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SYSTEMATIC STUDY OF BACTERIAL ORGANISMS ISOLATED FROM A VARIETY OF NATURAL SOURCES OF KOREA

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SUMMARY

Recent molecular and polyphasic taxonomic approaches toward bacterial systematics have played a significant role in revolutionizing our insight in the taxonomy of bacterial organisms. This advance has also contributed greatly to delineation of new taxa from bacterial organisms isolated from a variety of natural sources of Korea. Recently, many bacterial organisms have isolated from soil, seawater, foods, wastewaters and humans of Korea and have been subjected to polyphasic taxonomic study. From the results of this study, some isolates have been found to be members of new genera and new species.

INTRODUCTION

For a long time, bacterial classification and identification was mainly performed based on a few subjectively chosen phenotypic properties. The monothetic definition derived from these data readily led to bacterial classification and identification becoming wrong. However, bacterial taxonomy or systematics has progressed rapidly over the last several years. In particular, recent development of molecular systematics and polyphasic taxonomy is a milestone in modern bacterial taxonomy. Polyphasic taxonomic study aims at the integration of different kinds of data and information (phenotypic, genotypic and phylogenetic) for the purposes of accurate, objective and stable classification of the prokaryotes. This has largely been made possible as a result of recent progress in analytical chemistry and database management techniques together with spectacular development in molecular biology. Polyphasic techniques and data are now being widely applied for the elucidation of the evolutionary relationships as well as the classification of prokaryotic organisms. This study also has revolutionized our insight in the point of view of biological diversity of prokaryotes (1). Recently, molecular systematic and polyphasic data have contributed greatly to delineation of some new taxa in our laboratory. The descriptions of a new genus *Kribbella* together with some new species became possible from the results of these studies (2,3,4,5).

Recently, we have isolated many bacterial organisms from a variety of natural sources of Korea, such as soil, seawater, foods, industrial wastewater and humans. In the present study, these bacterial isolates were subjected to molecular systematic and polyphasic taxonomic study and

were characterized with data obtained.

MATERIALS AND METHODS

Bacterial strains. Bacterial strains were isolated from soil, seawater, foods, industrial wastewater and humans. Each strain was cultivated in suitable conditions.

Morphological and physiological characterizations. The morphology of cells was examined by light microscopy, scanning electron microscopy and transmission electron microscopy. Most physiological characteristics were determined as described previously (2,3,4,5).

Isolation of DNA. Chromosomal DNA was isolated and purified according to the method described previously (6).

Chemotaxonomic characterizations. Cell wall, menaquinones and polar lipids were analyzed as described previously (7) using reversed-phase HPLC. Fatty acids were extracted and analyzed following the instructions of the Microbial Identification System (MIDI). The G + C content was determined by HPLC method.

DNA-DNA hybridization. DNA-DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (8) using photobiotin-labelled DNA probes and microdilution wells.

16S rDNA sequencing and phylogenetic analysis. The 16S rDNA was amplified by PCR using the two universal primers as described previously (9). The sequencing of 16S rDNA was performed as described previously (9) or with ABI PRISM BigDye Terminator Cycle sequencing kit and automatic DNA sequencer. Phylogenetic analysis was mainly performed with CLUSTAL W software and the PHYLIP package.

RESULTS AND DISCUSSION

For the last several years, many bacterial organisms have been isolated from soil, seawater, foods (Kimchi and Jeotgal), industrial wastewater, humans and etc, and they have been characterized by polyphasic taxonomic study. From the results of these studies, members of some new species together with one genus have been found. The members belonging to the new taxa are grouped below according to source.

(1) Soil

Description of *Janibacter terrae* sp. nov. *Janibacter terrae* (ter'rae. M. L. gen. n. *terrae* of the earth) (10). Cells are non-spore-forming and non-motile cocci. Colonies are circular, opaque, glistening and convex and cream-colored on R agar. Neither substrate mycelia nor primary mycelia are seen. The optimal growth temperature is 28-30°C. The optimal pH for growth is 7.0-8.0. Growth occurs in presence of 8% (w/v) NaCl. No growth occurs under anaerobic conditions. Catalase- and DNase-positive. Urease-negative. Oxidase is weakly positive only on R agar. Cell wall contains *meso*-daminopimelic acid. The predominant menaquinone is MK-8(H_4). The major fatty acids are iso-$C_{16:0}$, $C_{18:1}$ ω9c and anteiso-$C_{17:0}$. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. The G+C content is 69 mol% (determined by HPLC). Isolated from a soil around a wastewater treatment plant, Korea. The type strain is $CS12^T$.

