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Insights into the Potential Role of *Gordonia alkanivorans* Strains in Biotechnologies †

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Abstract: Members of many species of the genus *Gordonia* are known for their significant metabolic potential, including the ability to utilize compounds of different structures. The aim of the work was to study the ability of nine *G. alkanivorans* strains to degrade persistent organic pollutants and to analyze the genomic peculiarities of these strains. The genomes of nine *Gordonia alkanivorans* strains were sequenced and assembled. The utilization of these strains as alkane and benzoate degraders in environmental biotechnologies, coupled with their capacity to potentially produce diverse secondary metabolites, holds promising prospects for both environmental and pharmaceutical applications.

Keywords: *Gordonia alkanivorans*; genome sequencing; benzoate; alkanes; biodegradation

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1. Introduction

The genus *Gordonia* is an aerobic Gram-positive bacteria of the order *Mycobacteriales*. Currently, this genus comprises 52 validly published and correctly named species [1]. *Gordonia* species have been isolated from various sources such as soil, marine samples, wastewater, clinical specimens, human skin, and even from the bark of rubber trees [2–9]. It can be said that *Gordonia* strains are ubiquitous. The studied genus exhibits a broad geographical distribution, with occurrences reported in the USA [10,11], Puerto Rico [12], Colombia [13], Brazil [14], Argentina [15], Israel [16], India [8], Namibia [17], South Africa [18], Germany [19], Russia [20,21], China [22–24], Taiwan [25], Thailand [26], South Korea [27,28], and Japan [29]. This diverse geographic range underscores the genus's ability to adapt to different climatic and ecological conditions.

Their extensive metabolic capabilities due to the remarkable plasticity of the genomes of this genus [30–33] make *Gordonia* strains promising for application in the field of environmental biotechnology. Furthermore, it is known that *Gordonia* strains can adapt to various environmental conditions, including high salt concentrations. For example, *Gordonia iterans* Co17 is capable of growing in a 12% salt concentration, while its ability to degrade alkanes remains intact within the range of C_8 to C_{32} under a 10% NaCl concentration [2].

Members of many species of the genus *Gordonia* are known for their ability to utilize compounds of a different structure, including persistent organic pollutants. For instance, the degradation process of petroleum hydrocarbons has been observed in the strain *Gordonia sihwaniensis* lys 1–3. Furthermore, the authors have, for the first time, analyzed and visualized the pathway degradation process of n-hexadecane by this species [34]. Regarding aromatic hydrocarbons, they constitute a group of primary organic pollutants

critical to the environment and public health due to their toxic, carcinogenic properties, and widespread prevalence. They include monocyclic (benzene, toluene) and polycyclic aromatic hydrocarbons (from naphthalene to pyrenes), as well as alkyl-substituted isomers. The literature frequently reports the ability of *Gordonia* to utilize substances such as anthracene, naphthalene, phenanthrene, pyrene, and benzene [35]. For instance, the *G. iterans* Co17 strain can degrade aromatic compounds such as naphthalene and anthracene by 55.3% and 63.2%, respectively [2]. The degradation of phenanthrene is a complex metabolic process. However, the ability of the *Gordonia* sp. SCSIO19801 strain to utilize phenanthrene (100 mg/L) as the sole carbon source at pH 8 and 28 °C has been studied. It was observed that after 7 days of the experiment, the SCSIO19801 strain had utilized over 80% of the phenanthrene through the salicylate metabolic pathway [36].

The strains *Gordonia hongkongensis* RL-LY01 [37] and *Gordonia* sp. GZ-YC7 [38] exhibit a strong potential for environmental bioremediation due to their ability to effectively degrade various phthalate esters or phthalic acid esters (PAEs). Hu et al. (2022) reported that *Gordonia* sp. GZ-YC7 efficiently removed more than 45% of di-(2-ethylhexyl) phthalate with an initial concentration of 500 mg/kg within 5 days, even when soil microbial activity is typically inhibited at lower di-(2-ethylhexyl) phthalate (DEHP) concentrations. For the strain *Gordonia* sp. GONU, the ability to hydrolyze high-molecular-weight phthalates has been observed. Specifically, DEHP was completely utilized within 24 h and di-n-octyl phthalate (DnOP) within 20 h. Dhar and colaborators (2023) have determined that the DEHP and DnOP degradation pathways are inducible. The information about the regulation of genes involved in the degradation of phthalate diesters forms the basis for further studies on the purification, characterization, and detection of PAEs [39]. Members of the genus *Gordonia* can also be considered as potential candidates for polyethylene biodegradation. For instance, the strain *Gordonia polyisoprenivorans* B251 demonstrated the ability to degrade polyethylene (PE), which was confirmed through a 30-day incubation with a PE film by detecting specific chemical changes and surface cracks [40].

This genus is of great importance in removing sulfur from fossil fuels and has been extensively covered by a number of authors [41], where the mechanisms and future prospects for the development of sustainable, environmentally friendly oil recycling technologies have been investigated.

The anabolic capabilities of strains within this genus are also recognized. For instance, several authors have noted the ability of *Gordonia* representatives to produce L-lysine, various polysaccharides, biosurfactants, carotenoids, and antimicrobial compounds [3,42–48]. Microbial surfactants are frequently employed in environmental remediation methods to address pollution. For example, the bioemulsans produced by *Gordonia* sp. BS29 are efficient in eliminating crude oil and PAHs from soil, while also providing a modest boost to the biodegradation of stubborn branched hydrocarbons; however, their most favorable outcomes are achieved when applied in the process of soil washing to remove hydrocarbons [49].

