidation and antioxidant potential of *Daucus carota* L. *Colloids Surf B Biointerfaces.* 60: 180-186.

21. Gray, N. D., Miskin, I. P., Kornilova, O., Curtis, T. P. and Head, I. M. 2002. Occurrence and activity of archaea in aerated activated sludge wastewater treatment plants. *Environ. Microbiol.* 4: 158-168.

22. Haigler, B. E., Nishino, S. F., and Spain, J. C. 1988. Degradation of 1, 2-dichlorobenzene by a *Pseudomonas* sp. *Appl. Environ. Microbiol.* 54: 294-301.

23. Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41: 95-98.

24. Jackson, M. J., Line, M. A. and Hasan, O. 1996. Microbial degradation of a recalcitrant plant growth retardant - paclobutrazol (PP333). *Soil Biol. Biochem.* 28: 1265-1267.

25. Jacyna, T. and Dodds, K. G. 1995. Some effects of soil-applied paclobutrazol on performance of 'Sundrop' apricot (*Prunus armeniaca* L.) trees and on residue in the soil. *New Zealand J. Hort. Sci.* 23: 323-329.

26. Jennings, J. C., Coolbaugh, R. C., Nakata, D. A. and West, C. A. 1993. Characterization and solubilization of kaurenoic acid hydroxylase from *Gibberella fujikuroi*. *Plant Physiol.* 101: 925-930.

27. Kishorekumar, A., Jaleel, C. A., Manivannan, P., Sankar, B., Sridharan, R. and Panneerselvam, R. 2007. Comparative effects of different triazole compounds on growth, photosynthetic pigments and carbohydrate metabolism of *Solenostemon rotundifolius*. *Colloids Surf. B: Biointerf.* 60: 207-212.

28. Kumar, S., Tamura, K. and Nei, M. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief. Bioinformatics* 5: 150-163.

29. Kuske, C. R., Barns, S. M. and Busch, J. D. 1997. Diverse uncultivated bacterial groups from soils of the arid southwestern United States that are present in many geographic regions. *Appl. Environ. Microbiol.* 63: 3614-3621.

30. Lin, K. H. R., Tsou, C. C., Hwang, S. Y., Chen, L. F. O. and Lo, H. F. 2006. Paclobutrazol pre-treatment enhanced flooding tolerance of sweet potato. *J. Plant Physiol.* 163: 750-760.

31. Luster, D. G. and Miller, P. A. 1993. Triazole plant growth regulator binding to native and detergent-solubilized plant microsomal cytochrome P450. *Pestic. Biochem. Phys.* 46: 27-39.

32. Macur, R. E., Wheeler, J. T., Burr, M. D. and Inskeep, W. P. 2007. Impacts of 2, 4-D application on soil microbial community structure and on populations associated with 2, 4-D degradation. *Microbiol. Res.* 162: 37-45.

33. Menhenett, R. 1984. Comparison of a new triazole retardant paclobutrazol (PP333) with ancymidol, chlorophonium chloride, B-9, and inflorescence development in *Chrysanthemum morifolium* Ramat. *Sci. Hort.* 24: 349-358.

34. Muyzer, G., de Waal, E. C. and Uitterlinden, A. G. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* 59: 695-700.

35. Norris, T. B., Wraith, J. M., Castenholz, R. W. and McDermott, T. R. 2002. Soil microbial community structure across a thermal gradient following a geothermal heating event. *Appl. Environ. Microbiol.* 68: 6300-6309.

36. N?bel, U., Engelen, B., Felske, A., Snaide, J., Wieshuber, A., Amann, R. I., Ludwig, W. and Backhaus, H. 1996. Sequence heterogeneities of genes encoding 16S rRNA in *Paenibacillus polymyxa* detected by temperature gradient gel electrophoresis. *J. Bacteriol.* 178: 5636-5643.

37. Reineke, W. and Knackmuss, H. J. 1984. Microbial metabolism of haloaromatics: isolation and properties of a chlorobenzene-degrading bacterium. *Appl. Environ. Microbiol.* 47: 395-402.

