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Studies of the biological characteristics and halophilism of a *Streptomyces* strain TA4-1

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Abstract. An actinomycete strain, TA4-1, was isolated from soil samples from the Tansui River estuary mangrove forest. This isolate was able to grow in media containing 3–10% salt, and showed a morphological similarity to *Actinopolyspora mortivallis* 13635, a previously reported halophilic actinomycete (Yoshida et al., 1991). The biological characteristics of strain TA4-1 were studied for taxonomic classification, and were compared with those of *A. mortivallis* 13635 and *Actinopolyspora halophila* 13403 to distinguish characteristics related to halophilism. No significant morphological difference among these three strains was observed with scanning electronic microscopy, but tests for cultural, physiological and biochemical characteristics, sugar utilization, and the whole cell chemotype (e.g. diaminopimelic acid, sugars, phospholipids, fatty acids, and DNA homology) suggested that strain TA4-1 belongs to the genus *Streptomyces*, and also revealed great differences among the three strains studied. Consequently, strain TA4-1 is distinguished from *A. mortivallis* and *A. halophila*, and is more likely a NaCl tolerant *Streptomyces* strain.

Keywords: Halophilic actinomycetes; *Streptomyces*; Taxonomy.

Introduction

Most halophilic microorganisms are bacteria (Larsen, 1967). The well-known halobacteria and halococci are extreme halophiles, which require a minimum concentration of 12% NaCl for growth and can grow in saturated NaCl. The moderate halophiles can grow in 5–20% NaCl, and the slightly halophilic microorganisms can grow only in the presence of 2–3% NaCl.

The halophilism of microorganisms is believed to be associated with highly unusual biochemical and physiological properties (Larsen, 1967; Kates et al., 1966) and metabolic functions. Novel antibiotics might be produced by microorganisms growing in environments that are very different from terrestrial conditions, e.g. marine habitats (Okazaki and Okami, 1976). The first extremely halophilic actinomycete reported, *Actinopolyspora halophila*, had distinguishing characteristics different from those of other actinomycetes and extremely halophilic bacteria (Gochnauer et al., 1975). Another novel species, *Actinopolyspora mortivallis* is moderately halophilic and can produce nucleoside antibiotics (Yoshida et al., 1991). In the course of our screening for new antibiotics, an actinomycete, designated strain TA4-1, was isolated from soil samples from the Tansui River estuary mangrove forest, Taipei, Taiwan. This isolate produces water soluble antibiotics that strongly inhibit the growth of Gram-positive and Gram-negative bacteria. Interestingly, it is able to grow in media containing 3–12% NaCl. This paper compares strain TA4-1 with *A. halophila* and *A. mortivallis*.

Materials and Methods

Microorganisms

Strain TA4-1 was isolated from a soil sample collected in the mangrove forest of the Tansui River estuary, Taipei, Taiwan. A 2 g sample of soil was added to 10 ml of phosphate buffer (pH 7.0), shaken for 10 minutes, and diluted 10-, 100-, and 1,000-fold in phosphate buffer. The dilutions were plated on TYG agar consisting of 5 g tryptone, 3 g yeast extract, 10 g glucose, 3 g NaCl, 1 g K_2HPO_4, 1 g KH_2PO_4, and 20 g agar-agar in 1,000 ml of distilled water. Plates were incubated at 28°C for 14 days. Colonies 2 mm in size were isolated. Taxonomic studies of the microbial isolates were conducted according to Arai (1976), Krasilinikov (1966), Waksman (1967), Sykes and Skinner (1973), Alexander (1961), and Williams et al. (1989).

The strains used for comparison were *A. halophila* 13403 (ATCC 27976) grown on YE+CM agar plate at 37°C and *A. mortivallis* 13635 (JCM 7550) grown on YE+CM agar plate at 45°C. All stock cultures were freeze-dried and kept at 4°C.

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Scanning electron microscopy

Samples for observation with a scanning electron microscope were prepared from one-, two-, and three-week-old cultures of strain TA4-1 grown on water agar with 3% NaCl at 28°C, *A. mortivallis* on YM+CM agar at 45°C, and *A. halophila* on YM+CM agar at 37°C, respectively. Colonies of strains on agar cultures were cut out and fixed in 2.5% glutaraldehyde buffer solution (pH 7.4) for 2 hours. After dehydration through a graded ethanol series, drying with a critical point apparatus, and coating with gold-palladium, the colonies and spores were examined using Hitachi S-520 and Zeiss DSM 950 microscopes (Wu et al., 1988).

Cultural characteristics

Spores of strain TA4-1 were collected from 21-day-old cultures grown on TYG agar with 3–10% NaCl and suspended in pH 7.0 phosphate buffer saline solution. Each of the various media was inoculated with one drop of the suspension, according to Waksman (1967), Shirling and Gottlieb (1966), Gochnauer et al. (1975), and Komagata and Suzuki (1987). Cultural characteristics were observed after 7, 14, and 21 days of incubation on various agar media at 28°C.

