羽毛分解菌應用於微生物肥料之開發研究

曾宥綡[1,2] 郭雅紋[2] 陳鴻堂[2]

摘要

本研究篩選具溶磷能力之羽毛分解菌株，經 16S rDNA 序列分析其菌株 TCC-2 及 TC4-1C 屬於節桿菌屬 (Genus *Arthrobacter*)，而菌株 PiA 則屬於巨大芽孢桿菌 (*Bacillus megaterium*)。菌株 TCC-2、TC4-1C 及 PiA 經培養於 1%羽毛之礦物鹽培養基 (BHF medium)至第 4 天，其羽毛分解率分別為 65.3%、62.9%及 44.3%，且羽毛分解液之 pH、EC、銨離子及全氮含量皆明顯提升。篩選之菌株具有溶解磷礦石粉與磷酸三鈣之能力，其中以菌株 PiA 溶解磷酸三鈣能力較佳 (71.3 mg/L，培養 4 天)，而菌株 TC4-1C 溶解磷礦石粉能力較佳 (5.04 mg/L，培養 4 天)。菌株 TCC-2 及 TC4-1C 可於 3% 氯化鈉濃度下生長，其中菌株 TC4-1C 可於 1%台肥即溶 5 號 (10-20-20)中有效分解羽毛，具有應用生成高養分有機液肥之潛力。篩選之 3 菌株皆可於磷礦粉及草木灰之培養基中分解羽毛，可應用於有機農業之養分補充。

關鍵詞:羽毛分解菌、溶磷菌、有機液肥

前言

氮為作物生長所需，有機栽培常以海鳥糞或豆粕做為氮肥補充，然而因價格高昂，羽毛可做為替代氮源，提供作物所需氮素 (Hadas and Kautsky, 1994)，然而因羽毛角蛋白質含量豐且其雙硫鍵多結構緊密，因此不易分解，無法快速釋放氮素供作物利用，另應用於飼料添加之研究指出，以高溫高壓處理羽毛以作為飼料添加，常因胺基酸結構破壞，如 methionine、lysine 及 tryptophan 易受破壞，而導致其作為飼料添加之營養價值減損 (Wang and Parsons, 1997)。微生物轉換羽毛廢棄物將可避免胺基酸遭破壞，可提高飼料添加之價值或作為相對速效之氮質肥料施用 (Bertsch and Coello, 2005; Choi, and Nelson, 1996; Gupta and Ramnani, 2006; Hadas and Kautsky, 1994; Kim et al., 2005; Onifade et al., 1998)。

磷為作物生長所需之必要元素，然因磷肥施用至土壤後易導致磷酸鈣、磷酸鋁及磷酸鐵沉澱而降低磷之植物有效性 (Alam and Ladha, 2004; Vassilev and Vassileva, 2003)，若因磷之植物有效性低而大量施用磷肥，則會導致施肥成本增加且造成環境汙染 (Reddy et al., 2002)。溶磷菌可經由產酸、鉗合及氧化還原等作用而溶解難溶性磷(Chung et al., 2005; Gulati et al.,

1.通訊作者
2.農委會臺中區農業改良場助理研究員、助理研究員、助理研究員

2010)。近來由於肥料價格高漲及其他產業對磷礦的需求增加，如食物防腐添加、殺真菌劑應用、製陶業、冶金業等，使得高品質磷礦分配至農業應用的機會降低，因此以溶磷菌進行土壤磷之溶出以提高作物利用率，有利於達農業之永續發展 (Gyaneshwar et al., 2002)。