38. Ridgway, H. F., Safarik, J., Phipps, D., Carl, P. and Clark, D. 1990. Identification and catabolic activity of well-derived gasoline-degrading bacteria from a contaminated aquifer. *Appl. Environ. Microbiol.* 56: 3565-3575.

39. Sharma, D. and Awasthi, M. D. 2005. Uptake of soil applied paclobutrazol in mango (*Mangifera indica* L.) and its persistence in fruit and soil. *Chemosphere* 60: 164-169.

40. Silva, C. M. M. S., Vieira, R. F. and Nicoletta, G. 2003. Paclobutrazol effects on soil microorganisms. *Appl. Soil Ecol.* 22: 79-86.

41. Singh, V. K. and Bhattacharjee, A. K. 2005. Genotypic response of mango yield to persistence of paclobutrazol in soil. *Sci Hort.* 106: 53-59.

42. Smit, E., Leeflang, P., Gommans, S., van den Broek, J., van Mil, S. and Wernars, K. 2001. Diversity and seasonal fluctuations of the dominant members of the bacterial soil community in a wheat field as determined by cultivation and molecular methods. *Appl. Environ. Microbiol.* 67: 2284-2291.

43. Smith, M. D., Hartnett, D. C. and Rice, C. W. 2000. Effects of long-term fungicide applications on microbial properties in tallgrass prairie soil. *Soil Biol. Biochem.* 32: 935-946.

44. Spain, J. C. and Nishino, S. F. 1987. Degradation of 1, 4-dichlorobenzene by a *Pseudomonas* sp. *Appl. Environ. Microbiol.* 53: 1010-1019.

45. Steffens, G. L. and Wang, S. Y. 1985. Persistence of several triazole GA biosynthesis inhibitors for retarding growth of young apple plants. *Proc. Plant Growth Regul. Soc. Am.* 12: 248.

46. Sterrett, J. P. 1985. Paclobutrazol: a promising growth inhibitor for injection into woody plants. *J. Am. Soc. Hortic. Sci.* 110: 4-8.

47. Storey, G. K. and Gardner, W. A. 1986. Sensitivity of the entomogenous fungus *Beauveria bassiana* to selected plant growth regulators and spray additives. *Appl. Environ. Microbiol.* 52: 1-3.

48. Sun, H. Y., Deng, S. P. and Raun, W. R. 2004. Bacterial community structure and diversity in a century-old manure-treated agroecosystem. *Appl. Environ. Microbiol.* 70: 5868-5874.

49. Tayama, H. K. and Carver, S. A. 1992. Residual efficacy of uniconazole and daminozide on potted 'Bright Golden Anne' *Chrysanthemum*. *HortScience.* 27: 124-127.

50. Thompson, I. P., Bailey, M. J., Ellis, R. J., Maguire, N. and Meharg, A. A. 1999. Response of soil microbial communities to single and multiple doses of an organic pollutant. *Soil Biol. Biochem.* 31: 95-105.

51. Thompson, I. P., Higgins, D. G. and Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research.* 22: 4673-4680.

52. Torsvik, V., Goksoyr, J. and Daae, F. L. 1990. High diversity in DNA of soil bacteria. *Appl. Environ. Microbiol.* 56: 782-787.

53. Wang, L. W. and Lin, C. H. 1992. The effect of GA-Biosynthesis inhibitors on plant physiology. *Weed Sci. Bull.* 13: 31-39.

54. Wang, S.

Y., Sun, T., Ji, Z. L. and Faust, M. 1987. Effect of Paclobutrazol on water stress-induced abscisic acid in apple seedling leaves. *Plant Physiol.* 84: 1051-1054.55. Ward, D. M., Weller, R. and Bateson, M. M. 1990. 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. *Nature.* 345: 63-65.56. Xi, H. F., Ye, Q. F., Shen, H. C. and Zhou, W. J. 1995. Uptake of MET by the leaf and its residue in the rapeseed plant and the soil. *Oil Crops of China* 17: 33-35.