Description of *Paenibacillus chinjuensis* sp. nov. *Paenibacillus chinjuensis* (chin.juen'sis. M.L. adj. *chinjuensis* referring to chinju, the city of Korea where the isolate was originated). Cells are facultatively anaerobic rods that are 0.6-0.9 μm wide and 3.0-6.0 μm long in 24 h culture on trypticase soy agar. Ellipsoidal spores are formed in swollen sporangia. Motile by means of peritrichous flagella. Colonies are light pink, smooth, glossy, circular and convex after 3-4 days on TSA. Catalase-, oxidase- and DNase-positive. Urease-negative. No growth occurs in the presence of more than 2% (w/v) NaCl. Growth occurs at 20 and 45°C. Optimal temperature for growth is 30-37°C. Optimal pH for growth is 6.5-7.3. Cell wall peptidoglycan contains *meso*-diaminopimelic acid. The predominant menaquinone is MK-7. The major fatty acid is anteiso-$C_{15:0}$. The G+C content is 53 mol% (determined by HPLC). Isolated from a soil of chinju, Korea. The type strain is strain $WN9^T$.

(2) Industrial wastewater

Description of *Nocardioides nitrophenolicus* sp. nov. *Nocardioides nitrophenolicus* (nitr.o.phen.o'li.cus. L. masc. adj. *nitro*, containing nitrogen; N. L. n. *pheno*, phenol; L. fem. adj. *nitrophenolicus*, relating to nitrophenols) (4). Cells are aerobic, non-acid-fast, non-spore-forming rods that are 0.5 to 0.8 μm wide and 1.0 to 3.0 μm long in two days culture on nutrient agar. Cells exhibit coccus in the stationary phase of growth. Motile by means of a single polar flagellum. Colonies are smooth, glossy and yellowish white with irregular edges on nutrient agar. Neither substrate mycelium nor primary mycelium is formed. Growth occurs at pH 6 and 10 and at 15 and 40°C. Optimal pH and temperature for growth are 8 and 30°C, respectively. Catalase and oxidase positive. Degrades phenol and *p*-nitrophenol. The G+C content is 71.4 mol% (as determined by HPLC). Isolated from an industrial wastewater of Cheong-ju, Korea.

The type strain is strain NSP41, which has been deposited in the Korean Collection for Type Cultures as KCTC 0457BP.

Description of *Rhodococcus koreensis* sp. nov. *Rhodococcus koreensis* (ko.re.en'sis. M. L. adj. *koreensis*, referring to Korea, the country where strain DNP505^T was isolated and taxonomically studied) (11). Cells are gram-positive and non-motile. Cells are rods, form filaments or show elementary branching at early growth phase and mostly cocci in stationary phase. Colonies are cream-colored, opaque and convex with slightly irregular edges on trypticase soy agar (TSA). Forms a substrate mycelium that fragments into rod to coccus elements. Grow optimally at pH 7.0-7.8 and at 25 to 30°C. Oxidase negative and catalase- and urease-positive. Degrade 2,4-dinitrophenol. Cell wall contains *meso*-daminopimelic acid, arabinose and galactose (wall chemotype IV). The predominant menaquinone is MK-8(H_2). The major fatty acid is $C_{16:0}$. Mycolic acids contain 43 to 53 carbon atoms. The G+C content of the DNA is 66 mol% (as determined by HPLC). Isolated from an industrial wastewater of Cheong-ju, Korea. The type strain is strain DNP505^T.

Description of *Gordonia nitida* sp. nov. (ni.ti'da. L. adj. *nitida*, bright, shining, glossy, referring to the shining and glossy colonies) (12). Cells are gram-positive, non-motile. Cells in the early growth phase are rods and cells in the exponential and stationary growth phases are cocci. Colonies are orange colored, glossy and low convex with slightly irregular edges on TSA. Neither substrate mycelia nor primary mycelia were found. Grow well at broad range of pH 6.0-9.0 but grew better at alkalic condition of pH 8.0-9.0. Grow optimally at 30-37°C and the growth was very slow or inhibited at 7°C and did not occur at 45°C. Catalase, urease and DNase positive. Oxidase negative. Cell wall contains *meso*-daminopimelic acid, arabinose and galactose (wall chemotype IV). The predominant menaquinone is MK-9(H_2). The major fatty acids are $C_{16:0}$, $C_{18:1}$ ω9c, and 10-methyl-$C_{18:0}$ (TBSA). Mycolic acids contain 47 to 55 carbon atoms. The G+C content of the DNA is 67 mol% (as determined by HPLC). Isolated from an industrial wastewater of Taegu, Korea. The type strain is strain LE31^T.