Microbial secondary metabolites, which are one of the main sources of bioactive natural products, are increasingly in demand for various biotechnological applications, with a primary focus on discovering new pharmaceuticals. Ma et al. (2021) isolated the strain *Gordonia* WA 4-31, capable of synthesizing secondary metabolites, from which the following compounds with antimicrobial activity were extracted: actinomycin D, actinomycin X2, mojavensin A, and cyclic (leucine-leucine) dipeptide. These compounds exhibited both anti-fungal and antiproliferative activities [50]. Additionally, the strain *Gordonia* sp. VO29-3, isolated from marine sediments, showed moderate antibacterial activity, inhibiting the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* [51]. Liu and colaborators (2023) also noted the ability of the *Gordonia terrae* WA8-44 strain to synthesize three active compounds (collismycin A, actinomycin D and actinomycin X2), with the capability of *Gordonia* to synthesize collismycin A and its potential as an effective anti-filamentous fungi agent being reported for the first time. Based on their research, the authors present collismycin A from *Gordonia* as a promising candidate for the development

of oral medications [52]. Furthermore, the strain *Gordonia* sp. 647W.R.1a.05 was found to possess the ability to synthesize neuroactive small-molecule natural products [53]. However, the genomes of *Gordonia* strains often contain BGCs that are not identified in databases with known metabolites [54]. This suggests that there is merit in further advancing research and development in this field.

The species *Gordonia alkanivorans* was introduced in 1999 due to the isolation of a type strain of this species, HKI 0136T, from tar- and phenol-contaminated soil [19]. Several recent studies have shown that representatives of this species can be used for oil pollution remediation, including those with a high sulfur content, as well as for cleaning natural systems contaminated with pesticides. Research is also being conducted to study the production of secondary metabolites, particularly carotenoids. For example, Silva et al. (2022) investigated the enhancement of carotenoid production using *G. alkanivorans* strain 1B through the optimization of cultivation conditions, such as the choice of the carbon source, the influence of light, and salt concentration. The study demonstrated that carotenoid production is stimulated by the presence of light and the glucose content in the medium [55].

For the strain *Gordonia alkanivorans* W33, it has been demonstrated that it can degrade 56.3% of the petroleum in contaminated soil within 45 days [56]. Yang and collaborators (2023) note that the efficient remediation of oil-contaminated soil was achieved by increasing the bacteria concentration through high-density fermentation and using vermiculite powder as a carrier, in addition to the inclusion of sophorolipids and rhamnolipids. It is known that crude oil may contain up to 10% sulfur, with the majority of it existing in an organic form, primarily as condensed thiophenes [57]. Previously, it was thought that thiophene catabolism in *G. alkanivorans* was only carried out using the *dsz* operon [58,59]. However, in previous studies, we have shown that there are *G. alkanivorans* strains that utilize thiophenes without *dsz* genes [60,61]. In one of the studies (2023), the ability of the strain *Gordonia alkanivorans* GH-1 to degrade beta-cypermethrin (β-CY), a pesticide whose breakdown products can have adverse effects on human health, was investigated. It was found that the strain not only degrades the substance itself but also its intermediate metabolites (dibutyl phthalate, benzoic acid, and phenol) [62].

The aim of this work is to study the ability of nine *G. alkanivorans* strains to degrade persistent organic pollutants and to analyze the genomic features of these strains. The results obtained will help to expand the existing knowledge of this species and its contribution to the biodegradation of aliphatic and aromatic compounds and to the biosynthesis of new natural products.

2. Materials and Methods

2.1. Bacterial Strains and Cultivation Conditions

We used 9 bacterial strains (Table 1) isolated from oil-contaminated soils and *G. alkanivorans* strain 135 [20,60] as a reference to investigate the catabolic properties. During previous studies with *G. alkanivorans* strains, we observed that representatives of this species exhibit significant biotechnological potential as degraders of pollutants of various chemical natures. Consequently, we became interested in determining whether there are differences in the genomic organization of strains representing the same species (*G. alkanivorans*) with similar catabolic capabilities.

Table 1. Information about the strains used in the study.

Strain	Collection Number	Isolation Source	Previously Identified As
96	IEGM 96	crude oil-contaminated soil, Ukraine	*G. rubripertincta*
129	IEGM 129	crude oil-contaminated soil, Ivano-Frankovsk, Ukraine	*G. rubripertincta*
132	IEGM 132	crude oil-contaminated soil, Ivano-Frankovsk, Ukraine	*G. rubripertincta*
133	IEGM 133	crude oil-contaminated soil, Ivano-Frankovsk, Ukraine	*G. rubripertincta*
134	IEGM 134	crude oil-contaminated soil, oilfield, Lvov, Ukraine	*G. rubripertincta*

Table 1. *Cont.*

Strain	Collection Number	Isolation Source	Previously Identified As
142	IEGM 142	crude oil-contaminated soil, Ukraine	*G. rubripertincta*
144	IEGM 144	crude oil-contaminated soil, Lvov, Ukraine	*G. terrae*
12	-	oil-polluted soil, Moscow, Russia	*G. alkanivorans*
152	-	oil-polluted soil, Moscow, Russia	*G. alkanivorans*

