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学位論文内容の要旨

博士の専攻分野名称：博士（農学）　　氏　名：ISLAM MD SAMIUL

学位論文題名

Characterization of a novel soil ultramicrobacterium from the rarely cultured *Verrucomicrobiota* phylum

(難培養性の*Verrucomicrobiota*門に属する新規超微小土壌細菌の特性)

Verrucomicrobiota is a widespread but poorly recognized bacterial phylum found in soil, freshwater, and host-associated habitats. Despite efforts to cultivate uncultured microorganisms, members of this phylum are rarely isolated, with only 82 strains currently reported. While certain members contribute to biopolymer degradation, nitrogen fixation, and plant symbiosis, their physiological and genetic traits remain unclear. This study focused on the characterization and taxonomic classification of a novel ultramicrobacterium within the *Verrucomicrobiota* phylum.

1. Morphological, physiological, and phylogenetic characterization of a novel *Verrucomicrobiota* strain

This study characterized a recently isolated *Verrucomicrobiota* strain ASA1^T. The strain exhibited no growth on nutrient-rich media but formed colonies on lower nutrient media (R2A and 1/100× PYG) after 8-14 days of incubation. The colonies appeared whitish-yellow and smooth, while the cells were ultramicrobacterial (<0.1 μm^3), coccoid to oval in shape (0.05–0.07 μm^3), and formed cluster-like aggregates (<10 μm). ASA1^T was Gram-negative, aerobic, motile, and tested negative for catalase and oxidase activities. Growth occurred within the temperature range 20-40 °C (optimum 30 °C) and pH 7-11 (optimum 8.4), and NaCl tolerance 0-3% (optimum 0%). Chemotaxonomic analysis revealed anteiso-$C_{15:0}$ (53%) as the major fatty acid, followed by $C_{16:0}$ (15%), with menaquinone-7 (MK-7) as the sole respiratory quinone. The strain showed a limited substrate utilization and enzymatic activity spectrum. Phylogenetic analysis indicated that strain ASA1^T represents a novel lineage within the family *Alterococcaceae* of the class *Opitutia*, based on low 16S rRNA gene sequence similarity (<93%).

2. Genomic characterization and potential distribution of a novel *Verrucomicrobiota* strain

Strain ASA1^T exhibited low average nucleotide identity (ANI) values (<77%) with its closest described type of strain. Additionally, average amino acid identity (AAI) values were below 53%, far below the accepted genus-level threshold (65–70%), further supporting its designation as a new genus. Clusters of Orthologous Groups (COG) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of strain ASA1^T showed gene enrichment in amino acid and carbohydrate metabolism, energy production, and signal transduction, alongside a notable proportion of genes with unknown functions. Habitability analysis revealed that strain ASA1^T and its relatives are widely distributed across diverse terrestrial and aquatic environments. Genome mining identified six biosynthetic gene clusters (BGC), including non-ribosomal peptide synthetase (NRPS), polyketide synthase (PKS), and arylpolyene types, all showing low similarity (<1%) to known clusters. These findings suggest the potential of ASA1^T as a source of novel secondary metabolites.

3. Taxonomic Proposal: *Congregicoccus parvus* gen. nov., sp. nov.

Based on comprehensive phylogenetic, genomic, and chemotaxonomic analysis, a novel genus and species is proposed within the family *Alterococcaceae* of the phylum *Verrucomicrobiota*. The type of strain, ASA1^T (= JCM 36569^T = NCIMB 15508^T), represents *Congregicoccus parvus* gen. nov., sp. nov. The complete genome is 5.82 Mbp with a G+C content of 65.6% and is publicly available in DDBJ/ENA/GenBank under accession number AP028972.1 (BioProject: PRJDB16742, BioSample: SAMD00647589, DRA: DRA017385).

This study presents a comprehensive characterization of strain ASA1^T, a novel member of the family *Alterococcaceae* within the class *Opitutia*. Its physiological traits and genomic features provide a valuable understanding of the biology of ultramicrobacteria from this bacterial lineage. Notably, the findings of this study reveal a previously untapped source of bioactive compounds and offer new insights into the isolation of uncultured microorganisms within the phylum *Verrucomicrobiota*.