

記錄 編號	6317
狀態	NC094FJU00105013
助教 查核	
索書 號	
學校 名稱	輔仁大學
系所 名稱	生命科學系
舊系 所名 稱	
學號	493546144
研究 生(中)	李世軒
研究 生(英)	Shih-shuan Li
論文 名稱 (中)	台灣綠島地熱區新穎耐熱性 Exiguobacterium sp. 之研究
論文 名稱 (英)	Studies on Novel Thermotolerant Exiguobacterium sp. Isolated from Geothermal Areas of Lutao, Taiwan
其他 題名	
指導 教授 (中)	呂誌翼 蔡珊珊
指導 教授 (英)	Jyh-yih Leu San-san Tsay
校內 全文 開放 日期	不公開
校外 全文	不公開

開放日期	
全文不開放理由	
電子全文送交國圖.	同意
國圖全文開放日期.	2006.08.09
檔案說明	電子全文
電子全文	01
學位類別	碩士
畢業學年度	94
出版年	
語文別	中文
關鍵字(中)	耐熱菌 菌種鑑定 綠島 微小桿菌
關鍵字(英)	Exiguobacterium thermotolerant green island
摘要(中)	<p>台灣擁有許多地熱資源，而這些高溫環境中蘊含著豐富的嗜熱性微生物。本研究自台東縣綠島鄉柚子湖地區的高溫海水水樣中，分離純化出耐熱性細菌。根據 16S rDNA gene sequence 定序比對結果篩選出 4 株序列比對較接近的菌株 NTU-1329、NTU-1330、NTU-1331 及 NTU-1337 進行本實驗。本研究菌株在 modified Thermus medium 固體培養基上培養兩天後，呈現直徑 2-3 mm 的橘黃色、凸面具有光澤的菌落。經分析皆為格蘭氏陽性菌，有周鞭毛具移動性，不產孢子的好氧菌。在對數成長期呈現桿狀，穩定期時會變成球狀，細菌長約 1.16-1.45 <math>\mu\text{m}</math> 寬約 0.65-0.82 <math>\mu\text{m}</math>。最適生長條件為 30 oC，pH 7-11；在生化分析方面，4 株菌皆具有</p>

