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BIOCHEMICAL, NUTRIENT AND INHIBITORY CHARACTERISTICS OF *STREPTOMYCES* CULTURED FROM A HYPERSALINE ESTUARY, THE LAGUNA MADRE (TEXAS)

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ABSTRACT

Streptomyces are common soil bacteria that produce secondary metabolites, including several antibiotics; however, the characteristics of marine *Streptomyces* are largely unknown. Sediment samples were taken from 3 sites in the Laguna Madre to isolate marine *Streptomyces*. Sediment was diluted, spread onto synthetic seawater media to estimate the total bacterial density of the samples and spread onto starch casein agar to isolate *Streptomyces*. Isolated *Streptomyces* were tested for salinity tolerance and optimal growth pH. Isolates were assayed using API 20E® test strips and BIOLOG™ plates to construct biochemical profiles and assess nutrient utilization abilities of the bacteria, respectively. Individual *Streptomyces* were tested for the ability to inhibit the growth of other isolated *Streptomyces* (i.e., interference competition) and putatively identified by DNA sequencing. Results showed that there was no significant difference in microbial density in sediments from the 3 sampling sites. Eleven (11) *Streptomyces* pure cultures were obtained in total; most tolerated salinity up to 60 ppt and grew optimally at pH 7.5. Biochemical profile comparisons showed that the *Streptomyces* were only at least 74% similar; most (8/11) were >90% similar. Isolates could use between 87-95 carbon sources. Three (3) isolates displayed interference toward other isolates. Ten (10) isolates were identified as *Streptomyces griseus* by DNA sequencing. Laguna Madre *Streptomyces* organisms display some diverse characteristics with regards to their halotolerance, biochemical profiles, carbon source utilization and inhibition toward other organisms. Further investigations may yield greater understanding of these organisms in this and other marine environments and may be a reservoir of novel microorganisms and secondary metabolites.

Keywords: *Streptomyces*, Bacteria, Laguna Madre, Hypersaline

1. INTRODUCTION

The genus *Streptomyces* is a diverse group of more than 500 species of Gram-positive, spore-forming, high G+C bacteria within the phylum Actinobacteria. Streptomycetes produce networks of long-chain filaments that resemble the mycelia produced by fungi but are true bacteria and common inhabitants of terrestrial soils. Streptomycetes have been reported to represent a large percentage (10-28%) of the bacteria of some soils (Barton and Northrup, 2011). A few members of the genus are plant pathogens; however, streptomycetes are best known for their production of secondary metabolites such as pigments and a variety of antimicrobial compounds that have been shown to inhibit the growth of other microorganisms (Madigan *et al*., 2000).

Although streptomycetes are abundant in soils, their occurrence, distribution and ecology in other environments, such as aquatic ecosystems, is not as well

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known. Members of the genus *Streptomyces* have been described as occurring widely in lake water (Rheinheimer, 1992) and marine streptomyetes have also been isolated (Jensen *et al*., 1991; Moran *et al*., 1995; Mincer *et al*., 2002). There are still many marine ecosystems that have not been explored for streptomycetes. In light of the vast secondary metabolism of streptomycetes, aquatic environments and marine ecosystems may be new sources of novel products produced by these bacteria.

The Laguna Madre is a shallow, hypersaline estuary in South Texas (USA) and Northern Mexico. Because it is partially land-locked between the Texas mainland and barrier islands in the Gulf of Mexico, high salt concentrations develop in the lagoon as a result of high evaporation rates and limited freshwater inputs (Tunnell and Judd, 2002). The Laguna Madre has an average depth of approximately 1.5 m and salinity of approximately 40 ppt. The Laguna Madre is along the migration route of several migratory and endangered species of birds and the macroecology of the ecosystem has been studied for decades. The microbial ecology of the Laguna Madre is largely undescribed.

The Laguna Madre may be a potential source of previously unstudied streptomycetes and streptomycetes isolated from the Laguna Madre may produce novel compounds or provide insights into the ecology of aquatic *Streptomyces* species that have been hitherto unknown. In this study, we sought to successfully isolate *Streptomyces* species from sediments of the Laguna Madre and characterize the isolated organisms using biochemical and nutrient utilization assays, inhibition experiments and DNA sequencing.

