JOURNAL OF BACTERIOLOGY, Oct. 1993, p. 6459–6466
0021-9193/93/206459-08$02.00/0
Copyright © 1993, American Society for Microbiology
Vol. 175, No. 20

A New Lipopeptide Biosurfactant Produced by *Arthrobacter* sp. Strain MIS38

MASAAKI MORIKAWA,[1] HIROMI DAIDO,[1] TOSHIFUMI TAKAO,[2] SATORU MURATA,[3] YASUTSUGU SHIMONISHI,[2] AND TADAYUKI IMANAKA[1]*

Department of Biotechnology,[1] Institute for Protein Research,[2] and Department of Applied Chemistry,[3] Osaka University, Yamadaoka, Suita, Osaka 565, Japan

Received 17 May 1993/Accepted 9 August 1993

A biosurfactant termed arthrofactin produced by *Arthrobacter* species strain MIS38 was purified and chemically characterized as 3-hydroxydecanoyl-D-leucyl-D-asparagyl-D-threonyl-D-leucyl-D-leucyl-D-seryl-L-leucyl-D-seryl-L-isoleucyl-L-isoleucyl-L-asparagyl lactone. Surface activity of arthrofactin was examined, with surfactin as a control. Critical micelle concentration values of arthrofactin and surfactin were around 1.0×10^{-5} M and 7.0×10^{-5} M at 25°C, respectively. Arthrofactin was found to be five to seven times more effective than surfactin. The minimum surface tension value of arthrofactin was 24 mN/m at a concentration higher than the critical micelle concentration. According to the oil displacement assay, arthrofactin was a better oil remover than synthetic surfactants, such as Triton X-100 and sodium dodecyl sulfate. Arthrofactin is one of the most effective lipopeptide biosurfactants.

Biosurfactants are surface active substances derived from living organisms, mainly from microorganisms (2, 5). The biological function of biosurfactants is thought to be participation in the solubilization of hydrophobic substances (hydrocarbons, lipids, and sterols, etc.), promoting enhanced cell assimilation. Emulsification which increases the surface area between two immiscible phases, results in small oil drops in water. It is generally concluded that microorganisms growing on water-insoluble hydrocarbons benefit from the presence of a surfactant (6, 8, 13). Biosurfactants are usually complex lipids, with more chemically complicated structures than synthetic surfactants. Lipopeptide biosurfactants are structurally more heterogeneous than glycolipid types.

The minimum surface tension and critical micelle concentration are parameters used to measure the efficiency of surfactant systems. The best-known lipopeptide biosurfactant is surfactin, which lowers the surface tension of 0.1 M $NaHCO_3$ from 71.6 to 27.0 mN/m (1). Recently, some strains of *Bacillus licheniformis* were shown to produce quite similar biosurfactant to surfactin (9, 15). *Arthrobacter* species have been reported to produce extracellular glycolipids (12, 20), none of which lowers the surface tension of water to 30 mN/m. We describe the chemical structure of a new lipopeptide-type biosurfactant.

MATERIALS AND METHODS

Screening of biosurfactant-producing bacteria. Biosurfactant-producing strains were selected as described previously (16), except that the cultivation temperature was 30°C. A slimy colony (strain MIS38) surrounded by a large halo on an oil-agar plate was obtained and used for further experiments.

Production and purification of biosurfactant. Strain MIS38 was cultivated in 3 liters of L broth (1% Bacto tryptone, 0.5% yeast extract, 0.5% NaCl [pH 7.2]) at 30°C for 48 h. The culture was centrifuged (10,000 × *g* for 10 min), and the supernatant fluid was concentrated by ultrafiltration (exclusion molecular size, 10 kDa).

The concentrated biosurfactant was extracted three times with an equal volume of hexane. After evaporation, the biosurfactant-containing extracts were purified by thin-layer chromatography on silica 60 (2 mm thickness; Merck Co., Berlin, Germany). Chloroform-methanol-water (65:25:4 [vol/vol/vol]) was used as the developing solvent, with visualization of separate components by either I_2 vapor or 30% sulfuric acid spray. A component with an R_f value of 0.3 was scraped and eluted with developing solvent. This component was purified further by thin-layer chromatography on silica gel 60 with a solvent system consisting of chloroform–methanol–25% ammonium hydroxide, (65:35:5 [vol/vol/vol]), yielding a surface active component with an R_f value of 0.44.

Further purification was done by C_{18}-reversed-phase high-performance liquid chromatography (HPLC). The major peak fraction was collected and dried, with a final yield of 50 mg of biosurfactant. This biosurfactant was dissolved in 50% acetonitrile at 60°C, cooled to room temperature, and stored at 4°C. Rod-shaped crystals (ca. 0.2 by 0.2 by 1 mm) formed after a couple of weeks.

Infrared spectrometry. A sample of biosurfactant was applied to a KRS-5 cell, and the Fourier transform-infrared spectrum was measured with a JIR-AQS20M (JEOL, Tokyo, Japan) spectrometer.

Amino acid analysis. The biosurfactant peptide was hydrolyzed with distilled 6 N HCl (containing 0.2% phenol) at 110°C for 24 h. The amino acid composition was analyzed with a Hitachi (Tokyo, Japan) type 835 amino acid analyzer.

NMR analysis. After the hydrolysis of purified biosurfactants (about 100 mg) with HCl, the fatty acids were extracted with ether and purified by thin-layer chromatography on silica 60 with the solvent system hexane-ether-acetic acid (90:10:1 [vol/vol/vol]). The fatty acids fraction (R_f = 0.16) was recovered from the plate, and the sample (6.3 mg) was dissolved in 0.7 g of $CDCl_3$. Tetramethylsilane was used as an internal standard. ^{1}H-nuclear magnetic resonance (NMR) of this fatty acid was analyzed by 400-MHz NMR (JNM-GSX-400; JEOL, Tokyo, Japan).

The intact biosurfactant was also dissolved in dimethyl sulfoxide-d_6 and analyzed by ^{1}H-NMR.

GC/MS analysis. A portion of fatty acid was esterified by diazomethane. Gas chromatography-mass spectrometry (GC/

* Corresponding author.

MS) of fatty acid methyl ester was carried out with a JEOL JMS-DX303 mass spectrometer at 70 eV on a capillary column (0.53 mm by 5 m) corresponding to OV-1.

