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Survival Strategy, Metabolic Potential and Taxonomic Reframe of *Kocuria polaris*

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Abstract

Antarctica is renowned as the most inhospitable environment where microorganisms are thriving in the frontiers of life. In the past few years, many novel bacterial species have been reported from the Antarctic environment. During taxonomic re-evaluation of novel bacterial species from Antarctica, it was noticed that *Kocuria polaris* shared high 16S rRNA gene sequence similarity with *Kocuria rosea*. In the present study, the taxonomic position, metabolic potentials, and stress survival strategy of *K. polaris* were evaluated through genome analysis. *K. polaris* encodes genes for glycolysis, citrate cycle, pentose phosphate pathway, dissimilatory nitrate reduction, assimilatory sulfate reduction, etc. In addition, *K. polaris* also encodes genes for cold and salt stress. The 16S rRNA gene sequence extracted from *K. polaris* and *K. rosea* genomes showed 99.7% similarity. In the phylogenomic tree, *K. polaris* and *K. rosea* clustered together. The average nucleotide identity and digital DNA–DNA hybridization values between *K. polaris* and *K. rosea* exceeded the threshold (95-96% for ANI and 70% for dDDH) value for distinguishing species, showing that they are similar species. The present study shed light on *K. polaris* survival strategy in extreme conditions. We further propose to reclassify *Kocuria polaris* as a later heterotypic synonym of *Kocuria rosea.*

Keywords: *Kocuria polaris*, *Kocuria rosea*, Reclassification, Heterotypic Synonym, Metabolic Potentials

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INTRODUCTION

Antarctica is renowned as one of the planet's most inhospitable environments, defined by its extreme cold, arid conditions, relentless winds, intense UV radiation, and extremes of light and darkness.[1] Despite being on the frontiers of life´s limits, the frozen realm likely harbors an extensive and undiscovered array of microorganisms.[2] The distinctive and inhospitable environment leads to the preferential selection of microbial species exhibiting atypical metabolic capabilities and/or the synthesis of uncommon metabolites and substances.[3,4] In the past few years, many novel microbial species from the Antarctic environment have been reported.[5-7] In this regard, a Gram-positive, orange-pigmented novel psychrophilic bacterium *Kocuria polaris* (*K. polaris*) was reported from an Antarctic cyanobacterial mat.[8] During the taxonomic assessment of Antarctic bacteria, an intriguing observation emerged, *K. polaris* exhibited a high degree of 16S rRNA gene sequence similarity to *Kocuria rosea* (*K. rosea*). As a result, this study aims to provide a comprehensive clarification of the taxonomic classification of *K. polaris* employing genome analysis. Further, its metabolic potential and survival strategy in the cold environment were evaluated through genome analysis.

MATERIALS AND METHODS

Genome attributes

To evaluate the taxonomic position of *K. polaris,* all the type species genomes of the genus *Kocuria* were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/). *Nesterenkonia flava* CCTCC AB 207010^T (GCF_031432335.1) genome was also downloaded to use as an outgroup for the construction of a phylogenomic tree. The quality of the genomes was evaluated using CheckM.[9] Since *K. polaris* exhibited a high degree of 16S rRNA gene sequence similarity to *K. rosea* their genomes were visualized and compared using Proksee.[10,11] The tRNAs were predicted using tRNAscan-SE.[12] Average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values were determined to evaluate the genomic relatedness between *K. polaris* and *K. rosea*. Pyani with ANIm parameter.[13] and the Genome-to-Genome Distance Calculator (http://ggdc.dsmz.de/ggdc.php version 3.0; local alignment tool BLAST+ using formula 2).[14,15] were used to estimate the ANI and dDDH values, respectively.

Phylogenomic tree construction

Phylogenomic tree was constructed using the Anvi'o tool (version 7.1).[16,17] The process of converting FASTA files into contigs-db and identifying open reading frames and matching genes in the contigs to single-copy core genes was carried out using the program anvi-gen-contigs-database and anvi-run-hmms.[18,19] The genes present in HMM source 'Bacteria 71' [20] were taken and aligned using MUSCLE.[21] The generated tree was displayed using MEGA version 7.0.[22]

16S rRNA gene comparison and functional annotation

To compare *K. polaris* and *K. rosea* the 16S rRNA gene from the genomes was extracted using the script "anvi-get-sequences-for-hmm-hits" (hmm-source Ribosomal RNA 16S) (https://github.com/tseemann/barrnap). The EzBioCloud server's pairwise alignment function was used to assess the 16S rRNA gene (extracted from the genome) sequence similarity between *K. polaris* CMS 76or^T and *K. rosea* ATCC 186^T (www.ezbiocloud.net/tools/pairAlign). To evaluate *K. polaris* metabolic potentials and survival strategy in the cold environment functional annotation was performed by KofamKOALA[23] using the anvi-run-kegg-kofams program.