2. MATERIALS AND METHODS

2.1. Study Site and Sample Collection

Sediment samples were taken from 3 sites in the Laguna Madre, a hypersaline estuary in South Texas, in June 2006. Samples (approximately 50 g) were collected from the top 10 cm of sediment. The sample locations were along a north to south transect. The sample locations were designated LMT056 [N26°30′42.1″, W97°22′23.6″], LMT055 [26°27′46.5″, W97°21′51.2″] and SB [N26°02′48″, W97°11′3.3″]. Samples LMT056 and LMT055 were closer in proximity to each other compared to sample SB, which was approximately 150 km south of the other sample sites. Samples were transported on ice to the University of Texas-Pan American Coastal Studies Lab on South Padre Island, Texas, USA and processed within 6 h of collection. Water temperature, pH and salinity were measured at the time of collection using a standard thermometer, a handheld pH meter and a handheld refractometer, respectively.

2.2. Culturing and Population Density

Ten-fold serial dilutions of sediment were made in Phosphate-Buffered Saline (PBS) Sambrook and Russell (2001) adjusted to pH 8. An aliquot of 100 µL of the diluted sample was spread onto a synthetic marine medium, Marine 2216 (Difco/BBL, Detroit MI) and Starch Casein Agar (SCA). Marine 2216 agar was used to estimate the total culturable microbial density of the sediment samples. SCA was only used to isolate members of the genus *Streptomyces* (Kuster and Williams, 1964); the density of *Streptomyces* in the samples was not determined. SCA was amended with 30 g NaCl L^{-1} to enhance marine *Streptomyces* growth. All media contained pentachloronitrobenzene (PCNB) at a final concentration of 1 g L^{-1} to minimize fungal contamination. Cultures were grown for 3-4 days in an incubator at 28°C. After incubating, the density of the microbial community was estimated by multiplying the number of colonies that arose on the agar plate by the dilution. Microbial density was reported as colony forming units per gram (cfu per gram) wet sediment.

2.3. *Streptomyces* Quantification

Colonies exhibiting characteristic streptomycete colony morphology (Bowers *et al*., 1996) on SCA were selected for further study. *Streptomyces* isolated from the Laguna Madre were inoculated onto Oatmeal Agar (OA) plates and allowed to grow for 4 days at 28°C. The composition of OA plates was per liter: 40 g of ground Quaker Oats® oatmeal, 15 g Bacto agar and 1 g casamino acids (Anderson and Wellington, 2001). The isolates were then transferred into sterile cryotubes containing 2 ml of 20% glycerol to create working spore suspensions of each of the marine *Streptomyces* isolates. Each isolate was diluted to approximately 4.5×10^6 spores/mL. Suspensions were quantified by measuring the OD_{600} using a spectrophotometer. The spore suspensions were used in salinity, pH and inhibition experiments.

2.4. Salinity and pH Assays

Streptomyces isolated from the Laguna Madre were assayed for their salinity and pH tolerances. SCA plates were made with seven salt concentrations (0 ppt, 5 ppt, 30 ppt, 40 ppt, 60 ppt, 90 ppt and 120 ppt) by adding the appropriate amount of NaCl prior to autoclaving. All media was adjusted to pH 8.0. *Streptomyces* isolates were grown in triplicate on separate plates by dotting 10

µl of quantified stock spore solution on each of the seven salinity plates. Cultures were incubated at 28°C for 3 days. A subjective scale was used to determine salinity tolerance based on ["No growth" or "Visible growth"] of the *Streptomyces* isolates.

The pH of SCA was adjusted with lactic acid or NaOH pellets to the following pH values: 6.8, 7.0, 7.3, 7.5, 7.8, 8.0 and 8.2. *Streptomyces* isolates were dotted (10 µL of quantified spore suspensions) on plates of the different pH values. Cultures were incubated at 28°C for 3 days. Assays were performed in triplicate. A subjective scale was used to rate the growth of the isolates (e.g., weak/little growth, fair growth, good growth and excellent growth).

