Rarobacter faecitabidus gen. nov., sp. nov., a Yeast-Lysing Coryneform Bacterium

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Phenotypic and chemotaxonomic characteristics of four isolates of yeast-lysing bacteria isolated from wastewater treatment systems were examined. The isolates were nonspore-forming, gram-positive, facultative anaerobic, irregular rods that were motile by multitrichous flagella. The isolates had an absolute requirement for hemin or hemoproteins for aerobic growth. Under anaerobic conditions, the isolates did not need heme compounds, but needed carbon dioxide. The deoxyribonucleic acid (DNA) base composition was 65.7 to 66.1 mol% guanine plus cytosine. The amino acid composition of the cell wall peptidoglycan was D-alanine, L-alanine, D-glutamic acid, L-ornithine, and D-serine (1:1:2:1:1). The major fatty acid of whole cells was 12-methyltetradecanoic acid. The major isoprenoid quinone was menaquinone with nine isoprene units. DNA-DNA hybridizations revealed clear separation of the isolates from known genera of the coryneform group. Therefore, Rarobacter faecitabidus gen. nov., sp. nov., is proposed for the isolates. The type strain is YLM-1 (JCM 6097).

In previous papers (5, 30) we reported the isolation of novel yeast-lysing bacteria from the wastewater treatment systems of alcoholic beverage factories. These bacteria adhered to and lysed viable cells of Saccharomyces, Hansenula, and Candida species. These bacteria hardly grew on nutrient agar but grew well on media containing extracts of viable yeast cells. Further, an essential factor for aerobic growth of the bacteria was hemin or hemoprotein.

This paper deals with the phenotypic characteristics, chemotaxonomic characteristics, deoxyribonucleic acid (DNA) base composition, and DNA-DNA hybridization of four yeast-lysing bacteria. Rarobacter faecitabidus gen. nov., sp. nov., is proposed for these strains, with strain YLM-1 (JCM 6097) as the type strain.

MATERIALS AND METHODS

Microorganisms and cultural conditions. Four strains of the yeast-lysing bacteria, YLM-1T (T, type strain), YLM-11, YLM-39, and YLM-113, were used. They were isolated from activated sludge of wastewater treatment systems of alcoholic beverage factories (YLM-1T, YLM-11, and YLM-113) and from the discharged water of an alcoholic beverage factory (YLM-39) (5, 30). They were maintained on YM-catalase agar, which contained (per liter) 5 g of yeast extract (Oxoid Ltd., London, United Kingdom), 5 g of malt extract (Difco Laboratories, Detroit, Mich.), 5.74 g of K2HPO4, 205 mg of MgSO4·7H2O, 1.15 g of NH4H2PO4, 60 mg of catalase (C-10; Sigma Chemical Co., St. Louis, Mo.), and 15 g of agar. Catalase and MgSO4·7H2O were separately sterilized by filtration. Cells used for chemotaxonomic analyses were cultivated in YM-catalase broth on a rotary shaker at 30°C for 2 days. Curtobacterium citreum JCM 1345T, Aureobacterium testaceum JCM 1353T, Cellulomonas fim1 JCM 1341T, Cellulomonas flavigena JCM 1489T, Oerskovia turbata JCM 3160T, Oerskovia xanthineolytica JCM 3164T, and Pseudomonas aeruginosa KS 0025T (ATCC 10145T) were used as references. They were cultured on nutrient agar or in nutrient broth (Nissui Seiyaku, Tokyo, Japan).

Morphological characteristics. The morphology of cells grown on YM-catalase agar and nutrient agar containing 60 mg of catalase per liter (nutrient-catalase agar) was investigated. To examine pleomorphism, 2% (wt/vol) sodium citrate was added to nutrient-catalase agar (28). Gram stain characteristics were examined by the method of Hucker and Conn (6). Motility was observed by the hanging-drop method, and flagellation was confirmed by transmission electron microscopy with a JEOl model 200CX microscope at 100 kV after negative staining with 1% (wt/vol) phosphotungstic acid. For thin-section electron micrographs, cells were fixed in 3% (wt/vol) glutaraldehyde–1% (wt/vol) OsO4, dehydrated, and embedded in Spurr resin.

Cultural characteristics. Colonial appearance was observed after incubation for 2 days at 30°C. YM-catalase agar was used for aerobic cultures, and nutrient agar was used in a disposable anaerobic system (Anaeromate; Nissui) for anaerobic cultures.

Physiological characteristics. Growth temperature, pH, and tolerance to NaCl were tested in nutrient-catalase broth for 3 days. Growth temperature was tested at pH 7, and effect of pH on growth was tested at 30°C. Tolerance to NaCl was tested at 30°C and pH 7. The heme requirement was examined by using nutrient agar. For studying vitamin requirements, the media of Keddie et al. (12) were used with 10 mg of hemin (Wako Pure Chemical Industries Ltd., Tokyo, Japan) per liter instead of catalase.