RESULTS AND DISCUSSION

The genus *Kocuria* was proposed by Stackebrandt *et al.*[24] and at the time of writing the genus includes 26 validly published species names.[25] Among *Kocuria* species, *K. polaris* was isolated from the Antarctic cyanobacterial mat, and as mentioned above it was reported to share high 16S rRNA gene sequence similarity with the type strain of *K. rosea,*[8] hence the present study evaluates its taxonomic position. We further evaluated the metabolic potentials and survival strategy of *K. polaris* in a cold environment.

The genome size of *K. polaris* CMS 76or^T was 3779800 (bp) with 72.8% G+C content while the genome size of *K. rosea* ATCC 186^T

was 3946651 (bp) with 72.7% G+C content. The genome completeness of *K. polaris* CMS 76or^T and *K. rosea* ATCC 186^T was 99.1 and 98.6%, respectively with zero contamination. A total of 48 tRNAs were predicted in both *K. polaris* CMS 76or^T and *K. rosea* ATCC 186^T. The graphical representation of the genome's comparison is mentioned in Figure 1.

Metabolic potential and survival strategy of *K. polaris*

K. polaris CMS 76or^T encodes genes for glycolysis, citrate cycle, and pentose phosphate pathway. Nitrate stands as the most highly oxidized variant among fixed nitrogen compounds, constituting a vital nutrient crucial for the sustenance of microbial and plant life.[26] In prokaryotes, dissimilatory nitrate reduction mechanisms have been extensively explored.[26-28] Reduction of nitrate to nitrite by respiratory membrane-bound *NarG* or periplasmic nitrate reductase *NapA* is the first step in dissimilatory nitrate reduction. Nitrite is next reduced to ammonia by cytoplasmic nitrite reductase *NirB* or periplasmic nitrite reductase *NrfA*.[27] In the present study, the genes encoding dissimilatory nitrate reduction (*NarGHI* and *NirBD*) were noticed in *K. polaris* CMS 76or^T. In addition, genes related to nitrate assimilation were also noticed in *K. polaris* CMS 76or^T.

Microorganisms use assimilatory sulfate reduction to convert inorganic sulfate to sulfide.[29] In the present study, it was noticed that *K. polaris* CMS 76or^T encodes genes (*CysND*, *CysH* and *Sir*) for assimilatory sulfate reduction. *K. polaris* CMS 76or^T encodes genes for various amino acid metabolism (like betaine, methionine, lysine, ornithine, arginine biosynthesis, etc). A detailed list of metabolic potentials of *K. polaris* CMS 76or^T is mentioned in Table S1.

Low temperatures significantly limit cellular function by impacting cell structure, water thickness, solute movement, membrane flexibility, enzyme activity, and large molecule interactions.[30] Microorganisms that survive in cold environments rely on adaptive strategies to keep their fundamental cellular processes intact.[30] In reaction to a quick temperature