2.5. API 20E® Strips

Biochemical profiles for *Streptomyces* isolates were generated using API 20E® test strips (bioMérieux Inc., Durham, NC). API 20E® strips include enzymatic tests for the fermentation or oxidation of glucose, mannitol, inositol, sorbitol, rhamnose, saccharose, melibiose, amygdalin and arabinose, along with nitrate reduction to nitrite and nitrate reduction to nitrogen gas. API 20E® strips also test for the presence of β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, urease, tryptophan deaminase, indole production, acetoin production (Voges-Proskauer) and gelatinase. API 20E® tests were performed according to the manufacturer's instructions. The number and types of positive tests were tabulated for the isolates and used to construct biochemical phenotype profiles of the cultures which were compared amongst the isolates. A similarity dendogram among profiles was constructed using the program NTSYSpc (Exeter Software, Setauket, NY).To construct the similarity dendogram, an input matrix was constructed with the 22 API 20E® tests. If a bacterial isolate was positive for that test, the matrix input was '1'. If the isolate was negative for the test, the matrix input was '0'. The NTSYSpc software was then used according to the manufacturer's instructions to produce a similarity matrix and tree. Each isolate's profile was compared to the profile of all the other isolates and reported as a Coefficient of Similarity on a scale of 0.00-1.00 with 1.00 equal to 100% similarity.

2.6. BIOLOG™ Carbon Source Utilization

Nutrient (carbon source) utilization profiles were determined for the *Streptomyces* isolates using BIOLOG SFP2™ plates. BIOLOG SFP2™ plates were 96 well plates that have 95 carbon sources and a water control. Spore suspensions for each isolate were made in 0.2% Carrageenan (Sigma, St. Louis MO) by harvesting an inoculated OA plate after 8 days growth at 28°C. Spore concentrations were adjusted to an optical density of 0.22-0.24 at 590 nm. The adjusted spore suspension (1.5 mL) was diluted in 13.5 mL of 0.2% Carrageenan and 100 µL of the resulting suspension was inoculated into each well of the BIOLOG SFP2™ plate using an eight channel micropipettor. Plates were incubated at 28°C. At 48 h, plates were measured using the BioRad® Model 680 microplate reader. The absorbance of each well at an optical density of 590 nm was recorded. The test was performed in duplicate BIOLOG SFP2™ plates for each isolate. The absorbance of the water control well was was subtracted from absorbances for each of the 95 carbon sources to account for background interference. Mean absorbance values for each substrate were calculated and negative mean absorbances were set to zero for subsequent analyses. Substrate richness was determined for each isolate (Zak *et al*., 1994; Vahjen *et al*., 1995). Substrate richness was defined as the total number of substrates with a positive mean absorbance value. A similarity dendogram among profiles was constructed as described above. Correspondence between similarity matrices for API and BIOLOG™ results was evaluated using Mantel's test (NTSYSpc; Exeter Software, Setauket, NY).

2.7. Interference Competition Assays

The ability of each *Streptomyces* isolate to inhibit and resist all others in the collection was evaluated following the methods of Davelos *et al*. (2004a). An aliquot (10 µL) of each of the quantified *Streptomyces* spore suspensions were dotted onto SCA plates and allowed to grow at 28°C. After three days, plates were turned over 4 mL of chloroform in a watch glass for 1 h in a fume hood to kill the isolate. Then the plates were allowed to vent dry for 30 min in a laminar flow hood. After venting dry, each plate had 15 mL of 1% water agar (5 g agar, 500 mL dH_2O) pipetted onto each plate. Once solidified, 100 µL of one isolate was spread over the water agar layer and allowed to grow at 28°C for 3 days. This experiment was performed in all possible pair wise combinations for each the *Streptomyces* isolates. Each combination was replicated three times to ensure that observed results were not due to chance. Since all zones of inhibition observed did not have a uniform circular appearance, the mean zone of inhibition was obtained by taking the average of the largest and smallest points of the inhibition zone. All data were analyzed by taking the mean of the three replicated plates.