Description of *Rhodococcus pyridinivorans* sp. nov. *Rhodococcus pyridinivorans* (py.ri.di.ni.vo'rans. M.L. n. *pyridin* pyridine; L. v. *vorare* to devour; M.L. adj. *pyridinovorans* devouring pyridine) (13). Cells are non-spore-forming, non-motile and gram-positive that is gram variable in old cultures. A substrate mycelium that penetrates into agar media is visible and they fragment into short rod to coccus elements. Cells are rods and branched filament at early growth phase and fragment into short rods or cocci. Colonies are light orange-colored, opaque and raised with slightly irregular edges on trypticase soy agar. Colonies have irregularly

round wrinkles. Grow well at pH 6.0-9.0 and optimal pH is 7.5-8.5. Optimal temperatures are 30-37°C. Catalase- and urease-positive. Oxidase- and DNase- negative. Cell wall type is chemotype IV sensu Lechevalier & Lechevalier (1970) containing meso-diaminopimelic acid, arabinose and galactose. The predominant menaquinone is MK-8(H_2). The major fatty acids are $C_{16:0}$, $C_{18:1}$ cis 9, 10-methyl-$C_{18:0}$ (TBSA). Mycolic acids with 36-46 carbon atoms are present. The G+C content of the DNA is 66 mol% (as determined by HPLC). The type strain is strain PDB9^T.

(2) Food

Description of *Planomicrobium* gen. nov. *Planomicrobium* (Pla.no.mi.cro'bi.um. Gr. n. *planos* wanderer; Gr. adj. *micros*, small; Gr. n. *bios*, life; M. L. n. *Planomicrobium*, motile microbe).

Description of *Planomicrobium koreense* sp. nov. *Planomicrobium koreense* (ko.re.en'se. M. L. adj. *koreense* referring to Korea, the country where strain JG07^T was isolated and taxonomically studied). Cells are cocci or short rods in the early growth phase but soon change to rods. Strictly aerobic and non-spore-forming. Motile by means of a single polar flagellum. Colonies are circular, smooth, low convex and yellow orange. Catalase positive. Oxidase- and urease-negative. Optimal temperatures are 20-30°C. Optimal pH for growth is 7.0-8.5. Growth occurs in the presence of 0-6% (w/v) The optimal concentration of NaCl for growth is 1-4%. The peptidoglycan type is A4α (L-Lys-D-Glu). The predominant menaquinones are MK-8, MK-7 and MK-6. The cellular fatty acid profile contains major amounts of saturated, unsaturated and branched fatty acids. The cellular phospholipids are phosphatidylethanolamine, phosphatidylglycerol, bisphosphatidylglycerol. The G+C content is 47 mol%. Isolated from Jeotgal. The type strain is strain JG07.

Description of *Lactobacillus kimchii* sp. nov. *Lactobacillus kimchii* (kim'chi.i. M.L. n. *kimchii* of Kimchi, Korean vegetables-fermented food) (14). Cells are short, slender rods measuring 0.6-0.8 by 1.5-3.0 μm on MRS medium at 30°C, which occur singly, in pairs or occasionally short chains. Gram-positive, non-spore-forming and non-motile. After 3 d incubation on MRS agar, colonies are white, circular to slightly irregular, convex, smooth, opaque. Catalase-negative. L(+)-lactic acid and D(−)-lactic acid are produced. Facultatively heterofermentative. Facultatively anaerobic. Growth occurs at 10 and 40°C. Optimal temperature and pH for growth are approximately 30°C and 6.0-7.0, respectively. Grow in the presence of 8% NaCl. The major fatty acid is the unsaturated fatty acid, $C_{18:1}$ ω9c. The G+C content is 35 mol% (determined by HPLC). Isolated from Kimchi. The type strain is strain MT-1077^T.