The ability of the strains to utilize thiophenes as the sole sulfur source was tested using the method described by Delegan (2021) [60]. The growth of bacterial strains was visually assessed based on the turbidity of the medium. The ability of the strains to grow on even alkanes (C_6–C_{20}) and aromatic compounds (naphthalene, phenol, benzoate, catechol) was tested using a mineral medium of the following composition: K_2HPO_4—8.71 g/L; 5 M NH_4Cl solution—1 mL/L; 0.1 M Na_2SO_4 solution—1 mL/L; 62 mM $MgCl_2$ solution—1 mL/L; 1 mM $CaCl_2$ solution—1 mL/L; 0.005 mM of $(NH_4)_6Mo_7O_{24} \times 4\ H_2O$ solution; micronutrients—1 mL (micronutrient composition in g/l: ZnO—0.41 g; $FeCl_3 \times 6\ H_2O$—5.4 g; $MnCl_2 \times 4\ H_2O$—2 g; $CuCl_2 \times 2\ H_2O$—0.17 g; $CoCl_2 \times 6\ H_2O$—0.48 g; H_3BO_3—0.06 g), and pH 7.0. The medium is balanced in such a way that *Gordonia* strains, when grown on it, yield maximum biomass output. The influence of $CaCl_2$ and $MgCl_2$ on biomass growth was described by Yang (2023) [56]. Alkanes were added at 7.5 mL/L, naphthalene, phenol, and benzoate at 1 g/L, and catechol at 0.1 g/L.

2.2. Genome Sequencing and Analysis

The genomic DNA of the strains was isolated from biomass grown on LB [63] agar using a DNeasy Blood and Tissue Kit (QIAGEN, 69506) and processed following a standard protocol as per the manufacturer's instructions. Whole-genome sequencing (WGS) was performed on a MGI platform (DNBSEQ-G400) using the DNBSEQ-G400RS High-throughput Sequencing Set (FCL PE150) (2 × 150 bp). A paired-end library was prepared with the MGIEasy Universal DNA Library Prep Set in accordance with the protocol. The information of the generated data is presented in Table 2.

Table 2. Number of sequencing data before and after filtration.

Strain	Read Pairs before Filtration	Read Pairs after Filtration	Read Pairs after Filtration (%)
96	2,962,813	2,835,521	95.70
129	3,147,953	3,003,848	95.42
132	4,354,967	4,162,088	95.57
133	5,192,473	5,019,737	96.67
134	5,815,565	5,558,107	95.57
142	3,330,918	3,171,608	95.22
144	5,382,881	5,150,561	95.68
12	9,985,963	8,692,673	87.05
152	6,985,286	4,928,247	70.55

The raw reads were filtered using Trimmomatic v. 0.39 [64] with the following parameters: LEADING:15 TRAILING:15 SLIDINGWINDOW:4:15 MINLEN:100; and assembled using SPAdes v. 3.15.4 [65]. Contigs shorter than 500 bp were removed.

To clarify species affiliations, the Average Nucleotide Identity (ANI) value with the type strain *G. alkanivorans* NBRC16433 (BACI00000000.1) was determined using the EzBioCloud ANI Calculator [66] with the OrthoANIu algorithm, and digital DNA–DNA hybridization (dDDH) was calculated using the Genome-to-Genome Distance Calculator (GGDC) [67]. Some authors present ANI as a more modern approach that correlates with DDH data, thereby eliminating the need for using both methods [68]. Nevertheless, there are instances where the ANI value surpasses the threshold, while the DDH value does

not reach it [69]. Furthermore, although ANI offers a more precise assessment of genomic relatedness, the heterogeneity of DDH values can provide valuable insights in certain cases.

The initial annotation was conducted using Prokka [70] and RAST [71] to enhance accuracy in gene function determination while minimizing errors. Subsequently, when the genomes were submitted to the GenBank database, additional annotation was carried out using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) version 4.6 [72]. The functional annotation of the genome was carried out using KEGG [73] and BlastKOALA [74], which is suitable for annotating fully sequenced genomes. The identification and analysis of secondary metabolite biosynthesis gene clusters were carried out using antiSMASH 7.0 [75], which is advantageous for its extensive dataset and analytical tools. For pangenome analysis and unique genes search, we used OrthoVenn3 [76] due to its enhanced accuracy in identifying orthologous clusters and providing clear data visualization.

3. Results

3.1. Identifying the Strains

Based on the results of the whole genome sequencing data, several strains were reidentified (Table 3).

Table 3. Species identification of the strains.

Strain	ANI Value with the Type Strain of *G. rubripertincta*, %	DDH Value with the Type Strain of *G. rubripertincta*, %	ANI Value with the Type Strain of *G. alkanivorans*, %	DDH Value with the Type Strain of *G. alkanivorans*, %	Taxonomic Position of the Strain
96	92.58	76.20	98.45	89.70	*G. alkanivorans*
129	92.45	76.00	98.42	88.60	*G. alkanivorans*
132	92.40	76.30	98.42	88.60	*G. alkanivorans*
133	92.40	76.10	98.37	88.50	*G. alkanivorans*
134	92.25	76.80	98.23	87.60	*G. alkanivorans*
142	92.54	76.30	98.23	91.00	*G. alkanivorans*
144	92.42	66.80	98.63	78.10	*G. alkanivorans*
12	92.38	76.20	98.40	90.10	*G. alkanivorans*
152	92.48	76.60	98.34	88.00	*G. alkanivorans*

Thus, all the strains reliably belong to *Gordonia alkanivorans*. The difference in the DDH value between the studied strains and the type of strain *G. alkanivorans* NBRC 16433 indicates some heterogeneity of the species, but all the strains pass the species threshold by both ANI (>96%) and DDH (>70%) [77]. Both of these methods fall under the OGRI (overall genome related index) category, which is used to determine if a strain belongs to a known species by assessing the relatedness between the genome sequences of the strains and the type strain of a species. When the sequences of two genome sequences are fragmented and the fragments are compared to identify homologous regions, the percentage of nucleotide matches in such a region is referred to as the identity value, and the average identity value is calculated for all aligned fragments, resulting in ANI. DDH values using GGDC are determined through in silico techniques without the necessity of actual DNA hybridization experiments. Similar to ANI, genome sequences of two strains are compared after fragmentation into shorter segments. GGDC computes the intergenomic distances between the fragments of the two genomes and measures the degree of similarity between corresponding fragments in the two genomes. This serves as the basis for employing various mathematical models to generate the DDH hybridization value, offering an alternative to the traditional DDH determination. Heterogeneity of DDH values may indicate evolutionary divergence among different strains within the same species and is often an indicator of the species genomic plasticity, allowing it to adapt to changing environmental conditions.