材料與方法

具溶磷能力之羽毛分解菌株之篩選

本研究採取中興大學中興湖周邊之土壤，添加10 g土壤於95 ml含有1% 雞羽毛 (取自興中台股份有限公司)、及0.5%磷酸三鈣 (Calcium phosphate tribasic, KATAYAMA) 之去離子水中，經室溫培養5天後以十倍連續稀釋塗抹於BHF (1% 羽毛， w/v) 固態培養基中，BHF培養基為參考BH基本礦物鹽培養基 (Bushnell and Haas, 1941)，並經修改其配方為每1L去離子水含有 1 g 磷酸氫二鉀、1 g 磷酸二氫鉀、0.2 g 硫酸鎂 (含7個結晶水)及0.02 g 氯化鈣並添加1% (w/v)之羽毛。羽毛先經由刀片切割機切割約20秒後備用。經培養3-5天後，進行純化培養並挑選單一菌落接種至液態磷酸三鈣或磷礦石粉 (Rock-P, CCM FERTILIZER Sdn Bhd) 培養基，經培養4天後以鉬藍法 (Watanabe and Olsen, 1965)進行水溶性磷含量分析，以篩選溶磷菌株並分析其溶磷量。磷酸三鈣培養基及磷礦石粉培養基之配方參照肥料檢驗方法 (方法編號AFS3183-1)。接種篩選之溶磷菌株單一菌落至BHF液態培養基中，於室溫培養4天後觀察羽毛分解狀況，以篩選出具溶磷能力之羽毛分解菌。上述培養基以高溫高壓滅菌20分鐘後備用。固態培養基配製則添加17 g/L 洋菜膠。

篩選之菌株16S rDNA基因序列分析

篩選菌株接種於5 mL NB (nutrient broth, Difco) 培養基中，於室溫培養24小時後，以MO BIO 公司之商業套組 (UltraClean^{TM} Microbial Genomic DNA isolation Kit, MO BIO Laboratories, INC., USA) 進行細菌 DNA 萃取。抽取之DNA以引子1F (5’ GAG TTT GAT CAT GGC TCA G 3’)及9R (5’ AAG GAG GTG ATC CAA CCG CA 3’) (Kämpfer et al., 2003; Shen et al., 2005) 進行PCR以放大16S rDNA。委外進行定序分析，取得序列後於 GenBank (NCBI) 進行序列比對，以初步確認篩選菌株之菌屬。

篩選菌株之耐鹽能力分析

菌株耐鹽能力分析以配製1%、2%、3%、4%、5%及6%之氯化鈉之NB (nutrient broth)培養基，經接種單一菌落培養18小時後測定其培養液之OD600值。

羽毛分解率及分解液之pH、EC、元素養分分析

本研究接種篩選菌株之單一菌落於50 mL羽毛培養基 (BHF)中，於30℃及120 rpm 震盪培養4天後進行分析。其培養液經預先以70℃烘乾至恆重之Whatman No.1濾紙過濾後，其濾液以電極分析pH及EC，銨態氮及全氮以微量擴散法測定 (Keeney and Nelson, 1982)，鉀以火焰光度計測定 (Sherwood flam photometer 410)，磷以鉬藍法測定 (Watanabe and Olsen. 1965)而鈣

及鎂則用原子吸收光譜儀 (Hitachi Polarized Zeeman Atomic absorption spectrophotometer Z-5000) 分析。濾紙上殘留羽毛經70℃烘乾至恆重後秤重，經與對照處理 (未接菌之羽毛培養基) 之殘留重量比較後，計算羽毛分解率。以上處理皆以3重複進行試驗。並分析羽毛於不同資材中之分解率及濾液元素養份分析。

