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Sequencing of the Whole Genome of a Bacterium of the Genus Achromobacter Reveals Its Potential for Xenobiotics Biodegradation

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Abstract: The isolation of new bacterial strains from the natural environment can lead to the detection of microorganisms of potential practical importance. The characterization of such microorganisms can be carried out using classical microbiological and molecular biology methods. Currently, studies of newly detected microorganisms are based on sequencing techniques. Sequencing of the full genome can provide information about the origin of the strain, its taxonomic status, and phenotypic characteristics. The studies were conducted using the bacteria *Achromobacter* sp. 77Bb1 isolated from the maize crop rhizosphere. The bacterial genome was sequenced using Illumina 2 × 150 nt technology. The obtained sequences were analyzed using bioinformatics methods, resulting in 57 contigs and genome containing 6,651,432 nt. Phylogenetic analysis based on *16S rRNA* gene sequences enabled the assignment of the analyzed bacteria to the genus *Achromobacter*. The obtained genome contained genes for 4855 proteins with functional assignment. Some of these genes were connected with xenobiotics biodegradation and metabolisms. All genes for aminobenzoate degradation and almost all for benzoate and styrene degradation were found in the analyzed genome, suggesting that the isolated strain has the potential to be used in natural bioremediation methods.

Keywords: *Achromobacter*; whole-genome sequencing; functional pathways; Illumina; xenobiotics

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1. Introduction

Endophytic bacteria can colonize healthy host tissues without causing disease symptoms. Under laboratory conditions, these microorganisms have been isolated from surface-sterilized plant organs. The vast majority of endophytic bacteria described so far are classified into the *Alpha*, *Beta-*, and *Gammaproteobacteria* groups and *Enterobacteriaceae*, *Pseudomonaceae*, and *Burkholderiaceae* families, with *Gammaproteobacteria* being the most diverse and dominant group [1–3]. Endophytic bacteria can colonize all plant organs both above and below the ground; however, the diversity and abundance of endophytic bacteria vary along the plant axis [4,5], and soil is the main reservoir for bacteria [6]. Microorganisms perform a number of positive functions in ecosystems, including contributing to the circulation of elements in the ecosystem, influence on plant health, soil structure and productivity, and decomposition of harmful substances present in the environment [7].

Isolation of endophytic microorganisms from plant parts is crucial for understanding their genetic and phenotypic properties. Advances in next-generation sequencing technologies have made it possible to sequence and characterize the complete genomes of microorganisms [8,9]. In addition, genome annotation, looking for potential metabolic pathways, and comparative analysis against other genomes have made it possible to predict the physiology and morphology of microorganisms [10,11]. Analysis of the entire bacterial genome allows us to determine the sequences of genes that may encode proteins of industrial importance (antibiotic resistance, ability to degrade toxic compounds, and ability to survive under stress conditions) [12,13].

The continued increase in the human population is causing increasing environmental pollution from industrial products and their derivatives. Environmental pollution with harmful compounds, such as xenobiotics, has negative effects on living organisms [14]. Scientists and manufacturers are seeking safe ways to control this problem [15]. For several years, research has been carried out on the possibility of using microorganisms that can degrade xenobiotics for this purpose [11].

The genus *Achromobacter* belongs to the class *Betaproteobacteria* and the family *Alcaligenaceae*, and contains 29 species, isolated from different environments, including clinical samples, water, soil, and plant tissues [16]. Previous studies have shown various beneficial properties of *Achromobacter*, including the promotion of plant growth [17–19] and decomposition of harmful substances [20–22]. Some strains of the genus *Achromobacter* are considered to have bioremediation potential. Various examples of bioremediation properties of this bacterial genus have been described, including the degradation of arsenite [23], biphenyl [24], haloaromatic acids [25], and polycyclic hydrocarbons [26]. Many genome sequences of *Achromobacter* spp. have been identified; however, few studies have included a genome-wide analysis to clarify the metabolic pathways involved in the breakdown of xenobiotics and the synthesis of secondary metabolites.

Most of the published data, based on strains of the *Achromobacter* genus, involved laboratory experiments. Analysis of the full genome of bacteria to determine its potential functions is still rarely applied. In this study, we present information on the full genome of the strain *Achromobacter* sp. 77Bb1. We focus on genes involved in the biosynthesis of secondary metabolites (among others, ectoine and squalene) and the biodegradation and metabolism of xenobiotics (styrene, aminobenzoate, and benzoate). This study can also provide a basis for developing an environmentally friendly and cost-effective ecological method for remediating xenobiotic-contaminated environments.

2. Materials and Methods

2.1. Bacterial Culture, Genomic DNA Isolation, and Sequencing

The 77Bb1 strain was primarily isolated from maize roots in the Baltic dunes near Swinoujscie and Sobieszewo, Poland. The roots of the extracted plants were surface sterilized using ethanol (70%) and sodium hypochlorite (5%). Sterile root fragments were spread on sterile microbial culture medium. Single cultures of microorganisms were transferred to new medium to isolate a pure bacterial culture. The obtained bacteria was stored in a bank of strains from the Department of Agricultural Microbiology of IUNG in Puławy. The strain was cultured in LB Universal Medium at 28 °C. DNA was isolated from the liquid culture (120 rpm for 72 h). The liquid culture was centrifuged and the pellet was used for DNA isolation using a commercial Bead-Beat Micro AX Gravity kit (A&A Biotechnology, Gdansk, Poland). The quality and concentration of the obtained DNA were checked using a Quantus™ Fluorometer and a QuantiFluor® ONE dsDNA System kit (Promega, Germany). Library preparation and sequencing were carried out using an external service (Eurofins Genomics, Germany) on Illumina NovaSeq, with paired end reads 2 × 150 bp.