Saponification of intramolecular lactone ring of biosurfactant. The biosurfactant was dissolved in 50 μl of 60 mM NaOH–40% methanol containing 40 atom% $H_2{}^{18}O$ (Isotec Inc.) and incubated at 37°C for 18 h. After the pH was adjusted to 3.0 with 1 M HCl, the sample was loaded on a Disposil C_{18} column (Nacalai Tesque, Kyoto, Japan), and the column was washed with distilled water. Saponified biosurfactant was eluted with 80% acetonitrile containing 12 mM HCl.

FAB-MS. Mass spectra were obtained in a JMS-HX100 double-focusing two-sector mass spectrometer (JEOL) equipped with a fast atom bombardment (FAB) ion source, a collision cell, and a data acquisition system (JMA-DA5000). Collision-induced dissociation and linked-scan measurements were carried out as described previously (21). Biosurfactant was dissolved in methanol and mixed with a liquid matrix (a mixture of dithiothreitol and dithioerythritol, 5/1 [wt/wt]) on the target.

Amino acid sequence analysis by Edman degradation. The biosurfactant was dissolved in 3% formate solution and incubated at 110°C for 24 h for site-directed hydrolysis at aspartic residues. This hydrolyzate was purified by C_4-reversed-phase HPLC. With monitoring at 215 nm, the main peak was collected and analyzed with a peptide sequencer ABI 476A (Applied Biosystems, Foster, Calif.). Peptide resulting from the formate hydrolysis was so hydrophobic (containing 2 mol of isoleucine and 3 mol of leucine of eight amino acid residues) that an arylamine-modified membrane (SequelonAA; Millipore Corp., Bedford, Mass.) was used to covalently bind the carboxyl-terminal amino acid.

Optical configuration analysis of amino acids. Total ratios of D and L amino acids in the biosurfactant were determined with an automatic amino acid analysis system (AMINOMATE; ICL Instruments, Victoria, Australia). The positions of D-Asp, L-Asp, D-Leu, and L-Leu were determined by the following method. Arthrofactin was hydrolyzed partially with concentrated HCl at 37°C for 3 days. The hydrolyzate was separated by C_4-reversed-phase HPLC (μBONDASPHERE C_4 column; 5-μm diameter; 300 Å [0.3 nm]; 3.9 by 150 mm) with elution with a 10 to 90% acetonitrile (containing 0.1% trifluoroacetic acid) linear gradient (5 to 35 min). After hydrolysis of the peptide, the amino acid residues were analyzed. Each fraction containing Leu and Asp was modified with a chiral FLEC [(+)-(9-fluorenyl) ethyl chloroformate; Eka Nobel AB, Surte, Sweden] (4) reagent to determine the D/L configuration. The retention time and each derivatized amino acid and standards were compared by C_{18}-reversed-phase HPLC Wakopak $5C_{18}$ column; 4.6 by 250 mm) with eluent A (tetrahydrofuran [THF]–0.15% sodium acetate [pH 4.0] [25:75]) or eluent B (THF–0.15% sodium acetate [pH 5.1], [50:50]), with 0 to 60% of gradient B for 0 to 40 min and 60 of gradient B for 40 to 60 min. The detector was set at λex of 260 nm and λem of 315 nm.

Surface tension. The surface tension was measured at 25°C with a Traube's stalagmometer (TOP Co. Kyoto, Japan). γ (surface tension) = $F(\gamma_0/m_0) \times m$, where γ_0 is the surface tension of distilled water, m_0 is the weight of distilled water per one drop, and m is the weight of sample per one drop. Samples were dissolved in 10 mM phosphate buffer (pH 8.0). The surface tension values for distilled water and the buffer were almost the same.

Assay for oil displacement activity of surfactants. We improved a convenient microassay system for surfactants. This method was based on the feature of the biosurfactant to change the contact angle at the oil-water interface. The surface pressure of the biosurfactant displaced the oil. Crude oil (15 μl) (Nikko Biotech Co., Shizuoka, Japan) was put onto the surface of 40 ml of distilled water in a petri dish (150 mm in diameter). A thin membrane of oil formed immediately. Then, 10 μl of the sample solution was gently put on the center of the oil membrane. A clear halo was visible under light. The area of this circle was measured. The sensitivity of this method enabled assay of at least 10 μg (about 10 nmol) of biosurfactant.

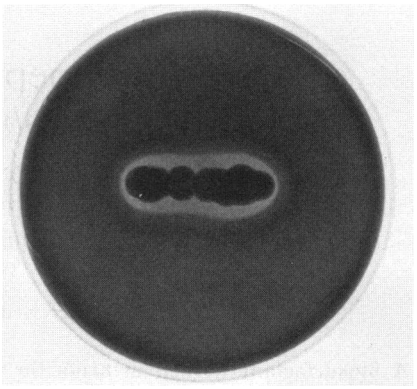

FIG. 1. Biosurfactant production of strain MIS38. Cells were grown at 30°C for 2 days on the blood agar plate (Eiken Chemical Co., Tokyo, Japan). A clear cytolytic halo was formed around the colony.

RESULTS

Identification of strain MIS38. Strain MIS38 is a nonspore-forming, gram-positive, strict aerobe which grows normally at 25 to 40°C. The strain is catalase positive and exhibits a rod-coccus cycle. According to *Bergey's Manual of Systematic Bacteriology* (19), strain MIS38 is grouped in section 15. There were two genera, *Arthrobacter* and *Brevibacterium*, in this section which show a cell morphology typical of the rod-coccus cycle. Finally, strain MIS38 was determined to be an *Arthrobacter* sp., because the strain showed motility and a lysine residue was found in the peptidoglycan. We named the biosurfactant produced by strain MIS38 arthrofactin. The production of surfactants was also confirmed by formation of a clear zone on blood agar plates (Fig. 1).

Structural analysis of arthrofactin. The Fourier transform-IR spectrum chart of purified arthrofactin is shown in Fig. 2. Characteristic absorption valleys at 1,540, 1,650, and 3,300 cm^{-1} indicated that arthrofactin contained some peptides. The lactone ring structure of arthrofactin was also suggested by the absorption at 1,740 cm^{-1}. Valleys at wavenumbers of 2,950 to 2,850 resulting from the C-H stretching mode suggested the presence of an aliphatic chain. These patterns were similar to those of surfactin (16). The total amino acid composition was determined to be Asp-Thr-Ser-Ile-Leu at a ratio of 2:1:2:2:4. The molecular mass of arthrofactin was estimated to be 1,354 by FAB-MS analysis (Fig. 3).