結果與討論

本研究篩選 3 株具溶磷能力之羽毛分解菌株，經 16S rDNA 序列分析，其中菌株 TCC-2 及 TC4-1C 屬於節桿菌屬 (genus *Arthrobacter*)，而菌株 PiA 經 16S rDNA 序列分析及委託食品工業發展研究所進行菌種鑑定為巨大芽孢桿菌 (*Bacillus megaterium*)，另 2 菌株之菌種仍在食工所分析中。此 3 菌株於磷礦石粉固態培養基中可顯示出透明環 (圖 1)，於磷酸三鈣及磷礦石粉液態培養基之溶磷能力如表一，以菌株 PiA 溶解磷酸三鈣效果較佳而菌株 TC4-1C 溶解磷礦石粉能力較佳。菌株培養於基本礦物鹽羽毛培養基之羽毛分解率分析 (表二)，顯示以節桿菌屬之菌株 TCC-2 及 TC4-1C 之羽毛分解能力較佳，其羽毛分解液之 pH、EC、銨離子及全氮含量皆明顯提升，顯示菌株可以羽毛為碳及氮源，並逐步分解羽毛角蛋白質。菌株耐鹽特性分析顯示菌株 TCC-2 及菌株 TC4-1C 可於 3%氯化鈉 NB 培養基中生長，其 OD600 值與不添加氯化鈉者相近，顯示於此氯化鈉濃度下不影響菌株本身生長特性，其中以菌株 TCC-2 之耐鹽能力最佳，於 6% 氯化鈉之培養基中，其 OD600 值仍有 1.5，其次為菌株 TC4-1C 而菌株 PiA 則於 1%氯化鈉培養基中生長明顯受抑制，於 3%氯化鈉濃度下則完全無法生長，不具耐鹽能力 (圖 2)，顯示菌株 TCC-2 及 TC4-1C 具有應用於與化學肥料混合施用之潛力，另經研究發現菌株 TC4-1C 可於 1%台肥即溶 5 號培養基中有效分解羽毛 (表三)，以羽毛作為碳源，並在含可溶性氮源情況下有效分解羽毛，可應用於生成高養分並富含胺基酸之液肥，有利提高此菌株於田間之應用性，推測此耐鹽菌於田間高化肥濃度培養環境下亦具有降低雜菌影響之效果，有助於此菌株功能之表現。另此 3 菌株皆可於 1%羽毛 0.5%磷礦粉及 0.5%草木灰中分解羽毛 (表四)，可生成適用於有機栽培之液肥，推測可藉由菌株之蛋白質分解能力及溶磷能力，提高有機栽培中豆粕之分解率及土壤溶磷效果。本研究篩選之菌株可應用於羽毛轉化再利用並以菌株本身之溶磷能力，提高土壤磷肥有效性，達到農業環境資源永續利用之目標。

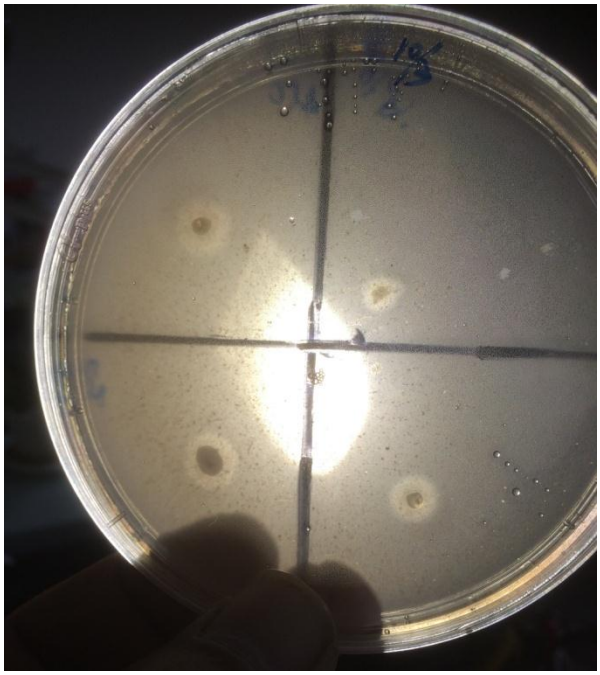

圖 1.篩選菌株於磷礦石粉培養基之溶磷狀況，左上為菌株 TC4-1C、右上為菌株 PiA、左下為菌株 TCC-2

Fig. 1. Isolates inoculated in the rock phosphate medium, top left is TC4-1C, top right is PiA and down left is TCC-2

