Draft genome sequence of *Micrococcus luteus* strain O'Kane implicates metabolic versatility and the potential to degrade polyhydroxybutyrates

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**A B S T R A C T**

*Micrococcus luteus* is a predominant member of skin microbiome. We here report on the genomic analysis of *Micrococcus luteus* strain O’Kane that was isolated from an elevator. The partial genome assembly of *Micrococcus luteus* strain O’Kane is 2.5 Mb with 2256 protein-coding genes and 62 RNA genes. Genomic analysis revealed metabolic versatility with genes involved in the metabolism and transport of glucose, galactose, fructose, mannose, alanine, aspartate, asparagine, glutamate, glutamine, glycine, serine, cysteine, methionine, arginine, proline, histidine, phenylalanine, and fatty acids. Genomic comparison to other *M. luteus* representatives identified the potential to degrade polyhydroxybutyrates, as well as several antibiotic resistance genes absent from other genomes.

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1. Introduction

*Micrococcus luteus* species are phylogenetically affiliated with the phylum Actinobacteria and characterized by a high genomic GC content (> 70%). Fifteen *Micrococcus luteus* are currently deposited in GenBank. The ecological distribution of the *Micrococcus luteus* genomes shows wide habitat preferences including soil [1,2], hydrocarbon-impacted environments [3,4] and human skin-associated [5] sources. Here, the strain O’Kane was isolated (by an undergraduate student, SDO) from an elevator surface with frequent human use in Stillwater, OK. Isolation efforts were part of the Student Initiated Microbial Discovery (SIMP) project at OSU (introduced in [6]). This project aims at increasing undergraduate student retention through participation in a two-semester course-embedded research endeavor.

*Micrococcus* is a phylogenetically and physiologically diverse genus with members ubiquitously found as part of skin microbiome [7]. Infections have been reported in patients with lowered immunity [8–11]. Genomic analysis of strains belonging to the genus *Micrococcus* can contribute to our understanding of the molecular mechanisms of opportunistic pathogenesis and infection and subsequently reduce the occurrence and/or mitigate the severity of such infections. Here we report on the draft genomic sequence and detailed analysis of the genome of *Micrococcus luteus* strain O’Kane.

2. Materials and methods

2.1. Genome project history

*Micrococcus luteus* O’Kane was isolated during an introductory microbiology course at Oklahoma State University. The course was modified to serve as part I of a project funded by the Howard Hughes Medical Institute aimed at improving undergraduate student persistence through authentic research. During part II of the project, genomes of selected strains are sequenced and analyzed by a team of undergraduate (KB and CM) and graduate (RAH) students during an upper division microbial genomics class. The quality draft assembly and annotation were completed in 2015–2016. Table 1 shows the genome project information.

2.2. Growth conditions and genomic DNA preparation

*Micrococcus luteus* O’Kane was grown overnight at 30 °C on tryptic soy agar (TSA) plates. A total of 17 μg of genomic DNA of high sequencing quality was isolated using the MPBio PowerSoil® DNA extraction kit according to manufacturer’s instructions. Negative stain TEM micrographs were obtained using the services of the Oklahoma State University Microscopy Lab. Briefly, the sample was placed on a carbon film TEM grid and allowed to incubate for 2 min, after which the excess liquid was wicked off. Phosphotungstic acid (PTA; 2% w/v) was then added to the grid followed by a 45-s incubation. Excess PTA was wicked off and the grid was allowed to dry before it was visualized using JOEL JEM-2100 transmission electron microscope.

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2.3. Genome sequencing and assembly

The genome of Micrococcus luteus O’Kane was sequenced using the Illumina MiSeq platform at the University of Georgia Genomics Facility using 2 × 300 paired end chemistry and an average library insert size of 700 bp. Quality filtered sequence data were assembled with the short read de Brujin graph assembly program Velvet 1.1 [12] using the settings kmer value of 101 bp and a minimum contig coverage value of 7×. The genome project is deposited in GOLD (Genomes On-Line Database) and this Whole Genome Shotgun (WGS) project has been deposited in GenBank under the accession MAYP00000000. The version described in this paper is version MAYP01000000.