2.8. DNA Extraction and Sequencing

DNA was extracted from the *Streptomyces* isolates using a commercial kit for Gram-positive bacteria (Wizard Genomic DNA Purification Kit; Promega, Madison WI) according to the manufacturer's instructions. Extracted DNA was verified by electrophoresis using 1% agarose gels. Genomic DNA concentration was quantified by reading the absorbance in a UV-VIS spectrophotometer at 260 nm. Purity of the extracted DNA was determined by the ratio of the absorbances at 260 and 280 nm. Ratios of 260/280 absorbance measurements were between 1.7 and 2.0 for all samples; thus, the extraction yielded mostly DNA (Sambrook and Russell, 2001).

Molecular identification of the isolated bacteria was performed by the Polymerase Chain Reaction (PCR) amplification and sequencing of the 16S ribosomal RNA (16S rRNA) genes (Sambrook and Russell, 2001) using primers designed for *Streptomyces*. *Streptomyces* specific primers SSU139F (5´-ACAAGCCCTGGAAACGGGGT-3´) and SSU657R (5´-CACCAGGAATTCCGATCT-3´) located at positions 139-158 and 640-657 (*S. ambofaciens* numbering) were used (Malkawi *et al*., 1999). PCR reaction mixtures (25 μL) consisted of 50 ng of DNA, 10 nmol of each primer and PCR Promega® GoTaq™ SuperMix. PCR was performed with a BioRad® MyCyclerTM thermal cycler following the Takeuchi *et al.* (1996) protocol. Amplified PCR products were purified with the QIAquick PCR purification kit following the manufacturer's instructions (QIAGEN Inc., Valencia, CA). A second round of PCR was done for sequencing using a commercially-available sequencing kit (Genome Lab DTCS Quick Start Kit; Beckman Coulter, Fullerton CA) according to the manufacturer's instructions and the SSU139F primer. Dye-tagged dideoxynucleotides (ddUTP, ddGTP, ddCTP and ddATP) were added to terminate elongation (Sambrook and Russell, 2001). The resulting PCR product was loaded into an automated DNA sequencer (CEQ 8000 Genetic Analysis System; Beckman Coulter, Fullerton, CA). The sequences were compared to known bacterial sequences available in the National Center for Biotechnology Information Basic Local Alignment Search Tool (BLASTN) database to identify the microorganisms (Altschul *et al*., 1997).

3. RESULTS

The salinity and pH of the Laguna Madre samples were as follows: LMT056, salinity 29.8 ppt and pH 8.87; LMT055, salinity 30.5 ppt and pH 8.63; SB, salinity 32.4 and pH 8.9. The estimated density of the total culturable microbial populations grown on Marine 2216 agar at each site were not statistically different (**Fig. 1**). A total of 11 distinct *Streptomyces* isolates were cultured from SCA plates. Of the 11 isolates, 5 were from LMT056, 1 was from LMT055 and 5 were from SB (**Table 1**). The 11 *Streptomyces* were characterized physiologically and genetically.

The majority of *Streptomyces* isolates displayed a maximum growth salinity of 60 ppt and a pH growth optimum of 7.5 (**Table 1**). With regards to salinity, the lowest salinity maximum was observed in isolate 56.2 which did not grow above 40 ppt NaCl. The pH optima for 2 isolates, 56.2 and SB.5, were slightly higher; these isolates grew best at pH 7.8 but did display growth at other pH values. Isolate 56.1 was the most halotolerant (120 ppt) and alkalitolerant of the 11 isolates (8.0) (**Table 1**).

The 11 cultured *Streptomyces* isolates were screened for biochemical phenotypes using API 20E® test strips. A suite of 22 tests was used to construct a phenotype profile for each isolate. The number of positive tests displayed by the isolates ranged from 3-11 (**Table 2**). All 11 *Streptomyces* isolates showed an API® profile similarity coefficient of at least 0.74 meaning that their profiles were at least 74% similar (**Fig. 2**). Eight of the 11 isolates displayed greater than 90% similarity when profiled with API 20E test strips and 2 isolates, 56.1 and 55.1, had identical profiles. Results of carbon source utilization based on BiOLOG™ assays are shown in **Fig. 3**. The number of carbon sources utilized ranged from 87-95. All 11 of the *Streptomyces* isolates had a minimum carbon source utilization similarity of 0.91 or 91%. Several isolates had identical profiles. Isolate SB.5 utilized the lowest number of carbon sources (87) and displayed the least similarity to the other isolates (**Fig. 3**). Mantel's test showed that there was no correspondence between API® and BIOLOG™ phenotypes ($r = 0.12$, $p>0.05$).

