Extracellular Accumulation of Mono- and Di-Succinoyl Trehalose Lipids by a Strain of Rhodococcus erythropolis Grown on n-Alkanes

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A bacterium, strain SD-74, which was isolated from soil under alkaline conditions, was found to abundantly produce acidic exolipids from n-alkanes. The strain proved to be alkali-resistant rather than alkalophilic and was identified as Rhodococcus erythropolis.

The acidic exolipids (15 g) were isolated from culture broth (1 l) containing n-hexadecane as the sole carbon source and were found to be composed of two new succinoyl trehalose lipids (STL-1 and STL-2). After purification, STL-1 showed mp 169 to 171°C and \([\alpha]_D^{20} +92.3^\circ (c=0.6, \text{CHCl}_3/\text{MeOH}=2:1)\), and STL-2 mp 161 to 163°C and \([\alpha]_D^{20} +75.0^\circ (c=0.6, \text{CHCl}_3/\text{MeOH}=2:1)\). On the basis of the results of chemical degradation and methylation, STL-1 and STL-2 were concluded to be 2,3,4,2'-di-O-succinoyl-di-O-alkanoyl-\(\alpha,\alpha\)-trehalose and 2,3,4-mono-O-succinoyl-di-O-alkanoyl-\(\alpha,\alpha\)-trehalose, respectively.

Many papers on the production of interesting enzymes by alkalophilic microorganisms have been reviewed by Horikoshi and Akiba.1) Few papers on the extracellular production of metabolites by alkalophilic microorganisms, however, have been published. We searched for alkalophilic microorganisms that produce extracellular metabolites from n-alkanes under alkaline conditions. In the course of this screening, a bacterium, strain SD-74, isolated from soil was found to produce acidic exolipids abundantly. The exolipids showed good surface-active properties.2) This paper describes the isolation and identification of strain SD-74, the isolation and purification of the exolipids produced from n-hexadecane, and the characterization of the two main new succinoyl trehalose lipids (STL-1 and STL-2). The effects of the culture conditions on the production of the exolipids will be described in the next paper.

Materials and Methods

Microorganisms. Strain SD-74 was isolated from an alkaline soil sample collected at Sado Island, Niigata Prefecture, Japan. Rhodococcus erythropolis IAM 12122 (ATCC 4277), the type strain of the species, and IFO 12682 were also used as references. The strains were maintained on nutrient agar supplemented with 0.1% yeast extract and 1% glycerol.

Isolation of microorganisms capable of using n-alkanes under alkaline conditions. For enrichment of microorganisms capable of using n-alkanes under alkaline conditions, about 200 soil samples were collected from 7 different places. Each sample (ca. 1 g) was suspended in sterile water (5 ml) and then each suspension (0.2 ml) was added to a test tube (25 x 200 mm) containing a selective medium (5 ml), which was composed of 5% (v/v) n-alkane mixture (C\(_{12}\)-C\(_{16}\)) as the carbon source, 0.15% NH\(_4\)NO\(_3\), 0.1% KH\(_2\)PO\(_4\), 0.02% MgSO\(_4\)-7H\(_2\)O, 0.1% yeast extract, 0.01% Span 80, 0.01% Tween 20, 0.5% Na\(_2\)CO\(_3\), 0.5% NaHCO\(_3\) and tap water (pH 10). Carbonates were sterilized separately by filtration. The cultures were incubated on a test-tube shaker for several days at 30°C. After visible growth had been observed, the cultures were transferred to
new test tubes containing the same medium and then incubated under the same conditions. After 3 to 4 repetitions of these steps, the cells were streaked on agar plates. For the plates, the same selective medium containing 2.0% agar was used, but the n-alkanes were soaked in pieces of flattened cotton put on the lids of the Petri dishes. After incubation at 30°C for 3 to 5 days, colonies were isolated.

**Screening tests.** Each of the isolated strains was inoculated into a 300-ml Erlenmeyer flask containing the selective medium (30 ml), followed by incubation for 7 days at 30°C on a rotary shaker (220 rpm). The culture broth was centrifuged at 10,000 × g for 20 min and then the resulting supernatant was examined for the presence of fermentation products.

