ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2007, p. 397–400 Vol. 51, No. 1
0066-4804/07/$08.00+0 doi:10.1128/AAC.01118-06

Letters to the Editor

Sophorolipids Having Enhanced Antibacterial Activity▽

Sophorolipids (SLs) are glycolipids produced fermentatively by yeasts such as *Candida bombicola*, *Candida apicola*, and *Wickerhamiella domercqiae* and composed of a dimeric sugar linked with a glycosidic bond to a hydroxyl fatty acid. SLs have generated interest in the pharmaceutical arena because of their wide array of therapeutic benefits. Shah et al. (5) first reported that natural SLs and their first-generation chemical derivatives are efficient microbicidal spermicides, having activities similar to that of nonoxynol-9. Bluth et al. (2) showed that natural SLs are effective septic shock antagonists. Additionally, SLs have been demonstrated to be effective anticancer agents against cancerous cell lines (4). One major drawback of SLs so far has been their cytotoxic activities. While efforts are being made to further chemically modify the molecule to increase its potency and decrease its toxicity, no improvements have been reported. The second generation of SL molecules was generated by conjugating amino acids to a fatty acid side chain (1). However, these molecules were less effective as microbicidal spermicides than first-generation compounds.

In the present study, we report the fermentative production of novel SL molecules that have higher antibacterial activities and offer a new backbone for further chemical modifications. The fermentation medium used to produce SLs by *Candida bombicola* uses glucose as the primary carbon source. Thus, the SL formed has two glucose molecules. We replaced glucose in the fermentation medium with the other sugars described in Table 1. The SLs formed in each of the media were extracted and purified per standard protocol (1). Liquid chromatography-mass spectrometry (LC-MS) analyses were carried out for the compounds, and the MIC extracts of compounds with masses from 500 to 800 are shown in Fig. 1. The peak appearing at 24.5 min in all the chromatograms is caused by the three sophorolipids, viz., a diacetylated lactonic SL with a C_{18} monounsaturated fatty acid (M+H, 689), a diacetylated lactonic SL with a C_{18} saturated fatty acid (M+H, 691), and a diacetylated open-ring SL with a monounsaturated fatty acid (M+H, 707). The peak at 25.3 min is also caused by compounds having the same mass. Two peaks for the same mass at different retention times have been reported earlier and are attributed to the attachment of a dimeric sophorose to the hydroxyl group at either the penultimate or the terminal fatty acid carbon, where the later is the minor component for the regioisomers (3). The peak at 33.1 min in all chromatograms is caused by a diacetylated open-ring SL with a saturated C_{18} fatty acid (M+H, 709). New peaks in the region of 35 to 50 min are caused by SL compounds having M+H values of 603 and 605, corresponding to an unacetylated lactonic SL with a diunsaturated C_{18} fatty acid and an unacetylated lactonic SL having a monounsaturated C_{18} fatty acid, respectively.

The appearance of new peaks for SLs produced from different sugars in comparison to that produced from glucose in the fermentation medium can be explained by the presence of a different sugar head group in the compound and the inability of the cellular machinery of *C. bombicola* to acetylate the new sugars. The unacetylated lactonic SLs reaffirm our hypothesis regarding the presence of new sugar heads in the SLs. Since the acetyl group is added to the glucose in the SL head group by the enzyme acetyltransferase, the presence of the unacetylated SLs indicates that the new sugar present in the head group is not glucose. Glucose in the SL head group is the natural substrate for acetyltransferase, and the new sugar head group could be presumed to be composed of molecules different from glucose, on which the enzyme cannot act. It is important to note that all the fermentation media had oleic acid as the lipidic substrate, and thus, no difference in structure could be expected for the fatty acid side chain. Further purification and characterization studies are needed to understand the precise chemical structure of each SL analog.

The antibacterial activities of the purified compounds show that SLs are more effective against gram-positive bacteria than gram-negative bacteria (Table 1). Comparing the antibacterial activities of SLs obtained in glucose-containing medium, it could be inferred that SLs obtained from different sugar-containing media differ in their activities against the tested organisms. SLs from arabinose-containing medium are more effective against three of the four gram-positive bacteria tested and against *Moraxella* sp. than SLs from glucose-containing medium. However, SLs from arabinose show no inhibition of the growth of *Escherichia coli.* SLs from lactose-containing me-

TABLE 1. Antibacterial activities

Microorganism	MLD_{50} (mg/ml)[a] for indicated sugar							
	Glucose	Fructose	Xylose	Ribose	Lactose	Mannose	Arabinose	Galactose
Rhodococcus erythropolis	0.098	0.024	0.006	0.024	0.098	0.098	0.006	>6.25
Bacillus subtilis	0.098	0.098	0.39	0.098	0.024	0.09	0.024	0.39
Staphylococcus epidermis	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25
Streptococcus agalactiae	0.098	0.024	0.024	0.098	0.098	0.39	0.024	0.39
Moraxella sp.	0.098	0.098	0.024	0.098	0.39	0.098	0.024	0.39
Pseudomonas putida	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25
Enterobacter aerogenes	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25
Escherichia coli	>6.25	>6.25	>6.25	>6.25	>6.25	>6.25	NI	NI

[a] The 50% minimum lethal doses (MLD_{50}) were determined by a broth microdilution method in 96-well microtiter plates. NI, no inhibition.