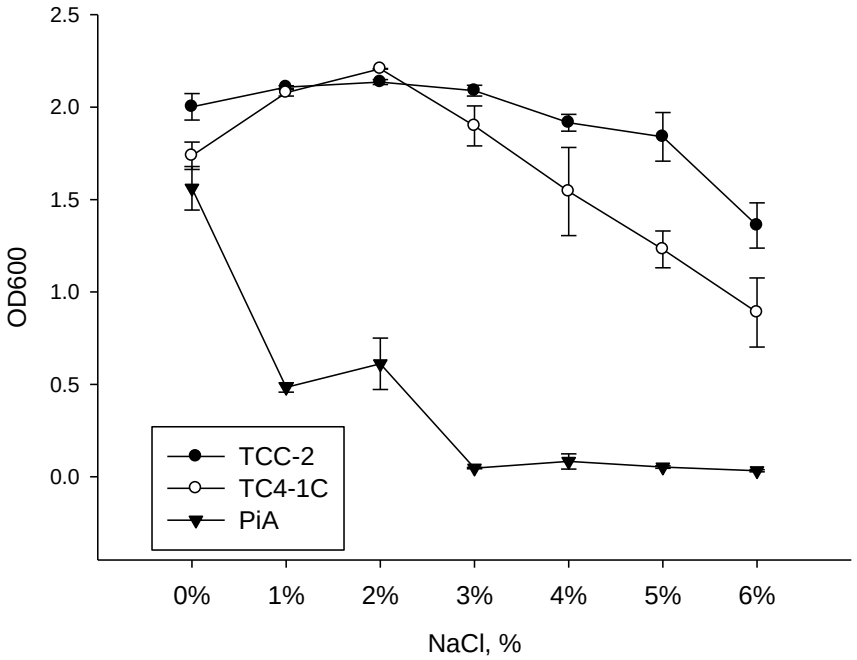

圖 2.篩選菌株於不同氯化鈉濃度 (%)之 NB 培養基中，經培養 18 小時之 OD600 吸光值

Fig. 2. The OD600 value of nutrient broth medium contained different concentration of NaCl after inoculation of the isolates.

表 1.菌株經培養於磷酸三鈣及磷礦石粉培養基至第 4 天之溶磷能力分析

Table1. The solubilization analysis of phosphate by incubation of the isolates in calcium phosphate and rock phosphate medium at the fourth day.

Isolates	Ca-P (mg/L)	R-P (mg/L)
TCC-2	8.51±0.007	2.34±0.22
TC4-1C	17.50±0.36	5.04±0.34
PiA	71.33±8.60	2.45±0.18

表 2.菌株於基本礦物鹽羽毛培養基 (BHF)至第 4 天之羽毛分解液成分分析及羽毛分解率

Table2. The feather degradation rate and nutrient analysis of filtrate by incubation of the isolates in the BHF medium at the fourth day.

Isolates	pH	EC	NH4+ (mg/L)	Tot-N (%)	P (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	Degradation rate %
C-2	8.32 ±0.06	4.09 ±1.11	535.95 ±38.25	0.55 ±0.015	533.43 ±11.92	767.5 ±13.44	1.8 ±0.14	4.7 ±0.57	65.3±0.7
4-1C	8.39 ±0.15	4.04 ±1.04	534.45 ±6.43	0.54 ±0.001	418.28 ±72.37	750.9 ±0.14	1.6 ±0.14	1.6 ±0.64	62.9±3.4
PiA	7.99 ±0.05	3.37 ±0.84	383.67 ±26.58	0.40 ±0.032	562.26 ±62.71	748.8 ±11.03	1.5 ±0.14	1.3 ±0.25	44.8
BK	6.78 ±0.04	1.69 ±0.59	11.4 ±0.85	0.014 ±0.001	413.50 ±7.78	717.6 ±41.65	1.85 ±0.35	17.4 ±0.14	-

表 3.菌株培養於 1%台肥即溶 5 號、1%羽毛、0.5%草木灰至第 4 天之羽毛分解液成分分析及羽毛分解率

Table3. The feather degradation rate and nutrient analysis of filtrate by incubation of the isolates in the 1% feather, 1% instant water soluble fertilizer #5 (10-20-20) and 0.5% plant ash medium at the fourth day.