2.2. Genome Assembly and Annotation, Phylogenetic Analysis, Comparative Genome Analysis

Fastq data obtained after sequencing were checked using quality control (*FastQC*) [27]. Adapter and primer sequences were removed in a trimming step (*Trim Galore* v.0.6.7, *Cutadapt* v.3.4) [28,29]. Trimmed sequences were used for assembly and contig generation (*Unicycler* v.0.4.8) [30]. Assembled sequences were used in *quast* and *metaquast* analyses for the generation of basic genome stats and comparison with reference genome [31,32]. Genome annotation was performed on the BV-BRC v.3.26.4 platform using the RASTtool kit (RASTtk) [33]. Identified genes were annotated into Enzyme Commission (EC) numbers [34], Gene Ontology (GO) [35], KEGG pathways [36], genus-specific protein families (PLFams), cross-genus protein families (PGFams) [37], and subsystem databases [38]. Mash/MinHash was used to identify the closest reference genomes on the BRC v.3.26.4 [39].

RaxML v. 8.0.0. software was used to analyze the resulting protein matrix, and fast bootstrapping was used to generate affinity values in the tree [40,41]. The PROKSEE (https://proksee.ca/, accessed on 3 February 2023) server was used to generate the circos graph. The genome was assigned to genus level using GTDBtk. Based on this analysis, the nine closest phylogenetically isolated from soil strains from genus *Achromobacter* were selected (Table 1). For each genome, global PATRIC protein families (PGFams) were determined and compared using the MUSCLE algorithm [42,43]. A chord diagram was prepared in R v.3.4.3 (R Core Team, 2016) using *circlize* v.0.4.15 [44].

Table 1. Basic information of compared genomes.

Strain_ID	Strain_Name	BioSample
A1	*Achromobacter* sp. 77Bb1	SAMN31831554
A2	*Achromobacter arsenitoxydans* SY8	SAMN02469904
A3	*Achromobacter marplatensis* B2	SAMN07270369
A4	*Achromobacter ruhlandii* LMG1866	SAMNEA6647237
A5	*Achromobacter* sp. B7	SAMN09690389
A6	*Achromobacter* sp. UMC46	SAMN05245113
A7	*Achromobacter* sp. UMC71	SAMN05245114
A8	*Achromobacter spanius* UQ283	SAMN10643443
A9	*Achromobacter xylosoxidans* GD03	SAMN15929548

The raw fastq files from genome sequencing were deposited in the NCBI Sequence Read Archive (SRA) under accession number SRP140180 (the genome was submitted into NCBI database under submission nr SUB12314981, BioProject PRJNA904146).

3. Results and Discussion

3.1. Genome Assembly and Annotation

The obtained genome consisted of 57 contigs with an N50 of 855,260 bp and a total length 6,651,432 of bp (Figure 1). Previous genomic studies of bacteria of the genus *Achromobacter* isolated from natural environments have resulted in genomes of lengths 6,441,875 bp [18], 5,532,918 bp [22], and 6,687,826 bp [19]. On average, bacteria in this genus have genome lengths ranging from 5.67 Mb to 7.21 Mb [45]. The genome of *Achromobacter* sp. 77Bb1 consisted of 57 contigs. The longest contig measured 1,018,060 nucleotides. The number and length of the obtained contigs depended on the starting material, sequencing process, and overall quality of the obtained sequences [46]. The coarse and fine consistency for this assembled genome were 99.2 and 97.3, respectively. Annotation analysis revealed the presence of 6026 coding sequences (CDS), of which 4855 were assigned protein function. A total of 55 tRNA-encoding sequences were found corresponding to 21 amino acids (Leu, Arg, Ser, His, Trp, Thr, Gly, Tyr, Asn, SeC, Met, val, Cys, Ala, Gln, phe, Pro, Glu, Asp, Lys, Ile) (Table 2). Scientists have identified 5162 genes in *Achromobacter* sp. HZ01 [22], 5914 protein-coding sequences in *A. spanius* UQ283 [19], and 5848 protein-coding genes in *A. xylosoxidans* SQU-1 [18]. Therefore, we can conclude that bioinformatic processing has resulted in a final genome comparable to that of other species of this genus.

Table 2. Basic statistics and characteristics of the analyzed genome.

Attribute	Value
Genome size (bp)	6,651,435
Number of contigs	57
GC content [%]	64.023
CDS	6026
Protein function assigned	4855
Hypothetical proteins	1171
tRNA	55