^{1}H-NMR analysis of the purified fatty acid portion arthrofactin demonstrated a single peak at δ = 0.85 ppm (methyl), indicating a straight-chain fatty acid (Fig. 4). The ratio of this integrated peak area and peaks at δ = 1.1 to 1.6 ppm (methylene) was 3:10. This value indicated that the aliphatic chain of this fatty acid was a normal hexyl residue. Other peaks were assigned as shown in Fig. 4.

Fatty acid was methylated and analyzed by GC/MS. Two peaks were detected at scan numbers 650 and 768 (data not shown) by total ion chromatography. Each electron ionization-MS pattern is shown in Fig. 5. The parental peak

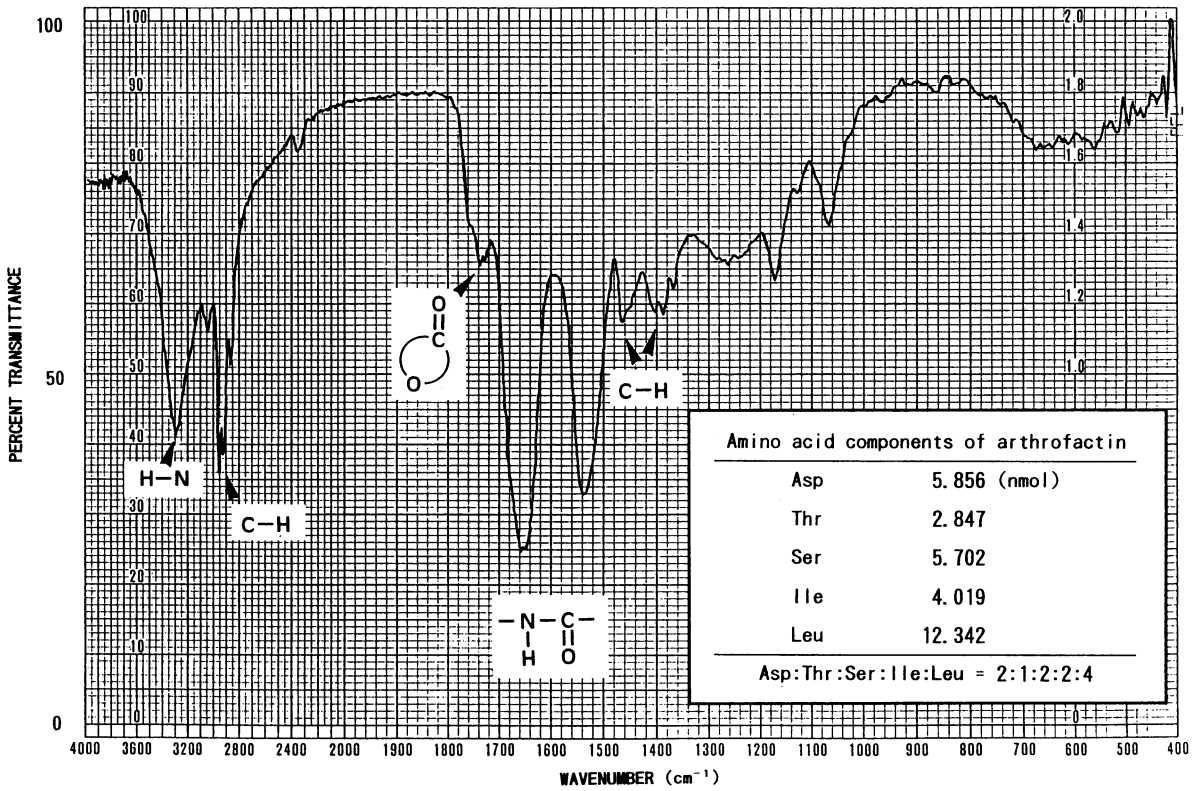

FIG. 2. Fourier transform-IR spectrum and amino acid analysis of arthrofactin.

of methyl ester was difficult to detect by this ionization method. The basal peak at *m/z* of 87 and 103 was due to $(CH_2-CH=COH-OCH_3]^+$ and $[CH(OH)CH_2COOCH_3]^+$, respectively. Other peaks were also assigned as shown in Fig. 5. The data from ^{1}H-NMR and GC/MS indicated that the fatty acid fraction was a mixture of (i) $C_7H_{15}CH=CHCOOCH_3$ and (ii) $C_7H_{15}CH(OH)CH_2COOCH_3$. The double bond in the former must have been formed during the hydrolysis of arthrofactin. It was concluded that the practical structure of the fatty acid portion of arthrofactin was that of 3-hydroxydecanoic acid, which was commonly found in the cells.

To determine the amino acid sequence, a collision-induced dissociation method in FAB-MS was applied to the peptide. The FAB mass spectra of intact and saponified biosurfactant gave signals at *m/z* of 1,354.9 (Fig. 3) and 1,373.4 (inset of Fig. 6), respectively. The latter value corresponds to the theoretical value calculated from the amino acid composition with lipid moiety at the N terminus. The shift in mass by 18.5 supported the presence of an intramolecular lactone ring in arthrofactin.

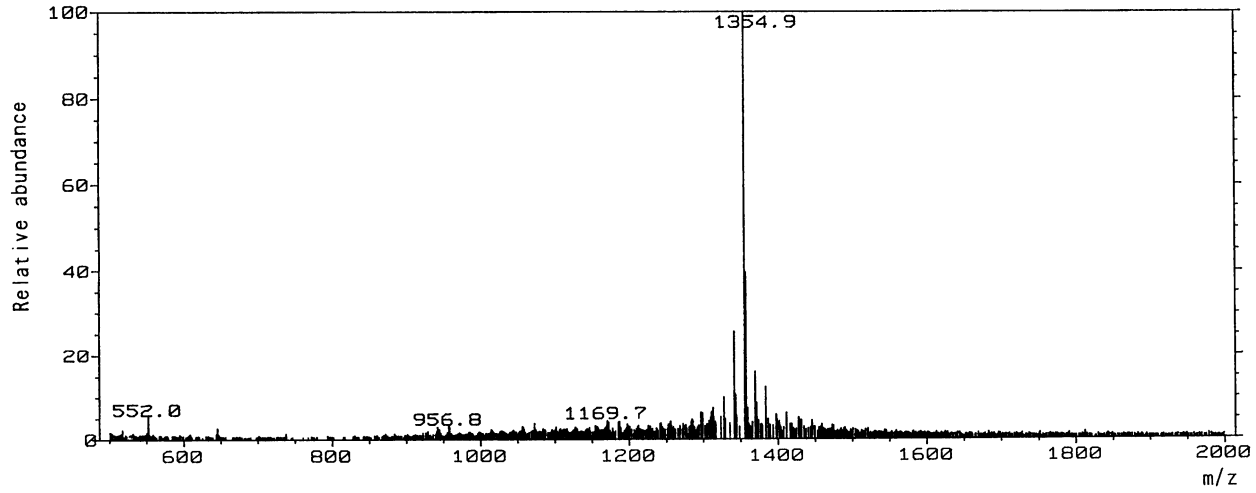

FIG. 3. FAB-MS spectrum of arthrofactin.