The genomes of the *G. alkanivorans* strains used in this work have sizes ranging from 4.9 to 5.1 Mb, with the exception of strain 144 (Table S1). Despite the fact that this strain

reliably belongs to the *G. alkanivorans* species, it has a smaller genome size and a higher number of contigs in the assembly compared to the other strains.

3.2. Physiological and Biochemical Characteristics of Strains

On agarized rich media (LB), all the strains form small round colonies of a pink-orange color. When grown on mineral media with alkanes as a carbon source or thiophenes as a sulfur source, lighter orange colonies are formed. The color change may indicate that when growing on mineral media with poorly accessible energy sources, the microorganisms use energy for basic metabolic processes and substrate utilization, but not for the biosynthesis of secondary metabolites (carotenoids) that give the cells their color.

Crude oil is a complex mixture of components, those being aliphatic and aromatic hydrocarbons and their sulfur-, nitrogen-, and oxygen-containing derivatives. We assumed that the strains isolated from areas contaminated with crude oil would be able to utilize compounds from each of these groups. All strains were able to utilize alkanes from C_{10} to C_{20} and benzoate; some strains are able to use dibenzothiophene (DBT) as the sole source of sulfur (Table 4).

Table 4. Substrate specificity profile of strains.

Strain	Alkanes C_{10}–C_{16}	Alkanes C_{18}–C_{20}	Benzoate	Phenol	Naphthalene	Catechol	DBT
96	++	+	+	-	-	-	±
129	+	+	+	-	-	-	-
132	+	+	+	-	-	-	±
133	+	+	+	-	-	-	-
134	++	++	+	-	-	-	-
142	+	+	+	-	-	-	-
144	+	+	+	-	-	-	-
12	++	+	+	-	-	-	±
152	++	+	+	-	-	-	-
135	++	++	+	-	-	-	+

++ very good growth, + good growth, ± weak growth, - no growth.

Thus, the strains are capable of degrading a wide range of alkanes and some aromatic compounds, such as benzoate; however, none of the strains can utilize PAHs (naphthalene) or their metabolites (catechol).

Gene encoding enzymes of the complete pathway of benzoate degradation, including both subunits of benzoate 1,2-dioxygenase, which is part of the group of enzymes responsible for the initial transformation of persistent organic pollutants and other aromatic compounds, were also found in all strains. The presence of these genes may suggest adaptation to high levels of aromatic compound pollution [78]. For example, chlorinated benzoates are released into the environment through herbicide use or as metabolites and intermediates of other compounds [79]. Furthermore, benzoate is a metabolite of the transformation of polychlorinated biphenyls, which belong to a category of enduring organic contaminants that were extensively utilized in industrial applications and continue to endure in the soil [80]. Therefore, the studied strains can be used as part of a consortium for the remediation of these pollutants.

3.3. Osmoprotectant Metabolism

Bacteria use the accumulation of compatible solutes as a prevalent defense mechanism against the harmful effects of high osmolarity. These compounds, referred to as compatible solutes, can be acquired through uptake systems or biosynthesis. The accumulation of these substances can be achieved both through biosynthesis and by absorption from the surrounding environment. For all examined strains, complete pathways for trehalose biosynthesis have been identified. However, one of the key reasons why uptake systems are crucial to cellular responses to osmotic stress is that they allow the energy-efficient

scavenging of osmostress protectants from the environment. Most of the currently known transporters of compatible soluble substances can be assigned to one of two groups of transport systems: ABC transporters and secondary transporters.

Among the binding-protein-dependent ATP-binding cassette (ABC) systems, *opu* genes were found. Secondary transporters: members of the major facilitator superfamily (MFS)—ProP; members of the betaine-choline-carnitine transport (BCCT) system—BetT; and members of the sodium-solute-symporter (SSS).

The most effective participants in protecting the microorganism from osmotic stress are glycine betaine and ectoine. One of the pathways for betaine biosynthesis occurs through the oxidation of choline in two stages, catalyzed by choline dehydrogenase (BetA) [EC 1.1.99.1] and betaine aldehyde dehydrogenase (BetB) [EC 1.2.1.8]. The functionality of this pathway depends on the presence of the BetT transporter. It should be noted that *bet*A was not found, but *cod*A was identified (Table 5), which encodes choline oxidase [EC 1.1.3.17], an enzyme with a similar function. CodA catalyzes the two-step oxidative process, converting choline into glycine-betaine with betaine aldehyde as an intermediate (Figure 1).

Table 5. The number of copies of genes in certain osmoprotective systems.