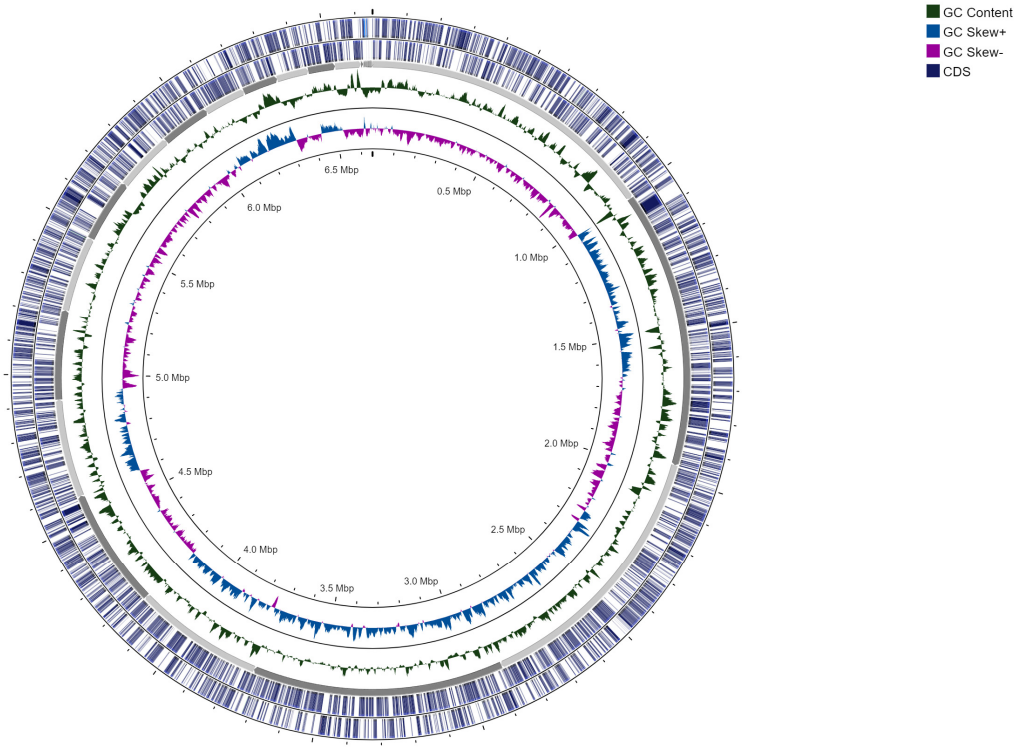

Figure 1. Circos graph represents contigs in the genome of *Achromobacter* sp. 77Bb1 generated using the Proksee tool. Purple and blue represent the GC skew on the − and + strains, respectively. Green represents the GC content. Dark blue represents the CDS in the forward (inner circle) and reverse (outer circle) directions.

Table 3 shows the distribution the of *Achromobacter* sp. 77Bb1 genes into functional classes. Each class is divided into several functional pathways. A total of 2396 genes were assigned to 10 functional superclasses (Table 2). The highest number of genes was assigned to metabolic pathways, from which the coding proteins involved in amino acid metabolism occurred most frequently. The lowest number of genes was assigned to the regulatory and cell signaling pathways (Figure 2). The same trend was observed for other bacteria of the genus *Achromobacter* [22].

Table 3. Number of genes associated with the analyzed functional classes.

Functional Superclass	No. of Genes
Cell envelope	45
Membrane transport	195
Cellular processes	155
DNA processing	84
RNA processing	52
Protein processing	257
Regulation and cell signaling	9
Energy	352
Metabolism	1067
Stress response, defense, virulence	180

Membrane transport is a fundamental aspect of cell function that enables the release of harmful substances or uptake of necessary nutrients [47]. A total of 27 different ABC transporter encoding genes were found in the genome of *Achromobacter* sp. 77Bb1 involved in many functions, such as transport of taurine, spermidine, putrescine, phosphate, phospholipid, ectoine, ferric iron, glycerol-3-P, urea, molybdenum, cyanate, excinuclease,

glycerol, lipopolysaccharide, and glutathione. Similar ABC transporters have been found in the genome of *Achromobacter xylosoxidans* adapted to salinity [18].

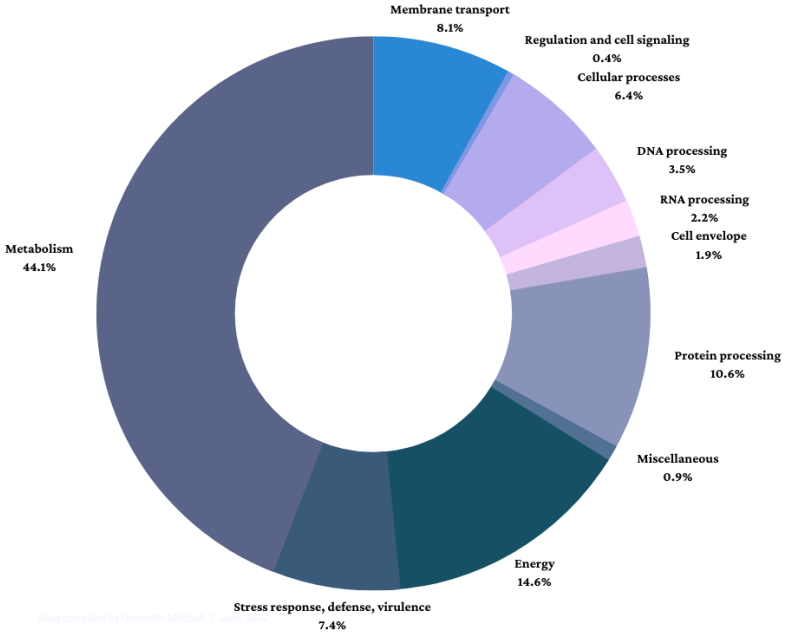

Figure 2. An overview of genes assigned to analyzed functional superclasses.

The genome was assigned to the genus *Achromobacter* using the GTDBtk. Based on this analysis, the eight phylogenetically closest strains from the genus *Achromobacter* were selected for comparison analysis. All strains analyzed were of environmental and soil origin. The basic statistics and differences between all the analyzed genomes are presented in Table 4.

Table 4. Basic statistics and characteristics of the analyzed genomes (A1—*Achromobacter* sp. 77Bb1; A2—*Achromobacter arsenitoxydans* SY8; A3—*Achromobacter marplatensis* B2; A4—*Achromobacter ruhlandii* LMG1866; A5—*Achromobacter* sp. B7; A6—*Achromobacter* sp. UMC46; A7—*Achromobacter* sp. UMC71; A8—*Achromobacter spanius* UQ283; A9—*Achromobacter xylosoxidans* GD03).