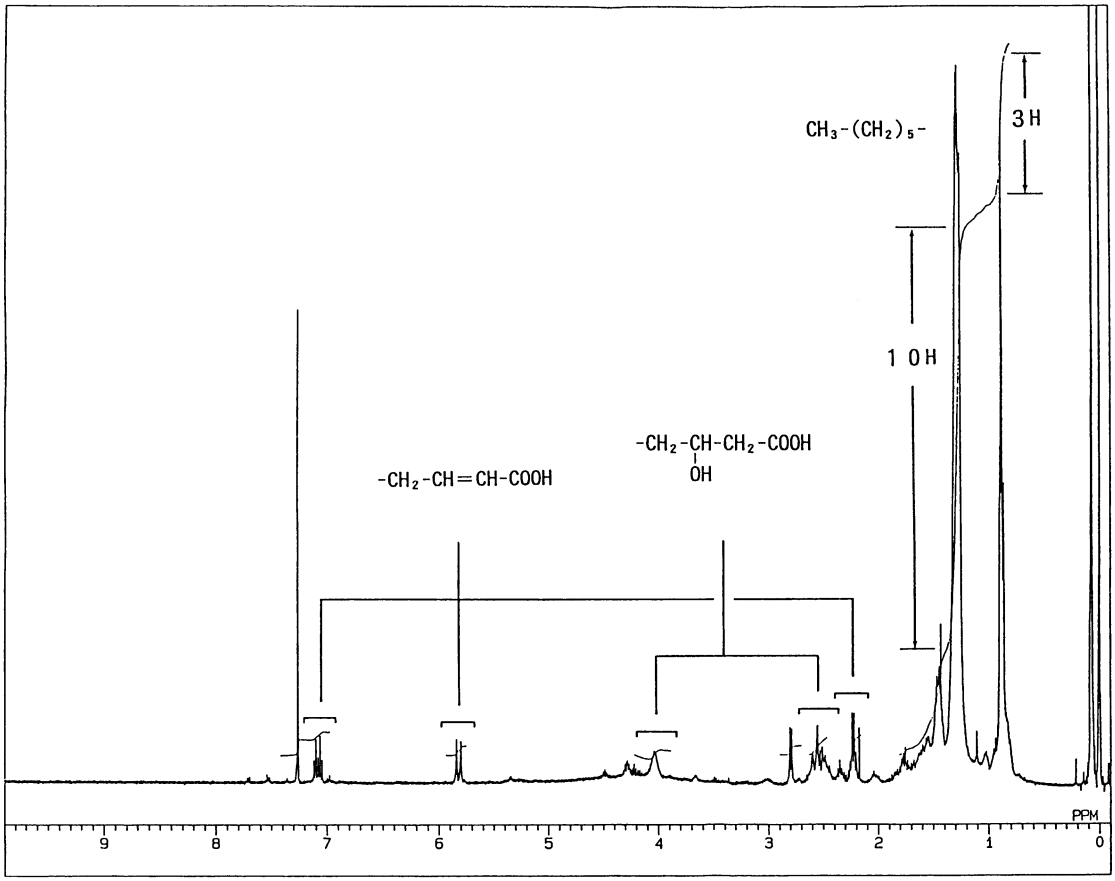

FIG. 4. ^{1}H-NMR spectrum of fatty acid portion of arthrofactin.

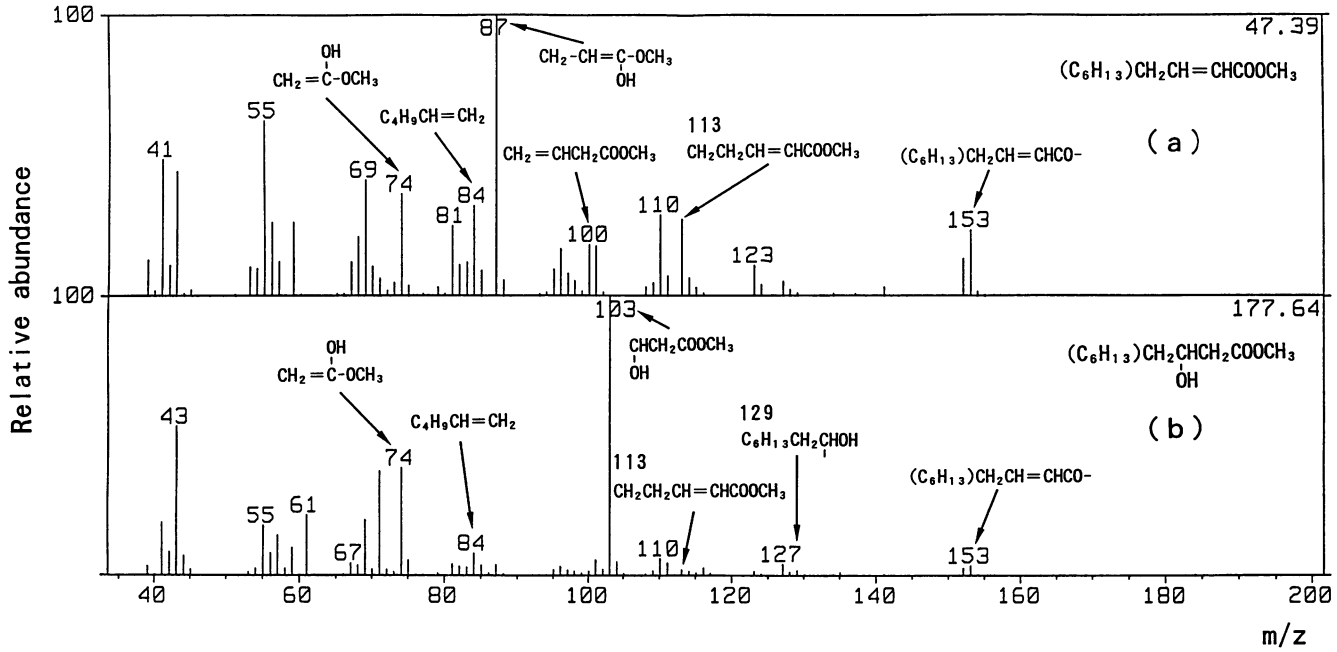

FIG. 5. Electron ionization-MS spectrum of fatty acid methyl ester. Fatty acid methyl ester was separated in two peaks scan number 650 (a) and 768 (b) at by total ion chromatography. Each peak was analyzed by electron ionization-MS. Peak at scan number 650 (a) and 768 (b).

