行政院國家科學委員會補助專題研究計畫成果報告

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計畫類別：■個別型計畫 □整合型計畫
計畫編號：NSC89－2313－B－002－233－
執行期間：89 年 08 月 01 日至 90 年 07 月 31 日

計畫主持人：謝文陽

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計畫參與人員：林育德 邱秀慧 鄭予珊

1、中文摘要

自台灣綠島沿岸熱泉分離出一株專性好氣的異營性菌株，為具好熱性的革蘭氏陰性海洋桿菌，編號為 T5054**。菌體在液體培養時不會產生孢子，並以一到數根極性鞭毛運動。線毛則常可見生於細胞兩端。菌株** T5054 **須要鈉離子以生長，在溫度約為** 45 C**, 酸鹼值** 7 **和氯化鈉濃度為** 4% **時為其最適生長條件。脂肪酸主要成分為** *iso*-$C_{15:0}$**，**ubiquinone-8 (Q-8)**則是其唯一的細胞** quinone **組成。**DNA G+C **含量為** 63.5 mol%**。此菌株生長時不須要供給任何維生素或其他有機生長因子；並能利用葡萄糖、甘露糖醇、多種有機酸和氨基酸作為單一碳源。依照菌株各項生理和生化特性，以及其** 16S rDNA **之親源分析，顯示菌株** T5054 **應該歸類於新屬中的一個新菌種。在這裡提議將此新菌種命名為** *Aeromarinobacter lutaoensis* gen. nov., sp. nov.**。標準株為** T5054 (= CCRC 17087^T = JCM 11179^T)

關鍵詞：*Aeromarinobacter lutaoensis***、好熱性、好鹽性、海洋細菌**

Abstract

A heterotrophic, slightly thermophilic, marine bacterium, designated strain T5054 was isolated from a hot spring on the coast of Lutao, Taiwan. It was a strictly aerobic, Gram-negative rod. Cells grown in broth cultures were non-spore-forming and motile by means of one to several polar flagella. Pili were commonly produced from both poles of the cells. Strain T5054 required sodium ions for growth, and exhibited optimal growth at about 45 C, pH 7 and 4% NaCl. It contained *iso*-$C_{15:0}$ as the most abundant fatty acid and ubiquinone-8 (Q-8) as the only isoprenoid quinone. Its genomic DNA G+C content was 63.5 mol%. The strain did not require either vitamins or other organic growth factors for growth; it grew on glucose, mannitol and a variety of organic and amino acids as sole carbon sources. Characterization data, together with the results of a 16S rDNA-based phylogenetic analysis, indicated that strain T5054 should be classified as a species of a new genus. The name *Aeromarinobacter lutaoensis* gen. nov., sp. nov. is proposed for this new bacterium. The type strain is T5054 (= CCRC 17087^T = JCM 11179^T).

Keywords: *Aeromarinobacter lutaoensis*, thermophile, halophile, marine bacteria

2、Introduction

Heterotrophic, halophilic, Gram-negative, marine bacteria generally require 70-700 mM sodium ions for optimal growth and yield in laboratory media (Baumann et al. 1971). They can be divided into two large groups based on their ability to ferment carbohydrates. The fermentative group is mainly composed of facultatively anaerobic, motile rods that are currently placed in the genera *Vibrio* (Baumann et al. 1984), *Photobacterium* (Baumann and Baumann 1984), *Listonella* (MacDonell and Colwell 1985) and *Moritella* (Urakawa et al. 1998). The non-fermentative group includes motile and non-motile aerobes. The motile members of this group are readily isolated from various marine sources and probably comprise a major component of the bacterial flora of the sea (Baumann et al. 1972; Nair and Simidu 1987; Shieh and Simidu 1986; Simidu et al.1982). They have been assigned to a large number of genera, and all valid species included in these genera are either mesophilic

or psychrophilic. Their optimal growth temperatures invariably are below 40 although a few species can grow at 45 even up to 50 et al. 1992; Nguyen et al. 1999).

Five strains of heterotrophic, strictly aerobic, marine bacteria that grow optimally at 45 C have been isolated by us previously from a hot spring on the coast of Lutao, Taiwan. All of them require sodium ions for growth and have the ability to grow in the absence of vitamins and other organic growth factors (Shieh and Jean 1994). In the present study it is reported that one representative strain of these bacteria, strain T5054^T, is significantly different from any previously described taxon to be placed in the species of a new genus. The name proposed is *Aeromarinobacter lutaoensis* gen. nov., sp. nov.

