Description of *Gordonia metalliredigo*, a heavy metal(loid) resistance bacterium isolated from the sediment of a stream impacted by acid mine drainage

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Introduction

The genus *Gordonia* was proposed in 1971 by Tsukamura (originally 'Gordona') (Tsukamura, 1971), with species of this genus belong to the mycolic acid-containing group of the actinomycetes (Goodfellow et al., 1998; Stackebrandt et al., 1997). At the time of writing, the genus *Gordonia* is comprised of 47 validly published and named species (Parte et al, 2020). Members of the genus are quite widespread in nature (Goodfellow 1992; Arenskotter, Broker, and Steinbuchel 2004). However, they have also been found in locations impacted by anthropogenic actions, such as sewage treatment plants, biofilters used for odour control (Lemmer and Kroppenstedt 1984; Bendinger et al 1995; Klatte et al 1996), fuel, and creosote/tar contaminated soils indicating their ability to adapt to various environments (Kummer, Schumann and Stackebrandt 1999; Chatterjee and Dutta 2003). Many members of this genus are also known for their ability to degrade various xenobiotic pollutants (phthalic acid esters, s-triazine, alkylpyridines and dibenzothiophene) as well as natural pollutants, such as rubbers (Arenskotter, Broker, and Steinbuchel 2004; Linos et al 2002; Yoon et al 2000; Zhang et al 2020). These abilities suggest they may be prime candidates for bioremediation/bioaugmentation and a potentially important player in the environmentally friendly clean-up of contaminated environments (Arenskotter, Broker, and Steinbuchel 2004; Jung et al 2019; Kong et al 2019; Zhang et al 2020). Another class of pollutants this genus has been known to tolerate are metals (Gurbanov et al 2019). For example, 4 genes have been identified in *G. westfalica* Kb1 which are putatively involved in heavy metal resistance (Broker et al 2004) and *G. alkanivorans* YC-RL2 also contains putative genes for heavy metal metabolism (Nahurira et al 2019). In regards to metalloids, genome sequencing has predicted some possible tellurium-resistance genes in *G. alkanivorans* and *G. polyisoprenivorans* (Wang et al 2014; Vivod et al 2017). However, as of yet, no *Gordonia* spp. has been shown to have the ability to remove/reduce metalloid oxyanions from the environment. This ability would help to further their appeal and add to their ability to remediate a broad range of compounds. Overall, this genus is of great interest for its biotechnological potential (Drzyzga 2012) In this study we set forth to taxonomically classify strain TSed Te^T, a new *Gordonia* species with high level resistance to Te and Se oxyanions through reduction to elemental form. This ability suggests not only are *Gordonia* spp. promising for bioremediation, but possibly bioreclaimation of these industrially relevant elements.

Materials and Methods

Physiological and Biochemical Experiments

Strain TSed Te1^T was isolated from the sediment of a stream contaminated by acid mine drainage, and grown aerobically on rich organic (RO) medium (Yurkov and Gemerden, 1992) at 28^{o}C and pH 7.8 in the dark unless otherwise noted. Physiological and taxonomic tests were carried out as published (Yurkov et al, 1994). Cells were collected and sent to Microbial ID Inc. for whole cell fatty acid analysis. Aerobic tolerance levels to tellurite and selenite were analyzed by initiating liquid culture with 5 % inoculum at different concentrations of metal(loid) oxyanion, including 0, 100, 250, 500, 750, 1000, 1500, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, and 10000 µg / mL. All experiments were performed in triplicate.

Phylogenetic Analysis

DNA was extracted as described (Chen and Kuo, 1993) and sent to Azenta for 16S rRNA gene Sanger sequencing using universal primers for a 1455 bp fragment (GenBank accession number: OL582960). A Maximum Likelihood phylogenetic tree was created using MEGA 11 phylogenetic and pairwise aligned sequences collected from NCBI Genbank (Tamura, Stecher, and Kumar, 2021). Genomic DNA was fully sequenced using Illumina MiSeq technology at MR DNA Lab, Shallowater, Texas, USA. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JAJQJP000000000. The version described in this paper is version JAJQJP010000000. Average nucleotide identity (ANI) and digital DNA:DNA hybridization (dDDH) values were determined using ChunLab's online ANI calculator (Yoon et al, 2017) and formula d_4 from the Genome-Genome Distance Calculator from DSMZ (Meier-Kolthoff et al, 2021) respectively.