Physiological characteristics

The physiological characteristics of strain TA4-1 were studied using the methods of Waksman (1967), Luedemann (1971), Luedemann and Brodsky (1964), Neyra et al. (1977), and Gochnauer et al. (1975). All cultures were inoculated with mature spores and mycelia on agar cultures and incubated at 28°C for 21 days, except for the gelatin liquefaction, which was incubated at 15°C. Cultures for testing NaCl tolerance and growth temperature were grown on TYG agar with 3% NaCl as a basal medium. Before observation, all cultures were incubated at 28°C for 1 to 2 weeks.

Utilization of carbon and nitrogen sources

Utilization of carbon and nitrogen sources was studied by the methods of Waksman (1967), Pridham and Gottlieb (1948), Shirling and Gottlieb (1966), and Lechevalier and Lechevalier (1970, 1980). Growth and nutrient utilization of strain TA4-1 were measured 21 days after incubation at 28°C.

Cell wall composition and chemotaxonomic studies

Determination of the cell wall composition (including sugars, lipids, amino acids, fatty acids, and DNA homology) were performed with the methods of Komagata and Suzuki (1987), Boone and Pine (1968), Pine and Boone (1967), Larsen (1967), Kawamoto et al. (1981), Lechevalier and Lechevalier (1970, 1980), and Yoshida et al. (1991).

Growth characteristics

The growth characteristics of strain TA4-1 were compared with those of *A. halophila* and *A. mortivallis* under various cultivation conditions, including temperature (10–50°C), pH (4–12), and media containing NaCl and metal ions in concentrations of 0–20% (Sehgal and Gibbons, 1960; Larsen, 1967; Novitsky and Kushner, 1975; Yoshida et al., 1991).

Antibiotic susceptibility

Susceptibility to various antibiotics was determined by the methods of Fingeld et al. (1978) and Yoshida et al. (1991).

Antimicrobial activities

Antimicrobial activities were studies according to the USA Code of Federal Regulation for Foods and Drugs (1981). Soft nutrient agar, Czapek-Dox agar, and Sabouraud agar were melted at 45 to 50°C. The indicator bacteria were added (10^6/ml) and mixed well. Four milliliters of this agar were poured onto hard agar plates. After cooling, 4 to 6 standard steel cylinders were put on each plate. The organism culture solution was then placed on these cylinders and the plate was incubated at 28, 30, and 37°C, respectively, for 24 to 48 hours. The appearance of an inhibition zone around the steel cups showed that the tested organism produced antibiotics that inhibited the indicator bacteria. we also used the paper disc method (8.0 mm diameter, Toyo Seisakusho Co. Japan) of antibiotic sensitivity testing.

Results

Scanning electron microscopy

Scanning electron micrograph of strain TA4-1 revealed smooth-surfaced spores with oblong shape (0.5–0.8 μm wide and 0.8–1.0 μm long). Over twenty spores might form rectum-flexible, spiral chains (Figure 1A), suggesting that it may belong to the genus *Streptomyces*. The spores of *A. mortivallis* showed smooth surface, oval or kidney-like shape (0.5–0.8 μm wide and 1.2–1.5 μm long). Typically, 10–12 spores formed chains without spiral structures (Figure 1B), which distinguished it from strain TA4-1. The spores of *A. halophila* also showed a smooth surface, but with circular to rectangular shape (0.5 μm wide , 0.5–0.7 μm long). Typically, 5–10 spores formed short chains without spiral structure (Figure 1C). Table 1 compares these microscopic structures. There appeared to be morphological similarity among these three strains, but they may still be distinguished from each other.

Cultural characteristics

The cultural characteristics of strain TA4-1 grown on various media are shown in Table 2. It grew well on enriched organic media, such as TYG agar. The colonies turned brown, and white spores developed in 2 weeks with the formation of brown, soluble pigment that indicated the production of melanin. Poor growth was observed on oatmeal, minimum actinomycete medium, and carrot plug. There was no pigment production.

Table 1. Comparison of the microscopic morphology of strain TA4-1, *A. halophila*, and *A. mortivallis*.