	<p>alkaline phosphatase、esterase、leucine arylamidase、acid phosphatase、<math>\alpha</math>-glucosidase、<math>\beta</math>-glucosidase、catalase、oxidase 等酵素活性以及具有 skim milk 與 starch 水解的能力。在碳源利用上，主要可利用 citrate、D-mannose、D-xylose、L-glutamate、malate、pyruvic acid、L-proline、L-alanine、L-ornithine。脂肪酸組成主要為 C16:0、isoC16:0、aiC17:0 以及 C18:0。由 16S rDNA 結果分析顯示此 4 菌株與 <i>Exiguobacterium aestuarii</i> 的相似度在 97.9 ~ 98.7 % 之間，在 DNA G+C 組成上為 60.3 ~ 62.9 mol %。根據形態、生理生化以及遺傳特性分析的結果發現，本研究的 4 株菌株與比對最相近的菌株 <i>E. aestuarii</i> 確實有差異，在酵素活性測試部分，最相近菌種 <i>E. aestuarii</i> 無 oxidase 與 starch 水解能力，本研究菌株皆有活性。本研究菌株 DNA G+C 組成皆大於 60 % 以上，而 <i>Exiguobacterium</i> 菌屬中目前無發現高於 60 % 的菌種。本研究菌株生長溫度最高可達 50 oC，為目前此屬中最高溫，而耐鹽度最高可達 15 %。因此推測本研究的四株菌株可能成為 <i>Exiguobacterium</i> 菌屬中的新菌種。</p>
<p>摘要 (英)</p>	<p>There are many geothermal areas which include variable microorganisms in Taiwan. The study is aimed to isolate the thermophiles from the sample of warm sea water located on Lyudao Township, Taitung County. According to the results of 16S rDNA sequences analyses, four isolates, NTU-1329, NTU-1330, NTU1331, and NTU-1337 were selected for further investigation. Colonies are 2-3 mm in diameter after incubation for 2 days on modified Thermus agar at 40 oC and yellow-orange convex with entire margin and glossy. Cells are Gram positive, non-spore forming, motile with peritrichous flagella. Cells are short rods (0.73 <math>\mu</math> m x 1.23 <math>\mu</math> m) in the early stationary phase. Growth occurs at 30 oC and 50 oC, with optimal growth at 30 oC. The optimal pH for growth is pH 7-11 and growth does not occur in the presence of 16 % NaCl. All strains hydrolyze skim milk and starch. They show alkaline phosphatase, esterase, leucine arylamidase, acid phosphatase, <math>\alpha</math>-glucosidase, <math>\beta</math>-glucosidase, catalase and oxidase activity. All strains utilize citrate, D-mannose, D-xylose, L-glutamate, malate, pyruvic acid, L-proline, L-alanine and L-ornithine. Major fatty acids are C16:0, isoC16:0, C18:0 and anteisoC17:0. The 16S rDNA sequence similarities of NTU-1329, NTU-1330, NTU1331, and NTU-1337 to <i>Exiguobacterium aestuarii</i> are between 97.9 ~ 98.6 %. The DNA G+C contents of the isolates are between 60.3 ~ 62.9 %. On the basis of morphological, physiological and genetic data, there are differences between the four strains and <i>E. aestuarii</i>. It is found oxidase-negative and no hydrolysis of starch in <i>E. aestuarii</i> but not in four strains. The maximal growth temperature and pH of four strains are the highest in the genus of <i>Exiguobacterium</i>. As a result, the four strains might be a novel species in the genus of <i>Exiguobacterium</i>.</p>
<p>論文 目次</p>	<p>中文摘要 I 英文摘要 III 縮寫對照表 IV 第一章 前言 1 一、嗜熱性微生物 1 二、台灣與綠島的地熱環境 2 三、台灣嗜熱性微生物之研究 3 四、<i>Exiguobacterium</i> 4 五、細菌的分類 6 六、研究目的與架構 10 第二章 材料與方法 11 一、材料 11 (一) 菌種 11 (二) 藥品與酵素 11 (三) 培養基 11 (四) 儀器 12 二、方法 13 (一) 樣本採集 13 (二) 嗜熱菌之分離、純化與保</p>

存 13 1. 菌種之分離與純化 13 2. 菌種之保存 14 2.1 菌種短期與中期保存 14 2.2 菌種長期保存 14 (三) 菌種分析 15 1. 形態觀察 15 1.1 菌落型態 15 1.2 格蘭氏染色 15 1.2 內孢子染色 16 1.3 移動性測試 16 1.4 穿透式電子顯微鏡觀察 16 2. 生理生化特性分析 16 2.1 溫度生長範圍測試 17 2.2 酸鹼度生長範圍測試 17 2.3 耐鹽度測試 17 2.4 單一碳源的利用能力 17 2.5 API-ZYM 酵素活性檢測 18 2.6 澱粉水解能力測試 18 2.7 蛋白?活性測試 19 2.8 幾丁質水解測試 19 2.9. 幾丁聚醣水解測試 19 2.10 Tween 20 與 Tween 80 的水解測試 20 2.11 氧化?活性測試 20 2.12 觸?活性測試 20 2.13 脂肪酸組成分析 21 2.14 抗生素測試 21 3. 菌種遺傳特性分析 22 3.1 DNA G+C 含量分析 22 3.2 16S rDNA 序列分析 23 第三章 結果 27 一、嗜熱菌之分離與純化 27 二、菌種鑑定 27 (一) 形態分析 27 1. 菌落形態 27 2. 格蘭氏染色 28 3. 內孢子染色 28 4. 移動性測試 28 5. 穿透式電子顯微鏡觀察 28 (二) 生理特性分析 29 1. 溫度生長範圍測試 29 2. 酸鹼度生長範圍測試 29 3. 耐鹽度測試 29 4. 單一碳源利用能力 30 5. API-ZYM 酵素活性檢測 31 6. 澱粉水解測試 31 7. 蛋白?活性測試 31 8. 幾丁質水解測試 32 9. 幾丁聚醣水解測試 32 11. 氧化?活性測試 32 12. 觸?活性測試 33 13. 脂肪酸組成分析 33 14. 抗生素測試 34 (三) 遺傳分析 34 1. DNA G+C 含量分析 34 2. 16S rDNA 序列分析 34 第四章 討論 36 一、Exiguobacterium 菌種鑑定 36 二、綠島嗜熱菌之研究 38 三、嗜鹼嗜熱細菌的發展與應用 38 第五章 結論 40 參考文獻 41 圖表 51 附錄一、10X Castenholz basal salt solution 74 附錄二、API-ZYM 酵素套件呈色對照表 75 附錄三、抗生素測試對照表 76 附錄四、分離菌株 16S rDNA 序列 77