Biochemical characteristics. Unless otherwise indicated, methods described in the Manual of Clinical Microbiology (14) were used for the tests. Catalase (60 mg/liter) was added to the test media. Nitrate reduction and denitrification were examined by using potassium nitrate broth. Utilization of inorganic nitrogen was tested by using yeast carbon base (Difco). An oxidation and fermentation test was conducted as described by Hugh and Leifson (7). The method of Yamada and Komagata (28) was employed to examine production of acid from carbohydrates and assimilation of organic acids. Production of gas was checked in 1% (wt/vol) peptone broth containing 1% (wt/vol) glucose. Hydrolysis of...
starch and casein was tested on nutrient agar plates containing 0.2% (wt/vol) soluble starch or 1% (wt/vol) casein. Hydrolysis of gelatin was tested on agar plates and stab cultures. Hydrolysis of cellulose was tested by disintegration of filter paper and depolymerization of carboxymethyl cellulose (23). Hemolysis was tested on sheep blood agar (Nissui). Acetoin production was tested by using MR-VP medium (Difco), and indole production was examined by using 1% (wt/vol) peptone broth and Kovac reagent. For production of hydrogen sulfide, triple sugar iron agar (Difco), Kligler iron agar (Difco), and lead acetate test paper were used. Extracellular deoxyribonuclease (DNase) was tested for 20 days, using DNase test agar (Difco); urease was tested for 6 days, using Christensen's urea agar (Difco), and oxidase was tested with oxidase test paper (Nissui). For the catalase test, hemin (10 mg/liter) instead of catalase was added to nutrient agar, and bubbling was observed after pouring 3% hydrogen peroxide on the colonies.

**Isolation of DNA.** DNA was isolated by the phenol method (16) with some modifications. A mixture of phenol and chloroform (1:1, vol/vol) was used instead of phenol to remove proteins, and 50 μg of ribonuclease A (Sigma) per ml was used for hydrolysis of ribonucleic acid.

**Determination of DNA base composition.** DNA base composition was determined by reversed-phase high-performance liquid chromatography (HPLC) after enzymatic hydrolysis of DNA into nucleosides (22).

**Cell wall analysis.** Cell walls were prepared as described by Yamada and Komagata (26) with some modifications. Freshly cultured cells were disrupted by a mechanical cell homogenizer (B. Braun Melsungen AG, Federal Republic of Germany). The crude cell wall fraction was collected by centrifugation and purified by digestion with trypsin, pronase E (Protease Type XIV; Sigma) (1 mg of pronase E per ml of 50 mM phosphate buffer [pH 7.6] at 37°C for 5 h), and pepsin. After acid hydrolysis, amino acids were determined by an amino acid analyzer (model 835; Hitachi Ltd., Tokyo, Japan). The configuration of the amino acids was determined as described by Kandler and König (9). Sugars of purified cell walls were analyzed by paper chromatography (1). The acyl type of cell wall was determined by a colorimetric method (25).

**Determination of cellular fatty acid composition.** Fatty acid composition of whole cells was determined as described by Suzuki and Komagata (19).

**Determination of quinone system.** Isoprenoid quinones were extracted from lyophilized cells with chloroform-methanol (2:1, vol/vol) and purified by thin-layer chromatography. The purified quinones were analyzed by HPLC (21). The abbreviations used for menaquinones are in the form MK-\(n\)(H\(m\)), with \(n\) indicating the number of isoprene units in the side chain and \(m\) indicating the number of hydrogen atoms saturating the isoprenoid chain.

**DNA-DNA hybridization.** DNA-DNA relatedness values were determined by the membrane filter method (11). DNA of strain YLM-1\(^T\) was labeled by the nick translation method, using kits TRK.625 and TRK.5000 (Amersham International, Buckinghamshire, United Kingdom). A DNA filter of *P. aeruginosa* KS 0025\(^T\) was used as the negative control.

**RESULTS**

**Morphological characteristics.** Cells of the four isolates were irregular rods (0.2 to 0.3 by 0.8 to 1.0 μm) and were pleomorphic, especially on the sodium citrate supplemented medium. Branching of cells was not observed. V-forms were observed. They were gram positive, but were gram variable in old cultures and not acid fast. Cells were motile and had multitrichous flagella. No spores were observed, and the cell cultures did not survive when heated at 80°C for 10 min. A thin-section electron micrograph showed a cell wall typical of gram-positive bacteria (Fig. 1).