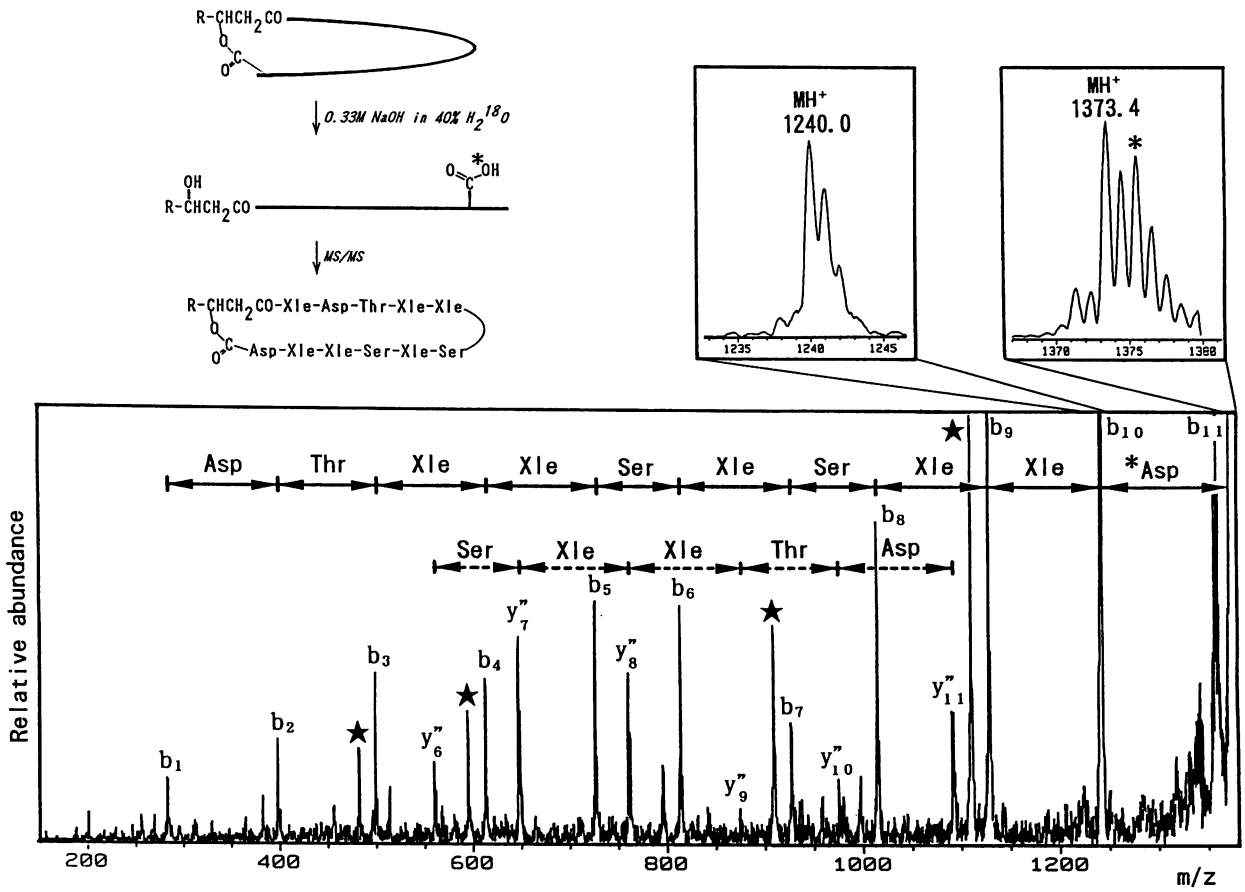

FIG. 6. Collision-induced dissociation and linked-scan spectrum of saponified and ^{18}O-labeled arthrofactin. The solid and broken lines show b and y″ series sequence ions, respectively. The solid stars correspond to the b −18 ion fragmentation series (10). *Asp and the asterisk in the inset indicate ^{18}O-labeled Asp and ^{18}O-incorporated molecular ion species, respectively. A schematic flowchart of the method is also shown on the upper left. Xle is Leu or Ile.

The collision-induced dissociation spectrum of saponified and ^{18}O-labeled biosurfactant clearly showed the sequence ions of the y_n and b_n series (18) from the N and C termini, respectively (Fig. 6). Considering the mass differences among y_n and b_n sequence ions, the N- and C-terminal sequences were determined to be -Asp-Thr-Xle-Xle-Ser- and -Asp-Thr-Xle-Xle-Ser-Xle-Ser-Xle-Xle-Asp, respectively (Xle is Leu or Ile). Taking into consideration the amino acid composition and the residual mass of the lipid moiety, the amino acid sequence of arthrofactin was assigned to be Xle-Asp-Thr-Xle-Xle-Ser-Xle-Ser-Xle-Xle-Asp. Morever, the loss of the ^{18}O atom after cleavage of the C-terminal Asp indicated that the intramolecular ester bond was formed between the hydroxyl group and the carboxyl group of the C-terminal Asp (Fig. 6). ^1H-NMR (400 MHz) data suggested that the hydroxyl group of the fatty acid was involved in the lactone ring formation as reported by Matsuyama et al. (14) (data not shown). Ile and Leu were identified by Edman degradation of partially hydrolyzed peptide which had been digested at two Asp residues. The amino acid sequence was determined as (Leu)-Asp-Thr-Leu-Leu-Ser-Leu-Ser-Ile-Ile-Asp. The data of amino acid composition suggested that the N-terminal residue was Leu. No irregular peaks were detected during Edman degradation. This result also supported the conclusion that the hydroxyl groups of both Thr and two Ser residues were free and did not participate in the lactone ring formation.