	Strain TA4-1	*A. halophila*	*A. mortivallis*
Spore size (μm)	0.5-0.8 x 0.5-1.0	0.5 x 0.8	0.5-0.8 x 1.2
Spore shape	oblong	rectangular	kidney-like
Spore chain	over 20 in spiral chain	5-10 in straight chain	10-12 in straight chain

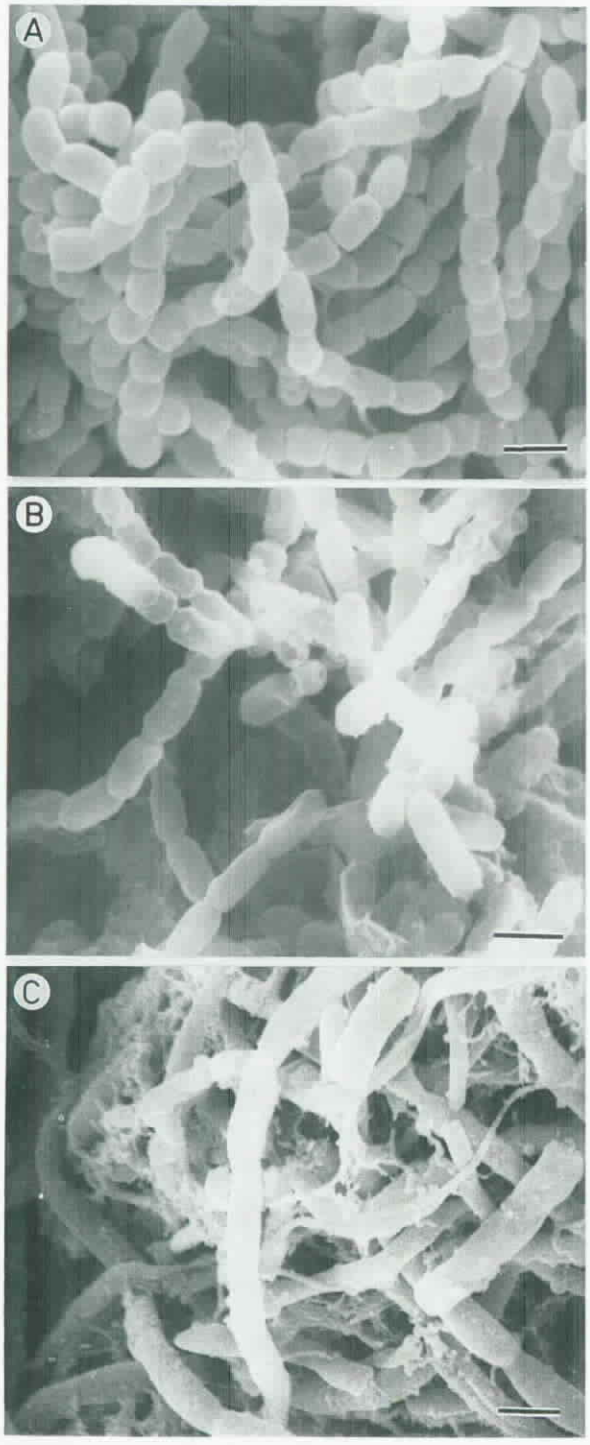

Figure 1. Scanning electron micrograph of (**A**) strain TA4-1; (**B**) *A. mortivallis*; (**C**) *A. halophila*.

The influence of NaCl on the growth of strain TA4-1 was compared to that on *A. halophila* and *A. mortivallis* (Table 3). On TYG agar, Strain TA4-1 grew well on plates containing 3–12% NaCl (Figure 2), but poor growth was observed below 3% and above 12% NaCl. No growth of strain TA4-1 appeared in YE+CM medium in which 20% NaCl was present, whereas *A. halophila*, because of it extreme halophilism, grew (with spore formation)only in YE+CM medium (Figure 3). The moderate halophile, *A. mortivallis*, did not grow in plain TYG, Czapek-Dox, or ISP9 medium, but did grow in TYG medium containing 10% NaCl (with spore formation) and also in YE+CM medium (Figure 4), corresponding to its growth requirement of 5–20% NaCl (Yoshida et al., 1991). Other media, including nutrient agar, ISP2, ISP7, and ISP8 also supported the growth and sporulation of *A. mortivallis*.

Physiological characteristics

Table 4 compares the physiological characteristics of the three strains. The distinctive characteristics are the optimal temperature and pH for growth (28°C and alkaline pH for strain TA4-1, 37°C and less alkaline pH for *A. halophila*, and 45°C and less alkaline pH for *A. mortivallis*). For strain TA4-1, the nitrate reduction and growth on carrot plug were negative and the other characteristic properties were positive. Some of the other physiological properties of strain TA4-1, e.g. starch hydrolysis, milk peptonization, and pigment production, were also distinguishable from those of *A. halophila* and *A. mortivallis*.

Utilization of carbon and nitrogen sources

As shown in Tables 5 and 6, strain TA4-1 was able to utilize half of the carbohydrates tested. Most of the amino acids, except cystine and tryptophan, could be used as a nitrogen source. The utilization of carbon and nitrogen sources by *A. halophila* and *A. mortivallis*, however, revealed significant differences when compared to that by strain TA4-1.

Cell wall composition and chemotaxonomic studies

The cell wall of strain TA4-1 contained LL type diaminopimelic acid (LL-A2Pm), which is characteristic of streptomycetes (Yamaguchi, 1965), whereas both *A. halophila* and *A. mortivallis* had meso type (Table 7). Paper chromatography of whole cell hydrolysate demonstrated a complete sugar pattern, including glucose, galactose, and ribose for strain TA4-1 (Figure 5), but only arabinose for both *A. halophila* and *A. mortivallis*.