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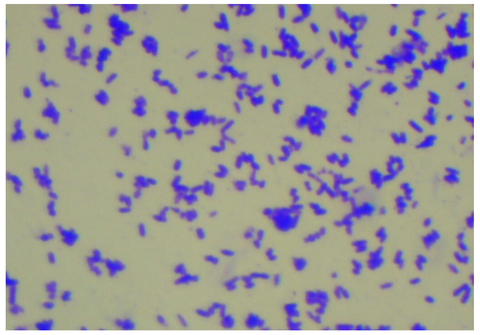

Figure 1. Crystal violet stained micrograph of strain TSed Te1^T after 7 days growth on a tryptic soy agar plate.

Table 1. Fatty acid profile of strain TSed Te1^T

Fatty acid	TSed Te1^T
$C_{14:0}$	3.74
$C_{16:0}$	34.28
$C_{17:0}$	2.48
$C_{17:1\ w9c}$	1.92
$C_{18:0}$	2.20
$C_{18:1\ w9c}$	26.91
10 methyl $C_{18:0}$	11.49
Sum Feature 3[a]	14.39
Sum Feature 6[b]	0.69

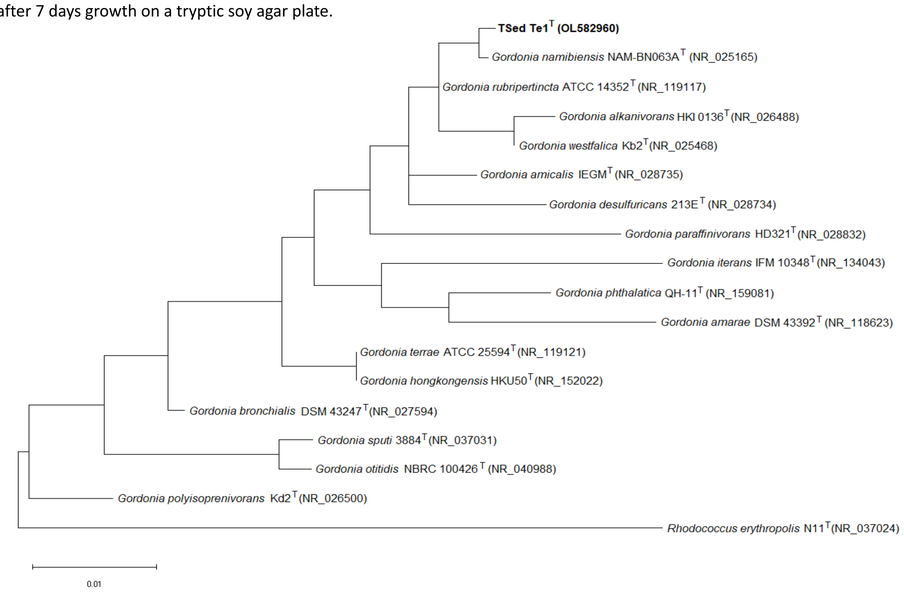

Figure 2. Maximum Likelihood phylogenetic tree of strain TSed Te1^T 16S rRNA gene sequence homology among type species of the genus *Gordonia*. Bar represents 0.01%.

Table 2. Physiological and biochemical features of TSed Te1^T compared to its nearest relatives

	TSed Te1^T	*G. nambiensis*	*G. rubripertincta*
Growth Range / Optimal			
Temperature (^{o}C)	25-47 / 35	20-40 / 28	20-45/ 15 – 20
pH	5.5 – 11.5 / 8.0	4.5-9.5/ 7.0	6.0 – 8.5 / 7.5
Utilization of			
Pyruvate	+	+	+
Acetate	+	+	+
Malate	+	+	+
Succinate	–	+	+
Glucose	+	+	+
Fructose	+	+	+
Ethanol	–	+	+
Glutamate	–	+	ND
Butyrate	–	–	ND
Citrate	+	ND	+
Hydrolysis of			
Gelatin	–	+	+
Tween 80	–	–	–
Starch	–	–	+
Enzyme Activities			
Nitrate Reductase	+	+	-
Urease	–	+	+
Oxidase	–	–	–
Catalase	+	+	+
ONPG	–	–	+
Arginine dihydrolase	–	–	ND
Lysine decarboxylase	–	ND	–

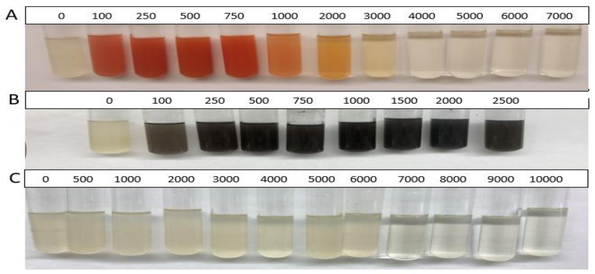

Figure 3. Growth of Tsed Te1 in the presence of varying concentrations of A) Selenite. B) Tellurite. C) Orthovanadate. Concentrations are in µg/ml.