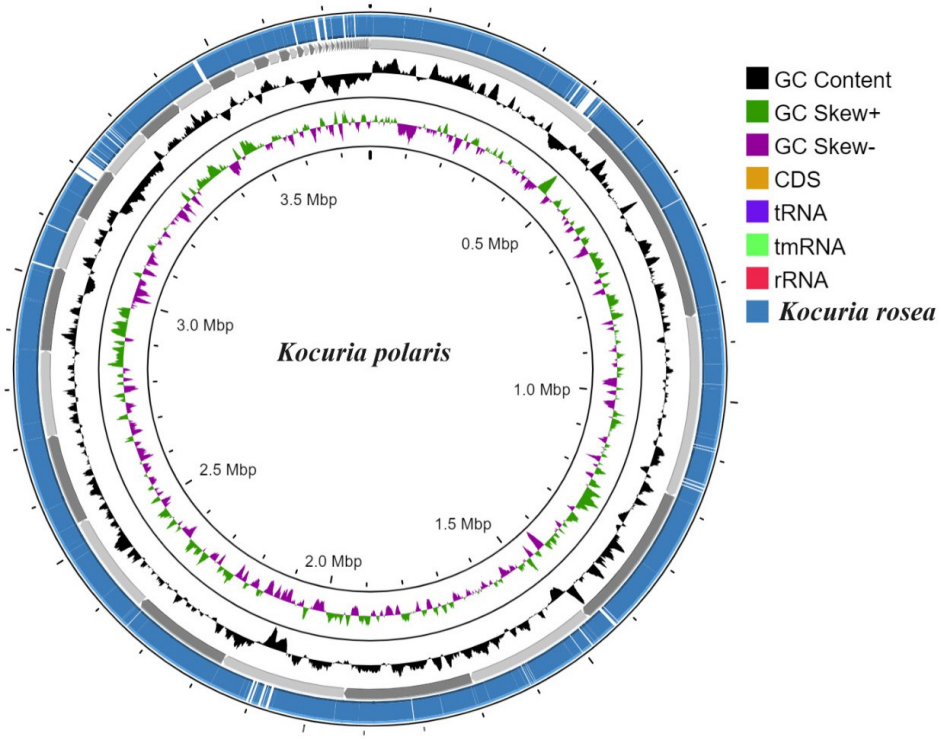

Figure 1. Genome graphical representation of *Kocuria polaris* CMS 76or^T and *Kocuria rosea* ATCC 186^T

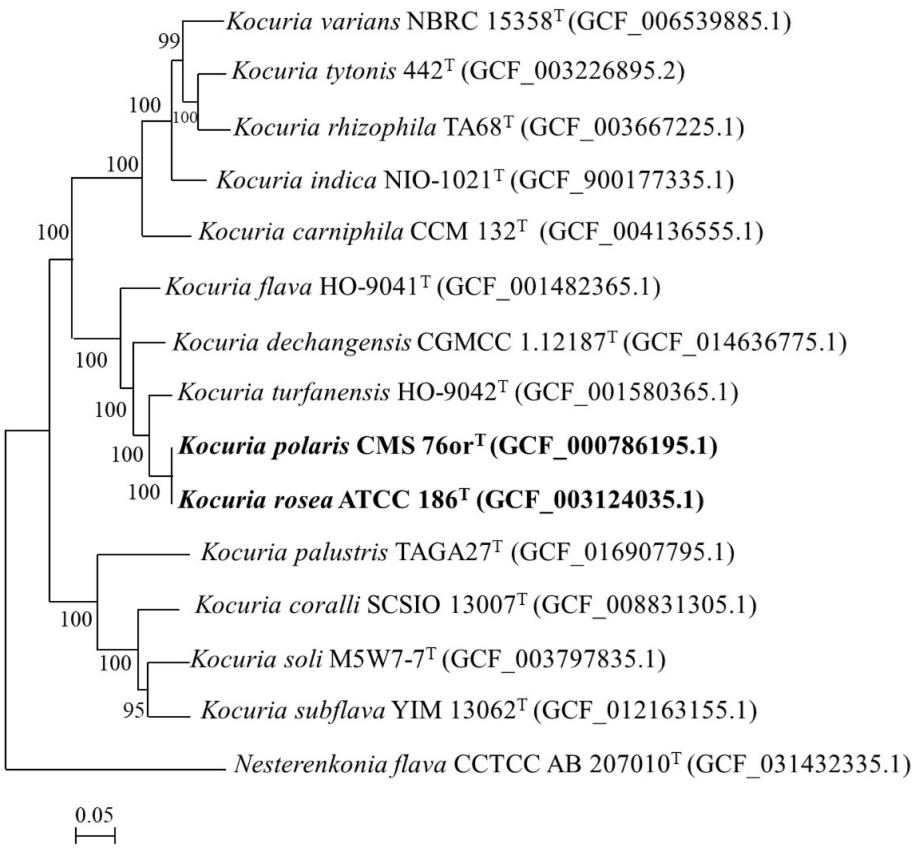

Figure 2. Phylogenomic tree (based on 71 bacterial single-copy genes) showing the relationships of *Kocuria polaris.* Bootstrap values greater than 50% are shown at branch points. Bar, 0.05 substitutions per nucleotide position

drop, many bacteria produce small cold shock proteins.[31] The cold-shock protein, CspA, was found to be significantly upregulated during the cold-shock response.[32] In the present study, CspA was also noticed in *K. polaris* CMS 76or^T. Universal stress proteins (USP) are key regulatory stress proteins that help bacteria survive in stressful environments.[33] In the present study, USP (ABCDEFG) was noticed in *K. polaris* CMS 76or^T. Trehalose production was reported to play a significant role in resistance to freezing in cold environments.[34] In the present study, The genes encoding trehalose biosynthesis were noticed in *K. polaris* CMS 76or^T.