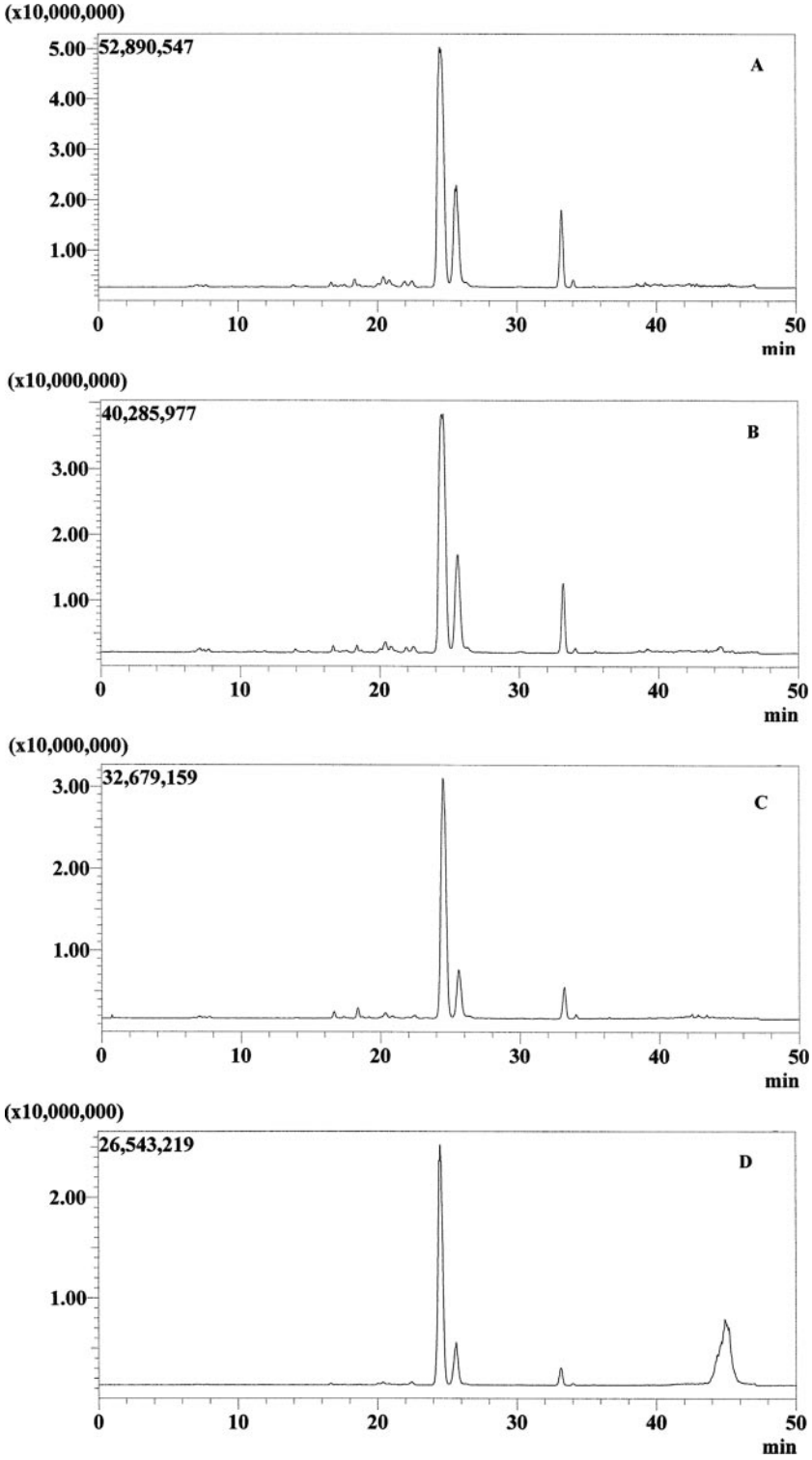

FIG. 1. LC-MS spectrum of sophorolipids obtained using different sugar sources in fermentation medium. A, glucose; B, fructose; C, xylose; D, ribose; E, lactose; F, mannose; G, arabinose; H, galactose. LC-MS experiments were conducted with a Shimadzu LCMS-2010EV apparatus. A C_8 column (150 by 3.0 mm; Princeton Chromatography Inc.) was utilized as the analytical LC column. Separations were achieved under gradient conditions by using 0.05% formic acid in water and 0.05% formic acid in acetonitrile as the mobile phase at a flow rate of 1 ml/min. MS analyses were performed on an instrument with positive polarity and an atmospheric pressure chemical ionization interface. The interface temperature was 300°C, the curved desolvation line temperature was 250°C, and the heat block temperature was 220°C. The detector voltage was 1.5 kV, and the scan speed was 4,000 amu/s.