Attribute	A1	A2	A3	A4	A5	A6	A7	A8	A9
Genome size (Mbp)	6.651	6.157	6.885	6.865	6.237	6.68	6.341	6.688	6.761
Number of contigs	57	105	61	86	1	1	1	1	1
CDS	6026	5646	6469	6458	5697	6238	5906	6331	6333
Contig N50 (bp)	855,260	106,744	471,181	181,399	-	-	-	-	-

There were 540 different genes present in all the analyzed samples (Table 5). The highest number of shared genes was observed in a comparative analysis of our strain and the bacteria *Achromobacter* sp. UMC46 which was isolated from soil and is able to metabolite terpene [48]. In contrast, the fewest number of shared genes was shown with the strain *Achromobacter arsenitoxydans* SY8, which was isolated from the soil of a pig farm and is resistant to arsenite [49].

3.2. Analysis of Genes from Different Metabolic Pathways

Figure 3 and Table 6 show the number of genes and proteins related to the different metabolic pathways in the genome of *Achromobacter* sp. 77Bb1 compared with the eight phylogenetically closest strains (Figure 3, Table 6). The highest number of genes was identified for amino acid metabolism, characterized by 13 different pathways, with 280 gene products. The highest number of these genes was present in the genome of *A. xylosoxidans* strain GD03

isolated from soil, and found to inhibit the growth of the *Phytophthora* sp. [50]. The genome of our strain contained 672 gene copies of 242 products in those metabolic pathways. The identified genes were related to metabolism of 14 amino acids and 13 nonproteogenic amino acids, biosynthesis of 7 amino acids, and degradation of 4 amino acids.

Table 5. The number of copies of genes associated with the analyzed functional classes in *Achromobacter* sp. 77Bb1 and 8 closest phylogenetic strains (A1—*Achromobacter* sp. 77Bb1; A2—*A. arsenitoxydans* SY8; A3—*A. marplatensis* B2; A4—*A. ruhlandii* LMG1866; A5—*Achromobacter* sp. B7; A6—*Achromobacter* sp. UMC46; A7—*Achromobacter* sp. UMC71; A8—*A. spanius* UQ283; A9—*A. xylosoxidans* GD03).

Functional Superclass	No. of Genes								
	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**
Cell envelope	45	41	39	37	47	39	36	42	37
Membrane transport	195	221	179	192	181	181	237	247	218
Regulation and cell signaling	9	9	19	9	9	11	9	20	9
Cellular processes	155	147	170	147	159	172	159	162	153
DNA processing	84	84	86	89	87	85	89	89	82
RNA processing	52	70	50	50	72	54	66	54	59
Protein processing	257	256	259	268	255	274	261	269	234
Energy	352	354	349	397	365	340	364	399	351
Metabolism	1067	938	998	1047	1040	959	964	1020	865
Stress response, defense, virulence	180	173	167	172	179	170	145	177	144

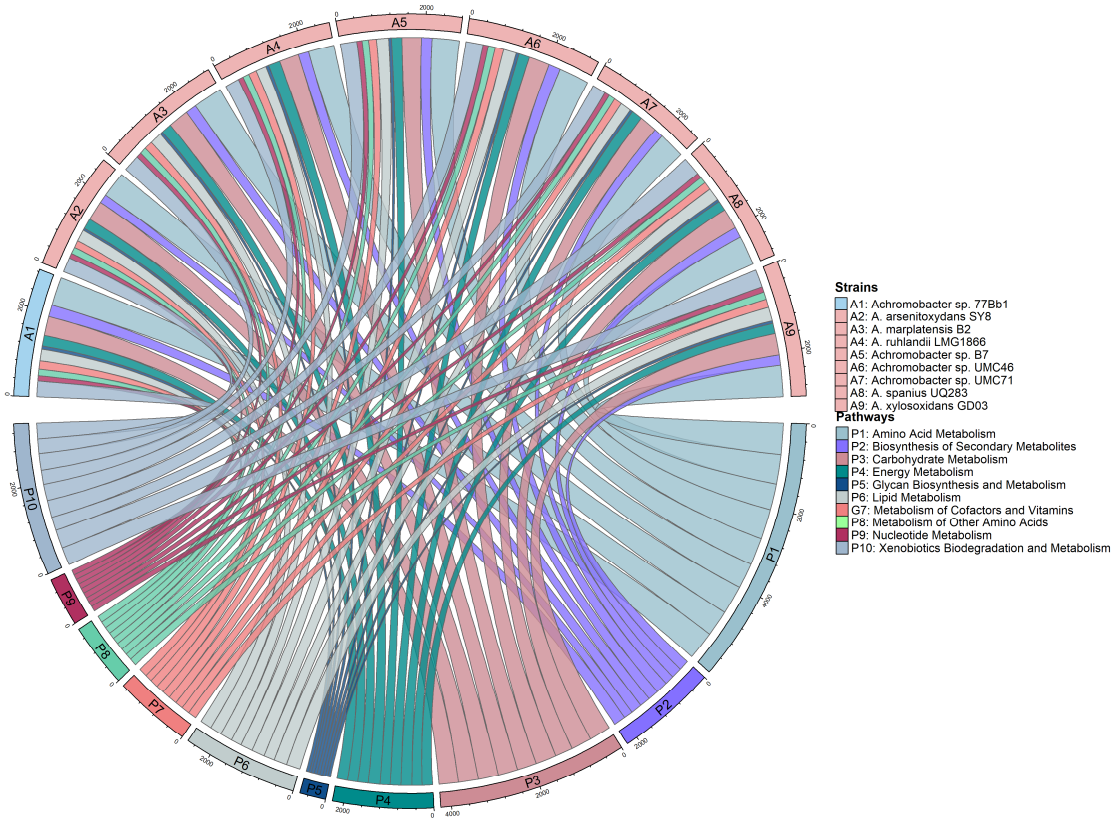

Figure 3. Chord diagram showing the number of genes from different metabolic pathways in the genome of *Achromobacter* sp. 77Bb1 compared to the eight phylogenetically closest strains.