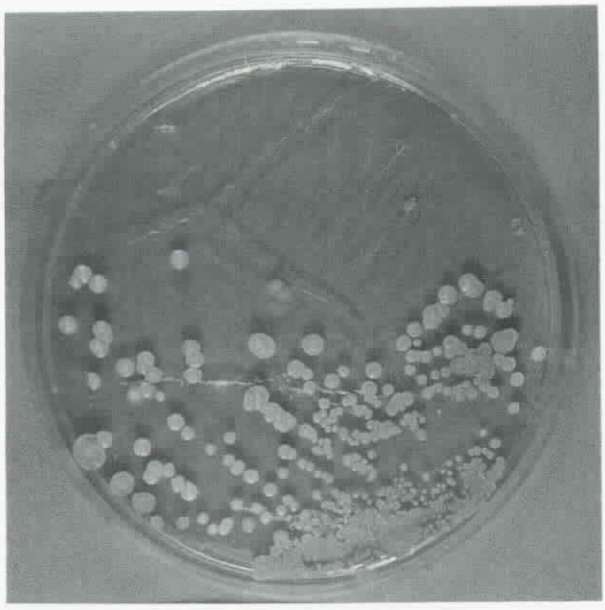

Figure 2. Strain TA4-1 grown on the TYG plus 10% NaCl agar plate at 28°C for 3 weeks.

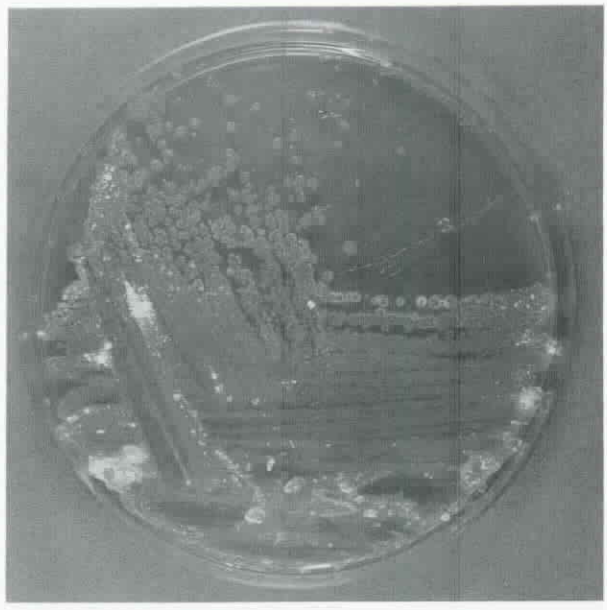

Figure 3. Strain *A. halophila* grown on YM+CM agar plate at 45°C for 3 weeks.

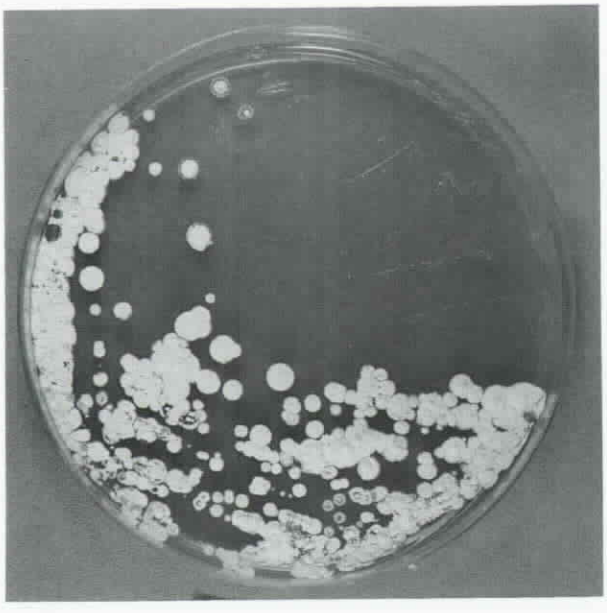

Figure 4. Strain *A. mortivallis* grown on YM+CM agar plate at 45°C for 3 weeks.