**Taxonomic studies on strain SD-74.** Cultural and physiological characterization of the selected strain, SD-74, was principally performed by the standard methods. The type of cell division was determined by a microculture technique. The chemical composition of cells, that is, amino acids, carbohydrates, fatty acids, mycolic acids and glycolate, was analyzed by the methods of Becker et al., Staneck and Roberts, Suzuki and Komagata, Alshamaony et al. and Uchida and Aida, respectively. The G+C content was estimated by the method of Kaneko et al. using a DNA-GC kit (Yamasa Shoyu Co., Ltd.).

**Culture conditions.** Seed cultures were prepared by inoculating loopfuls of cells of strain SD-74 grown on slants into 300-ml Erlenmeyer flasks containing a seed culture medium (30 ml), followed by incubation at 30°C for 2 days on the rotary shaker. The seed culture medium contained 2% fructose, 2% glucose, 0.3% KNO₃, 0.5% KH₂PO₄, 1.1% K₂HPO₄, 0.02% MgSO₄·7H₂O, 0.3% yeast extract and tap water. Fructose and glucose were sterilized separately. The seed cultures (4 ml) were transferred to twenty 500-ml Erlenmeyer flasks, each containing a fermentation medium (50 ml). The flasks were shaken at 30°C for 7 days on the rotary shaker. The fermentation medium contained 10% (v/v) n-hexadecane, 0.3% KNO₃, 1.5% KH₂PO₄, 3.3% K₂HPO₄, 0.01% MgSO₄·7H₂O, 0.1% yeast extract and tap water.

The effect of the initial pH on cell growth was examined in Monod’s test tubes. The medium contained 3% glycerol, 2% acid hydrolysate of casein (Sigma, type I), 0.2% yeast extract (Difco), 0.02% MgSO₄·7H₂O, 0.2 m KH₂PO₄, 0.1 m succinic acid, 0.1 m N,N-bis(2-hydroxyethyl)glycine (BICINCE) and tap water. After autoclaving, portions of the basal medium were adjusted to different pHs with a mixture of 2N NaOH and 10% Na₂CO₃, and then dispensed, at 7 ml each, into Monod’s test tubes. The tubes were inoculated with the cell suspension (0.5 ml) of strain SD-74, followed by incubation at 30°C on a Monod’s shaker. Growth was measured as the turbidity at 580 nm with a Shimadzu-Bausch & Lomb Spectronic 20 colorimeter.

**Isolation of the acidic exolipids.** Culture broths were combined (ca. 1 l) and then centrifuged at 10,000 × g for 30 min. Since the remaining n-hexadecane and cells came to the surface, the aqueous layer was carefully siphoned off and acidified with 6 N HCl to pH 3.0. The acidic exolipids were precipitated as a voluminous gel mass. After removal of the supernatant by centrifugation, the gel mass was washed twice with water and then mixed with hot methanol (300 ml). The mixture was washed three times with n-hexane to remove minor lipids and remaining n-hexadecane. The mixture was concentrated and the residue was dried over P₂O₅ under reduced pressure. A pale brown residue (ca. 15 g) was obtained and used as the mixture of the main exolipids (that is, STL-1 and STL-2) in the following experiments.

**Purification of STL-1 and STL-2.** The mixture of STL-1 and STL-2 (10 g) was dissolved in ethyl acetate (200 ml) and then stood in a cold room. STL-1 was mainly obtained as a precipitate and then recrystallized with ethyl acetate. The combined mother liquor, after condensation, was placed on a column (3 × 40 cm) of silica gel (Iatrobeads 6RS-8060, Iatron) and then the lipids were eluted with chloroform-methanol (10:1). The combined fractions containing STL-2 were further purified by repeated preparative thin-layer chromatography and recrystallization with ethyl acetate.

**Analytical methods.** Thin-layer chromatography (TLC) was carried out on Silica gel 60 plates (Merck, No. 5553) with the following solvent systems: A, chloroform-methanol-water (65:25:4); B, chloroform-methanol-7 N ammonium hydroxide (65:25:4); C, chloroform-acetone (5:1); and D, n-butanol-ethanol-water (6:2:2). Preparative TLC of STL-1 and STL-2 was carried out on Silica gel 60 plates (Merck, No. 13894) with solvent A. To distinguish the functional groups of the products, the following reagents were used: anthrone reagent for sugars, bromocresol green for free acid groups, Dittmer-Lester reagent for phospholipids, ninhydrin reagent for amino groups and hydroxylamine-ferric chloride reagent for ester groups.