參考  
文獻

時雨青 2005 台灣地區新穎嗜熱性蛋白細菌之研究。國立台灣大學植物學研究所碩士論文 陶光恆 2002 台灣嗜熱菌 *Thermus taiwanensis* WL219 含錳超氧歧化?之研究。國立台灣大學植物學研究所碩士論文 陳懋彥 2002 台灣地熱區嗜熱性細菌之研究。國立台灣大學植物學研究所博士論文 楊美桂、陳淵銓 1995 普通微生物學實驗。藝軒圖書出版社，台北 Alfredsson, G. A. & Kristjansson, J. K. (1995). Ecology, distribution, and isolation of *Thermus*. In *Thermus species*, pp. 43-63. Edited by R. Sharp & R. A. D. Williams. New York: Plenum Press. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* 215, 403-410. Bertoldo, C. & Antranikian G. (2002). Starch-hydrolyzing enzymes from thermophilic archaea and bacteria. *Curr Opin Chem Biol* 6(2): 151-160. Brock, T. D. (1986). *Thermophiles. General, Molecular and Applied Microbiology*. New York: John Wiley & Sons. Brock, T. D. & Freeze, H. (1969). *Thermus aquaticus* gen. n. and sp. n., a nonsporulating extreme thermophile. *J Bacteriol* 98(1): 289-297. Buyer, J. S. (2002a). Identification of bacteria from single colonies by fatty acid analysis. *J Microbiol Methods* 48, 259-265. Buyer, J. S. (2002b). Rapid sample processing and fast gas chromatography for identification of bacteria by fatty acid analysis. *J Microbiol Methods* 51, 209-215. Cappuccino, J. G. & Sherman, N. (1998). *Microbiology: A Laboratory Manual*. 5rd ed. The Benjamin/Cummings Publishing Company, Inc., New York. Chen, M. Y., Lin, G. H., Lin, Y. T. & Tasy, S. S. (2002 a). *Meiothermus taiwanensis* sp. nov., a novel filamentous, thermophilic species isolated in Taiwan. *Int J Syst Evol Microbiol* 52,

1647-1654. Chen, M. Y., Tsay, S. S., Chen, K. Y., Shi, Y. C., Lin, Y. T. & Lin, G. H. (2002 b). *Pseudoxanthomonas taiwanensis* sp. nov., a novel thermophilic, N<sub>2</sub>O-producing species isolated from hot spring. *Int J Syst Evol Microbiol* 52, 1-7.

Chen, M. Y., Wu, S. H., Lin, G. H., Lu, C. P., Lin, Y. T., Chang, W. C. & Tsay, S.S. (2004). *Rubrobacter taiwanensis* sp. nov., a novel thermophilic, radiation-resistant species isolated from hot springs. *Int J Syst Evol Microbiol* 54, 1849-1855.