2.4. Genome annotation

Gene models were created using the prokaryotic gene calling software package Prodigal [13]. A total of 2318 gene models of average gene size 1004 bp were predicted. To functionally annotate the predicted protein sequences, we used a combination of NCBI Blast C++ homology search and HMMER 3.0 [14] hmmscan against the PFAM 26.0 database [15]. Additional gene analysis and functional annotation were carried out through the Integrated Microbial Genomes Expert Review (IMG-ER) platform.

2.5. Comparative genomics

We compared the genome of Micrococcus luteus strain O’Kane to 11 closely related Micrococcus luteus genomes (IMG IDs: 2505679057, 2551306408, 644736390, 645951813, 2576861783, 2576861777, 2574179829, 2627854072, 647000274, 2627853625, 2623620484). We used the “Genome clustering” function on the IMG-ER analysis platform to conduct genomic comparisons based on the COG profile. We also used principal component analysis to compare the genomes based on several genomic features including the genome size, the number of genes, the number of transporters identified, the GC content, the number of non-coding bases, the number of genes belonging to COG categories, as well as the number of genes belonging to each COG category. The PCA analysis was conducted using the “princomp” function in the labdsv library of R [16]. The results were visualized using a biplot.
Fig. 2. A maximum likelihood phylogenetic tree constructed using multiple sequence alignments of 16S rRNA genes. Micrococcus luteus O’Kane sequence is shown in bold. Reference sequences are also shown and GenBank accession numbers are given in parentheses. The tree was obtained under “Tamura-Nei + G” model with a variable site γ shape parameter of 0.47. “Escherichia coli” was used as the outgroup. Bootstrap values, in percent, are based on 100 replicates and are shown for branches with >50% bootstrap support. Multiple sequence alignment, model selection, and maximum likelihood analysis were carried out in Mega [30].

3. Results and discussion

3.1. Classification and features

Cells of strain O’Kane appeared as Gram positive, non-motile, aerobic cocci that were arranged in tetrads (Fig. 1A). Colonies on TSA agar were bright yellow (Fig. 1B).

Within the genus Micrococcus, 17 species have been described with validly published names: M. agilis type strain ATCC 996T, M. alovera type strain DSM 20550T, M. antarcticus type strain JCM 11467T, M. cohni type strain DSM 23974T, M. endophyticus type strain DSM 7945T, M. flavus type strain JCM 14000T, M. halobius type strain ATCC 21727T, M. kristinae type strain DSM 20032T, M. lactis type strain DSM 23694T, M. luteus type strain ATCC 4698T, M. luteus type strain ATCC 25766T, M. nishinomiyaensis type strain DSM 29093T, M. roseus type strain ATCC 186T, M. sedentarius type strain ATCC 14392T, M. terrus type strain JCM 17489T, M. varians type strain ATCC 15306T, and M. yunnanaensis type strain DSM 21948T.

Strain O’Kane shares 93.4–99.7% 16S rRNA gene identities with other species in the genus Micrococcus: M. agilis (95.1%), M. alovera (99.2%), M. antarcticus (98.4%), M. cohni (97.9%), M. endophyticus (98.7%), M. flavus (98.1%), M. halobius (93.9%), M. kristinae (93.6%), M. lactis (96.2%), M. luteus (98.3%), M. nishinomiyaensis (93.6%), M. roseus (95.8%), M. sedentarius (93.4%), M. terrus (98%), M. varians (94.1%) and M. yunnanaensis (99.5%), and is 99.7% to M. luteus type strain.

Compared to other Micrococcus luteus strains with sequenced genomes, strain O’Kane shares 99% 16S rRNA gene similarity with strains NBD3Y10 AU359 (GenBank accession number LQAC01000000), CCH3-E2 (GenBank accession number LSKC01000000), RIT 324W, RIT 304, RIT 305 [17], SUBG006 (GenBank accession number JOKP01000000) [18], modasa (GenBank accession number AMYK02000000) [19], and 1058_MULTS86_000310304 (GenBank accession number JWEM01000000), and 100% similarity to strains 773_MLUTS351_6015661806 (GenBank accession number JUTN00000000) and strain SK58 (GenBank accession number ADCD00000000).

