Strain YM01 recognized as a species of *Kocuria* isolated from boiling water concentrates of queen crab(*Chionoecetes japonicus*)

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INTRODUCTION

A Gram-positive, aerobic coccus (YM01) was isolated and sequence comparisons of the 16S rDNA indicated this isolate to be phylogenetic neighbours of members of the genus *Kocuri*a, family *Micrococcacea*e.

The genus *Kocuria* (Stackebrandt *et al*., 1995) currently contains several species, i.e. the type species *Kocuria rose*a, *Kocuria varian*s, *Kocuria kristinae, Kocuria erythromyxa, Kocuria palustris* and *Kocuria rhizophila,* etc. All were originally placed in the genus *Micrococcu*s. The first three species were identified as individual species. *K. erythromyxa*, strain UWO 1045, was first called '*Sarcina erythromyxa*', then recognized as a strain of *Micrococcus roseus* and subsequently described as *Deinococcus erythromyxa* (Brooks & Murray, 1981). Following 16S rDNA analysis the *Micrococcus* species were shown to form an individual cluster within the *Arthrobacter-Micrococcus* line of descent (Stackebrandt *et al*., 1980, 1995; Koch *et al*., 1994); a cluster later described as the family *Micrococcaceae* (Stackebrandt *et al*., 1997). Based on 16S rDNA analysis and pattern of chemotaxonomic properties the three *Micrococcus* species were transferred to a new genus *Kocuri*a. Later, *Deinococcus erythromyxa* was added to this genus as *K. erythromyxa* when information on the 16S rDNA sequence became available (Rainey *et al*., 1997). *Kocuria palustris* and *Kocuria rhizophila* were isolated from the rhizoplane of narrow-leaved cattail *(Typha angustifoli*a) collected from a floating mat in the Soroksar tributary of the Danube river, Hungary, They were classified and deposited at the DSMZ as strain DSM 11925 and DSM 11926, respectively in 1999.

Now, we report the description of the strain YM01 recognized as a species of *Kocuria.* The strain YM01 was isolated from boiling water concentrate of queen crab(학명) harvested from the Eastern Sea of Korea.

METHODS

Bacterial strains and cultural conditions. Strain YM01 was isolated using multi-tube serial dilution technique on nutrient agar plate (Difco) from boiling water concentrate of queen

crab(*Chionoecetes japonicus*) harvested from the Eastern Sea of Korea. The strains were grown on nutrient agar at 25 C.

Physiological characterization. All physiological tests was carried out at 25 C except for those done in the varing temperature condition. Physiological tests were carried out according to published methods.

Cellular fatty acid analysis. Cellular fatty acids were extracted from according to Korn-Wendisch *et al.* (1989) were analysed by GC.

DNA isolation and determination of G+C content of DNA.

The DNA was isolated as described by Cashion *et al.* (1977). The G+C content of the DNA was determined by high-performance liquid chromatography as described by Mesbah *et al.* (1989).

16S rDNA sequence determination and phylogenetic analysis.

Genomic DNA extraction, PCR mediated amplification of the 16S rDNA and sequencing of the PCR products carried out were as described by Rainey *et al.* (1996). The sequence reaction products were electrophoresed using ABI 310 genetic analyser. Evolutionary distances were calculated by the method of Jukes & Cantor (1969). Phylogenetic dendrograms were reconstructed using the treeing algorithm of De Soete (1983).

RESULTS AND DISCUSSION

The cell was spherical, occurring in pairs, tetrads and packets, 1.0-1.5 μm in diameter, Gram-positive and non-motile. Endospores were not produced. Not acid-fast. The colonies are 1.5-2.5 mm in diameter, opaque, smooth with irregular edges with yellow pigmentation. Aerobic.

Partial 16S rDNA sequences of strain YM01 was determined. The isolate contained all the signature nucleotides that define the family *Micrococcaceae* to which the genus *Kocuria* belongs phylogenetically (Stackebrandt *et al.*, 1997). The phylogenetic dendrogram was constructed and strains YM01 grouped amongst species of the genus *Kocuria*.

No growth above 40 C. Good growth between pH 5.7-7.7. Catalase positive. Gelatinase, phosphatase, Tween 80 hydrolysis, growth on Simmons' citrate are positive. Oxidase, starch hydrolysis, indol, urease, aesculin hydrolysis, arginine dihydrolase and phenylalanine deaminase reaction are negative. H_2S reaction weak. Nitrate not reduced to nitrite. Acid production from d-glucose, d-fructose, d-mannose and saccharose. No acid production from glycerol, mannitol, sorbitol, ribose, d-xylose, l-arabinose, galactose, lactose, maltose, b-gentiobiose, salicin, trehalose, melezitose or amidon. Utilization of adonitol, l-arabinose, d-fructose, l-fucose, d-glucose, turanose, xylitol, methylpyruvate, glucuronamide, dextrin, glycogen, Tween 40, Tween 80 and N-acetyl-d-glucosamine. No utilization of cellobiose, d-trehalose, N-acetyl-d-galactosamine, *meso*-inositol, maltose, d-mannitol, d-melibiose, sorbitol, inosine, glycerol, l-glutamic acid and l-alanine. Good growth at 10% NaCl.

The major fatty acids are ai-$C_{17:0}$, ai-$C_{15:0}$ and i-$C_{15:0}$. Predominant polar lipids are phosphatidylglycerol and diphosphatidylglycerol.

The DNA base composition is 69.4 mol% G+C.

Strain YM01 isolated from boiling water concentrate of queen crab(학명) harvested from the Eastern Sea of Korea was quite similar to DSM 11926, the type strain of *Kocuria rhizophila* sp. nov.

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