Strain	*bet*A/*cod*A	*bet*B	*bet*T	*opu*A	*opu*BD	*opu*C
96	1	4	1	1	2	2
129	-	5	1	1	3	2
132	1	3	1	1	2	2
133	1	3	1	1	2	2
134	1	3	1	1	2	2
142	1	4	1	1	2	2
144	1	3	1	1	2	2
12	1	4	1	1	2	2
152	1	3	1	1	2	2

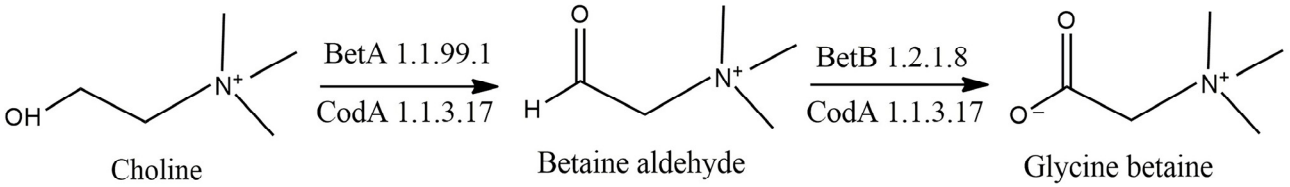

Figure 1. Betaine biosynthesis pathway.

It can use either choline or the intermediate betaine-aldehyde as a substrate [81]. Breisch (2021) demonstrated through mutagenesis that in the absence of *bet*A, choline oxidation to glycine betaine does not occur. Additionally, the *bet*A deletion mutant shows no impairment in utilizing glycine betaine as a compatible solute, as evidenced by the restoration of growth when glycine betaine is added to the medium [82]. Therefore, it can be assumed that in the studied strains, CodA may perform the function of BetA.

The biosynthesis of ectoine begins with aspartate through a series of sequential reactions catalyzed by diaminobutyrate-2-oxoglutarate transaminase EctB [EC:2.6.1.76], L-2,4-diaminobutyric acid acetyltransferase EctA [EC:2.3.1.178] и L-ectoine synthase EctC [EC:4.2.1.108] (Figure 2). The *ect*ABC gene cluster involved in the biosynthesis of ectoine has been found in all strains. Additionally, a stand-alone gene *ect*D, encoding ectoine hydroxylase [EC:1.14.11.55], which is involved in the conversion of ectoine to hydroxyectoine, has been identified.

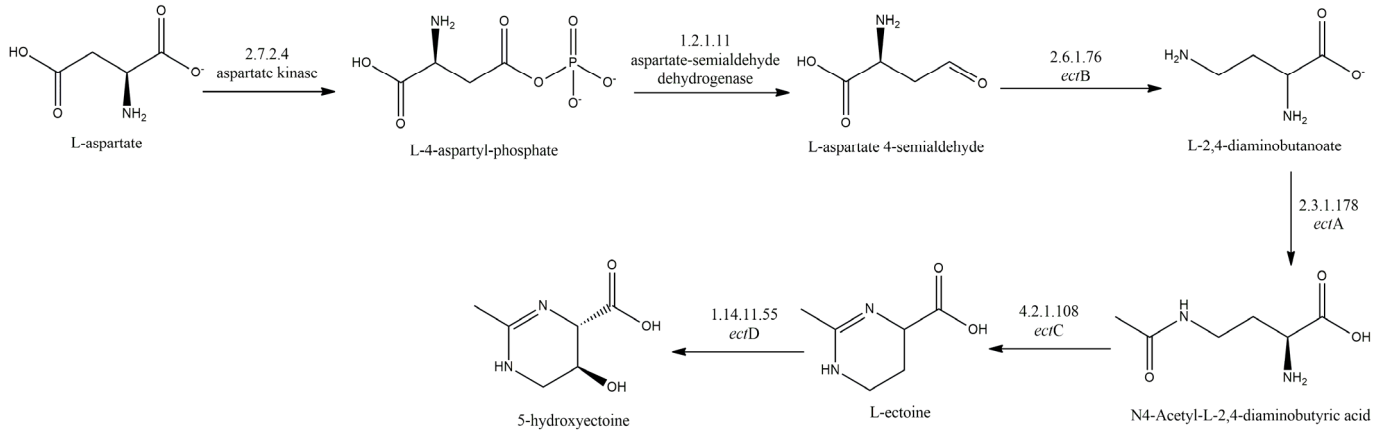

Figure 2. Ectoine biosynthesis pathway.

Thus, all the studied strains contain the genes of the most important solutes that can protect cell structures and metabolic processes at high salt concentrations. The ability of the strains to grow in a medium with an increased NaCl concentration was experimentally evaluated (Table 6).

Table 6. The ability to grow in high salt concentrations.

Strain	Concentration of NaCl			
	1%	**3%**	**7%**	**10%**
96	+	+	+	+
129	+	±	±	-
132	+	+	-	-
133	+	+	±	±
134	+	+	-	-
142	+	+	-	-
144	+	±	±	-
12	+	+	-	-
152	+	+	±	-

Almost all strains are capable of growing at salt concentrations of 1–3%. Strain 96 exhibited the highest resilience to saline environmental conditions, with the capacity to grow in a 10% NaCl solution.

3.4. Pangenome Analysis

The pangenome of the studied strains was analyzed in conjunction with the type strain *Gordonia alkanivorans* NBRC 16433 (Figure S1). It is represented by 4779 clusters, of which 3744 (78.34%) make up the core. As a result of the GO annotation of the core clusters, the highest number of proteins with known functions were assigned to «trehalose O-mycolyltransferase activity» and «antibiotic biosynthetic process».

Strain 144 contains the highest number of unique protein sequences (97) that are not found in the genomes of the other studied strains. These sequences include proteins with peptidase and hydrolase activity, Acyl-CoA dehydrogenases, as well as sequences involved in targeting and inserting nascent membrane proteins into the cytoplasmic membrane. Additionally, a viral sequence (*Caudovirales*, *Siphoviridae*) involved in peptidoglycan catabolic processes was detected. Furthermore, for strain 144, it was found to have the highest number of singletons (genes for which no orthologs could be found in any of the other species) (Table 7). Nevertheless, a substantial portion of unique sequences currently remains unannotated, potentially indicating gaps in existing databases concerning the genetic organization among prokaryotes.