Chen, T. L., Y. J. Chou, W. M. Chen, B. Arun, & Young, C. C. (2006). *Tepidimonas taiwanensis* sp. nov., a novel alkaline-protease-producing bacterium isolated from a hot spring. *Extremophiles* 10:35-40.

Chen, W. M., Chang, J. S., Chiu, C.H., Chang, S. C., Chen, W. C., Sheu, S. Y. & Jiang, C. M. (2005). *Caldimonas taiwanensis* sp. nov., a amylase producing bacterium isolated from a hot spring. *Syst Appl Microbiol* 28, 415-420.

Chien, A., Edgar, D. B. & Trela, J. M. (1976). Deoxyribonucleic acid polymerase from the extreme thermophile *Thermus aquaticus*. *J Bacteriol* 127, 1550-1557.

Choi, Y. J., Kim, E.J., Piao, Z., Yun, Y. C. & Shin, Y. C. (2004). Purification and characterization of chitosanase from *Bacillus* sp. strain KCTC 0377BP and its application for the production of chitosan oligosaccharides. *Appl Environ Microbiol* 70, 4522-4531.

Chung, A. P., Rainey, F. A., Valente, M., Nobre, M. F., & da Costa, M.S. (2000). *Thermus igniterrae* sp. nov. and *Thermus antranikianii* sp. nov., two new species from Iceland. *Int J Syst Evol Microbiol* 50, 209-217.

Collins, M. D., Lund, B. M., Farrow, J. A. E. & Schleifer, K. H. (1983). Chemotaxonomic study of an alkalophilic bacterium, *Exiguobacterium aurantiacum* gen. nov., sp. nov. *J Gen Microbiol* 129, 2037-2042.

Colwell, R. R. (1970). Polyphasic taxonomy of the genus *Vibrio*: numerical taxonomy of *Vibrio cholerae*, *Vibrio parahaemolyticus*, and related *Vibrio* species. *J. Bacteriol.* 104:410 – 433.

Cote, R. J. & Gherna, R. L. (1994). Nutrition and Media. In *Methods for General and Molecular Bacteriology*, pp. 655-682. Edited by P. Gerhardt. Washington, D.C.: American Society for Microbiology.

da Costa, M. S. (1995). The cell walls and lipids of *Thermus*. In *Thermus Species*, pp. 143-253. Edited by R. Sharp & R. A. D. Williams. New York: Plenum Press.

Farrow, J. A. E., Wallbanks, S. & Collins, M. D. (1994). Phylogenetic interrelationships of round-spore-forming bacilli containing cell walls based on lysine and the non-spore-forming genera *Caryophanon*, *Exiguobacterium*, *Kurthia*, and *Planococcus*. *Int J Syst Bacteriol* 44(1): 74-82.

Felsenstein, J. (2004). PHYLIP (Phylogeny Inference Package) version 3.61. Fruhling A, Schumann P, Hippe H, Straubler B & Stackebrandt E. (2002). *Exiguobacterium undae* sp. nov. and *Exiguobacterium antarcticum* sp. nov. *Int J Syst Evol Microbiol* 52(Pt 4): 1171-1176.

Gherna, R. L. (1994). Culture preservation. In *Methods for General and Molecular Bacteriology*, pp. 278-292. Edited by P. Gerhardt. Washington, D.C.: American Society for Microbiology.

Goodfellow, M. & O' Donnell, A. G. (1993). *Handbook of new bacterial systematics*. Academic Press Ltd., London.

Gupta, R., Berg, Q. K., & Lorenz, P. (2002). Bacterial alkaline proteases: molecular approaches and industrial applications. *Appl Microbiol Biotechnol* 59(1): 15-32.