Paper chromatography (PPC) was carried out on Toyosh. No. 51B filter paper. Sugars were separated with solvent E, n-propanol-ethyl acetate-water (7:1:2), and located by spraying Yoda’s reagent. High-performance liquid chromatography (HPLC) was performed with a Waters apparatus (M-45) equipped with an Aminex HPX-87H column (BIO-RAD). Sugars and the water-soluble acid component were eluted with 0.01 N H₂SO₄ at a column temperature of 60°C and a flow rate of 0.8 ml/min. Adipic acid was used as the internal standard (tᵣ, 12.1 min).

Gas chromatography (GC) was performed with a
Shimadzu Gas Chromatograph GC-8A equipped with a flame-ionization detector. Fatty acid methylesters were analyzed on a glass column (4 mm x 1 m) packed with 15% FFAP on Chromosorb WAW, the temperature being programmed from 120~220°C at 10°C/min.

GC-mass spectrometry (GC/MS) of fatty acid methylesters and partially methylated alditol acetates was carried out with a Hitachi M-80B GC/MS Spectrometer at 70 eV on a glass column (3 mm x 1 m) packed with Chemipack FP (Gasukuro Kogyo Inc.).

$^1$H-NMR spectra of the per-O-acetylated derivatives of STL-1 and STL-2 in CDCl$_3$ were measured with a JNM-FX-100 spectrometer (JEOL Ltd.).

Deacylation of the mixture of STL-1 and STL-2. The mixture of STL-1 and STL-2 (1 g) was mixed with 1 N NaOH (10 ml) and then kept in a boiling water bath for 1 hr. After acidification of the reaction mixture with 2 N HCl to pH 3, long-chain fatty acids were extracted with n-hexane. The resulting aqueous layer was continuously extracted with ether for 24 hr to obtain the water-soluble acid moiety. The remaining aqueous layer containing the sugar moiety was desalted with Amberlite IR-120B (H$^+$) and IRA-410 (OH$^-$), and then concentrated under reduced pressure (ca. 0.4 g). The concentrate (10 mg) was dissolved in 1 N H$_2$SO$_4$ (2 ml) and then hydrolyzed in a boiling water bath for 1 hr.

Determination of molar ratio of acyl residues to sugar moiety. STL-1 or STL-2 (100 mg each) was dissolved in 1 N NaOH (2 ml) and then deacylated under the same conditions as described above. After acidification of the reaction mixture with 2 N H$_2$SO$_4$ to pH 3, long-chain fatty acids were extracted with n-hexane. After removal of n-hexane, the residues were methylated with 10% (v/v) H$_2$SO$_4$ in methanol, and then analyzed by GC and GC/MS. The sugar and water-soluble acid moieties in the remaining aqueous layer were simultaneously analyzed by HPLC.

Methylation analyses of STL-1 and STL-2. After preliminary methylation of free carboxyl residues of STL-1 or STL-2 (7 mg each) with diazomethane, permethylation was carried out by the method of Deferrari et al. using diazomethane-boron trifluoride etherate. The permethylated STL-1 and STL-2 (Rf 0.65 and 0.61 in solvent C, respectively) were purified by preparative TLC with solvent C. After alkaline methanolysis of the products with 0.3 M sodium methoxide, the resulting methylated sugar components were hydrolyzed with 1 N H$_2$SO$_4$, followed by reduction with 1% NaBH$_4$, and acetylation to prepare partially methylated alditol acetates. The resulting methylated alditol acetates were separated and then identified by GC/MS analysis according to the methods of Bjorndal et al. and Jansson et al.

**Results**

Selection of strain SD-74 producing acidic exolipids

Twenty strains, which grew well on a mixture of n-alkanes, were isolated from alkaline soil samples collected at Sado Island and Chichibu.

These strains were screened as to the production of metabolites derived from the mixture of n-alkanes used as the sole carbon source. The culture broth of strain SD-74 was found to give a voluminous white precipitate as metabolites after acidification of the broth with 6 N HCl. This strain and its metabolites were extensively studied.

Taxonomical studies on strain SD-74

The strain was isolated from an enrichment culture under alkaline conditions, pH 10, but showed better growth at pH 7.0 ~ 7.5, as Fig. 1 shows.

Table 1 shows the characteristics of the strain. From these characteristics, this strain was tentatively identified as *Rhodococcus erythropolis*.