Table 7. Number of genes without orthologs.

Strain	Proteins	Clusters	Singletons
96	4446	4318	61
129	4380	4252	22
132	4521	4404	1
133	4520	4399	4
134	4587	4312	60
142	4530	4329	63
144	5390	4506	315
12	4480	4522	25
152	4421	4183	88
NBRC 16433	4445	4155	109

Based on the results of genome analysis and individual unique genes, the strains can be divided into two groups within the species. The representatives of the first group (strains 129, 132, 133, 144) are characterized by unique genes of tyrocidine and gramicidin biosynthesis [83]. The representatives of the second group have a greater catabolic potential: the genomes of the strains contained (1) operons for the biosynthesis of steroid compounds, (2) additional copies of genes involved in dibenzothiophene catabolism, and (3) genes for the catabolism of aromatic compounds, cytochrome P450-pinF2 and phenol hydroxylase P5.

In the first group of 4404 clusters, 4022 are part of the core, constituting 91.32% (Figure S2, Tables S2 and S3). The pangenome of the second group consists of 4369 clusters, with 3905 (89.37%) belonging to the core. Overall, it is evident that the second group is more heterogeneous (Figure S3). In the second group, among the core clusters (Figure S4, Tables S4 and S5), the highest number of proteins with known functions in a single cluster are associated with «antibiotic biosynthetic process», «vancomycin biosynthetic process», and «cholesterol catabolic process». The «steroid biosynthetic» and «steroid metabolic process» GO categories are represented by several clusters each (7 and 5, respectively). In general, both groups of representatives exhibit a large number of diverse clusters annotated as «biosynthetic process».

3.5. Features of the Genetic Organization of G. alkanivorans

3.5.1. Hydrocarbon Degradation Ability

Approximately 45% of the CDS (coding DNA sequences) in all strains were annotated and categorized into various functional groups. Genes responsible for the degradation of different organic pollutants were identified. In the «Degradation of aromatic compounds» category 21 orthologous functional groups (KO) were found for all strains, except for strains 144 and 134, which had 19 and 23 groups, respectively. Additionally, all strains, except for 96 and 142, were found to contain genes for the degradation of dibenzofuran (DF) and dibenzo-p-dioxin (DD), both of which are toxic compounds and are formed as by-products during the synthesis of pesticides and herbicides [84]. In the subcategory «Benzoate degradation» within the «Xenobiotics biodegradation and metabolism» category, 19 KOs were identified for all strains, except for strain 134, which had 20 groups. The ability to use benzoate as a source of carbon and energy is a characteristic feature of representatives of this genus [61,85].

Integral membrane non-heme iron monooxygenases AlkB were absent in the genomes of all examined strains. However, soluble cytochrome P450s, specifically the CYP153 genes, were found in multiple copies (Table 8). Furthermore, each strain contained one copy of alkane hydroxylase for long-chain length alkanes (>C_{18}), known as LadA, which is a thermophilic soluble monooxygenase from *Geobacillus* [86]. Gene clusters *prm*DCBA, encoding multiple components of propane monooxygenase, were also discovered in all strains.

Table 8. The number of copies of the CYP153 gene.

Strain	CYP153
96	2
129	1
132	3
133	3
134	2
142	2
144	3
12	2
152	2

Thus, all the *G. alkanivorans* strains studied lack *alk*B genes in their genomes; therefore, we assume that the ability to utilize alkanes in these strains is controlled by the CYP153 and LadA genetic systems. The absence of the *alk*B genetic system has also been noted in our previous studies [60].

The CYP153 hydroxylases have at least a 99% identity between the strains, and they have one amino acid substitution each: one hydroxylase has A/T variants at position 239, the other hydroxylase has A/S variants at position 7, and they are in the first and second strain groups, respectively (Figure 3).