Hobel, C. F., Hreggvidsson, G. O., Marteinson, V. T., Bahrani-Mougeot, F., Einarsson, J. M. & Kristjansson,

J. K. (2005). Cloning, expression, and characterization of a highly thermostable family 18 chitinase from *Rhodothermus marinus*. *Extremophiles* 9,53-64. Hoppert, M. & Holzenburg, A. (1998). *Electron Microscopy in Microbiology*. Guildford, UK: BIOS Scientific Publisher. Humble, M. W., King, A. & Phillips, I. (1977). API ZYM: a simple rapid system for the detection of bacterial enzymes. *J Clin Pathol* 30:275-277. Johnson, J. L. (1994). Similarity analysis of DNAs. In *Methods for General and Molecular Bacteriology*, pp. 655-682. Edited by P. Gerhardt. Washington, D.C.: American Society for Microbiology. Kim I. G., Lee M. H., Jung S. Y., Song J. J., Oh T. K. & Yoon J. H. (2005). *Exiguobacterium aestuarii* sp. nov. and *Exiguobacterium marinum* sp. nov., isolated from a tidal flat of the Yellow Sea in Korea. *Int J Syst Evol Microbiol* 55(Pt 2): 885-889. Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequence. *J. Mol. Evid.*, 16:111-120. Koyama, N. (1999). Presence of Na<sup>+</sup>-stimulated P-type ATPase in the membrane of a facultatively anaerobic alkaliphile, *Exiguobacterium aurantiacum*. *Curr Microbiol* 39(1): 27-30. Kristjansson, J. K. (1992). *Thermophilic Bacteria*. Boca Raton: CRC Press. Lapage, S. P., Shelton, J. E., Mitchell, T. G. & MacKenzie, A. R. (1970). Culture collections and the preservation of bacteria. In *Methods in Microbiology*, pp. 135-288. Edited by J. R. Norris & D. W. Ribbons. London ; New York: Academic Press. Lopez-Cortes A, Schumann P, Pukall R & Stackebrandt E. (2006). *Exiguobacterium mexicanum* sp. nov. and *Exiguobacterium artemiae* sp. nov., isolated from the brine shrimp *Artemia franciscana*. *Syst Appl Microbiol* 29(3): 183-190. Manaia, C. M. & da Costa, M. S. (1991). Characterization of halotolerant *Thermus* isolates from shallow marine hot springs on S. Miguel, Azores. *J Gen Microbiol* 137, 2643-2648. Marteinsson, V. T., Hauksdottir, S., Hobel, C. F., Kristmannsdottir, H., Hreggvidsson, G. O. & Kristjansson, J. K. (2001). Phylogenetic diversity analysis of subterranean hot springs in Iceland. *Appl Environ Microbiol* 67, 4242-4248. McNaught, A. D. & Wilkinson, A. (1997). *International Union of Pure and Applied Chemistry Compendium of Chemical Terminology ( 'The Gold Book' )*. 2nd ed, Blackwell Science, Oxford, UK Mesbah, M. & Whitman, W. B. (1989). Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine + cytosine of DNA. *J Chromatogr* 479, 297-306. Nielsen, P., Fritze, D. & Priest, F. G. (1995). *Microbiology (UK)* 141, 1745 - 1761. Oberhofer, T. R. & Maddox, L. (1970). Evaluation of a zone size chart for antibiotic susceptibility tests by disk diffusion. *Am J Clin Pathol.* 54(4), 596 - 601. Rainey, F. A., Fritze, D. & Stackebrandt, E. (1994). The phylogenetic diversity of thermophilic members of the genus *Bacillus* as revealed by 16S rDNA analysis. *FEMS Microbiol Lett* 115(2-3): 205-211. Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* 46(4): 1088-1092. Rothschild, L. J. & Mancinelli, R. L. (2001). Life in extreme environments. *Nature* 409(6823):

1092-1101. Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4, 406-425.