Description of *Bacillus jeotgali* sp. nov. *Bacillus jeotgali* (je.ot.ga'li. M.L. n. *jeotgali* of Jeotgal Korean traditional food). Cells are facultatively anaerobic, endospore-forming rods that are 0.8-1.1 μm wide and 4.0-6.0 μm long. Gram-variable. Motile by means of peritrichous flagella. Colonies are cream-yellow or light orange-yellow colored, smooth, irregular and flat on TSA+ASW. Catalase- and urease-positive. Oxidase-negative. Grow in the presence of 13% (w/v) NaCl. Growth occurs at 10 and 45°C. Optimal growth temperature is 30-35°C. Optimal pH for growth is 7.0-8.0. The growth is inhibited below pH 5.0. Cell wall peptidoglycan contains *meso*-diaminopimelic acid. The predominant menaquinone is MK-7. The major fatty acid is iso-$C_{15:0}$. The G+C content is 41 mol% (determined by HPLC). Isolated from Jeotgal. The type strain is strain YKJ-10^T.

Description of *Halomonas alimentaria* sp. nov. *Halomonas alimentaria* (a.li.men.ta'ri.a. L. adj *alimentaria* relating to food). Cells are cocci measuring 0.8-1.2 μm in diameter or short rods measuring 0.8-1.2 μm wide and 1.3-1.9 μm long. Gram staining reaction is negative. No flagellum is found. Facultative anaerobic. Colonies are smooth, glistening, circular and low convex after 3 d culture. Color of colonies is cream-yellow on marine agar and dark yellow on TSA+ASW. Catalase- and oxidase-positive. Urea is hydrolyzed. Grow in the presence of 23% (w/v) NaCl. Growth occurs at 4 and 45°C. Optimal growth temperature is 30°C. Optimal pH for growth is 6.5-7.5. The predominant isoprenoid quinone is ubiquinone-9. The major fatty acids are $C_{18:1}$ ω7c, $C_{16:0}$, $C_{19:0}$ cyclo ω8c and $C_{16:1}$ ω7c and/or iso $C_{15:0}$ 2OH. The G+C content is 63 mol% (determined by HPLC). Isolated from Jeotgal. The type strain is strain YKJ-16^T.

(3) Seawater

Description of *Bacillus aquamarinus* sp. nov. *Bacillus aquamarinus* (a.qua.ma.ri'nus. L. n. *aqua* water; L. adj. *marinus* of the sea; M.L. adj. *aquamarinus* pertaining to seawater). Cells are facultatively anaerobic, endospore-forming rods that are 0.9-1.2 μm wide and 2.0-3.0 μm long in 3 d culture on TSA. Round terminal spores are observed in swollen sporangia. Gram-variable. Motile by means of a single polar flagellum. Colonies are light orange colored, smooth, circular-irregular and raised on TSA. Catalase-, oxidase- and urease-positive. Grow in the presence of 13% (w/v) NaCl. Growth occurs at 4 and 37°C. Optimal growth temperature is 25°C. Optimal pH for growth is 6.5-7.0. The peptidoglycan type is A4α (L-Lys-L-Ala-D-Asp). The predominant menaquinone is MK-7. The major fatty acid is anteiso-$C_{15:0}$. The G+C content is 40 mol% (determined by HPLC). Isolated from a seawater. The type strain is strain SW28^T.

Description of *Halomonas marisflava* sp. nov. *Halomonas marisflava* (ma.ris.fla'va. L. gen. n. *maris* of the sea; L. fem. adj. *flava* yellow; M.L. gen. n. *marisflava* of the Yellow Sea). Cells are rod or oval measuring 0.9-1.2 μm wide and 1.7-2.3 μm long in 3 d culture on marine agar at 28°C. Gram staining reaction is negative. Cells were motile by means of a single polar flagellum. Colonies are yellow-orange, smooth, glistening, circular-slightly irregular with convex on TSA and flat-low convex on MA. Facultative anaerobic. Catalase positive. Oxidase- and urease-negative. Grow in the presence of 27% (w/v) NaCl. Growth occurs at 4- 37°C with an optimum of 25-30°C. Optimal pH for growth is 7.0-8.0. The predominant isoprenoid quinone is ubiquinone-9. The major fatty acids are $C_{18:1}$ and $C_{16:0}$. The G+C content is 57 mol% (determined by HPLC). Isolated from Jeotgal. The type strain is strain YKJ-6^T.

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