Isolates	pH	EC	NH4+ (mg/L)	Tot-N (%)	P (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)	Degradation rate %
C-2	7.89	16.32	1324	1.330	703.8	2565.0	0.2	0.2	11.13
4-1C	8.39 ±0.01	17.3 ±0.21	1571.5 ±6.36	1.51 ±0.09	749.5 ±88.6	2730.3 ±40.3	0.1 ±0.0	0.1 ±0.0	35.65 ±7.86
PiA	7.68	16.18	1252	1.203	881.2	2633.4	0.2	0.3	2.96
BK	7.15	14.89	1081	1.061	680.2	2542.2	0.3	6.8	-

表 4.菌株培養於 1%羽毛、0.5%磷礦石粉及 0.5%草木灰至第 4 天之羽毛分解液成分分析
Table4. The nutrient analysis of filtrate by incubation of the isolates in the 1% feather, 0.5% rock phosphate and 0.5% plant ash medium at the fourth day.

Strains	pH	EC	NH4+ (mg/L)	Tot-N (%)	P (mg/L)	K (mg/L)	Ca (mg/L)	Mg (mg/L)
C-2	8.6	4.30	205.33	0.274	8.22	1218.4	5.53	3.77
	±0.02	±0.50	±41.79	±0.037	±0.87	±122.7	±1.80	±0.64
4-1C	8.75	4.54	249.67	0.32	9.53	1232	2.83	3.67
	±0.01	±0.60	±32.31	±0.001	±0.30	±83.5	±0.25	±0.06
PiA	8.5	4.38	205.0	0.25	8.37	1255.8	5.37	4.47
	±0.17	±0.40	±24.25	±0.04	±2.18	±55.9	±2.98	±1.42
BK	8.85	3.27	12.00	0.052	5.82	1228.9	2.43	2.90
	±0.25	±0.39	±2.00	±0.016	±0.26	±105.8	±1.01	±2.14

參考文獻

1. Alam, M. M. and J. K. Ladha. 2004. Optimizing phosphorus fertilization in an intensive vegetable-rice cropping system. Biol. Fertil. Soils 40:277-283.
2. Bertsch, A. and N. Coello. 2005. A biotechnological process for treatment and recycling poultry feathers as a feed ingredient. Bioresour. Technol. 96:1703-1780.
3. Bushnell, L. D. and H. F. Haas. 1941. The utilization of certain hydrocarbons by microorganisms. J. Bacteriol. 41:653-73.
4. Choi, J. M. and P. V. Nelson. 1996. Developing a slow-release nitrogen fertilizer from organic sources: II. Using poultry feathers. J. Am. Soc. Hort. Sci. 121:634-638.
5. Chung, H., M. Park, M. Madhaiyan, S. Seshadri, J. Song, H. Cho, and T. Sa. 2005. Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. Soil. Boil. Biochem. 37:1970-1974.
6. Gulati, A., N. Sharma, P. Vyas, S. Sood, P. Rahi, V. Pathania, and R. Prasad. 2010. Organic acid production and plant growth promotion as a function of phosphate solubilization by *Acinetobacter rhizosphaerae* strain BIHB 723 isolated from the cold deserts of the trans-Himalayas. Arch. Microbiol. 192:975-983.
7. Gupta, R. and P. Ramnani. 2006. Microbial keratinases and their prospective applications: an overview. Appl. Microbiol. Biotechnol. 70:21-33.
8. Gyaneshwar, P., G. Naresh Kumar, L. J. Parekh, and P. S. Poole. 2002. Role of soil microorganisms in improving P nutrition of plants. Plant Soil 245:83-93.
9. Hadas, A. and L. Kautsky. 1994. Feather meal, a semi-slow-release nitrogen fertilizer for organic farming. Fertil. Res. 38:165-70.