Table 6. The number of proteins associated with the analyzed metabolic pathways in *Achromobacter* sp. 77Bb1 and 8 closest phylogenetic strains (A1—*Achromobacter* sp. 77Bb1; A2—*A. arsenitoxydans* SY8; A3—*A. marplatensis* B2; A4—*A. ruhlandii* LMG1866; A5—*Achromobacter* sp. B7; A6—*Achromobacter* sp. UMC46; A7—*Achromobacter* sp. UMC71; A8—*A. spanius* UQ283; A9—*A. xylosoxidans* GD03).

Metabolic Pathway	No. of Proteins								
	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**A7**	**A8**	**A9**
Amino acid metabolism	672	617	687	613	642	712	616	694	742
Biosynthesis of polyketides and nonribosomal peptides	50	32	40	45	41	48	40	43	43
Biosynthesis of secondary metabolites	282	212	262	248	253	279	220	253	240
Carbohydrate metabolism	455	472	482	465	458	495	418	484	508
Energy metabolism	252	246	245	277	242	252	259	237	245
Glycan biosynthesis and metabolism	75	53	67	68	67	70	64	59	64
Lipid metabolism	272	278	287	249	288	307	258	309	330
Metabolism of cofactors and vitamins	196	169	194	193	187	202	188	192	198
Nucleotide metabolism	123	118	123	122	124	128	116	120	128
Xenobiotics biodegradation and metabolism	371	345	392	334	390	406	318	439	419

The main pathways from the carbohydrate metabolism group were amino sugar and nucleotide sugar metabolism, ascorbate and aldarate metabolism, butanoate metabolism, C5-branched dibasic acid metabolism, citrate cycle (TCA cycle), fructose and mannose metabolism, galactose metabolism, glycolysis/gluconeogenesis, glyoxylate and dicarboxylate metabolism, inositol phosphate metabolism, pentose and glucuronate interconversions, pentose phosphate pathway, propanoate metabolism, pyruvate metabolism, and starch and sucrose metabolism. Genes for all of these metabolic pathways were present in the genome of *Achromobacter* sp. 77Bb1. The highest number of genes involved in carbohydrate metabolism was identified for butanoate metabolism and pyruvate metabolism. Pyruvate may be used by bacteria to generate acetate, acetyl-CoA, and L-lactate [51]. The genome of *Achromobacter* sp. 77Bb1 contains genes for pyruvate dehydrogenase and dihydrolipoyllysine-residue acetyltransferase, which catalyze the transformation of pyruvate into acetyl-CoA.

The lowest number of gene copies was identified for the biosynthesis of polyketides and nonribosomal peptides (426) and glycan biosynthesis and metabolism (655). For glycan metabolism, all the analyzed strains showed the activity of 10 metabolic pathways out of the 22 identified in the KEGG database. Two pathways assigned to nucleotide metabolism were present in all the analyzed strains (purine and pyrimidine metabolism). The same trend was observed for a bacterium belonging to the genus *Achromobacter* with potential phosphorus-removal capabilities [52]. Genes coding for proteins involved in nucleotide metabolism comprise the lowest percentage of core, accessory, and unique genes in the pan-genomic analysis of the genus *Achromobacter* [53].

Comparative analysis of the genome of *Achromobacter* sp. 77Bb1 and eight phylogenetically closest strains identified presence of 15 pathways in lipid metabolism. All analyzed strains contained genes coding for α-linolenic acid metabolism, arachidonic acid metabolism, ether lipid metabolism, fatty acid biosynthesis, fatty acid elongation in mitochondria, fatty acid metabolism, glycerolipid metabolism, glycerophospholipid metabolism, linoleic acid metabolism, primary bile acid biosynthesis, sphingolipid metabolism, and the synthesis and degradation of ketone bodies. The highest number of genes encoding proteins involved in lipid metabolism were identified for fatty acid metabolism (896), α-linolenic acid metabolism (374), and fatty acid elongation in the mitochondria (372). Fatty acids are the main breakdown products of alkanes and are converted to acetyl-CoA via β-oxidation.

In the genome *Achromobacter* sp. HZ01, similar to the genome of *Achromobacter* sp. 77Bb1, we identified genes assigned into fatty acid metabolism related to β-oxidation [22].

Methane, nitrogen, and sulfur metabolism accounted for 44, 50, and 10 proteins, respectively. Genes encoding 27 proteins participating in the reductive carboxylate cycle (CO_2 fixation) were present in genome of *Achromobacter* sp. 77Bb1. The analyzed strain also contained genes encoding proteins involved in carbon fixation in photosynthetic organisms (19), oxidative phosphorylation (37), and photosynthesis (10). Although several genes involved in the metabolic pathway of methane conversion were found in the genome of the analyzed bacteria, the presence of key enzymes responsible for its oxidation, such as methane monooxygenase, methanol dehydrogenase, and alcohol oxidase, has not been identified [22]. Several bacterial genomes of the genus *Achromobacter* have been shown to contain genes encoding proteins involved in the denitrification process. No such sequence has been shown to be present in the analyzed genome [52]. In the genome of *Achromobacter* sp. 77Bb1, genes taking part in the first steps of assimilatory sulfate reduction and dissimilatory sulfate reduction and oxidation were found. Moreover, these genes may be involved in plant growth-promoting traits [54]. To date, six pathways have been proposed for CO_2 fixation in bacteria [55]. In the genome of *Achromobacter* sp. 77Bb1, almost all genes for the reductive tricarboxylic acid cycle (rTCA) were present. We observed a lack of genes for fumarate reductase (catabolize reduction of fumarate to succinate), 2-oxoglutarate synthase (catabolize synthesis of oxoglutarate from succinyl-CoA), and ATP-citrate-lyase (catabolize reduction of citrate into oxaloacetate). There are also a few genes involved in the Wood–Ljungdahl (WL) pathway. Continuously rising atmospheric CO_2 levels exacerbate climate change. Conventional methods for fixing carbon dioxide are becoming ineffective. Therefore, attempts are being made to use microorganisms that have the ability to bind CO_2 and metabolize it into valuable products.

