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Genomic Analysis of a Novel Heavy Metal Resistant Isolate from a Black Sea Contaminated Sediment with the Potential to Degrade Alkanes: *Plantactinospora alkalitolerans* sp. nov.

Lorena Carro [1,*], Aysel Veyisoglu [2], Kiymet Guven [3], Peter Schumann [4], Hans-Peter Klenk [5] and Nevzat Sahin [6,*]

[1] Departamento de Microbiología y Genética, Facultad de CC Agrarias y Ambientales, Universidad de Salamanca, Plaza Doctores de la Reina, 37007 Salamanca, Spain
[2] Department of Medical Services and Techniques, Vocational School of Health Services, Sinop University, Sinop 57000, Turkey
[3] Biology Department, Faculty of Science, Eskisehir Technical University, Eskisehir 26470, Turkey
[4] Leibniz Institute DSMZ—German Collection of Microorganisms and Cell Cultures, Inhoffenstraße 7B, 38124 Braunschweig, Germany
[5] School of Natural and Environmental Sciences, Newcastle University, Ridley Building, Newcastle upon Tyne NE1 7RU, UK
[6] Department of Molecular Biology and Genetics, Faculty of Science, Ondokuz Mayis University, Samsun 55139, Turkey
* Correspondence: authors: lcg@usal.es (L.C.); nsahin@omu.edu.tr (N.S.)

Abstract: Microorganisms that grow in poorly studied environments are of special interest when new biotechnological applications are searched. The Melet river offshore sediments at the Black Sea have been described to contain an important number of contaminants from upstream industries which have been accumulating for years. Bacteria of such habitats must be adapted to the presence of those compounds and in some cases, are able to use them as carbon sources. In the analysis of some samples recovered from this environment, an actinobacterial strain was isolated, named as S1510^T, and its taxonomic position was determined using a combination of phenotypic and genotypic properties. Strain S1510^T presented phenotypic properties typical of members of the family *Micromonosporaceae* and was assigned to the *Plantactinospora* genus, based on the phylogenetic analyses of the 16S rRNA gene and whole-genome sequences. Low dDDH (digital DNA-DNA hybridization) values with other members of the genus confirmed that *Plantactinospora* sp. S1510^T represents a novel species, and is proposed with the new name *Plantactinospora alkalitolerans*. The strain presented characteristics not previously described for other species in the genus, such as its high tolerance to alkaline pHs, the presence of genes related to the production and degradation of alkanes (*ole*ABCD, *ssu*AD, *alm*A), the degradation of several aromatic compounds, and the tolerance to high heavy metal concentrations. In addition, *Plantactinospora* sp. S1510^T presents several bioclusters to produce nonribosomal peptide-synthetases, terpenes, polyketide synthases, and bacteriocins, that possess low similarities with known compounds.

Keywords: *Micromonosporaceae*; alkane; heavy metal; contaminant; marine sediment; biocluster

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1. Introduction

The Black Sea is a special area of study, due to some unique characteristics of this body of water. Between them is its limited connection with the Atlantic Ocean as well as the high number of watersheds from different countries that drain into it. Since industry development in the 1960s, contamination has influenced the evolution of native species and probably has influenced the microbial structure and population in several areas of this sea. Some of the river mouths accumulate part of the contaminants transported by the rivers, and this has been detected for the Melet river, increasing the amount of heavy metal and

other compounds from the petroleum industry in the sea sediment [1,2], mainly proceeding from mining activities, various domestic and agricultural wastes. The *Actinomycetota* can tolerate and degrade toxic compounds, since they presented a high metabolic diversity, they are able to develop in a great number of substrates [3]. In addition, their natural capacity to produce a significant number of antibiotics, generates additional interest in looking for new strains of these microorganisms in poorly studied habitats. During this study, several samples of the sea sediment of the Black Sea at the Melet river mouth were recovered to isolate members of the phylum *Actinomycetota* capable of surviving in those conditions (high heavy metal and other contaminants concentrations), that could produce novel compounds not previously studied, including antibiotics and enzymes to degrade toxic substrates. Within the isolated bacteria, a microorganism that could represent a new species within the family *Micromonosporaceae* was selected for a polyphasic study, confirming that it represents a novel taxon within the *Plantactinospora* genus. The genomic analysis shows the ability to produce several biocompounds and tolerate toxic compounds present in the isolation area.