The 11 *Streptomyces* isolates were examined for interference competition toward the other isolates. Interference competition plate assays were performed to determine whether an isolate could inhibit the growth of other isolates. A representative plate assay is shown in **Fig. 4**. Only 3 of the 11 *Streptomyces* strains could inhibit other isolates and eight isolates were resistant to inhibition by other isolates (**Table 3**). Isolate 55.1 was inhibitory toward isolate 56.1, isolate SB.3 could inhibit isolate SB.1 and isolate SB.4 could inhibit growth of isolates 56.1 and 56.5. These three inhibitory isolates were also resistant to inhibition by all other isolates. All other combination failed to produce inhibited growth. The five isolates from location LMT056 did not inhibit any other isolates; however two of these isolates were susceptible to inhibition from the other locations (**Table 3**).

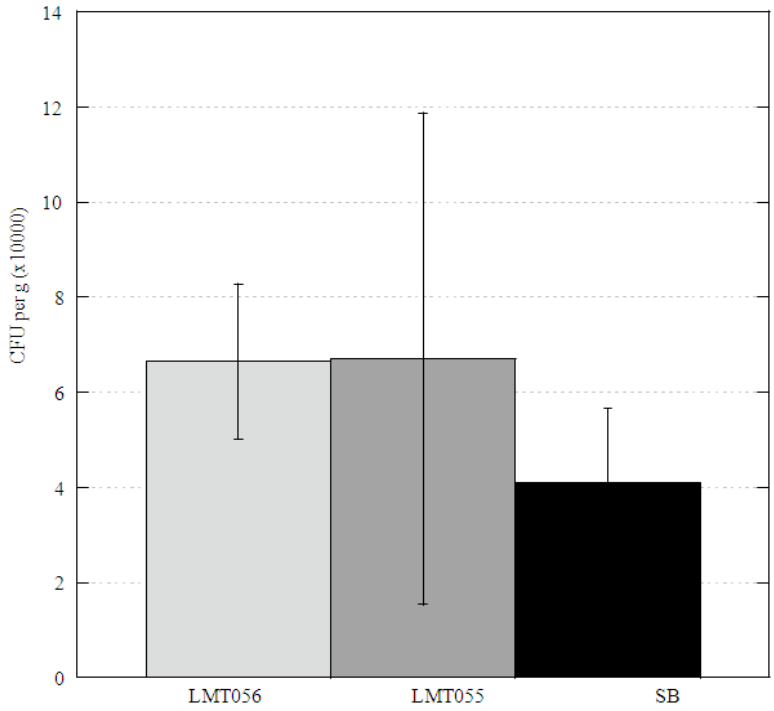

Fig. 1. Estimated density of bacteria in Laguna Madre sediment samples collected in June 2006. Sediment was diluted and spread on Marine 2216 agar. Values are reported as colony forming units per gram (cfu per gram ×10,000) wet sediment. Shown are the means of triplicate incubations with standard deviation