**Cultural characteristics.** Colonies grown aerobically on YM-catalase agar were about 1 to 2 mm in diameter, pale yellow, opaque, circular, convex, entire, and smooth. The strains grew more weakly under anaerobic rather than aerobic conditions. Colonies grown anaerobically on YM

![FIG. 1. Electron micrograph of thin sections of *R. faecitubidus JCM 6097\(^T\)* cells. Bar, 1 μm.](image-url)
TABLE 1. G+C contents of DNA and DNA-DNA hybridization of isolates and some coryneform and related bacteria

<table>
<thead>
<tr>
<th>Strain designation</th>
<th>G+C content mol%</th>
<th>% DNA complementarity with [3H]DNA of YLM-1T</th>
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<tr>
<td>Rarobacter faecitabidus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>YLM-1T</td>
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<td>100</td>
</tr>
<tr>
<td>YLM-11</td>
<td>65.8</td>
<td>54</td>
</tr>
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<td>YLM-39</td>
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<td>62</td>
</tr>
<tr>
<td>YLM-113</td>
<td>65.8</td>
<td>73</td>
</tr>
<tr>
<td>Curtobacterium citreum JCM 1345T</td>
<td>71.5</td>
<td>2</td>
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<td>2</td>
</tr>
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<td>Oerskovia turbata JCM 3160T</td>
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<td>4</td>
</tr>
<tr>
<td>Oerskovia xanthineolytica JCM 3164T</td>
<td>74.3</td>
<td>3</td>
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* Data from reference 27.

TABLE 2. Cellular fatty acid compositions

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<tr>
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<th>a-15</th>
<th>a-17</th>
<th>a-19</th>
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<td>2</td>
<td>10</td>
<td>Tr</td>
<td>14</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
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<td>Tr</td>
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<td>1</td>
<td>2</td>
<td>1</td>
<td>13</td>
<td>Tr</td>
<td>9</td>
<td>3</td>
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<td>5</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>12</td>
<td>Tr</td>
<td>10</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>YLM-113</td>
<td>1</td>
<td>51</td>
<td>2</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>13</td>
<td>Tr</td>
<td>12</td>
<td>4</td>
<td>11</td>
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</table>

* Abbreviations for fatty acids are as follows: a-13, anteiso-branched tridecanoic acid; i-14, iso-branched tetradecanoic acid; and n-13, straight-chain tridecanoic acid.

Rarobacter faecitabidus gen. nov., sp. nov.

Agar in an atmosphere containing carbon dioxide were about 0.5 to 1 mm in diameter, beige, translucent, circular, flat, entire, and smooth. Water-soluble pigments were not produced.

Physiological and biochemical characteristics. The isolates grew at 20 to 35°C, but did not grow below 15 or above 41°C. The optimal temperature was 30°C. They grew well at pH 6 to 8 but did not grow below pH 5 or above pH 9. They grew in the presence of 3% (wt/vol) NaCl but not in 5% (wt/vol) NaCl. The isolates grew well on nutrient agar containing catalase (60 μg/ml), hemoglobin (1 mg/ml), or hemin (10 μg/ml) but not on nutrient agar without these ingredients. In anaerobic conditions, they grew in an atmosphere containing carbon dioxide on nutrient agar containing NaCl. The isolates grew well on nutrient agar containing NaCl but not in 5% (wt/vol) NaCl. Inorganic nitrogen but not potassium nitrate. Acid was produced from glucose in aerobic and anaerobic conditions, but not from L-arabinose, D-xylose, L-rhamnose, D-galactose, L-sorbosine, succrose, lactose, trehalose, raffinose, inulin, glycerol, erythritol, adonitol, mannotol, dulcitol, D-sorbitol, inositol, esculin, or α-methyl-D-glucoside. Production of gas from glucose was not detected. The following 22 organic acids were not assimilated: acetic acid, pyruvic acid, t-lactic acid, l-lactic acid, malic acid, succinic acid, fumaric acid, 2-oxoglutaric acid, citric acid, formic acid, n-propionic acid, n-butyrinic acid, oxalic acid, malonic acid, glutaric acid, adipic acid, pimelic acid, glycolic acid, glyoxylic acid, gluconic acid, hippuric acid, and uric acid. The organisms hydrolyzed starch, casein, and gelatin but not cellulose or carboxymethyl cellulose. Hemolysis was not detected. The isolates did not produce acetoin, indole, or hydrogen sulfide. Reactions of extracellular DNase and urease were negative, and those of oxidase and catalase were positive.

Chemotaxonomic characteristics. The four strains were similar in DNA base composition, cell wall composition, cellular fatty acid composition, and major isoprenoid quinone. The guanine-plus-cytosine (G+C) contents of DNA were 65.7 to 66.1 mol% (Table 1). The amino acid composition of the cell wall peptidoglycan was as follows: α-alanine (D-Ala), L-Ala, D-glutamic acid (D-Glu), L-ornithine (L-Om), and D-serine (D-Ser) (1:1:2:1:1). No sugars, except for N-acetylmuramic acid and N-acetylglucosamine, were detected in the purified peptidoglycan. The acyl type of the cell wall was acetyl. The major fatty acid of whole cells was 12-methyltridecanoic acid (anteiso-C12:0) (about 50% of the total acid) (Table 2). The major isoprenoid quinone was MK-9 (more than 90%); small amounts of MK-8 and MK-7 were detected.