The optical configurations of all amino acids were determined (Table 1). A little racemization occurred in the case of Leu. Partially hydrolyzed peptides were isolated and analyzed to determine the positions of D-Asp, L-Asp, D-Leu, and L-Leu (Fig. 7).

Finally, the complete structure of arthrofactin is shown in Fig. 8. Aspartic acid has two carboxyl groups in the molecule, at α and β positions. Although it remains to be elucidated which one participates in the formation of the lactone ring, it is most probable that the carboxyl group bound to an α carbon is likely to form a lactone ring from the analogy of other lactone-type biosurfactants.

TABLE 1. Optical configurations of amino acids

Amino acid	Optical configuration[a]:	
	D	L
Ser	200.89	0.00
Asp	117.00	116.83
Thr	4.33	0.00
Leu	266.35	127.29
Ile	0.00	125.34

[a] Each value is the number of picomoles of the amino acid contained in 100 pmol of arthrofactin. The reason for the low value of Thr is not clear.

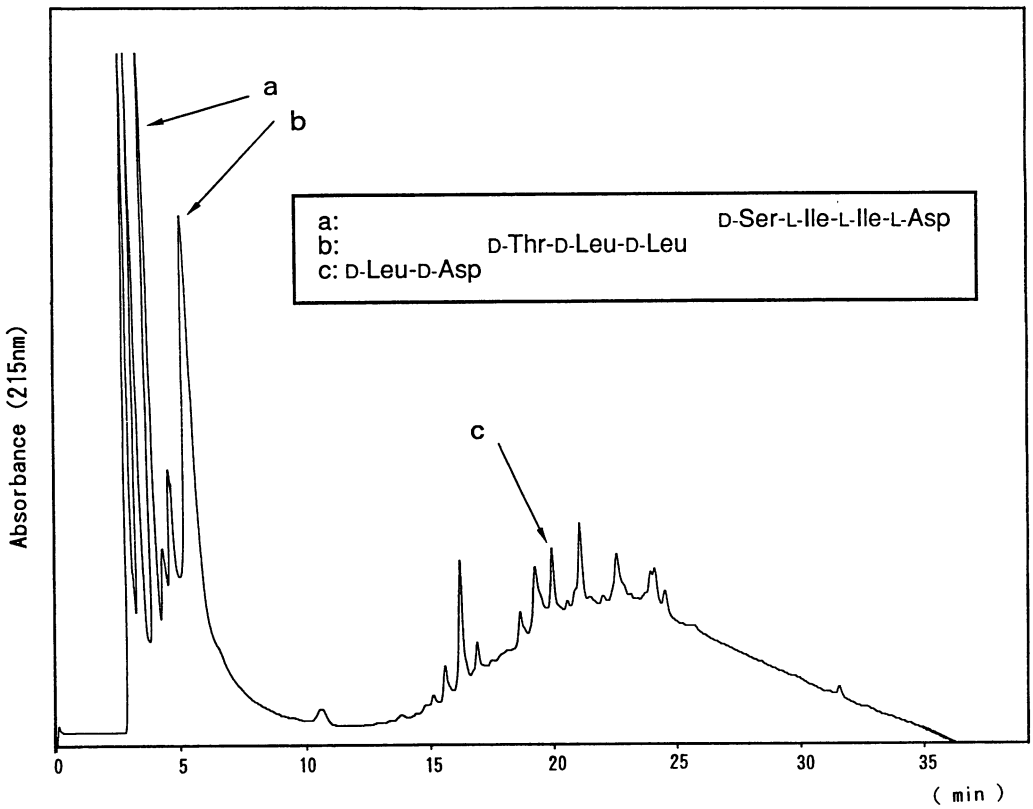

FIG. 7. Separation pattern of partially hydrolyzed peptides on C_4-reversed-phase HPLC and their amino acid sequences. Arthrofactin was hydrolyzed partially with concentrated HCl at 37°C for 3 days. Each fraction containing Leu and Asp was selected and modified with a chiral FLEC reagent to determine the D or L configuration.

Surface activity of arthrofactin and surfactin. The surface tension of each sample was measured by using a Traube's stalagmometer (Fig. 9). The critical micelle concentration values of arthrofactin and surfactin were estimated to be around 1.0×10^{-5} M and 7.0×10^{-5} M, respectively. The minimum surface tension value of arthrofactin was 24 mN/m at a concentration of 1.0×10^{-4} M. This value was obviously lower than that of surfactin (27 mN/m at 5.0×10^{-4} M). The concentrations of biosurfactants required to lower the surface tension of water from 72 to 40 mN/m were 7.5×10^{-6} M (arthrofactin) and 4.0×10^{-5} M (surfactin). This proved that the surface activity of arthrofactin was at least five times higher than that of surfactin.

Comparison of oil displacement activity of arthrofactin and surfactin. We noticed that biosurfactant-producing bacteria had formed a clear circle around the colony on oil-L agar plates (16), and we took advantage of this phenomenon to measure the activity of surfactants conveniently. The area of the oil-displaced circle was in good proportion to the concentration of the surfactants (Fig. 10). The specific activity of arthrofactin was 1.7 and 2.0 times higher than that of surfactin on the basis of weight and the number of molecules, respectively. Arthrofactin also showed higher activity than did well-known chemical surfactants such as Triton X-100 and sodium dodecyl sulfate.

DISCUSSION

There are numerous reports on the isolation of biosurfactant-producing bacteria, of which only a few have well-defined chemical structures. Surfactin and iturin (17) are well-characterized lipopeptides produced by *Bacillus subtilis* (Fig. 8). Both biosurfactants contain the same fatty acid and seven amino acid residues in their peptide rings. Iturin contains Asn, Gln, and Ser and no acidic amino acid residues. Recently, the chemical structure of serrawettin W2 has been analyzed (14) (Fig. 8). The fatty acid is the same as that of arthrofactin. Serrawettin contains only Ser and Thr as hydrophilic amino acid residues. In comparison with surfactin and arthrofactin, the relatively low surface activities of iturin (37.5 mN/m) and serrawettin (33.9 mN/m) suggest that Asp and Glu (in surfactin or arthrofactin) are more effective hydrophilic residues than Asn, Gln, Ser, or Thr (in iturin or serrawettin) in exhibiting high surface activity. Arthrofactin has even higher surface activity than surfactin, though each biosurfactant has two acidic amino acid residues in common. This may be due to the delicate balance of hydrophilic and hydrophobic parts, that is, additional Ser, Thr, and Ile residues and a short, straight structure in the fatty acid part of arthrofactin. Viscosin (11), an effective lipopeptide biosurfactant produced by *Pseudomonas fluorescens* (surface tension, 26.5 mN/m) also contains Glu, alloThr, and two Ser residues, and the fatty acid length is the same as that for arthrofactin. It is a general tendency that a straight-chain fatty acid is more effective for higher surface activity than a branched-chain fatty acid. With longer lengths of the $-CH_2-$ chain, smaller critical micelle concentration values were observed (3). The critical micelle concentration value of arthrofactin was lower than that of surfactin, in spite of a larger peptide portion and short fatty acid chain length. This indicates that hydrophobic amino acid residues, 4-Leu