3、Results and discussion

Determination of growth parameters

Strain T5054 could be considered as a slightly thermophilic bacterium since it grew in PY broth over a temperature range of 25-50 C and most rapidly at approximately 45 C (Fig. 1A). The cultures growing at 45 C reached an OD_{600} level of about 0.9 within 3 d after which no significant change of the OD_{600} was monitored in the following 2-3 d (not shown). Growth was normally weak or absent at 55 at 20 and 60 e strain could grow over a pH range from 5 to 9, with an optimum around 7. Growth was not observed at pH 4 or 10. Fig.1B shows the effect of NaCl on growth. Strain T5054 grew in PY broth at NaCl levels of 0.5-12% (ca. 0.085-2.05 M) with optimal growth occurring at 4% (ca.0.68 M). Growth was absent in NaCl concentrations of 0 and 15%. Substitution of KCl (0.17-0.85 M) for NaCl did not support bacterial growth (not shown), indicating that the strain required Na^+ but not Cl^- for growth and that the Na^+ requirement was not for osmotic function. Halophilic microorganisms that grow best in media containing 0.2-0.5 M (ca. 1.2-3%) NaCl and 0.5-2.0 M (ca. 3-12%) NaCl are defined as slight halophiles and moderate halophiles, respectively (Kushner and Kamekura 1988). Thus strain T5054^T could be categorized as a moderate halophile. The possible function(s) of Na^+ in the cells of the strain await further investigation.

Bacterial characterization

Strain T5054 was Gram-negative according to the staining and KOH testing methods. It produced circular, off-white and non-luminescent colonies on PY plate medium after 1-2 d incubation. Cells grown in PY broth or GM medium appeared as straight or slightly curved rods in shape, motile, and about 2.0-3.5 μm long and 0.6-1.0 μm wide, with one to several polar flagella as revealed by transmission electron microscopy (Fig. 2). When grown in the GM medium the cells commonly produced numerous fine pili, mainly from both poles of the cells (Fig. 2A). Fewer pili were produced when grown in PY broth (Fig. 2B and 2C). Endospores were not produced by the cells under the growth conditions described in this work. Neither endospore-like structures nor sporulation was ever observed or confirmed by phase contrast microscopy or spore staining in the present study. Indole production, nitrate reduction and denitrification were not detectable. Strain T5054 did not ferment any of the test carbohydrates (glucose, cellobiose, dulcitol, galactose, lactose, mannitol, mannose, saccharose, trehalose, xylose). Activities of C (not oxidase, catalase and lipase but not agarase, amylase, caseinase, DNase gelatinase, arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase were detected. The strain could grow on glucose, mannitol, acetate, fumarate, â-hydroxybutyrate, DL-malic acid, malonate, pyruvate, L-alanine, L-aspartate, L-glutamate and L-phenylalanine as sole carbon sources, but it was unable to grow on arabinose, cellobiose, galactose, lactose, melibiose, mannose, saccharose, xylose, adonitol, dulcitol, inositol, citrate, tartrate, L-arginine, L-glycine, L-lysine, L-methionine, L-ornithine, L-threonine, L-tryptophan or L-valine as sole carbon sources. Strain T5054 was further found able

to grow on ammonium and the amino acids L-alanine, L-arginine, L-aspartate, L-glutamate, L-glycine, L-lysine, L-methionine, L-ornithine, L-phenylalanine, L-threonine, L-tryptophan and L-valine but not on nitrate and nitrite as sole nitrogen sources.

Bacteria inhabiting the marine environment include halophiles and non-halophiles while only the halophiles are considered to be autochthonous organisms. Halophilic, marine bacteria isolated prior to 1985 are exclusively mesophilic or psychrophilic. However, a variety of thermophilic, halophilic, marine bacteria including autotrophs and heterotrophs have been reported since 1986. Among them *Rhodothermus marinus* (Alfredsson et al. 1988), *Rhodothermus obamensis* (Sako et al. 1996) and *Thermaerobacter marianensis* (Takai et al. 1999) are strictly aerobic heterotrophs, and the others are either autotrophs or heterotrophs of either strict anaerobes or facultative anaerobes. Strain T5054, like *R. marinus*, *R. obamensis* and *T. marianensis* are characterized as a strictly aerobic, rod-shaped, heterotrophic bacterium. The strain, however, is distinguished from *R. marinus* and *R. obamensis* in that it does not produce reddish-colored colonies on plate media and is a motile, flagellate bacterium unable to grow at 60-75 C. It is also differentiated from *T. marianensis* since *T. marianensis* is an immotile, non-flagellate and Gram-variable bacterium capable of growing at 65-75 C but not at 25-45 C.