3.3. Analysis of Genes Taking Part in Biosynthesis of Secondary Metabolites

Secondary metabolism plays a crucial role in the vitality of any bacterium, allowing it to survive in certain, sometimes harmful, environmental conditions, or enabling it to form interactions with other organisms [56]. Secondary metabolites for the biosynthesis of which genes have been found in *Achromobacter* sp. 77Bb1 include ectoine, anthocyanin, betalain, carotenoid, diterpenoid, sesquiterpenoid, terpenoid, and insect hormones. Ectoine is an osmoprotectant that allows survival under osmotic stress [18]. The genome of *Achromobacter* sp. 77Bb1 contains all ectoine biosynthetic gene clusters (*ectB*, *ectA*, *ectC*, *ectD*, *lysC*, *asd*), which enables bacteria to synthetize L-ectoine from L-aspartate. The lipid membrane of bacteria is stabilized by squalene which can be synthetized by few bacteria. Our *Achromobacter* strain contains genes involved in the conversion of farnesyl-PP into squalene [57].

In the genome of *Achromobacter* sp. 77Bb1, some genes for plant growth hormones (brassinosteroid, flavone, flavonol, flavonoid, isoflavonoid, isoquinoline, phenylpropanoid, stilbenoid, diarylheptanoid, gingerol, tropane, piperidine, pyridine alkaloid, and zeatin) and antibiotics (novobiocin, penicillin, cephalosporin, puromycin, streptomycin, and tetracycline) were found. This indicates the ability of this bacterium to influence plant growth and development by increasing its ability to survive in a given environment [56,58]. Genes encoding aldehyde dehydrogenase, nitrilotriacetate monooxygenase (components A and B), 3-hydroxyacyl-CoA dehydrogenase, enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, acyl-CoA synthetase, flavodoxin reductases, and probable poly(beta-D-mannuronate) O-acetylase, present in genome of *Achromobacter* sp. 77Bb1, can take part in limonene and pinene degradation. Pinene is an organic chemical compound derived from a group of cyclic monoterpenes and is the main component of pine resin. Several bacteria have been shown to contain all or some genes encoding enzymes involved in the degradation of this compound in their genome [59]. However, the presence of genes encoding proteins involved in the degradation of the abovementioned substances in the genome does not

prove the ability of the analyzed strain to perform such a process. Nevertheless, it has been proven that the decomposition of cyclic monoterpenes is rare in the environment [60,61].

3.4. Analysis of Genes Taking Part in Xenobiotics Biodegradation and Metabolism

We showed that our strain has almost all genes encoding proteins involved in the aerobic degradation of styrene (Figure 4c, Table 7). The ability of bacteria to break down harmful substances, such as xenobiotics, could become the basis for the application of such microorganisms in the biotechnology and industry. Bacteria capable of degrading styrene can potentially be used in the bioremediation of its pollution. Styrene is the simplest alkenylbenzene and is used both as a production substrate for synthetic polymers and as a solvent in this industry, and it is released into the environment. Styrene has been proven to have toxic effects on genes, and its oxide is classified as a probable carcinogen and immunomodulatory agent in humans [62]. Similar studies have been conducted on bacteria from the genera *Pseudomonas*, *Bacillus*, *Bradyrhizobium*, and *Thauera* [63]. The ability to degrade styrene was identified in *Exiguobacterium* sp. [64], *Bacillus* sp. [65], *Lysinibacillus* sp., and *Pseudomonas aeruginosa* [66].

Benzene and its derivatives have a negative impact on the environment. Searching for bacteria that can potentially degrade these compounds is of great importance. The genome of *Achromobacter* sp. 77Bb1 contains genes for enoyl-CoA hydratase (EC 4.2.1.17), probable poly(beta-D-mannuronate) O-acetylase(EC 2.3.1.-), 3-hydroxybutyryl-CoA dehydrogenase (EC 1.1.1.157), and acetyl-CoA acetyltransferase (EC 2.3.1.9), which enable the metabolism of benzene and its derivatives into acetoacetyl-CoA and acetyl-CoA which proceed to the citric acid cycle. The genes encoding 3-ketoacyl-CoA thiolase (EC 2.3.1.16) and probable poly(beta-D-mannuronate) O-acetylase (EC 2.3.1.-), which is responsible for decomposition of benzoyl acetyl-CoA into benzoyl-CoA and acetyl-CoA, were found. *Achromobacter* sp. 77Bb1 has the potential to transform E-phenylitaconyl-CoA into benzoyl-CoA, and toluene into 3-methylcatechol. *Achromobacter* sp. 77Bb1 has all the genes for the conversion of thiobenzamide and benzoyl phosphate into benzoate in its genome (Figure 4a, Table 7), almost all genes for the production of succinyl-CoA from benzene (Figure 4b, Table 7), and all genes needed to catabolize the benzene-to-catechol conversion reaction.

Fourteen of the genes shown in Table 7 were present in the genomes of all the *Achromobacter* strains analyzed. The *A. arsenitoxydans* SY8 strain lacks genes encoding nitrilotriacetate monooxygenase component A, nitrilotriacetate monooxygenase component B, and phenylacetaldehyde dehydrogenase. Genes for nitrilotriacetate monooxygenase subunit A were also absent in genomes of *Achromobacter* sp. B7, *Achromobacter* sp. UMC46, *Achromobacter* sp. UMC71, *A. spanius* UQ283, and *A. xylosoxidans* GD03, respectively.