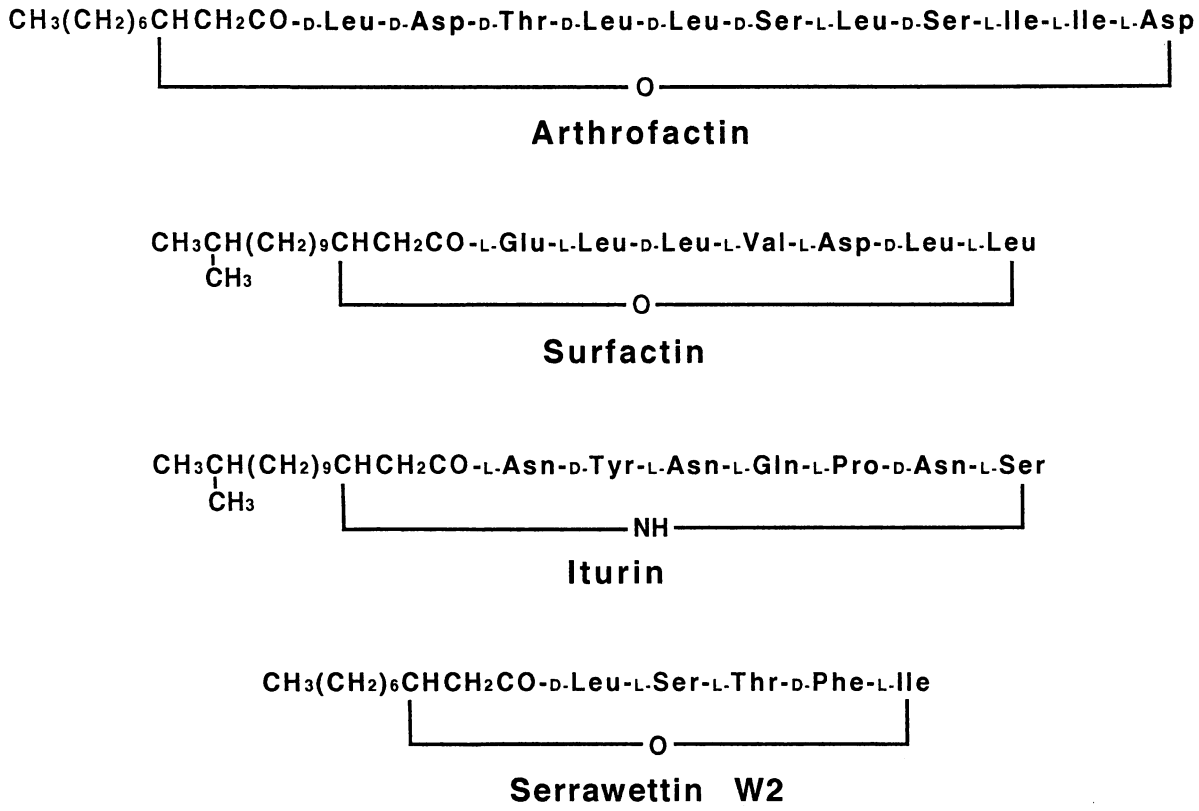

FIG. 8. Structure of lipopeptide-type biosurfactants. The structure of arthrofactin was determined in this study.

and 2-Ile, contribute to the total hydrophobicity of arthrofactin rather than hydrophilicity even though they exist in the peptide part. Surfactant BL 86 is another effective lipopeptide (critical micelle concentration, 10 μg/ml) produced by *B. licheniformis* 86 (7). The complete chemical structure remains to be determined. It should also be noted that few lipopeptide biosurfactant contains basic amino acid residues. The modification of various biosurfactants would elucidate the structure and activity relationships.

In this report we have also proposed a new method to estimate the oil displacement activity of surfactants. This phenomenon cannot be explained clearly because a crude oil

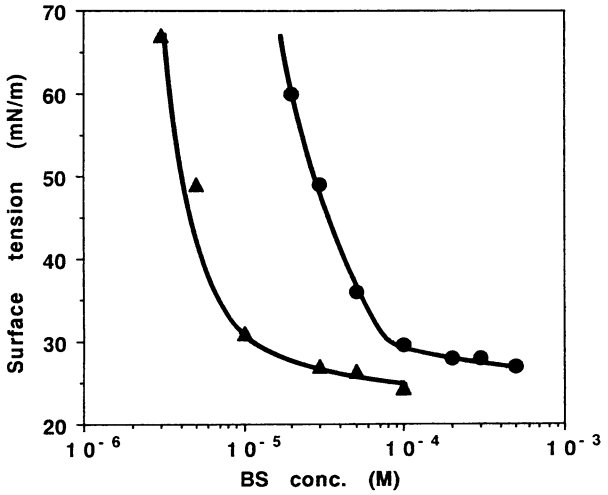

FIG. 9. Surface activities of arthrofactin and surfactin. Surface tension was measured with a Traube's stalagmometer. Each droplet was formed slowly (about 10-s interval). The temperature was at 25°C. ▲, arthrofactin; ●, surfactin; BS, biosurfactant.