Susceptibility to antibiotics

According to the diameters (mm) of the inhibition zones of strains T5054 in response to various antibiotics, and with reference to the standards from NCCLS (1990), strain T5054 was susceptible to ampicillin, carbenicillin, cephalothin, chloramphenicol, colistin, gentamicin, kanamycin, neomycin, novobiocin, oxacillin, penicillin G, polymyxin B and tetracycline, intermediate to erythromycin, nalidixic acid and streptomycin, and resistant to clindamycin and lincomycin.

Detection of diaminopimelic acid and cell-wall murein type

Cell hydrolysates of strain T5054 contained *meso*-diaminopimelic acid and the acyl type rather than glycol type of murein. Detection of *meso*-diaminopimelic acid revealed that the strain might contain a cell-wall peptidoglycan of the directly cross-linked *meso*-diaminopimelic acid type which is commonly found in the majority of Gram-negative bacteria as well as most species of the genus *Bacillus* (Claus and Berkeley 1986; Schleifer and Kandler 1972).

Quinone type analysis

Ubiquinone-8 (Q-8) was the only isoprenoid quinone detected in the cells of strain T5054.

Cellular fatty acids

Strain T5054 contained *iso*-$C_{15:0}$ as the most abundant fatty acid in the cells (46.4-54.3 mol%). Other cellular fatty acids normally detected at levels greater than 5.0 mol% included *iso*-$C_{17:0}$ (11.1-18.2 mol%), *anteiso*-$C_{15:0}$ (17.7-22.0 mol%) and *anteiso*-$C_{17:0}$ (5.6-8.4 mol%). The contents in parenthesis represent ranges of five replicated measurements.

DNA base composition

The DNA G+C content of strain T5054 was 63.5 mol%.

16S rDNA-based phylogeny

More than 99% of 16S rDNA sequence (99.2%; estimated by comparison with the *E. coli* sequence J01859) of strain T5054 was determined. The sequence was aligned and compared with all bacterial 16S rDNA sequences available in the GenBank database. The 16S rDNA-based phylogenetic analysis has revealed that strain T5054 is a member of the gamma *Proteobacteria*. The closest species of the strain are *Marinobacter hydrocarbonoclasticus* and *Marinobacter aquaeolei* (levels of sequence similarity, 96.5-96.8%); no other species have exhibited sequence similarity levels greater than 90% with the strain. Fig. 3 shows the phylogenetic position of strain $T5054^T$ among selected

representatives of non-fermentative, motile, marine bacteria and some reference organisms belonging to the gamma *Proteobacteria*. Results of the 16S rDNA-based phylogenetic analysis indicate that strain T5054 represents either a new genus or a new species of the genus *Marinobacter*. The strain is considered to represent a new genus rather than a new species of *Marinobacter* since its G+C content (63.5 mol%) is significantly different from that of *Marinobacter* (ca. 53-56 mol%). The strain is also distinguished from *Marinobacter* in that it contains *iso*-$C_{15:0}$ and not $C_{16:0}$, nor $C_{18:ù\,19c}$ as the major fatty acid.. The name *Aeromarinobacter* is proposed for this new genus. *Aeromarinobacter lutaoensis* is the type species of this genus and strain T5054 (= CCRC 17087 =JCM 11179) is the type strain of this species.

Aeromarinobacter lutaoensis is considered to be of marine origin since it is unable to grow in the absence of Na^+ and requires a sea salt level similar to that of natural seawater for optimal growth. The capability for growth in the absence of organic growth factors in this species is favorable for life in the natural environment. Members of *Aeromarinobacter lutaoensis*, however, may be confined to inhabiting only the coastal and submarine hot springs due to their requirements for oxygen, Na^+, organic matter and a slightly high temperature.