Genes for the benzene-to-succinyl-CoA metabolic pathway have been detected in the genome of *Oceanimonas* sp. GK1 [11], whereas they were not shown in the analyzed genomes of strains of the genus *Pseudomonas* [67]. Genes for benzoate and aminobenzoate degradation have been found in the genome of *S. paucimobilis* [68].

The genome of *Achromobacter* sp. 77Bb1 contains 16 genes for methyl-accepting chemotaxis proteins that participate in the accommodation of bacteria into the environment and the transduction of external signals [69]. In addition to genes that determine alkane degradation, the genome contains sequences encoding cytochrome o ubiquinol oxidase, which inhibits metabolic pathways for alkane degradation when more preferred carbon sources are present [70]. Not all bacterial genomes capable of decomposing hydrocarbon sources have such regulatory mechanisms [71–74]. *Achromobacter* sp. 77Bb1 also has genes for methyl-accepting chemotaxis sensor/transducer protein, methyl-accepting chemotaxis protein, chemotaxis protein methyltransferase CheR, chemotaxis signal transduction protein, chemotaxis response regulator protein-glutamate methylesterase CheB, chemotaxis regulator CheY, chemotaxis response-phosphatase CheZ, and chemotaxis protein CheD.

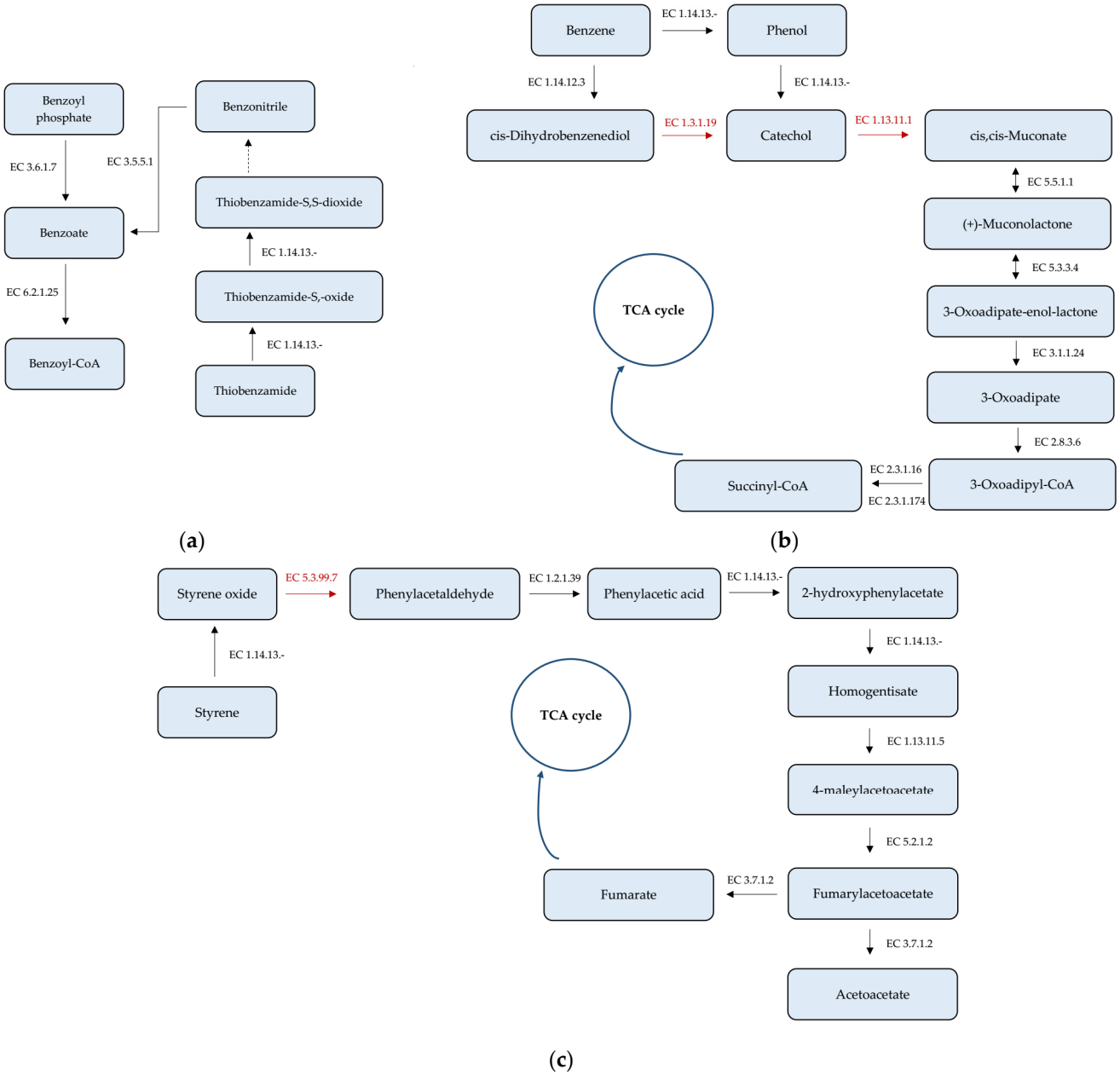

Figure 4. Metabolic pathways of (**a**) aminobenzoate degradation; (**b**) benzoate degradation; (**c**) styrene degradation. Arrows and enzyme symbols marked in black indicate genes present in the genome of *Achromobacter* sp. 77Bb1. Red color indicates the lack of a given gene.