2. Material and Methods

2.1. Isolation

In a study to understand the actinobacterial diversity in a contaminated area of the Black Sea, a marine sediment was collected by a dredge at a depth of 12 m, offshore of the Melet river (GPS coordinates for the sampling site are 40°59.650′ N and 37°58.953′ E). Sediment samples were subsampled aseptically and stored at −20 °C, until used. The sample was processed using the dilution-plating method. One gram of sediment was diluted in 9 mL of NaCl 0.85% (*w*/*v*), mixed and serially 10-fold diluted until 10^{-6}, then 100 microliters of each dilution was plated over media. SM3 medium [4] was used for isolation of the strain, supplemented with filter sterilised cycloheximide (50 μg mL^{-1}), nalidixic acid (10 μg mL^{-1}), novobiocin (10 μg mL^{-1}) and nystatin (50 μg mL^{-1}). The plates were incubated at 28 °C for 30 days and the colonies were isolated as pure cultures and maintained on yeast-malt extract agar (ISP 2) [5] slopes at room temperature and stored for long-term maintenance in glycerol suspensions (20%, *v*/*v*) at −80 °C. Following the growing on SM3 medium plates for 4 weeks, several strains were observed on the surface of the medium. From these, a strain showing morphological characteristics of the family *Micromonosporaceae* [6], filamentous growth and orange colonies, was selected for a further study, the strain S1510^{T}.

2.2. 16S rRNA Gene Phylogeny

The genomic DNA and the 16S rRNA gene amplification was performed following the protocol previously described [7]. The resulting 16S rRNA gene sequence was used for comparison with close published species, using the EzBioCloud server [8]. The phylogeny, based on the 16S rRNA gene sequences of *Plantactinospora* sp. S1510^{T} and other members of the genus *Plantactinospora* was generated with MEGA X [9], using several approaches, the neighbour-joining [10], the maximum parsimony [11] and the maximum likelihood [12] methods, with a bootstrap test of 1000 [13]. The evolutionary distances were computed using the Kimura 2-parameter method [14] and the Tamura–Nei models [15], respectively.

2.3. Genome Sequencing, Annotation and Analysis

The genomic DNA was obtained from MicrobesNG company (University of Birmingham), following their standardized protocol. Briefly, forty microlitres of microbial suspension were lysed with 120 μL of TE buffer containing lysozyme (final concentration 0.1 mg/mL) and RNase A (final concentration 0.1 mg/mL), and incubated for 25 min at 37 °C. Proteinase K (final concentration 0.1 mg/mL) and SDS (final concentration 0.5% *v*/*v*) were added and incubated for 5 min at 65 °C. The genomic DNA was purified using an equal volume of SPRI beads and resuspended in EB buffer (Qiagen, Dusseldorf, Germany). DNA was quantified with the Quant-iT dsDNA HS kit (ThermoFisher Scientific,

Waltham, MA, USA, EEUU) assay in an Eppendorf AF2200 plate reader (Eppendorf UK Ltd., Stevenage, UK). Illumina HiSeq 2500 platform was used for the amplification of the genome sequences using 250 paired-end reads from the MicrobesNG company for *Plantactinospora* sp. S1510^T. The reads were assembled into contigs using SPAdes software [16] and the contigs, under 500 bp were discarded. The genome sequence data of *Plantactinospora* sp. S1510^T was uploaded to the Type (Strain) Genome Server (TYGS, https://tygs.dsmz.de (accessed on 7 July 2022)) for the phylogenomic reconstruction and dDDH calculations [17]; the phylogenomic tree was edited with the iTOL pipeline [18]. The genome analysis was carried out using the SEED-viewer [19] after the annotation with the RAST server [20], and the capacity of the strain to produce secondary metabolites was determined through the antiSMASH server [21].

2.4. In Vitro Tests to Evaluate the Alkane Degradation and Heavy Metal Tolerance

To evaluate the capacity of *Plantactinospora* sp. S1510^T to degrade alkanes, a minimum salt medium M3 [22] was prepared and supplemented with 1% (*v*/*v* or *w*/*v*) of the following compounds decane, tetradecane, hexadecane, and eicosane (Fluka®). The solid media were inoculated with *Plantactinospora* sp. S1510^T, using four replicates per treatment, and left at 28 °C for two weeks. The negative controls, without any carbon source, and the positive controls, using glucose as a carbon source, were grown in parallel. The tolerance to the presence of several heavy metals was evaluated by growing the strain on M65 media (DSMZ Medium nº 65: glucose 4 g/L, yeast extract 4 g/L, malt extract 10 g/L, $CaCO_3$ 2 g/L, agar 18 g/L; pH 7.2), supplemented with several concentrations of toxic heavy metals that could be present in the habitat of the strain of study (Cd, Co, Cr, Cu, Hg, Pb, Zn) [2] in ppm (parts per million) of metal (1, 10, 100, 300, 500, 1000, 2000, 5000). The reagents used for the metals were $CdCl_2$, $CoCl_2$, $CrCl_3$, $CuCl_2$, $HgCl_2$, $PbCl_2$, $ZnCl_2$; Sigma-Aldrich®). The plates were incubated for 14 days at 28 °C. For both tests, inoculum was prepared using seven day-old cultures of *Plantactinospora* sp. S1510^T and resuspended in 0.85% NaCl (*w*/*v*) solutions until it reached the McFarland 6 concentration, 10 µL of that suspension was inoculated over the plates for each replicate.