Characterization of the exolipids

The precipitate obtained from the acidified broth of strain SD-74 was analyzed by GC and GC/MS. The fatty acid composition of the precipitate was determined by GC in Fig. 1. Effect of pH on Cell Growth.

Cells were inoculated into Monod's test tubes containing 7 ml of media, which contained 3% glycerol, 2% acid hydrolysate of casein (Sigma, type I), 0.2% yeast extract (Difco), 0.02% MgSO$_4$, 7H$_2$O, 0.2 M K$_2$HPO$_4$, 0.1 M succinic acid and 0.1 M BICINE, and which were adjusted to different pHs with a mixture of 2 N NaOH and 10% Na$_2$CO$_3$. The cultures were incubated with shaking at 30°C. ●●●, 12 hr; ----, 16 hr; ———, 20 hr.
Table I. Description of Strain SD-74

<table>
<thead>
<tr>
<th>Morphological characteristics</th>
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<tbody>
<tr>
<td>Short rods, (0.4 ~ 0.6) x (0.8 ~ 1.5) μm, to long and branched cells, (0.4 ~ 0.6) x (6 ~ 15) μm, and filaments cells, but no aerial mycelium.</td>
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<tr>
<td>Bending-type cell division. Gram-positive.</td>
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<td>Non-motile. No sporulation.</td>
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<th>Cultural characteristics</th>
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<tr>
<td>Nutrient agar colonies: weak growth, orange.</td>
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<tr>
<td>Nutrient broth: weak growth, membranous, slightly turbid.</td>
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<tr>
<td>Nutrient agar containing glycerol: good growth, dull orange to pink, opaque.</td>
</tr>
<tr>
<td>Nutrient broth containing glycerol: vigorous growth with precipitate of floculent cells.</td>
</tr>
<tr>
<td>YM agar colonies: good growth, orange.</td>
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<td>Litmus milk: neutral.</td>
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<th>Physiological characteristics</th>
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<tr>
<td>Nitrate reduction: (+).</td>
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<tr>
<td>Nitrate respiration: (−).</td>
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<tr>
<td>Liquefaction of gelatin: (−).</td>
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<tr>
<td>Hydrolysis of starch: (−).</td>
</tr>
<tr>
<td>Indole production: (−).</td>
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<tr>
<td>Catalase: (+). Oxidase: (−).</td>
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<tr>
<td>DNase: (+). O/F test: oxidative.</td>
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<tr>
<td>Decomposition: adenine (+); tyrosine (−); urea (+).</td>
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<td>Grew on fructose, glucose, maltose, sucrose, inulin, glycerol, mannitol, propane-1,2-diol, ethanol, acetate, propionate, butyrate, pyruvate, citrate, fumarate, malate, pimelate, gluconate, p-hydroxybenzoate and salicin, but not on galactose, l-arabinose, l-sorbose, lactose, l-rhamnose, trehalose, erythritol, sorbitol, inositol, butane-1,4-diol, lactate, malonate, succinate, glutarate, adipate, benzoate, m-hydroxybenzoate or glycine.</td>
</tr>
<tr>
<td>Grew on acetamide, but not on serine or trimethylenediamine, as sole carbon and nitrogen sources.</td>
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<tr>
<td>Acid from fructose, glucose, glycerol and mannitol, but not from maltose, sucrose or inulin.</td>
</tr>
<tr>
<td>Growth range: pH, 5.5 ~ 10 (optimum, 7.0 ~ 7.5); temperature, 10 ~ 40°C (optimum, 30°C); not inhibited by 7% NaCl.</td>
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<tr>
<th>Chemical composition of cells</th>
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<tr>
<td>meso-Diaminopimelic acid, arabinose and galactose: (+).</td>
</tr>
<tr>
<td>Mycolic acids: (+, C_{35}^{-}C_{37}).</td>
</tr>
<tr>
<td>Major cellular fatty acids: saturated (C_{14}^{-}C_{18}) and monounsaturated (C_{16}^{-}C_{19}) straight-chain fatty acids, and 10-methyl fatty acids (C_{17}, C_{19}).</td>
</tr>
<tr>
<td>Glycolate test: (+).</td>
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<td>G+C content: 71 mol%.</td>
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</table>

![Fig. 2. Thin-layer Chromatogram of Exolipids.](image_url)

Solvent systems: (A), CHCl₃-CH₃OH-H₂O (65:25:4); (B), CHCl₃-CH₃OH-7n NH₄OH (65:25:4).