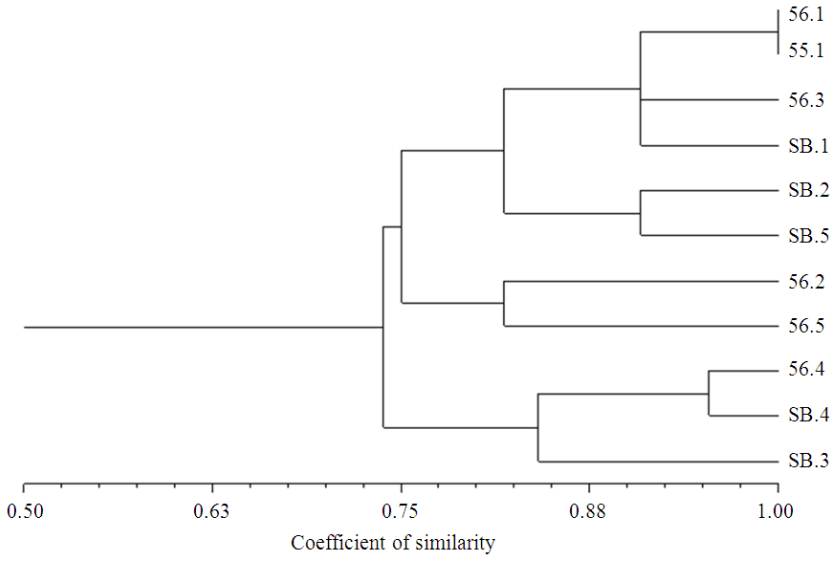

Fig. 2. Similarity of cultured *Streptomyces* microorganisms from the Laguna Madre based on API 20E® phenotype profiles.A total of 22 different enzymatic reactions were assayed using API 20E® strips. Based on the number of positive tests and which of the 22 tests were positive, a phenotype profile was created and compared to the profile of all other tested isolates. The similarity coefficient is shown on the x-axis

Table 1. Characterization and identification of *Streptomyces* isolates from the Laguna Madre. Salinity and pH tolerances were based on agar plate assays. Isolates were identified by sequencing of the 16S rRNA gene and comparing the sequencing to known sequences in the BLASTn database. The percent sequence match is shown

Isolate designation	Site of Origin	Maximum Growth Salinity (ppt)	pH Growth Optimum	Identity by 16S rDNA sequencing	ID Match (%)
56.1	LMT056	120	8.0	*Streptomyces griseus*	97
56.2	LMT056	40	7.8	*Streptomyces griseus*	96
56.3	LMT056	60	7.5	*Streptomyces scabiei*	94
56.4	LMT056	60	7.5	*Streptomyces griseus*	98
56.5	LMT056	60	7.5	*Streptomyces griseus*	99
55.1	LMT055	60	7.5	*Streptomyces griseus*	93
SB.1	SB	60	7.5	*Streptomyces griseus*	98
SB.2	SB	60	7.5	*Streptomyces griseus*	99
SB.3	SB	60	7.5	*Streptomyces griseus*	98
SB.4	SB	60	7.5	*Streptomyces griseus*	99
SB.5	SB	60	7.8	*Streptomyces griseus*	95

Table 2. Biochemical (API) profiles of *Streptomyces* bacteria from the Laguna Madre. Eleven (11) *Streptomyces* isolates were isolated from the Laguna Madre sediment. The isolates' metabolic activities were tested using API 20E® strips. A plus sign (+) indicates that the isolate was positive for the test; a negative sign (-) indicates a negative reaction for the test

	Isolate										
Test	56.1	56.2	56.3	56.4	56.5	55.1	SB.1	SB.2	SB.3	SB.4	SB.5
ONPG	–	–	–	–	–	–	+	–	–	–	–
ADH	–	+	–	–	+	–	–	–	+	–	–
LDC	–	+	–	–	+	–	–	–	+	–	–
ODC	+	–	–	–	+	+	+	+	–	–	+
CIT	+	+	+	+	+	+	+	+	+	+	+
H_2S	–	–	–	–	–	–	–	–	–	–	–
URE	–	+	–	+	+	–	–	+	+	–	–
TDA	–	–	–	–	–	–	–	–	–	–	–
IND	–	–	–	–	–	–	–	–	–	–	–
VP	–	–	–	–	–	–	–	–	–	–	–
GEL	+	+	+	+	+	+	+	+	+	+	+
GLU	+	+	+	–	–	+	+	–	–	–	–
MAN	+	+	+	–	+	+	+	–	–	–	–
INO	–	–	–	–	–	–	–	–	–	–	–
SOR	+	–	–	–	+	+	–	+	–	–	+
RHA	–	–	–	–	–	–	–	–	–	–	–
SAC	+	–	+	–	+	+	+	+	+	–	+
MEL	–	–	–	–	–	–	–	–	–	–	–
AMY	+	+	+	–	+	+	+	–	–	–	+
ARA	–	–	–	–	–	–	–	–	–	–	–
NO_2	+	+	+	+	+	+	+	+	+	+	+
N_2	–	–	–	–	–	–	–	–	–	–	–