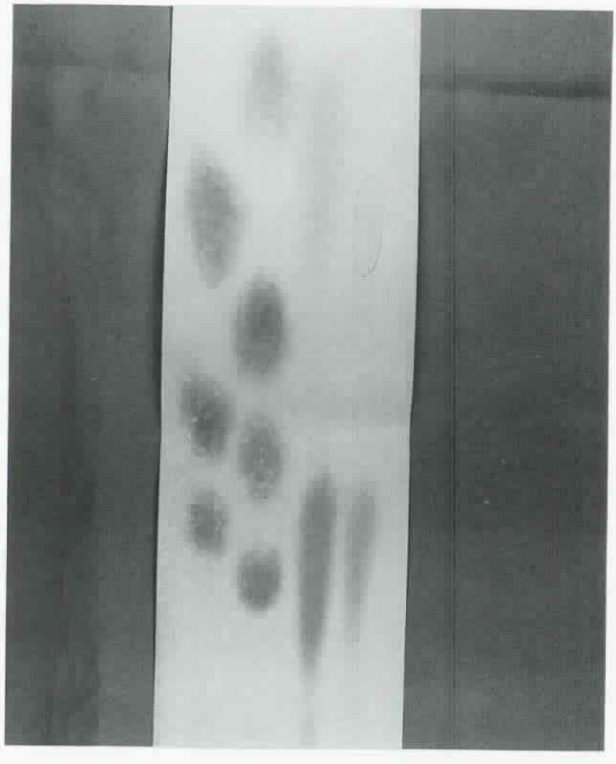

Figure 5. Thin layer chromatograph for the cell wall sugar composition of strain TA4-1.

Table 2. Cultural characteristics of strain TA4-1.

Medium	Characteristics				
	Colony growth	Vegetative mycelia	Aerial mycelia	Spore	Soluble pigment
Glycerol tyrosine agar	Moderate	Moderate, light brown	Moderate, white	Moderate, white	Brown
Peptone yeast agar	Moderate	Moderate, brown	Moderate, white brown	Moderate, white, powdery	Brown
Oatmeal agar	Poor, flat	Restricted growth, brown	Poor, light brown	Sparse, white	None
Glycerol asparagine agar	Moderate, flat	Restricted growth, light brown	Poor, white	Moderate, white, powdery	Greenish brown
Yeast-malt extract agar	Well	Abundant, light brown	Abundant, grayish white	Moderate, white	Brown
Starch agar	Well, flat	Moderate, light brown	Moderate, brown	Moderate, white, powdery	Greenish brown
Minimal actinomycetes medium	Moderate	Moderate, light brown	Poor	Sparse, white	None
Peptone agar	Moderate	Moderate, brown	Moderate, brown	Moderate, white	Brown
Tryptone yeast glucose agar	Well	Well, brown	Abundant, white	Abundant, white	Brown
Nutrient agar	Well	Moderate, brown	Abundant, white	Moderate, white, powdery	Brown
Potato plug	Moderate	Moderate, brown	Moderate, white	Moderate, white	Brown
Carrot plug	Moderate	Moderate, brown	Poor, white	Sparse	None

Table 3. Comparison of the halophilic growth of strain TA4-1, *A. halophila*, and *A. mortivallis* on different kinds of media.

Medium	Growth		
	Strain TA4-1	*A. halophila*	*A. mortivallis*
TYG	+	-	-
TYG with 10% NaCl	+	-	+/-
YE+CM	-	+	+

+: healthy growth with spore formation; +/-: growth without spore formation; -: no growth.

Table 4. Comparison of the physiological characteristics of strain TA4-1, *A. halophila*, and *A. mortivallis*.

Reaction	Medium	Response		
		Strain TA4-1	*A. halophila*	*A. mortivallis*
Gelatin liquefaction	Gelatin medium	+++	-	-
Starch hydrolysis	Starch agar	+++	+	-
Milk coagulation	Litmus milk	+++		
Milk peptonization	Litmus milk	+++	+	-
Nitrate reduction	Nitrate broth	-	-	-
Tyrosinase reaction	Tyrosine agar	++	-	-
H_2S reduction	Peptone iron agar	-	+/-	+
NaCl tolerance	TYG slant with NaCl	3-12%	10-25%	5-12%
Growth temperature	TYG slant	4-37°C	28-37°C	28-50°C
Melanin formation	PY slant	+++	-	-
Growth on potato plug	Potato plug	+	-	-
Growth on carrot plug	Carrot plug	-	-	-
Blood hemolysis	5% blood agar	+++	-	+/-
Pigment production	TYG and YE+CM	Brown	-	Brown
pH range	TYG slant	6-11	5-9	5-9

+++: abundant growth; ++: well growth; +: growth; +/-: poor growth; -: no growth.

Table 5. Comparison of the utilization of carbon source by TA4-1, *A. halophila,* and *A. mortivallis.*

Carbon source	Strain TA4-1	*A.halophila*	*A. mortivallis*
L-Arabinose	-	-	-
D-Xylose	+	-	-
D-Glucose	-	-	+
D-Fructose	-	+	-
Sucrose	+	-	-
L-Rhamnose	-	-	-
Raffinose	-	-	-
i-Inositol	-	-	-
D-Mannitol	+	-	-
Mannose	-	-	-
Galactose	-	-	-
Maltose	-	-	-
Lactose	+	-	-
Inulin	+	+	-
Starch	+	-	-
Dextrin	-	-	-
Glycerol	+	-	-
Sorbitol	+	-	+
Dulcitol	+	-	-
Salicin	+	-	+

++: well growth; +: growth; -: no growth.