(1) Precipitate obtained from the acidified culture broth; (2) 1, 12-dodecanedioic acid; (3) myristic acid.

The contents of STL-1 and STL-2 in the total anthrone-positive lipids were determined to be 67% and 18%, respectively, with a densitometer (TLC Scanner CS-920, Shimadzu).
centrate gave colorless crystals; mp 96°C, $[\alpha]_D^{20} +174^\circ$ (c = 5, H$_2$O). The per-O-acetyl derivative showed mp 97°C, $[\alpha]_D^{20} +160^\circ$ (c = 2, CHCl$_3$). The crystals and acetyl derivative showed no melting point depression on mixing with an authentic sample of $\alpha,\alpha$-trehalose dihydrate and one of $\alpha,\alpha$-trehalose octaacetate, respectively.

2) Water-soluble acid moiety. The ether layer of the deacylated mixture of STL-1 and STL-2 left a solid mass (0.3 g), on removal of the ether. The solid mass gave one peak (t$_R$ 0.77 min) on HPLC. This t$_R$ value corresponded to that of succinic acid. After recrystallization from acetone, the solid mass gave colorless prisms, mp 184°C. The crystals showed no melting point depression on mixing with an authentic sample of succinic acid.

Characterization of STL-1 and STL-2

The IR spectra of STL-1 and STL-2 had similar patterns; IR $\nu_{max}$ (film) cm$^{-1}$: 3400 (O–H), 1740 (C=O), 1210 and 1145 (C-O).

The colorless powder of STL-1 showed mp 169 to 171°C and $[\alpha]_D^{20} +92.3^\circ$ (c = 0.9, CHCl$_3$/MeOH = 2:1). The components of the long-chain fatty acids were $n$-hexadecanoate (87%) and $n$-tetradecanoate (13%). Anal. Found: C, 61.21; H, 8.97%. Calcd. for C$_{52}$H$_{90}$O$_{19}$ (hexadecanoate): C, 61.27; H, 8.90%.

The colorless powder of STL-2 showed mp 161 ~ 164°C and $[\alpha]_D^{20} +75.0^\circ$ (c = 0.6, CHCl$_3$/MeOH = 2:1). The components of the long-chain fatty acids were $n$-hexadecanoate (75%) and $n$-tetradecanoate (25%). Anal. Found: C, 60.53; H, 9.13. Calcd. for C$_{48}$H$_{86}$O$_{16}$·2H$_2$O (hexadecanoate): C, 60.40; H, 9.40%.

1) Molar ratio of succinic acid and fatty acids to trehalose. The molar ratios of succinic acid and long-chain fatty acids to trehalose in STL-1 were determined by GC and HPLC to be 1.90 of succinic acid and 1.83 of $n$-hexadecanoic acid plus 0.27 of $n$-tetradecanoic acid, respectively. These results showed that the molar ratio of trehalose–succinic acid–long-chain fatty acids was 1:2:2. Further evidence was provided by the $^1$H-NMR spectrum of per-O-acetylated STL-1; NMR $\delta_H$ (CDCl$_3$): 0.87 (6H, t, $\omega$-CH$_3$ of alkanoyl), 1.53 (4H, m, $\beta$-CH$_2$– of alkanoyl), 2.26 (4H, t, $\alpha$-CH$_2$– of alkanoyl), 2.62 (8H, br. s, COCH$_2$-CH$_2$CO), 1.9 ~ 2.1 (12H, m, O–COCH$_3$). After methylation of the acetyl derivative with diazomethane, a new signal appeared at $\delta$ 3.68 (6H, s, COOCH$_3$). Proton integration of each signal for acyl groups supported the above molar ratio.