Tests: ONPG, β-galactosidase activity; ADH, Arginine Dihydrolase; LDC, Lysine Decarboxylase; ODC, Ornithine Decarboxylase; CIT, Citrate Utilization; H2S, Hydrogen Sulfide Production; URE, Urease; TDA, Tryptophan Deaminase; IND, Indole Production; VP, Acetoin Production (Voges-Proskaur); GEL, Gelatinase; GLU, Glucose; MAN, Mannitol; INO, Inositol; SOR, Sorbitol; RHA, Rhamnose; SAC, Sucrose; MEL, Melibiose; AMY, Amygdalin; ARA, Arabinose; NO2, Nitrate Reduction to Nitrite; N2, Nitrate Reduction to Nitrogen Gas

Table 3. Interference competition assays amongst the 11 *Streptomyces* isolates from the Laguna Madre. Each isolate was tested to determine if it could inhibit the growth of another isolate by using an agar overlay inhibition method. Values are the mean of triplicate experiments. Inhibition of other isolates is shown in rows (0.00 indicates no inhibition); resistance to other isolates is shown in columns (0.00 indicates resistance to inhibition)

	Isolate overlaid inhibition Zone (mm)										
Isolate dotted	56.1	56.2	56.3	56.4	56.5	55.1	SB.1	SB.2	SB.3	SB.4	SB.5
56.1		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
56.2	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
56.3	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
56.4	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00
56.5	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00
55.1	8.13	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00
SB.1	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00
SB.2	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00
SB.3	0.00	0.00	0.00	0.00	0.00	0.00	6.88	0.00		0.00	0.00
SB.4	9.50	0.00	0.00	0.00	12.0	0.00	0.00	0.00	0.00		0.00
SB.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	

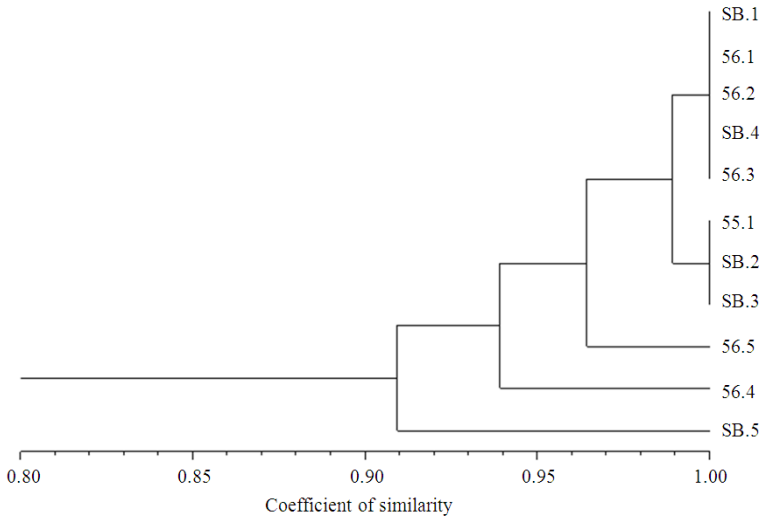

Fig. 3. Similarity of carbon utilization of cultured *Streptomyces* microorganisms from the Laguna Madre.A total of 95 carbon sources were tested using BIOLOG™ plates. Based on the number of positive tests and which tests were positive, a carbon utilization profile was created and compared to the profile of all other tested isolates. The similarity coefficient is shown on the x-axis

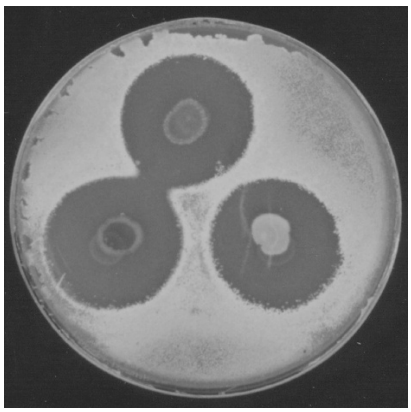

Fig. 4. Representative interference competition plate showing zones of inhibited growth

Sequencing of the 16S rRNA genes of the 11 isolates revealed that 10 of the isolates were at least a 93% match to *Streptomyces griseus* in the BLASTn database (**Table 1**). Isolate 56.3 most closely matched to *Streptomycesscabiei*, some strains of which are pathogenic to plants (Healy and Lambert, 1991).