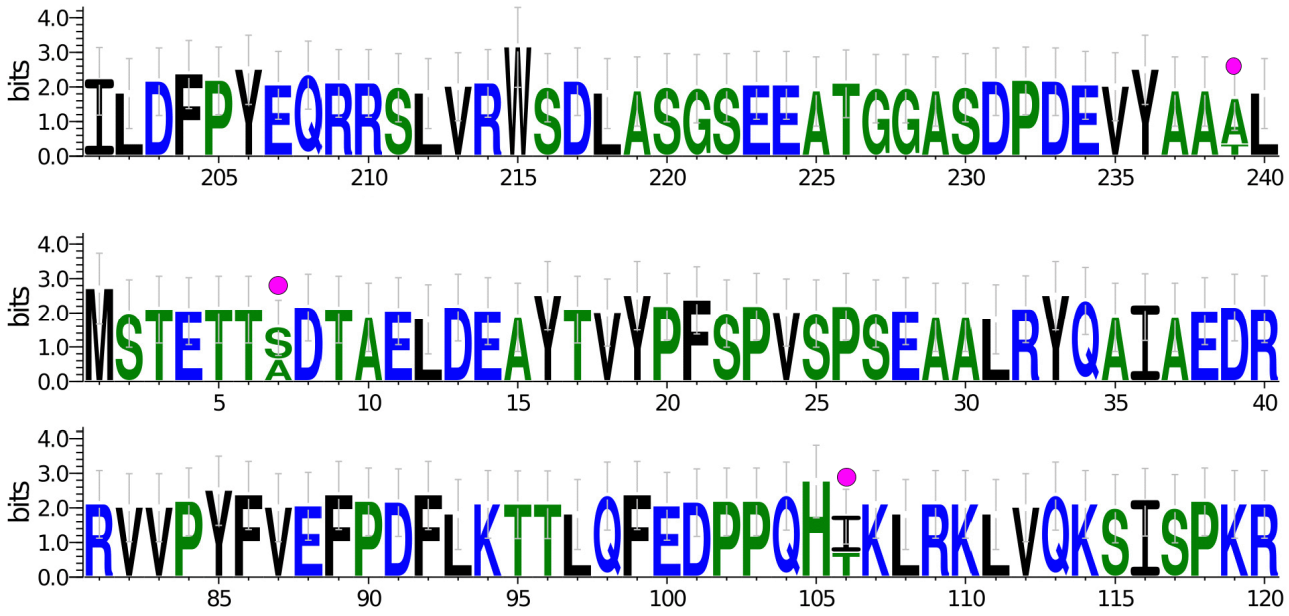

Figure 3. The CYP153 amino acid substitutions position (pink markers).

3.5.2. Plasmids

Of the nine strains of *G. alkanivorans*, two (strains 142 and 152) have plasmid elements. The plasmid of strain 142 (p142) is 67,219 bp in length; the plasmid of strain 152 (p152) is 44,937 bp. We assume that they are circular, since *Gordonia* is generally not characterized by the maintenance of large linear plasmids as, for example, in rhodococci.

In total, 98% of the entire length of plasmid p142 is a 99% repeat of plasmids pCP89 (CP094666.1) of the *Gordonia amicalis* G2 strain (percent identity (PI) 99.70%) and pCP86 (CP096597.1) of the *G. amicalis* 6-1 strain (PI 99.98%) (Figure 4). It is interesting to note this relationship between plasmids whose hosts are strains of different species.

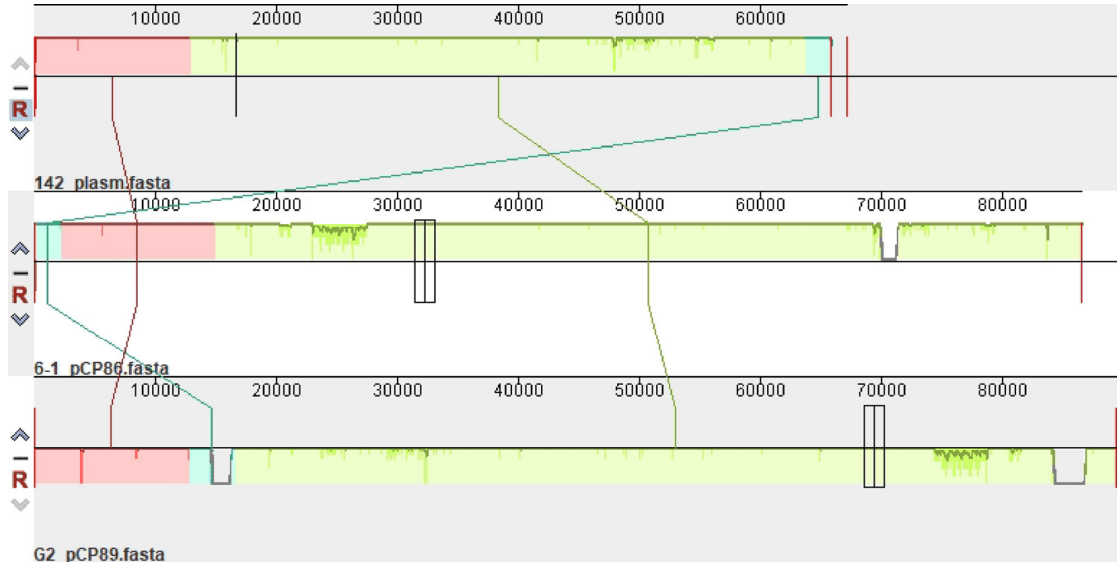

Figure 4. Mauve alignment demonstrating relatedness of the plasmids p142 (strain *G. alkanivorans* 142), pCP86 (*G. amicalis* 6-1), and pCP89 (*G. amicalis* G2). Vertical bars mark boundaries between elements. Colored blocks show regions of a genome aligned with parts of another genome.

The plasmid of strain 152 has a relationship with the plasmid pG135. The ANI value between the plasmids p142 and p152 is 80.00%.

3.5.3. Biosynthesis of Secondary Metabolites

The examined strains were found to have a significant number of biosynthetic gene clusters (BGCs), the majority of which showed no homology with gene clusters encoding the production of known natural products. Types of clusters that are common to all nine strains include:

- Terpene (SF2575 biosynthetic gene cluster: polyketide type II; carotenoid);
- Streptozotocin;
- Type I polyketide synthase;
- NRPS (non-ribosomal peptide synthetase);
- Non-alpha poly-amino acids (ε-Poly-L-lysine);
- Ectoine;
- Redox cofactor;
- NRPS-independent, *Iuc*A/*Iuc*C-like siderophores;
- Arylpolyene (primycin like most similar known cluster is found in all strains except for 134, 142, and 144, which do not exhibit similarity to this group).

The colibrimycin biosynthetic gene cluster (present in all strains except 144), nonribosomal peptide metallophores, namely madurastatin (except 96, 144 and 152), depsibosamycin (present only in strains 132, 133 and 144), cadaside (present in 134 and 152), speibonoxamine (present in 96 and 152) were also detected. Clusters identified only in individual strains are presented in Table 9.