4、Assessment of plan accomplishment

(A) This plan had completed results as following:
1. Morphological observation of bacterial isolates and colonies
2. Determination of bacterial isolates' physiological and biochemical characteristics
3. Utilization of bacterial isolates to different carbon and nitrogen sources
4. Susceptibility to various antibiotics
5. Confirmation optimum growth condition of bacterial isolates
6. Composition of fatty acids
7. DNA G+C content analysis
8. Sequence of 16S rDNA and phylogeny analysis

(B) Staff had learned the knowledge and technique about marine bacterial taxonomy study.

5、Reference

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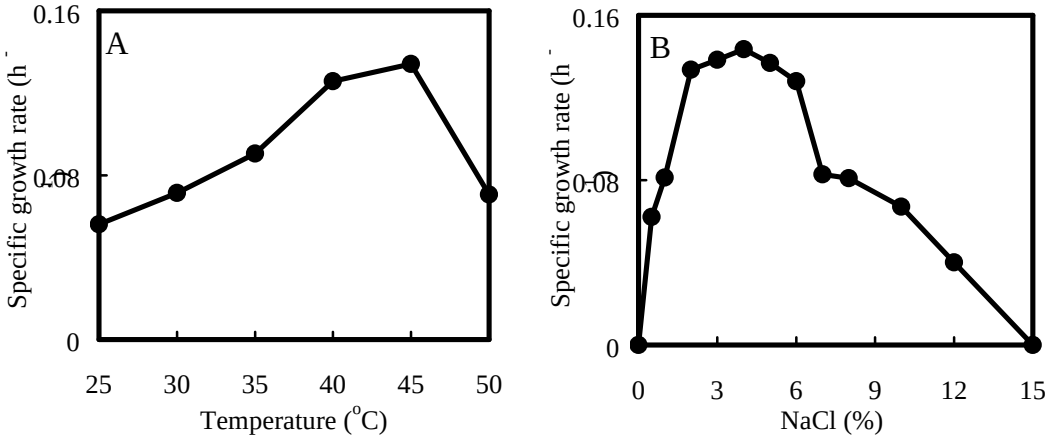

Fig. 1 Growth responses of strain T5054 in PY broth to different temperatures (A) and NaCl concentrations (B)

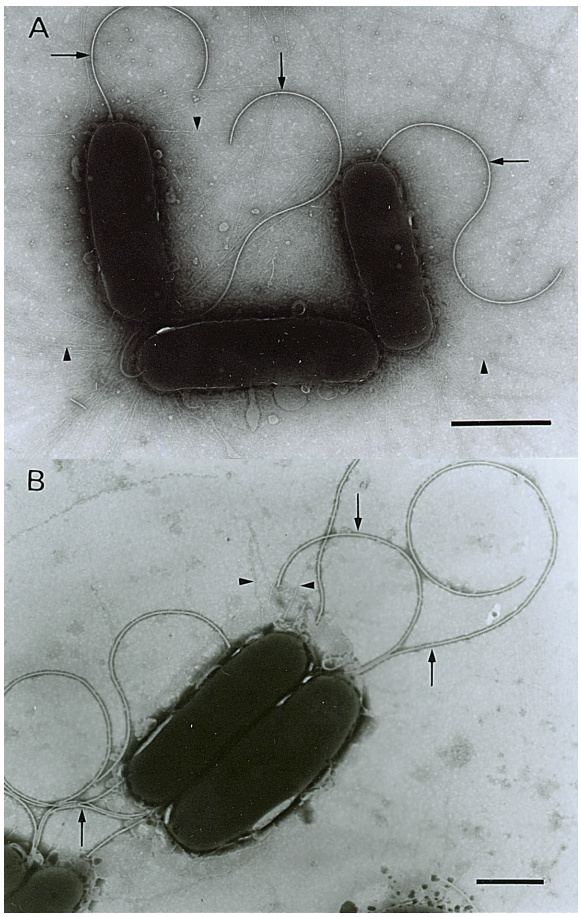

Fig. 2 Electron micrographs of strain T5054. (A) Cells grown in GM medium showing a single polar flagellum (arrows) and numerous pili (arrowheads). Scale bar, 1 ì m. (B and C) Cells grown in PY broth showing one to several polar flagella (arrows) and fewer pili (arrowheads). Scale bar, 1 ì m

Fig. 3 16S rDNA-based phylogenetic dendrogram showing the position of strain T5054 within the radiation of its related taxa in the gamma *Proteobacteria*. The scale bar represents 10 nucleotide substitutions per 100 nucleotides