Table 6. Comparison of the utilization of nitrogen source by strain TA4-1, *A. halophila*, and *A. mortivallis.*

Nitrogen source	Strain TA4-1	*A. halophila*	*A. mortivallis*
Cys.	+/-	+	+
Leu.	+	-	+/-
Ile.	++	-	+
Ser.	+	+	-
Glu.	+	+	-
Phe.	+	+	+
Lys.	+	+/-	+
Gly.	+	+/-	+
l-Asp.	++	+	+
Thr.	+	+/-	+
Tyr.	+	+/-	+/-
Asp.	+	+	+
Val.	+	+	+
Try.	+/-	+	+
Pro.	+	+	+
Met.	+	+/-	+/-

++: well growth; +: growth; +/-: poor growth; -: no growth.

Table 7. Comparison of cell wall composition of strain TA4-1, *A. halophila*, and *A. mortivallis.*

	Strain TA4-1	*A. halophila*	*A. mortivallis*
A2Pm	ll-A2Pm	meso-A2Pm	meso-A2Pm
Sugar*	C	A	A
Phospholipid**	PE	PC	PC

* C: complete; A: arabinose; G: galactose.
** PE: phosphatidylethanolamine; PC: phosphatidylcloline.

Table 8. Comparison of the effect of temperature on the growth of strain TA4-1, *A. halophila,* and *A. mortivallis.*

Incubation temperature (°C)	Growth		
	Strain TA4-1	*A. halophila*	*A. mortivallis*
20	++	+	+/-
37	+	+	+
45	+/-	-	++
50	-	-	+

++: well growth; +: growth; +/-: poor growth; -: no growth.

The phospholipid was found to be phosphatidylethanolamine (PE) for strain TA4-1, but phosphatidylcholine (PC) for *A. halophila* and *A. mortivallis*. This information, and particularly, the finding of arabinose in the cell walls of *A. halophila* and *A. mortivallis* but not in that of strain TA4-1, may significantly indicate a different taxonomy.

Testing for DNA homology (Berd, 1973) found no DNA-DNA homology among the three strains, suggesting the absence of taxonomic relation.

Growth characteristics

The growth characteristics of strain TA4-1 were further compared with those of *A. halophila* and *A. mortivallis* using agar plates or shake flask cultures. The incubation temperature affected growth significantly (Table 8). Strain TA4-1 grew well at 28–37°C, similar to *A. halophila*. *Actinopolyspora mortivallis*, however, preferred higher temperature (45°C) for growth, as previously reported by Yoshida et al. (1991).

Culture pH had no significant influence on the growth of any of the actinomycetes studied, although they typically preferred an alkaline environment. Strain TA4-1 grew well in a wider pH range (pH 6–11) than did *A. halophila* or *A. mortivallis* (pH 5–9).

The influence of NaCl was studied further with agar plate cultures supplemented with various amounts of NaCl (Table 9). Table 3 shows that strain TA4-1 grew in plain TYG medium, but could still grow in the presence of up to 10% NaCl, suggesting NaCl tolerance. *Actinopolyspora halophila* proved to be extremely halo-

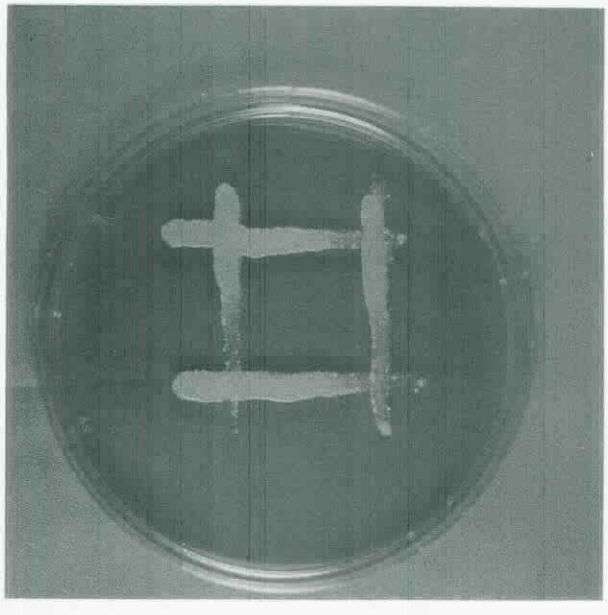

Figure 6. Strain TA4-1 grown on the TYG plus 14% KCl agar plate.