A sudden drop in temperature can cause phase separation of cell membrane phospholipids, resulting in decreased membrane fluidity and increased permeability.[35] Palmitoleate has been shown to increase the flexibility of cell membranes while decreasing the temperature at which phase transition occurs. This helps to mitigate the adverse effects of cold temperatures. When the temperature drops, in certain bacteria, the cold-induced acyltransferase LpxP induces the attachment of palmitoleate to lipid A.[36] The adaptation of membrane fluidity also involves the fast desaturation of fatty acids in pre-existing phospholipids. This is accomplished by the activation of fatty acid desaturase (Des), which is regulated by the sensor kinase DesK and the response regulator DesR.[37] Genome analysis of *K. polaris* CMS 76or^T revealed the presence of LpxP, DesK and DesR.

K. polaris CMS 76or^T was also reported to tolerate NaCl up to 2.9%.[8] Salt-in and salt-out mechanisms help microorganisms regulate

osmoregulation.[38,39] Microorganisms employ the salt-in strategy to maintain osmotic equilibrium through the accumulation of large amounts of inorganic salts or ions within their cytosol.[38,39] The salt-out strategy entails the removal of salt ions from the cytoplasm while concurrently accumulating large amounts of compatible solutes.[38-40] Genome analysis of *K. polaris* CMS 76or^T revealed the presence of genes related to potassium uptake protein (Trk system), and multicomponent Na^+:H^+ antiporter. In addition, it was also noticed that *K. polaris* CMS 76or^T encodes genes for compatible solutes like betaine, proline, and trehalose.

Taxonomic position re-evaluation

In the present study, the 16S rRNA gene sequence extracted from *K. polaris* CMS 76or^T and *K. rosea* ATCC 186^T genome showed 99.7% similarity to each other. Even in the original article *K. polaris* CMS 76or^T was reported to share 99.8 and 71% 16S rRNA gene sequence and DNA–DNA hybridization (DDH) similarity with *K. rosea* ATCC 186^T, respectively.[8] The 16S rRNA gene sequence similarity was above the threshold value (98.7%), while the DDH value was close to the threshold value (70%) for species delineation.[41] In the phylogenomic tree (Figure 2), *K. polaris* CMS 76or^T and *K. rosea* ATCC 186^T clustered together.

The proposed cut-off values for ANI and dDDH values for species delineation were 95-96% and 70%, respectively.[14,42,43] The ANI value and dDDH value between *K. polaris* CMS 76or^T and *K. rosea* ATCC 186^T were 98.7 and 87.6%, respectively which were above the cut-off value indicating they are the same species. Based on the above results we propose to reclassify *Kocuria polaris* as a later heterotypic synonym of *Kocuria rosea.*

CONCLUSION

In the present study, the survival strategy under cold stress, metabolic potential, and taxonomic position of *K. polaris* was evaluated through genome analysis. *K. polaris* encodes genes for glycolysis, citrate cycle, pentose phosphate pathway, dissimilatory nitrate reduction, assimilatory sulfate reduction, etc. To overcome cold stress, *K. polaris* encodes genes for cold shock proteins, universal stress proteins, and mechanisms to enhance membrane fluidity. In addition, it also encodes genes related to potassium uptake protein, multicomponent Na^+:H^+ antiporter, and genes related to the synthesis of compatible solutes like betaine, proline, and trehalose involved in overcoming salt stress. The ANI, dDDH, and phylogenomic analysis suggest that *K. polaris* and *K. rosea* are similar species.

SUPPLEMENTARY INFORMATION

Supplementary information accompanies this article at https://doi.org/10.22207/JPAM.18.3.11

Additional file: Additional Table S1.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript and/or in the supplementary files.

ETHICS STATEMENT

This article does not contain any studies on human participants or animals performed by the author.

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