10. Kämpfer, P., U. Dreyer, A. Neef, W. Dott, and H. J. Busse. 2003. *Chryseobacterium defluvii* sp. nov., isolated from wastewater. Int J Syst Evol Microbiol 53: 93–97.
11. Keeney, D. R. and D. W. Nelson. 1982. Nitrogen-Inorganic Form. P. 659-663. In: Methods of Soil Analysis, Part 2, 2nd edition.(Page A. L., R. H. Miller and D. R. Keeney,eds.) ASA, Madison, Wisconsin,
12. Kim, J. M., Y. M. Choi, and H. J. Suh. 2005. Preparation of feather digests as fertilizer with *Bacillus Pumilus* KHS-1. J. Microbiol. Biotech. 15:472-476.
13. Onifade, A. A., N. A. Al-Sane, A. A. Al-Musallam and S. Al-Zarban. 1998. A review: potentials for biotechnological applications of keratin-degrading microorganisms and their enzymes for nutritional improvement of feathers and other keratins as livestock feed resources. Bioresour. Technol. 66:1-11.
14. Reddy, M. S., S. Kumar, K. Babita, and M. S. Reddy. 2002. Biosolubilization of poorly soluble rock phosphates by *Aspergillus tubingensis* and *Aspergillus niger*. Bioresour. Technol. 84:187-189.
15. Shen, F. T., P. Kämpfer , C. C. Young, W. A. Lai, and A. B. Arun. 2005. *Chryseobacterium taichungense* sp. nov., isolated from contaminated soil. Int. J. Syst. Evol. Microbiol. 55:1301–1304.
16. Vassilev, N. and M. Vassileva. 2003. Biotechnological solubilization of rock phosphate on media containing agro-industrial wastes. Appl. Microbiol. Biotechnol. 61:435-440.
17. Wang, X. and C. M. Parsons. 1997. Effect of processing systems on protein quality of feather meals and hog hair meals. Poultry Sci. 76: 491-496.
18. Watanabe, F. S. and S. R. Olsen. 1965. Test of an ascorbic acid method for determining phosphorous in water and $NaHCO_3$ extracts from soil. Soil. Sci. Soc. Am. J. 29:677-678.

Application of Feather-Degrading Bacteria in Development of Biofertilizer

You-Hong Zeng[1,2], Ya-Wen Kuo[2], and Hong-Tang Chen[2]

Abstract

The dual function bacteria possessed feather degradation and phosphate solubilization were isolated as *Arthrobacter* sp. TCC-2, *Arthrobacter* sp. TC4-1C and *Bacillus megaterium* PiA by 16S rDNA sequence analysis. The feather degradation rate by incubation of isolates TCC-2, TC4-1C and PiA in the BHF medium at the fourth day were 65.3%, 62.9% and 44.3% respectively and the pH, EC, NH_4^+ and total-N in the feather hydrolysates were significantly increased compared with no inoculated ones . Among the three phosphate-solubilizing bacteria, the isolate PiA showed higher phosphate solubilization ability from the calcium phosphate (71.3 mg/L after incubated 4 days) and the isolate TC4-1C showed higher phosphate solubilization ability from the rock phosphate (5.04 mg/L after incubated 4 days). Isolates TCC-2 and TC4-1C can grow in the 3% NaCl nutrient broth medium and the isolate TC4-1C can efficiently degrade feather in 1% instant waster solube fertilizer #5 (10-20-20) medium, showed TC4-1C can be used to produce high nutrient contents of organic liquid fertilizer. The feather also can be degraded by the three isolates in rock phosphate and plant ash medium for applying in the nutrient amendment in the organic farming system.

Key words: Feather-degrading bacteria, phosphate-solubilizing bacteria, organic liquid fertilizer

---

1. Corresponding author.

2. Assistant researcher, Taichung District Agricultural Research and Extension Station, COA.