Phylogenetic analysis based on the 16S rRNA gene placed M. luteus strain PPL-S13, M. luteus strain CV39, and M. luteus strain DSM12 as the closest taxonomic relatives of Micrococcus luteus O’Kane (Table 2, and Fig. 2).

3.2. Genome properties

The genome assembly produced a contig N50 of 0.58 Mb and a total genome size of 2,501,088 bp. As expected for Micrococcus luteus genomes, the GC content was very high (73%). Out of the 62 RNA genes identified, 4 ribosomal RNA and 49 tRNA genes were detected. The ribosomal RNA operon showed the typical bacterial organization with the 5S, 16S, and 23S rRNA and the tRNAs modasa (GenBank accession number AMYK02000000) [19], and 1058_MULTS86_000310304 (GenBank accession number JWEM01000000), and 100% similarity to strains 773_MLUTS351_6015661806 (GenBank accession number JUTN00000000) and strain SK58 (GenBank accession number ADCD00000000).

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Table 3

| Attribute | Value | % of total |
|-----------|-------|------------|
| Genome size (bp) | 2,501,088 | 100.00 |
| DNA coding (bp) | 2,272,219 | 90.85 |
| DNA G + C (bp) | 1,825,734 | 73.00 |
| DNA scaffolds | 8 | 0.00 |
| Total genes | 2318 | 100.00 |
| Protein coding genes | 2256 | 97.33 |
| RNA gene | 62 | 2.67 |
| Genes in internal clusters | 404 | 17.43 |
| Genes with function prediction | 1810 | 78.08 |
| Genes assigned to COGs | 1606 | 62.28 |
| Genes with Pfam domains | 1897 | 81.84 |
| Genes with signal peptides | 79 | 3.41 |
| Genes with transmembrane helices | 554 | 23.80 |
| CRISPR repeats | 1 | 0.0004 |

Table 4

| Code | Value | % of total | Description |
|------|-------|------------|-------------|
| J | 175 | 9.85 | Translation, ribosomal structure and biogenesis |
| A | 1 | 0.06 | RNA processing and modification |
| K | 106 | 5.97 | Transcription |
| L | 92 | 5.18 | Replication, recombination and repair |
| B | 1 | 0.06 | Chromatin structure and dynamics |
| D | 19 | 1.07 | Cell cycle control, Cell division, chromosome partitioning |
| V | 36 | 2.03 | Defense mechanisms |
| T | 55 | 3.1 | Signal transduction mechanisms |
| M | 81 | 4.56 | Cell wall/membrane biogenesis |
| N | 5 | 0.28 | Cell motility |
| U | 19 | 1.07 | Intracellular trafficking and secretion |
| O | 90 | 5.06 | Posttranslational modification, protein turnover, chaperones |
| C | 105 | 5.9 | Energy production and conversion |
| G | 107 | 6.02 | Carbohydrate transport and metabolism |
| E | 187 | 10.52 | Amino acid transport and metabolism |
| F | 68 | 3.83 | Nucleotide transport and metabolism |
| H | 117 | 6.58 | Coenzyme transport and metabolism |
| I | 103 | 5.8 | Lipid transport and metabolism |
| P | 121 | 6.81 | Inorganic ion transport and metabolism |
| Q | 175 | 9.85 | Secondary metabolites biosynthesis, transport and catabolism |
| R | 150 | 8.44 | General function prediction only |
| S | 74 | 4.16 | Function unknown |

The total is based on the total number of protein coding genes in the genome.
in 71.1% of protein-coding genes. Psort [20] classified proteins as 53.6% cytoplasmic, 0.62% extracellular, and 27.1% associated with the membrane. Based on the presence of 139 single copy genes [21], the genome is predicted to be 79.14% complete. Genome statistics are shown in Table 3. The distribution of genes into COG functional categories is shown in Table 4.