The molar ratios of succinic acid and long-chain fatty acids to trehalose in STL-2 were determined to be 0.95 of succinic acid and 1.52 of $n$-hexadecanoic acid plus 0.51 of $n$-tetradecanoic acid, respectively. These results showed that the molar ratio of trehalose–succinic acid–long-chain fatty acids was 1:1:2. Further evidence was provided by the $^1$H-NMR spectrum of per-O-acetylated STL-2; NMR $\delta_H$ (CDCl$_3$): 0.88 (6H, t, $\omega$-CH$_3$ of alkanoyl), 1.62 (4H, m, $\beta$-CH$_2$– of alkanoyl), 2.26 (4H, t, $\alpha$-CH$_2$– of alkanoyl), 2.63 (4H, br. s, COCH$_2$CH$_2$CO), 1.9 ~ 2.1 (15H, m, O–COOCH$_3$). After methylation of the acetyl derivative with diazomethane, a new signal appeared at $\delta$ 3.68 (3H, s, COOCH$_3$).

2) Ester linkage of acyl groups to trehalose. After complete permethylation of STL-1 and STL-2, partially methylated alditol acetates were prepared and analyzed by GC/MS. The gas chromatogram of the alditol acetates derived from STL-1 showed two equimolar peaks, the fragmentation patterns of which on GC/MS corresponded to those of 1,2,5-tri-O-acetyl-3,4,6-tri-$\alpha$-methyl hexitol and 1,2,3,4,5-penta-O-acetyl-6-O-methyl hexitol.

The gas chromatogram of the alditol acetates derived from STL-2 showed two equimolar peaks, the fragmentation patterns of
which on GC/MS corresponded to those of 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl hexitol and 1,2,3,4,5-penta-O-acetyl-6-O-methyl hexitol.18

From these results, it was concluded that the acidic exolipids, STL-1 and STL-2, were 2,3,4,2'-di-O-succinoyl-di-O-alkanoyl-α,α-trehalose and 2,3,4-mono-O-succinoyl-di-O-alkanoyl-α,α-trehalose, respectively (Fig. 3).

Characterization of minor exolipids

The minor exolipids fraction obtained from the precipitate of the total acidic exolipids on hexane extraction was also analyzed by the same procedure as described above. The minor exolipids were found to consist of trehalose (1.0), succinic acid (1.7), acetic acid (0.1) and long-chain fatty acid (2.6; C₈-C₁₆) in molar ratio.

Discussion

Strain SD-74 was isolated by means of an enrichment culture under alkaline conditions, but the optimum pH for its growth was found to be 7.0~7.5. The strain, accordingly, was alkali-resistant rather than alkalophilic. From the taxonomical properties shown in Table I, the strain was tentatively identified as Rhodococcus erythropolis (Gray and Thornton) Goodfellow and Alderson, according to “Bergey’s Manual of Systematic Bacteriology,” Vol. 2. Strain SD-74, however, showed poorer growth on nutrient broth containing no glycerol, had a deep color and was rich in filaments, compared with the 2 reference strains of R. erythropolis. Strain SD-74 differed from them in some physiological characteristics, such as the failure to assimilate trehalose, sorbitol, lactate, succinate, adipate, etc. Furthermore, the 2 reference strains of R. erythropolis were both found to be able to produce an acidic exolipid different from STL-1 and STL-2 under selected culture conditions, as described elsewhere.

Trehalose lipids, especially α,α-trehalose dimycolate, have been of particular interest among many unusual lipids of pathogenic strains of mycobacteria and allied microorganisms, because of their biological activity, e.g., they act as immunostimulants.20,21 Several saprophytic microorganisms grown on n-alkanes have been reported to produce different types of surface-active trehalose lipids in cultures.22,23 Batrakov et al.24 reported that Mycobacterium paraffinicum grown on hexadecane intracellularly produced a new acidic trehalose-tetraester, 2-O-octanoyl-3,2'-di-O-decanoyl-6-O-succinoyl-α,α-trehalose, together with four other trehalose lipids. Ristau and Wagner25 reported that Rhodococcus erythropolis DSM 43245 extracellularly produced another acidic trehalose-tetraester with the same residues, which in contrast were localized in the sugar moiety at positions 2,3,4 and 2', with a yield of 0.1 g from 1 l of culture broth. Wagner et al.26 reported that the yield of this trehalose-tetraester was increased to 7.9 g/l by a temperature shift and nitrogen limitation. In contrast with these monosuccinoyl-trialkanoyl-trehaloses, the present STL-1 and STL-2 were found to be disuccinoyl-dialkanoyl- and monosuccinoyl-dialkanoyl-trehaloses, respectively. To our knowledge, these succinoyl trehalose lipids have not been found previously.

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References

Accumulation of Succinoyl Trehalose Lipids