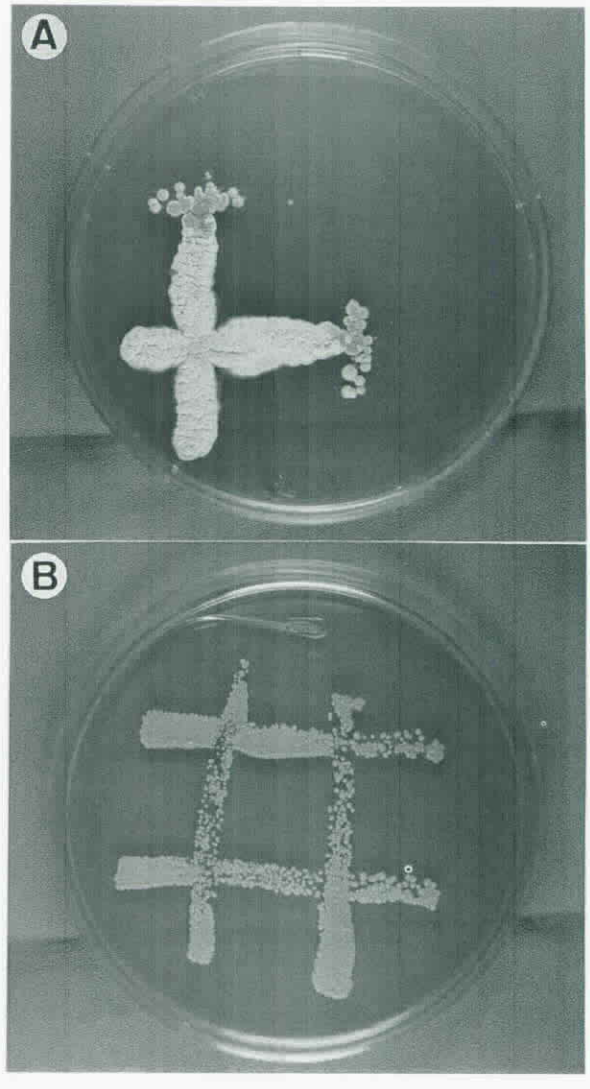

Figure 7. Strain TA4-1 grown on the TYG agar plates plus (**A**) 200 unit penicillin; (**B**) 4 unit streptomycin.

Table 9. Comparison of the effect of NaCl on the growth of strain TA4-1, *A. halophila*, and *A. mortivallis*.

NaCl level (% wt/v)	Growth		
	Strain TA4-1	*A. halophila*	*A. mortivallis*
0-7	++	-	+/-
10	+/-	-	+
10-15	-	+/-	+
15-18	-	+	+
18-20	-	+	+

++: well growth; +: growth; +/-: poor growth; -: no growth.

Table 10. Comparison of the effect of metal ions on the growth of strain TA4-1, *A. halophila*, and *A. mortivallis*.

Metal ions	Growth		
	Strain TA4-1	*A. halophila*	*A. mortivallis*
Na^+ (NaCl)	+	+	+
K^+ (KCl)	+ (<12%)	-	-
Mg^{++} ($MgSO_4$)	- (<18%)	-	-

+: significant affect on growth; -: no significant affect on growth.

Table 11. Antibiotic sensitivity test of strain TA4-1, *A. halophila*, and *A. mortivallis*.

Antibiotics	Concentration (mcg/ml)		
	Strain TA4-1	*A. halophila*	*A. mortivallis*
Penicillin[a]	> 300	1.2	2
Streptomycin[b]	4	< 1	> 128

[a]Penicillin as penicillin-G (1647 units/mg, Sigma)
[b]Streptomycin as streptomycin sulfate (Meiji Seika, Tokyo, Japan)

Table 12. Antimicrobial activities of antibiotics produced by strain TA4-1.

Test organisms	Inhibition zone (mm)
Staphylococcus aureus 209P	-
Sarcina lutea ATCC 9341	1.25
Escherichia coli NIHJ	-
Pseudomonas aeruginosa	-
Bacillus subtilis PCI 219	1.28
Bacillus sereus ATCC 11778	0.83
Candida albicans ATCC 10231	-
Saccharomyces cerevisiae ATCC 9763	-
Penicillium italicum Wehmer	-

philic and *A. mortivallis* to be moderately halophilic, corresponding to previous reports (Gochnauer et al., 1975; Yoshida et al., 1991).

The halophilism of bacteria suggests an ability to respond to osmotic stress induced by the growth environment (Csonka, 1989). This osmotic adjustment characteristic has been related to the cell envelope (Larsen, 1967) and to various inorganic or organic intracellular compatible solutes (Csonka, 1989; Meikle et al., 1988) which may be influenced by nutritional conditions (Robertson et al., 1992). A simple study compared the influence of metal ions on the growth of halophilic actinomycetes (Table 10). It was found that Na^+ influenced mycelial growth significantly at NaCl levels corresponding to their halophilism. Interestingly, the potassium cation influenced strain TA4-1 as Na^+ did (Figure 6), but poorly supported growth of *A. halophila* and *A. mortivallis*. Magnesium cations had no effect on the growth of all three strains studied.