The production of carotenoids by *Gordonia* is widely known and finds applications in various industries, including food, medical, cosmetic, and pharmaceutical [55]. However, many other products, such as streptozotocin [87] or colibrimycin [88], are primarily documented for other genera, particularly *Streptomyces*. It is noted that ulbactins F and G from *Brevibacillus* sp exhibit inhibitory effects on tumor cell migration within the submicromolar to micromolar range [89]. Echoside A and echoside B have demonstrated significant potential as antiviral agents. The authors propose that these compounds could be further explored as potential antiviral agents against SARS-CoV-2 [90]. Additionally, the discovery of cahuitamycins provides a critical foundation for future research endeavors aimed at developing drugs to prevent biofilm formation [91]. Of particular interest is the

complete similarity of the icosalide A biosynthetic gene cluster from *Burkholderia gladioli* in strain 152. Icosalide is a lipocyclopeptide antibiotic, which originally was isolated from a fungus of the *Aureobasidium* species. It has shown activity against entomopathogenic bacteria [92,93]. There is also information of icosalides, which shows certain inhibitory activity against the active stage of *Mycobacterium tuberculosis* and some cancer cell lines [94]. Therefore, considering the fact that actinomycete genomes often contain «silent» genes [95], members of the genus *Gordonia* may have significant potential for producing various novel natural products.

Table 9. Similarity with known clusters.

Strain	Most Similar Known Cluster	Similarity
96	sarpeptin	25%
129	ulbactin echoside	28% 11%
144	cahuitamycin	12%
12	leucomycin	3%
152	icosalide	100%

4. Discussion

There are many areas with a high salt content, including oil production sites, which are subject to hydrocarbon contamination. In such situations, an effective solution is the use of microorganisms capable of tolerating high salt concentrations. The studied strains show the ability to degrade a variety of aliphatic hydrocarbons and benzoate, while being tolerant to salty conditions. These features make them potential candidates for cleanup and remediation of contaminated areas. In general, we can say that the strains of *G. alkanivorans* species are similar in terms of their physiological properties and degradative potential. Nevertheless, representatives of this species do not have metabolic pathways for the degradation of naphthalene. Thus, there is still no strain of *G. alkanivorans* capable of utilizing PAHs (naphthalene) or their metabolites (catechol) known at this time. However, considering the presence of a greater number of catabolic genes and operons in the representatives of the subgroups (strains 96, 134, 142, 12, 152), we can hypothesize that these strains hold promise in the removal of steroid compounds. Our results, together with data from other studies [96], suggest that all currently sequenced *Gordonia alkanivorans* strains do not have a AlkB genetic system for the utilization of alkanes. Nevertheless, their biodegradation potential of these pollutants is not inferior to representatives of species containing CYP153. Furthermore, for some strains lacking the *dsz* gene cluster, growth on DBT is observed, which correlates with our previous research on this genus [60,61].

Furthermore, most of the examined strains exhibit the ability to synthesize various secondary metabolites, such as terpenes, saccharides, polyketides, ribosomal, and nonribosomal peptides. The capacity to produce diverse secondary metabolites is of significant importance in the food, feed, and fuel industries. Seeing that the 100% genetic cluster similarity of strain 152 with the known icosalide biosynthesis cluster, this strain may be considered for therapeutic and pharmaceutical purposes after further research. However, it is important to note that under conventional culture conditions, the majority of bioactive metabolites encoded in actinomycete genomes remain inactive. Therefore, to further study the production of new compounds, it is necessary to optimize cultivation conditions.

Moreover, the identified genes for degradation of pollutants, genes for the formation of osmoprotectors and synthesis of secondary metabolites can be used for mutagenesis to create more productive organisms. For example, Goel and collaborators (2011) successfully transferred the bacterial gene coda to create a transgenic plant [97]. Moreover, our earlier data (not published) show that the genetic material of *Gordonia* is easily transferred to closely related genera such as *Rhodococcus*.

5. Conclusions

The genomes of nine strains of *Gordonia alkanivorans* isolated from oil-contaminated soils were sequenced and assembled. Expanding the database of sequenced genomes may be useful for more accurate identification and development of omics studies. The genomes are about 5 Mb in size. Some of the strains contain plasmids, but the functions of these plasmids are currently not fully understood. It is interesting to note that *Gordonia* plasmids do not appear to be species-specific. The function of plasmid p142 in strain 142 is currently unclear, but the fact that copies of the plasmid have been observed in members of another species (*G. amicalis*) may indicate the importance of this plasmid for the vital activity of *Gordonia*. We plan to obtain a plasmid-free eliminant of strain 142 in the future in order to obtain a better understanding of the functions of this plasmid. At this point, we can assume that plasmids such as p142 are required by strains for metal transport and resistance.

As we found out, none of the *G. alkanivorans* strains studied in this work are capable of utilizing aromatic compounds. In this regard, we assume that in the future, there will be the creation of modified strains carrying gene clusters for PAH catabolism on their basis. Such strains will have greater potential for use in biotechnology.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr11113184/s1. Figure S1: A diagram showing the results of overlapping clusters of orthologous genes in strains (overlapping clusters containing proteins from different strains, sorted in descending order); Figure S2: GO annotation core clusters for the representatives of the first group (strains 129, 144, 132, 133); Figure S3: The representatives of the first group (a,c); of the second group (b,d): (a), (b)—Venn diagram depicting pangenome of two group strains; (c), (d)—UpSet table representing the number of orthologous clusters in each strains and the number of unique and shared homologous gene clusters; Figure S4: GO annotation core clusters for the representatives of the second group (96, 134, 142, 12, 152); Table S1: Assembly metrics of the strains; Table S2: Biological process list for the representatives of the first group; Table S3: Molecular function list for the representatives of the first group; Table S4: Biological process list for the representatives of the second group; Table S5: Molecular function list for the representatives of the second group.

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