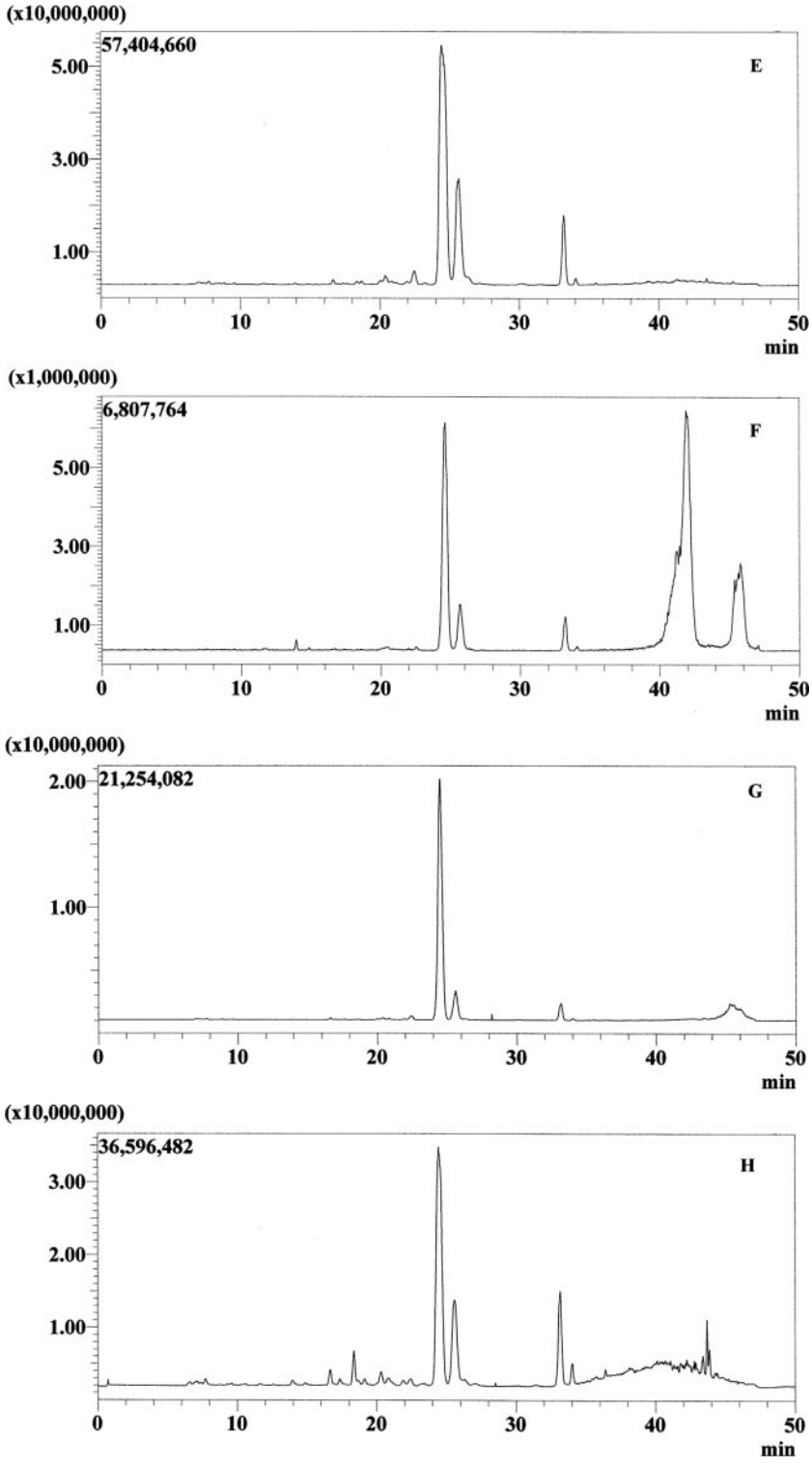

FIG. 1—*Continued.*

dium were the most effective compound against *Bacillus subtilis*.

All the efforts so far to generate a clinically viable compound by chemically modifying the SLs from glucose-containing medium have failed; we believe that the molecules generated through the present method will open a new door. With higher activities on their own, these molecules could be hypothesized to lead us to more potent compounds when chemistries similar to that carried out in developing first-generation SLs are used on them.

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▽ Published ahead of print on 6 November 2006.