Sambrook, J. & Russell, D. W. (2001). *Molecular Cloning: A Laboratory Manual*. 3rd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Sasser, M. (2001). Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids. Technical Note #101. May 1990, revised February 2001. Newark, DE: MIDI.

Sharp, R., Cossar, D. & Williams, R. A. D. (1995). Physiology and metabolism of *Thermus*. In *Thermus Species*, pp. 67-85. Edited by R. Sharp & R. A. D. Williams. New York: Plenum Press.

Sharp, R. J., Riley, P. W. & White, D. (1992). Heterotrophic thermophilic Bacilli. In *Thermophilic Bacteria*, pp. 20-50. Edited by J. K. Kristjansson. Boca Raton: CRC Press.

Shieh, W. Y. (1993). A halophilic thermophilic bacterium isolated from a coastal hot spring in Lutao, Taiwan. *J Gen Microbiol* 139, 2505-2510.

Shieh, W. Y. & Jean, W.D. (1998). *Alterococcus agarolyticus*, gen. nov., sp.nov., a halophilic thermophilic bacterium capable of agar degradation. *Can J Microbiol* 44, 637-645.

Shieh, W. Y., Jean, W. D. & Lin, Y. T. (2003). *Marinobacter lutaoensis* sp. nov., a thermotolerant marine bacterium isolated from a coastal hot spring in Lutao, Taiwan. *Can J Microbiol* 49, 244-252.

Shivvers, D. W. & Brock, T. D. (1973). Oxidation of elemental sulfur by *Sulfolobus acidocaldarius*. *J Bacteriol* 114(2): 706-710.

Sperandio, V., Torres, A. G. & Kaper, J. B. (2002). Quorum sensing *Escherichia coli* regulators B and C (QseBC): a novel two-component regulatory system involved in the regulation of flagella and motility by quorum sensing in *E. coli*. *Mol Microbiol* 43, 809-821.

Stackebrandt, E. & Liesack, W. (1993). Nucleic acids and classification, p.151 – 194. In M. Goodfellow and A. G. O’ Donnell (ed.), *Handbook of new bacterial systematics*. Academic Press Ltd., London.

Ueno, S., Kaieda, N. & Koyama, N. (2000). Characterization of a P-type Na<sup>+</sup>-ATPase of a facultatively anaerobic alkaliphile, *Exiguobacterium aurantiacum*. *J Biol Chem* 275(19): 14537-14540.

Vandamme, P., Pot, B., Gillis, M., de Vos P., Kersters, K., Swings, J. (1996). Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microbiol Rev* 60:407-438.

van den Burg, B. (2003). Extremophiles as a source for novel enzymes. *Curr Opin Microbiol* 6(3): 213-8.

Wiegel, J. & V. V. Kevbrin (2004). Alkalithermophiles. *Biochem Soc Trans* 32(Pt 2): 193-198.

Woese, C. R. & Fox, G. E. (1977). Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proc Natl Acad Sci U S A* 74, 5088-5090.

Woese, C. R., Kandler, O. & Wheelis, M. L. (1990). Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proc Natl Acad Sci U S A* 87, 4576-4579.

Yumoto, I., Hishinuma-Narisawa, M., Hirota, K., Shingyo, T., Takebe, F., Nodasaka, Y., Matsuyama, H. & Hara, I. (2004). *Exiguobacterium oxidotolerans* sp. nov., a novel alkaliphile exhibiting high catalase activity. *Int J Syst Evol Microbiol* 54: 2013-2017.

Zhilina, T.N., Garnova, E.S., Tourova, T.P., Kostrikina, N.A. & Zavarzin, G.A. (2001). *Microbiology (RU)* 70, 64 – 72.

附註	
全文 點閱 次數	
資料 建置 時間	
轉檔 日期	
全文 檔存 取記 錄	
異動 記錄	M admin Y2008.M7.D3 23:18 61.59.161.35