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Marine actinomycete *Streptomyces* sp. ISP2-49E, a new source of Rhamnolipid

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Keywords

Marine actinomycete; *Streptomyces* sp; Rhamnolipid; Surfactant

1. Subject and source

Streptomyces sp. ISP2-49E was isolated from marine sediment collected from Galveston Bay, Texas, as part of continued bioprospecting for novel therapeutics from marine-derived microbes. Dr. Xia Yan of Northwest A&F University isolated the bacteria ISP2-49E and identified this microbe as a member of the *Streptomyces* genus based on morphology and 16S rDNA sequence analysis (Figure 1). A voucher specimen (ISP2-49E) is deposited with Professor Mark T. Hamann, Department of Pharmacognosy, the University of Mississippi, USA. The investigation of biologically active metabolites from ISP2-49E yielded one rhamnolipid, Rha-Rha-C₁₀-C₁₀ (Figure 2).

2. Previous work

Rhamnolipids (RLs) are constructed of one or two rhamnoses conjugated to fatty acid chains. These metabolites were first discovered from *Pseudomonas pyocyanea* (now *P. aeruginosa*) by Bergström et al., and reported as bacteriostatic molecules in *Mycobacterium tuberculosis* assays (Bergstrom et al., 1947). While the pharmacological properties of RLs limit their potential as therapeutic agents, there is significant potential for development as an industrial biosurfactant (Desai et al., 1997; Lang et al., 1999). In addition to antibacterial activity, antiphytoviral (Haferburg et al., 2004) and antifungal (Stanghellini et al., 1997) activities have been reported. The most recent report on RLs demonstrated antimalarial activity of a rhamnolipid Rha-C₁₀-C₁₂ (Wang et al., 2013).

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3. Present work

The marine sediment derived actinomycete *Streptomyces* sp. ISP2-49E, was cultivated in 10 L ISP2 liquid medium (Shirling et al., 1966) supplemented with 2% artificial ocean with continuous agitation in a shaking incubator (28°C, 5 days). The cultures, including broth and cells, were then extracted with ethyl acetate, the organic phase separated, and ethyl acetate removed under vacuum. The crude extract was initially separated into enriched fractions using a sephadex LH20 column with an isocratic methanol: water (90: 10) mobile phase to yield 30 sub-fractions. Fractions 9 and 10 (260 mg) were further purified by preparative HPLC using a C₈ column (Phenomenex, 10 mm × 250 mm, 10µm) eluted with a gradient of methanol: water (5: 95~100: 0), followed by a C₅ column eluted with a gradient of methanol: water (70: 30~100: 0) to obtain 5 mg pure metabolite. The structure was determined by comparing 1D, 2D NMR and MS with literature (Sharma et al., 2007; Edwards and Hayashi, 1965) (Figure 2). This is the first report of a rhamnolipid from the genus *Streptomyces*.

4. Ecological significance

While rhamnolipids are best described from *Pseudomonas* sp., they are also a biologically active component produced by the actinobacterium *Renibacterium salmoninarum*, *Cellulomonas cellulans* and *Nocardioles* sp. (Arino et al., 1998; Christova et al., 2004; Soberon-Chavez et al., 2005; Vasileva-Tonkova et al., 2005). To the best of our knowledge, this is the first report that *Streptomyces* sp. is capable of producing rhamnolipids.

Research in rhamnolipids has been promoted by their potential roles in bioremediation, biomedicine, and lubrication (Chrzanowski et al., 2012; Muller et al., 2012). In microbial ecology they increase the uptake of hydrophobic carbon sources by acting as a biosurfactant and changing the hydrophobicity of the cell wall (Chrzanowski et al., 2012). These molecules have also been linked to cell motility, formation of biofilms and chemical defense (Chrzanowski et al., 2012). Many rhamnolipids were isolated from hydrocarbon contaminated environment or grown in the presence of oils, further supporting their role in hydrocarbon uptake. Production of rhamnolipids is found in different phyla, including Proteobacteria and Actinobacteria. Within Proteobacteria, production is spread within the classes of beta-proteobacteria, gamma-proteobacteria and delta-proteobacteria. (Table 1) While the taxonomic classification of rhamnolipid producers is diverse, the involvement of hydrophobic hydrocarbons somewhere in either isolation or growth cultures is a clear trend (Figure 3, Table 1). This trend is consistent in the actinobacteria, *C. cellulans* was isolated from hydrocarbon polluted soil, and *R. salmoninarum* and *Nocardioles* sp. were both grown on hydrophobic substrates, n-hexadecane and paraffin, respectively (Arino et al., 1998; Christova et al., 2004; Vasileva-Tonkova et al., 2005).