**Fig. 3.** (A) COG profile clustering of the genomes compared in this study. (B) Principal component analysis biplot of the genomic features and COG category distribution in the genomes compared. Genomes are represented by stars. Arrows represent genomic features or COG categories used for comparison. The arrow directions follow the maximal abundance, and their lengths are proportional to the maximal rate of change between genomes. The first two components explained 75% of variation.
3.3. Insights from the genome sequence

Genomic analysis of strain O’Kane revealed a Gram-positive microorganism with an atypical cell wall structure. Genomic evidences suggest the presence of a peptidoglycan layer lacking pentaglycine bridges and with meso-diaminopimelic acid as the third amino acid in the peptide linkage. Genomic analysis also identified the potential of biosynthesis of the polar lipids phosphatidic acid, phosphatidylserine, phosphatidylglycerol, CDP-diacylglycerol and Cardiolipin. No evidence of flagella was identified in the genome, which was confirmed in the TEM picture. Genomic evidence for pili (Flp system) was detected.

Further analysis identified a metabolically versatile microorganism in which almost complete to complete catabolic KEGG pathways were identified for the C-6 sugars glucose, galactose, fructose, and mannose, and some amino acids including alanine, aspartate, asparagine, glutamate, glutamine, glycine, serine, cysteine, methionine, arginine, proline, histidine, phenylalanine, as well as fatty acids. The presence of genes encoding a complete TCA cycle and electron transport chain in which almost complete to complete catabolic KEGG pathways were identified, the TEM picture. Genomic evidence for pili (Flp system) was detected.

Further investigation is required to assess the functionality of the enzyme. Comparison of the genome of strain O’Kane to the 11 other closely related genomes, as well as the number of genes belonging to each COG category to the other genomes. These included secretion systems (type I), and antibiotic resistance genes among others.

3.4. Insights from comparative genomics

When the genome of strain O’Kane was compared to 11 closely related Micrococcus luteus genomes based on their COG profile, strain O’Kane clustered with Micrococcus luteus strain RIT304 (Fig. 3A). We used genomic features including the genome size, the number of genes, the number of transporters identified, the GC content, the number of non-coding bases, the number of genes belonging to COG categories, as well as the number of genes belonging to each COG category to compare Micrococcus luteus O’Kane genome to the 11 other closely related genomes, and we show (Fig. 3B) that strain O’Kane clustered with two other M. luteus genomes, strain SKS8 and strain RIT304, presumably based on the enrichment in the number of COG-related genes identified in the genomes and their GC content. Comparison against the other M. luteus genomes also revealed that M. luteus strain O’Kane genome harbored several genes that did not have homologues in the other genomes. These were genes encoding for antibiotic resistance (for chloramphenicol, tetracyclin, and macrolide antibiotics), as well as a gene encoding for polyhydroxybutyrate (PHB) depolymerase. PHBs are polymers biosynthesized and stored intracellularly by a wide range of organisms to be used as carbon and energy sources when nutrients become limiting [24]. Following cell death and lysis, these PHBs are released in the environment and are subsequently degraded by other co-existing organisms [24] via secretion of an extracellular PHB depolymerase. Strain O’Kane PHB depolymerase harbors a signal peptide, which suggests that it would be secreted extracellularly. Previous reports on extracellular PHB depolymerases from Actinobacteria were mainly in the genus Streptomyces [24, 25]. To our knowledge, this is the first report of a PHB depolymerase from a Micrococcus species. Further investigation is required to assess the functionality of the enzyme.

4. Conclusions

This study presents the draft genome sequence and the first detailed annotation of a representative of the genus Micrococcus luteus. The genome revealed extensive sugar and amino acid degradation machineries, several genes with potential virulence-associated function including type I secretion system and Flp pili. Comparative genomics using general genomic features as well as the COG function profile agreed with the phylogenetic position predicted based on the 16S rRNA gene sequence. The presence of a predicted extracellular polyhydroxybutyrate depolymerase encoded in the genome represents the first report of such enzyme in a Micrococcus representative.

Competing interests

All authors declare no competing interests.

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Authors’ contributions

RAH, KB, CM, MBC, and NY contributed to the analysis. RAH, WDH, DPF, and NY wrote the manuscript. RAH, CB, and SDO performed the lab experiments.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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