4. DISCUSSION

This small pilot study was conducted to ascertain whether the Laguna Madre was a potential habitat of marine *Streptomyces* species. The Streptomycetaceae have been described in the literature for decades but it has been suggested that aquatic streptomycetes are terrestrial forms that have been washed into freshwater and/or marine environments and that these aquatic streptomycetes are not members of the native microbial community (Cross, 1981). It has also been suggested that *Streptomyces* inhabiting aquatic ecosystems may not be active but merely surviving in a dormant (e.g., endospore) state. The pH optima for growth by the *Streptomyces* we studied would seem to support this notion and suggests that our isolates may be of terrestrial origin. Most of the 11 cultures grew optimally at pH values that were lower than the water pH of the Laguna Madre at the time of sampling (see Results) and more closely aligned with the pH of terrestrial soils (**Table 1**; Atlas and Bartha, 1998). Several studies have shown the presence of streptomycetes from aquatic environments, including marine near-shore areas and deep sea sediments (Jensen *et al*., 1991; Moran *et al*., 1995; Mincer *et al*., 2002). Despite this, their abundance and activity in hypersaline marine ecosystems, like the Laguna Madre, has been largely neglected.

In this study, 11 *Streptomyces* isolates out of the tens-of-thousands of microorganisms present in the sediments of the Laguna Madre were cultured. The low number of isolated organisms may be due to ineffective sampling or culturing techniques, or it could indicate that *Streptomyces* are only minor constituents of the Laguna Madre's sediment microbial community. Given that most of the 11 isolates are presumptively the same or similar species (e.g., *Streptomyces griseus*) based on 16S rRNA sequencing, it is also possible that the species richness of *Streptomyces* in the Laguna Madre is limited. *Streptomyces griseus* is a spore-forming, alkaliphilic bacterium known to produce many types of secondary metabolites, including the antibiotic streptomycin (Liu *et al*., 2005). However, the results of the 16S rRNA gene sequencing should only be considered putative. There are a limited number of complete bacterial sequences in the BLASTn database and none of the isolates was a 100% match for known sequenced *Streptomyces* strains. As more sequences are added to the database, better identification of the organisms will become possible.

Despite the small number of isolates and their 16S rRNA gene similarity, the 11 *Streptomyces* display some diverse characteristics with regards to their biochemical profiles and carbon source utilization. Ten of the 11 isolates had distinct profiles when assayed with API 20E® strips and all could utilize a range of organic carbon substrates in BIOLOG™ assays. Many of the 11 isolates had identical BIOLOG™ profiles because they gave positive results for all 95 substrates tested (isolates 56.1, 56.2, 56.3, SB.1 and SB.4) or 94 out of 95 substrates tested (isolates 55.1, SB.2, SB.3). Despite this, the lack of correspondence between API® and BIOLOG™ phenotypes (Mantel's test) shows some diverse characteristics among this collection of isolates and suggests that utilizing both measures of physiological diversity provides independent information on isolate phenotype. The *Streptomyces* isolates also displayed a strong halotolerance, which would be necessary for survival in the hypersaline Laguna Madre and some isolates were able to inhibit the growth of others. Thus, although *Streptomyces* in the Laguna Madre may be a small population of phylogentically similar organisms, they are phenotypically and physiologically varied. This result of lack of correspondence between genetic identity and phenotype is consistent with other studies of soil bacteria in terrestrial systems (Bronstad *et al*., 1996; Bramwell *et al*., 1998; Davelos *et al*., 2004b).

5. CONCLUSION

Based on the results of this small project, a further investigation of *Streptomyces* in the Laguna Madre is warranted to gain a greater understanding of the density, activity and diversity of these organisms in this and other hypersaline environments, which may be a reservoir of novel microorganisms and their secondary metabolites.

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