DNA-DNA hybridization. Table 1 shows DNA-DNA relatedness among the strains and several coryneform bacteria. The four strains showed more than 50% DNA complementarity to strain YLM-1T, while the other coryneform bacteria showed less than 5% complementarity to strain YLM-1T.

DISCUSSION

The four isolates were nonsporeforming, gram-positive, non-acid-fast, irregular rods and showed V-forms. These characteristics showed that these isolates belong to the coryneform group of bacteria (15).

It has been reported that some gram-positive bacteria have the ability to lyse yeast cells (10, 13) and fungus cells (20). These bacteria satisfy the characteristics of the genus Oerskovia (2, 13), possibly O. xanthineolytica. Our isolates are quite different from Oerskovia strains not only in their morphological characteristics, but also in their chemotaxonomic features, especially in their isoprenoid quinone and diamino acid in peptidoglycan.

Table 3 shows the amino acid compositions of the peptidoglycan of the isolates and some other coryneform bacteria. Based on the amino acid composition, the peptidoglycan type of our isolates may be group A of Schleifer and Kandler (17). The genera of coryneform bacteria whose cell walls contain ornithine are Cellulomonas, Curtobacterium, and Aureobacterium (8). Among those genera, only strains of the
genus *Cellulomonas* have peptidoglycan of group A. However, there are no strains of the genus *Cellulomonas* which have the same amino acid composition as that of our isolates. Furthermore, the major isoprenoid quinone of the isolates is different from that of strains of the genus *Cellulomonas* (Table 4). On the basis of chemotaxonomic characteristics, there is not a suitable genus for the isolates among coryneform and related bacteria (Table 4). The results of DNA-DNA hybridization revealed that they formed an independent cluster within the coryneform group.

The isolates produced acid from limited kinds of sugars and sugar alcohols and did not assimilate the organic acids tested. These characteristics differ from those of the genera *Curtobacterium* and *Oerskovia* (Table 4). Among coryneform bacteria, the isolates produced acid from D-glucose and D-mannose, but not from L-arabinose or L-rhamnose. Organic acids studied thus far are not assimilated (see above). Starch, casein, and gelatin are hydrolyzed, but cellulose is not hydrolyzed. Acetoin and indole are not produced. Oxidase positive. DNA base composition ranges from 65.7 to 66.1 mol% G+C. Diamino acid of cell wall is L-ornithine. Cell wall acyl type is acetyl. Major cellular fatty acid is anteiso-C15. Major isoprenoid quinone is MK-9. Type species is *Rarobacter faecitabidus*.

**Description of Rarobacter faecitabidus sp. nov.** In addition to the characteristics given for the genus, the characteristics of *Rarobacter faecitabidus* (fae.ci.ta'bi.dus. L. fem. n. faex, dregs; L. adj. tabidus, dissolving; M. L. masc. adj. faecita-bidus, dreg dissolving) are as follows. Facultative anaerobic. Requires hemin or hemoproteins besides biotin and thiamine as growth factors in the aerobic condition. Requires carbon dioxide but not hemin or hemoproteins for anaerobic growth. Colonies on nutrient-catalase agar are pale yellow, opaque, circular, convex, entire, and smooth. Acid is produced aerobically and anaerobically from D-glucose, D-fructose, D-mannose, maltose, cellobiose, dextrin, arbutin, and salicin, but not from L-arabinose, L-rhamnose, D-galactose, L-sorbose, lactose, trehalose, raffinose, inulin, glycero1, erythritol, adonitol, mannitol, dulcitol, D-sorbitol, inositol, esculin, or α-methyl-D-glucoside. Hydrogen sulfide is not produced. DNase and urease negative. Catalase positive. DNA base composition is 65.7 to 66.1 mol% G+C (determined by the HPLC method). Amino acid composition of cell wall peptidoglycan is D-Ala, L-Ala, D-Glu, L-Orn, and D-Ser (1:2:1:1). Adheres to viable yeast cells of the genera *Saccharomyces* and *Hansenula*, agglutinates with them, and lyzes them. Type strain is YLM-1 (JCM 6097). The description of the type strain is the same as that of the species. DNA base composition of the type strain is 66.1 mol% G+C.

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We thank J. Sugiyama for his kind suggestion on etymology, K. Uchida for helpful suggestions on preparation of cell walls, and A. Hirata for technical assistance in electron microscopy. This research was supported by a grant from the Environment Agency, Tokyo, Japan.

**LITERATURE CITED**