The ability to break down drugs can have positive application. The genome of the bacterium analyzed showed the presence of genes encoding enzymes such as alcohol dehydrogenase: EC 1.1.1.1, glutathione S-transferase: EC 2.5.1.18, thiopurine S-methyltransferase: EC 2.1.1.67, inosine-5′-monophosphate dehydrogenase: EC 1.1.1.205, GMP synthase: EC 6.3.5.2, dihydropyrimidinase: EC 3.5.2.2, beta-ureidopropionase: EC 3.5.1.6, orotate phosphoribosyltransferase: EC 2.4.2.10, involved in the degradation of felbamate, cyclophosphamide, ifosfamide, azathioprine, 6-mercaptopurine, and fluorouracil. According to the BV-BRC database, these enzymes are commonly present in many bacteria [75,76].

Moreover, in the genome of *Achromobacter* sp. 77Bb1, a few proteins involved in decomposition of 1- and 2-methylnaphthalene, 7,12-dimethylbenz[a]anthracene, 1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (DDT), aflatoxin B1, atrazine, benzopyrene, bisphenol A, bromobenzene, coprolactam, ethylbenzene, fluorobenzoate, 1,1-dichloroethylene, 1,2-

dibromoethane, gamma-hexachlorocyclohexane, geraniol, 1-nitrophthalene, naphthalene, anthracene, trichloroethylene, tetrachloroethene, xylene, and trinitrotoluene were found.

Table 7. The loci of the genes present in the genome of *Achromobacter* sp. 77Bb1 involved in metabolic pathways of aminobenzoate degradation, benzoate degradation, and styrene degradation.

Gene	Contig No.	Strand	Start Position	End Position	NA Length (bp)
Nitrilotriacetate monooxygenase component A (1.14.13-)	17	+	49,978	51,315	1338
	5	–	114,628	115,914	1287
Nitrilotriacetate monooxygenase component B (1.14.13-)	3	+	322,411	323,079	669
	4	+	215,673	215,673	618
	5	+	14,315	14,833	519
	7	+	140,548	141,192	645
	3	–	68,595	69,236	642
	1	–	777	1409	633
	11	+	7978	8502	525
Plant-induced nitrilase (3.5.5.1)	4	+	214,093	215,052	960
	4	–	234,963	235,886	924
Acylphosphate phosphohydrolase (3.6.1.7)	3	–	329,557	329,865	309
Benzoate-CoA ligase (6.2.1.25)	9	+	136,740	138,419	1680
Aromatic-ring-hydroxylating dioxygenase (1.14.12.3)	3	+	902,075	902,608	534
Muconate cycloisomerase (5.5.1.1)	10	+	44,014	45,150	1137
	4	+	209,116	210,243	1128
Muconolactone isomerase (5.3.3.4)	8	+	91,217	91,492	276
Beta-ketoadipate enol-lactone hydrolase (3.1.1.24)	3	+	719,268	720,092	825
	8	+	91,855	92,634	780
	18	+	5051	5701	651
3-oxoadipate CoA-transferase subunit A (2.8.3.6)	3	+	723,751	724,416	666
	9	+	148,213	148,893	681
	1	–	662,567	663,400	834
	8	+	89,883	90,560	678
3-oxoadipate CoA-transferase subunit B (2.8.3.6)	8	+	90,557	91,201	645
	9	+	148,904	149,590	687
	18	+	5698	6378	681
	3	+	724,418	725,143	726
	1	+	661,840	662,574	735
3-ketoacyl-CoA thiolase (2.3.1.16)	1	–	974,655	975,839	1185
	3	–	415,601	416,782	1182
3-oxoadipyl-CoA thiolase (2.3.1.174)	1	–	282,888	284,093	1206
Phenylacetaldehyde dehydrogenase (1.2.1.39)	13	+	22,603	24,114	1512
Homogentisate 1,2-dioxygenase (1.13.11.5)	7	–	269,021	270,319	1299
	17	–	36,021	37,328	1308
Maleylacetoacetate isomerase (5.2.1.2)	17	+	20,488	21,198	711
	4	+	672,336	672,980	645
Fumarylacetoacetase (3.7.1.2)	7	–	267,582	268,901	1320
	17	–	37,667	38,980	1314

The presence of a given gene in the genome indicates its potential and does not ensure its capabilities. Genome analysis of a particular bacterial strain makes it possible to predict the functionality of several genes. These features suggest that the analyzed strain could

be used as a candidate for biopreparation for the degradation of xenobiotics and other harmful substances.

4. Conclusions

The analyzed genome was 6,561,435 bp long. Previous studies on *Achromobacter* isolated from natural environments have examined genomes ranging from 5.67 Mb to 7.21 Mb in length. Bioinformatics analysis revealed 6026 coding genes, 4855 of which were assigned a protein function, whereas previous studies identified an average of 5641 genes in the genomes of *Achromobacter* sp. Therefore, it can be concluded that bioinformatics processing resulted in a genome comparable to that of other genera. Phylogenetic analysis showed the greatest similarity between our strain and the reference genome of *Achromobacter piechaudii* ATCC 43553, but the greatest number of shared genes was shown by comparative analysis with *Achromobacter xylosoxidans* strain NCTC10807. Almost all the genes involved in the reductive tricarboxylic acid cycle were present in the genome of *Achromobacter* sp. 77Bb1. The presence of these genes indicated the potential ability of the analyzed bacteria to the CO_2-fixing process. The genome of *Achromobacter* sp. 77Bb1 contains all the genes involved in ectoine biosynthesis, which allows the bacterium to synthesize L-ectoine. This could potentially indicate the development of traits for survival in highly osmotic environments. We showed that in the genome of our strain, almost all genes encoding proteins involved in the aerobic degradation of styrene are present, which could potentially indicate its ability to carry out this process. In addition, we identified genes involved in the adaptation of bacteria to the environment and transduction of external signals. In silico analysis only indicates the potential of the bacterium to perform the described functions and does not ensure its ability to do so. These features suggest the possibility of using the analyzed strain to degrade xenobiotics and other harmful substances.

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