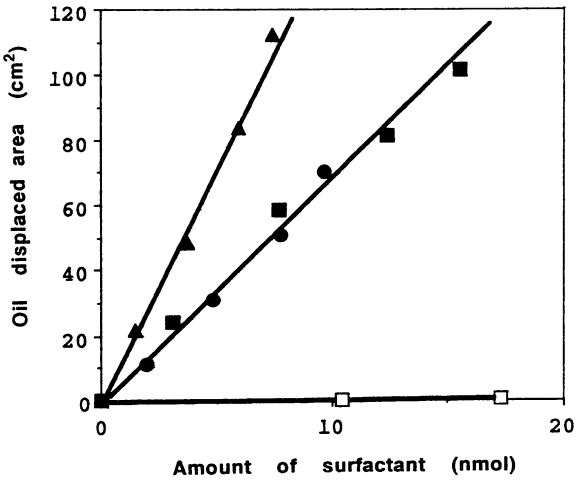

FIG. 10. Relationship between amount of surfactant and oil-displaced circle area. Surfactants were dissolved in water or buffer at each concentration, and the cleared circle areas were measured. ▲, arthrofactin; ●, surfactin; □, sodium dodecyl sulfate; ■, Triton X-100.

was adopted to the measurement. The hydrophile-lipophile balance value is sometimes useful to explain the property of surfactants. The hydrophile-lipophile balance value for Triton X-100 is 13.5, and it exhibited the same oil displacement activity as that of surfactin (Fig. 10). In contrast, no activity with sodium dodecyl sulfate was found. This might be explained by its exceptionally high hydrophile-lipophile balance value of 40. Nevertheless, this assay method is expected to be applied for the selection of surfactants when one needs to clean up oil.

ACKNOWLEDGMENTS

We thank S. Tsunazawa (Institute for Protein Research, Osaka University), M. Nomura, Y. Nakatsuji, and S. Masuyama (Faculty of Engineering, Osaka University) for helpful discussions.

REFERENCES

1. **Arima, K., A. Kakinuma, and G. Tamura.** 1968. Surfactin, a crystalline peptidelipid surfactant produced by *Bacillus subtilis*: isolation, characterization and its inhibition of fibrin clot formation. Biochem. Biophys. Res. Commun. **31:**488–494.
2. **Cooper, D. G., and J. E. Zajic.** 1980. Surface-active compounds from microorganisms. Adv. Appl. Microbiol. **26:**229–253.
3. **Dahanayake, M., A. W. Cohen, and M. J. Rosen.** 1986. Relationship of structure to properties of surfactants. J. Phys. Chem. **90:**2413–2418.
4. **Einarsson, S., B. Josefsson, P. Möller, and D. Sanchez.** 1987. Separation of amino acid enantiomers and chiral amines using precolumn derivatization with (+)-1-(9-fluorenyl)ethyl chloroformate and reversed-phase liquid chromatography. Anal. Chem. **59:**1191–1195.
5. **Fiechter, A.** 1992. Biosurfactants. Trends Biotechnol. **10:**208–217.
6. **Gutierrez, J. R., and L. E. Erickson.** 1977. Hydrocarbon uptake in hydrocarbon fermentations. Biotechnol. Bioeng. **19:**1331–1349.
7. **Horowitz, S., J. N. Gilbert, and W. M. Griffin.** 1990. Isolation and characterization of a surfactant produced by *Bacillus licheniformis* 86. J. Industr. Microbiol. **6:**243–248.
8. **Hug, H., H. W. Blanch, and A. Fiechter.** 1974. The functional role of lipids in hydrocarbon assimilation. Biotechnol. Bioeng. **16:**965–985.
9. **Jenny, K., O. Käppeli, and A. Fiechter.** 1991. Biosurfactants from *Bacillus licheniformis*: structural analysis and characterization. Appl. Microbiol. Biotechnol. **36:**5–13.
10. **Johnson, R. S., S. A. Martin, K. Biemann, J. T. Stults, and J. T. Watson.** 1987. Novel fragmentation process of peptides by collision-induced decomposition in a tandem mass spectrometer: differentiation of leucine and isoleucine. Anal. Chem. **59:**2621–2625.
11. **Laycock, M. V., P. D. Hildebrand, P. Thibault, J. A. Walter, and J. L. C. Wright.** 1991. Viscosin, a potent peptidolipid biosurfactant and phytopathogenic mediator produced by a pectolytic strain of *Pseudomonas fluorescens*. J. Agric. Food Chem. **39:**483–489.
12. **Li, Z., S. Lang, F. Wagner, L. Witte, and V. Wray.** 1984. Formation and identification of interfacial-active glycolipids from resting microbial cells. Appl. Environ. Microbiol. **48:**610–617.
13. **Mallee, F. M., and H. W. Blanch.** 1977. Mechanistic model for microbial growth on hydrocarbons. Biotechnol. Bioeng. **19:**1793–1816.
14. **Matsuyama, T., K. Kaneda, Y. Nakagawa, K. Isa, H. Hara-Hotta, and I. Yano.** 1992. A novel extracellular cyclic lipopeptide which promotes flagellum-dependent and -independent spreading growth of *Serratia marcescens*. J. Bacteriol. **174:**1769–1776.
15. **McInerney, M. J., M. Javaheri, and D. P. Nagle, Jr.** 1990. Properties of the biosurfactant produced by *Bacillus licheniformis* strain JF-2. J. Industr. Microbiol. **5:**95–102.
16. **Morikawa, M., M. Ito, and T. Imanaka.** 1992. Isolation of new surfactin producer *Bacillus pumilus* A-1, and cloning and nucleotide sequence of the regulator gene, *psf-1*. J. Ferment. Bioeng. **74:**255–261.
17. **Peypoux, F., M. Guinand, G. Michel, L. Delcambe, B. C. Das, and E. Lederer.** 1978. Structure of iturin A, a peptidelipid antibiotic from *Bacillus subtilis*. Biochemistry **17:**3992–3996.
18. **Roepstorff, P., and J. Fohlman.** 1984. Proposal for a common nomenclature for sequence ions in mass spectra of peptides. Biomed. Mass Spectrom. **11:**601.
19. **Sneath, P. H. A., N. S. Mair, M. E. Sharpe, and J. G. Holt.** Bergey's Manual of Systematic Bacteriology, vol. 2. 1986. Williams & Wilkins, Baltimore.
20. **Suzuki, T., H. Tanaka, and S. Itoh.** 1974. Sucrose lipids of *Arthrobacteria*, *Corynebacteria* and *Nocardia* grown on sucrose. Agric. Biol. Chem. **38:**557–563.
21. **Takao, T., H. Hori, K. Okamoto, A. Harada, M. Kamachi, and Y. Shimonishi.** 1991. Facile assignment of sequence ions of a peptide labelled with ^{18}O at the carboxyl terminus. Rapid Commun. Mass Spectrom. **5:**312–315.