Antibiotic susceptibility

Table 11 compares the susceptibility to antibiotics of strain TA4-1 with that of *A. halophila* and *A. mortivallis*. Strain TA4-1 was resistant to penicillin but sensitive to streptomycin. Growth on agar plates was observed in the presence of 300 mcg/ml penicillin or 4 mcg/ml streptomycin. (Figures 7A and 7B). *Actinopolyspora halophila* was very sensitive to both antibiotics and *A. mortivallis* was sensitive to penicillin but very susceptible to streptomycin. The difference in antibiotic susceptibility may be correlated to variations in their cell wall composition.

Antimicrobial activities

The antimicrobial activity of strain TA4-1 is shown in Table 12. Antibiotics produced by strain TA4-1 inhibited the growth of *S. lutea, B. subtilis*, and *B. sereus*, suggesting an antibacterial activity. *Actinopolyspora halophila* and *A. mortivallis*, however, showed no activity inhibiting the growth of either Gram-negative or Gram-positive bacteria.

Discussion

Although the morphology of strain TA4-1, observed by scanning electron microscopy, is not significantly different from that of *A. mortivallis* or *A. halophila*, it's biological characteristics suggest that strain TA4-1 is a streptomycete. This is supported by the whole cell chemotype analyses. Consequently, strain TA4-1 does not belong to the genus *Actinopolyspora*.

The isolation of strain TA4-1 is valuable and of interest. It is an antibiotic producer and, according to the physiological studies, it performs more enzymic reactions than do the other two known halophilic actinomycetes (Table 3). Enzymes from strain TA4-1 may be able to perform catalytic reactions under extreme conditions, e.g. high pH or salinity. This is an industrially useful potential.

The halophilism of strain TA4-1 is ambiguous because NaCl appears to be unnecessary for its growth. The fact that strain TA4-1 grows in media containing 3–12% NaCl does not prove it to halophilic, but strongly suggests that it is tolerant of NaCl. Various microorganisms are known to tolerate NaCl. In one survey, 18.8% of the 1300 *Streptomyces* strains in a culture collection could grow in 10% NaCl (Tresner et al., 1968). Okazaki and Okami (1976) isolated 13 marine streptomycetes from shallow sea mud, and found that marine isolates have higher tolerance to NaCl than do ISP cultures. Three strains out of the 13 isolates required 3–5% NaCl in the medium for their growth, showing halophilism according to Larsen's (Larsen, 1967) definition. Strain TA4-1 may be one such marine strain of *Streptomyces*, as it was isolated from an estuary, a marine habitat where *Streptomyces* are distributed (Weyland, 1981).

The NaCl tolerance of strain TA4-1 may be the result of years of evolution of an indigenous species under salinity stress (Yancey et al., 1982); or may be due to the adaptation to the marine habitats of a washed-in terrestrial organism (Goodfellow and Haynes, 1984; Wu, 1993). It remains to be seen whether strain TA4-1 is a true marine streptomycete requiring NaCl in the media for optimal growth (Goodfellow and Haynes, 1984). Assignment of strain TA4-1 to species level will require comparison with reported NaCl tolerant *Streptomyces* species.

Previous studies, performed mainly on bacteria such as halobacteria and halococci, have reported interesting metabolic apparatii and a characteristic cellular surface related to their halophilism (Larsen, 1967). A typical physiological response of these halophiles to osmotic stress is an elevated level of cellular compatible solutes, e.g. potassium ion, glutamate, proline, choline, etc., under a genetic level of control (Csonka, 1989). Unfortunately, very little is known about the biochemical, physiological, and genetic aspects of the NaCl tolerance or halophilism of actinomycetes. Knowledge of whether these actinomycetes respond to osmotic stress in a way similar to the halophilic bacteria, and if their compatible solutes are of the same nature awaits further study.

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鏈黴菌 TA4-1 之生物特性及嗜鹽性研究

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放線菌 TA4-1 是篩選新抗生素產生菌過程中自淡水河口，紅樹林土壤中分離出之一株抗生素產生菌，能生長在含鹽份 3-10% 的培養基中。TA4-1 菌之形態與嗜鹽放線菌 *Actinopolyspora motivallis* 13635 (Yoshida, 1991) 相似。為闡明 TA4-1 菌在分類學上之位置，乃進行生物特性研究，並同時採用 *A. motivallis* 13635 和 *Actinopolyspora halophila* 13403 做比較試驗，以求發現與嗜鹽性相關連之特性。結果發現，此三株菌之菌体形態在電子顯微鏡下觀察並無顯著之差異。但是培養特性，生理生化特性，醣類分解特性，whole cell chemotype 之試驗結果卻顯示 TA4-1 菌應屬於鏈黴菌屬，而三試驗菌株之特性有極大的差異。依此推之，TA4-1 菌株應不同於 *A. motivallis* 和 *A. halophila* 而較可能是 *Streptomyces* 屬之一耐鹽性菌株。

關鍵詞：鏈黴菌；分類學；嗜鹽放線菌。