Summary

A nearly complete 16S rRNA gene sequence confirmed TSed Te1^T is deeply branched within *Gordonia*, belonging to this genus (Fig. 1). Pairwise comparisons to type species revealed 16S rRNA homology of strain TSed Te1^T was 99.5 % similar to *G. namibiensis* NBRC 108229T (Brandao et al, 2001) and 99.3 % to *G. rubripertincta* NBRC 101908T (Tsukamura, 1971). Average nucleotide identity (ANI) between TSed Te1^T and *G. namibiensis* was 95.3 % and dDDH was 62.2 %. Both ANI and dDDH confirm this isolate to be a new species based on accepted cut-offs for species delineation (Chun et al, 2018). It is Gram positive, non-motile, oxidase negative and catalase positive, non-spore forming and obligately aerobic, showing small (1-2 mm), circular, raised, pink colonies with entire margins and a membranous surface on RO plates under aerobic conditions. Growth occurs between 20 and 40 °C and from pH 5.5 to 11.5 with the optimum being 35 °C and pH 8.0. Growth occurred up to 0.5% NaCl. Morphologically, it is rod shaped, an average of 2.1 µm in length with a varied arrangement (Fig. 2). The bacterium was susceptible to erythromycin, tetracycline, chloramphenicol, imipenem, streptomycin, and kanamycin but resistant to nalidixic acid, bacitracin, penicillin, and ampicillin (Table 1). Vitamins are not required for growth. The carbon sources that could promote growth were pyruvate, citrate, fructose, glucose, acetate, malate, butyrate, yeast extract, casamino acids, and bactopeptone. Glutamate, formate, methanol, ethanol, and lactate were not used. Tween 20, Tween 80, agar, starch and gelatin were not hydrolyzed. Taxonomic traits are summarized in Table 1. Whereas metal(loid) oxyanions such as tellurite are known as toxic to most microorganisms at 1 µg/ mL (Yurkov, Jappe, and Vermeglio, 1996), the ability to resist increased concentrations of Te and/or Se oxyanions has not been observed in members of this genus. Since high levels of resistance are rare, especially at extreme levels, they can be used as defining characteristics of bacteria. Here, strain TSed Te1^T aerobically tolerated as high as 2500 µg/mL of tellurite and 3000 ug/ml selenite, respectively (Fig. 3). Furthermore, reduction to elemental forms was visible as culture color change for each metal(loid) (Fig. 3). Regarding vanadate, no reduction was observed, however, growth still occurred up to 6000 µg / mL. In the case of selenite, one can see visually optimal growth appeared to occur at 250 µg / mL (Fig. 3), suggesting increased growth in the presence of this oxyanion. This effect has been previously reported for aerobic anoxygenic phototrophs for vanadate (Csotonyi et al, 2015) and tellurite (Maltman and Yurkov, 2019), where metal(loid) oxyanions are potentially acting in catalytic electron shuttling, promoting energy production and faster growth for the bacterium under those conditions. Whole cell fatty acid analysis revealed TSed Te1^T contained primarily $C_{16:0}$ and $C_{18:1\ w9c}$, and 10-methyl $C_{18:0}$ (TBSA), which is characteristic of *Gordonia* spp. (Yoon et al 2000). The G + C content of the DNA was 67.6 mol%, in line with members of this genus [new refs].

Species Description

G. metalliredigo (me.tal`li.re.di`go. L. adj. metalli metallic, related to metal, L. verb. redigo to reduce, N. L. n. metalliredigo, referring to the ability of the bacterium to aerobically reduce multiple metal(loid) oxyanions).
Non-motile, non-spore forming, Gram positive, obligate aerobe which forms small (1-2 mm), circular, raised, pink colonies with entire margins and a mucoid texture on RO plates. Cells are rod shaped and an average of 2.1 µm in length. Strain is catalase positive and oxidase negative. Growth occurs between 25 and 47 °C with 35 °C being optimal and from pH 5.5 to 11.0 with 8.0 preferred. Growth occurred up to 0.5% NaCl. Primary fatty acids are $C_{16:0}$, $C_{18:1\ w9c}$, and 10 methyl $C_{18:0}$. Can aerobically tolerate tellurite and selenite, and orthovanadate up to 2000, 3000, and 6000 µg/ mL, respectively and reduce these oxyanions to elemental forms. Remaining physiological and biochemical traits are as described in Table 1. The DNA G + C content is 67.6 mol. This bacterium inhabits the sediment of a stream contaminated with acid mine drainage.
Strain TSed Te1$_T$ ribosomal 16S rRNA gene sequence under GenBank accession number: OL582960. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JAJQJP000000000. The version described in this paper is version JAJQJP010000000. The type strain is TSed Te1^T.

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