Streptomyces sp. IA49E was isolated from Galveston Bay near the Inter-coastal Canal. This area is surrounded by petrochemical facilities and there is heavy shipping traffic in the canal, resulting in an increased probability of hydrocarbon pollutants. Indeed, the sediment sample from which ISP2-49E was isolated had visible signs of oil contamination. It is likely that the production of rhamnolipids is a trait that provides an environmental edge to *Streptomyces*

sp. IA49E by providing a mechanism to utilize the contaminant hydrocarbons as a carbon source and a potential chemical defense from other microbes.

Although bacterial species other than *P. aeruginosa* have been reported as RL producers, it is important to note that many have not been conclusively identified (e.g., through 16S ribosomal RNA gene sequencing), nor has their production of RLs been confirmed using high-precision analytical methods (e.g., HPLC-MS). Despite the promise of RL as industrial biosurfactants, pursuit of this application has been limited because the best characterized producer, *P. aeruginosa*, is also a human pathogen. Potentially, the most valuable aspect of our research is finding novel RL-producing species for commercial production purposes. The pathogenicity of the newly discovered RL-producers is uncertain and will require further investigation. Novel isolates, reported to be RL producers, are distributed within and outside the class gamma-proteobacteria. This emphasizes the need for wide spectrum screening programs for RL producers that encompass different orders and classes of bacteria. While some groups predict that gamma-proteobacteria will comprise the majority of novel RL-producing isolates (Rooney et al. 2009), identifying new RL-producing bacteria from broad screening methods is important to biotechnological development of RL's as this increases the potential for discovery of nonpathogenic producers. These screening efforts can potentially generate more appropriate candidates for the safe and industrially viable production of RLs.

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Highlights

- We identified *Streptomyces* sp. ISP2-49E from marine sediment of the Gulf of Mexico
- We isolated a biologically active rhamnolipid from the media of a ISP2-49E culture
- This is the first documented production of a rhamnolipid from *Streptomyces* spp.
- Rhamnolipids as an adaptation for hydrocarbon contaminated environments is proposed

Figure 1.

Phylogentic analysis of *Streptomyces* sp. IA49E with *Streptomyces griseolus* NBRC 3719, *S. cinereorectus* NBRC 15395, *S. sanglieri* NBRC 100784, *S. spiroverticillatus* NBRC 12821, *Micromonospora inyonensis* DSM 46123 (another actinomycete) and *Myxococcus xanthus* ATTC 25232as a non actinomycete. Clearly IA49E aligns within the *Streptomyces* spp.

Figure 2.
Structure of Rha-Rha-C₁₀-C₁₀

Figure 3. Phylogenetic analysis of rhamnolipid producers from Table 1. The main branches are between the Actinobacteria and Proteobacteria.

Table 1

Table of rhamnolipid producers and the presence of hydrophobic hydrocarbon. In all cases except *T. koreensis* and *Myxococcus* sp., there is involvement at either isolation or growth media level in strains that produce rhamnolipids.

Producing organism	Hydrophobic Hydrocarbon Involvement	Reference
<i>Pseudomonas aeruginosa</i>	molasses distillery waste water	Li et al., 2011
<i>Acinetobacter calcoaceticus</i>	petroleum contaminated soil isolate	Chen et al., 2012
<i>Pseudoxanthomonas</i> sp.	isolated from a coal sample	Nayak et al., 2009
<i>Enterobacter</i> sp.	isolated from biodiesel facility	Rooney et al., 2009
<i>Pantoea</i> sp.	grown on hydrocarbon	Vasileva et al., 2007
<i>Burkholderia</i> sp.	grown in soy bean oil	Andrä et al., 2006
<i>Myxococcus</i> sp.	soil isolate unknown hydrocarbon	Ohlendorf et al., 2009
<i>Renibacterium salmoninarum</i>	grown on <i>n</i> -hexadecane	Christova et al., 2004
<i>Cellulomonas cellulans</i>	isolated from hydrocarbon polluted soil	Arino et al., 1998
<i>Nocardioides</i> sp.	grown on paraffin	Vasileva et al., 2005
<i>Tetragenococcus koreensis</i>	isolated from kimchi	Lee et al., 2005