2.5. Phenotypic and Chemotaxonomic Characterizations

Other physiologic characteristics of *Plantactinospora* sp. S1510^T were also analysed, including the tolerance tests of growth under determined temperatures (4, 10, 20, 28, 30, 37, 40, 45, 50 and 55 °C), several pH conditions (4.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 10.0, 11.0 and 12.0), and different NaCl concentrations (1–10 % (*w*/*v*) at intervals of 1.0 NaCl units). All of these tests were determined using the ISP2 medium [5] as the basal medium at a pH of 7.2 at 30 °C (when this was not the characteristic to evaluate), for 14 days. The phosphate buffer systems (KH_2PO_4(0.2 M)/HCl(0.2 M), KH_2PO_4(0.2 M)/K_2HPO_4(0.2 M) and K_2HPO_4(0.2 M)/NaOH(0.2 M)) were used to maintain the pH values of the media. The capacity of growth and cultural characteristics of *Plantactinospora* sp. S1510^T were determined on several media (Czapek's agar [23], GPHF agar (DSMZ-medium 553), ISP media 2–7 [5], modified Bennett's agar [24], nutrient agar [25], N-Z-Amine agar (DSMZ-medium 554), and tryptic soy agar (TSA; Difco)) after two weeks of incubation at 30 °C. The ISCC-NBS colour chart was used to determine the colony colours [26]. The ability of the strain for the utilization of compounds, such as as carbon and nitrogen sources, as well as other degradation tests, were determined, as previously described [27]. ISP9 medium [5] was used as the basal medium for the carbon-source utilization test supplemented with 1% (*w*/*v*) of the carbon sources as the final concentration, while 0.1% (*w*/*v*) was used for the final concentration of the nitrogen sources.

A chemotaxonomic analysis of *Plantactinospora* sp. S1510^T was carried out from the freeze-dried biomass of cultures grown at 30 °C in N-Z-Amine broth (DSMZ medium 554), for 14 days unless stated otherwise. The isomers of diaminopimelic acid and whole-cell sugars were prepared, according to Lechevalier and Lechevalier [28] and analysed by thin layer chromatography [29]. The amount and type of isoprenoid quinones were obtained

following the method previously described [30] and analysed by HPLC. The method of Minnikin et al. [31], with the modifications of Kroppenstedt and Goodfellow [32], was applied for the extraction of the polar lipids. An Agilent Technologies 6890 N gas chromatograph, fitted with an autosampler and a 6783 injector, was used for the determination of the extracted, methylated cellular fatty acids, according to the standard protocol of the Sherlock Microbial identification (MIDI) system [33], using the TSBA 5.0 database.

3. Results

3.1. Identification of the Strain

3.1.1. Morphology

The strain *Plantactinospora* sp. S1510^T presented a filamentous morphology with single spores at the tip of the hyphae. The colonies were developed as substrate mycelium with rough surfaces, and a whole range of orange colours, from light to vivid orange, depending on the media used. A good growth was observed on the ISP2, ISP3, ISP4, and ISP7 agars, intermediate on the Bennett, ISP6, Nutrient, and TSA agars, and poor on the Czapek and ISP5 agars (Figure S1).

3.1.2. 16S rRNA Gene Analysis

The nearly complete 16S rRNA gene sequence was obtained for the strain of study, with a total of 1472 bp (accession number KF494804). A comparative analysis of this sequence with closely related strains deposited in public databases confirmed that the strain S1510^T was affiliated with the family *Micromonosporaceae* and had a high sequence similarity to the genus *Plantactinospora*, and was closely related to the type strains of *Plantactinospora soyae* and *Plantactinospora solaniradicis* with similarity values of 99.8% and 99.1%, respectively. The similarities with other strains in the database were all lower than 98.5%, below the threshold defined for novel species definitions in bacteria [34]. The phylogenetic position of *Plantactinospora* sp. S1510^T, based on the 16S rRNA gene, is shown in Figure 1, and is ubicated in an independent branch, together with the *P. soyae* NEAU-gxj3^T strain. The obtained tree is monophyletic for the genus *Plantactinospora* and the morphology is conserved under the different methods applied for the tree reconstruction.

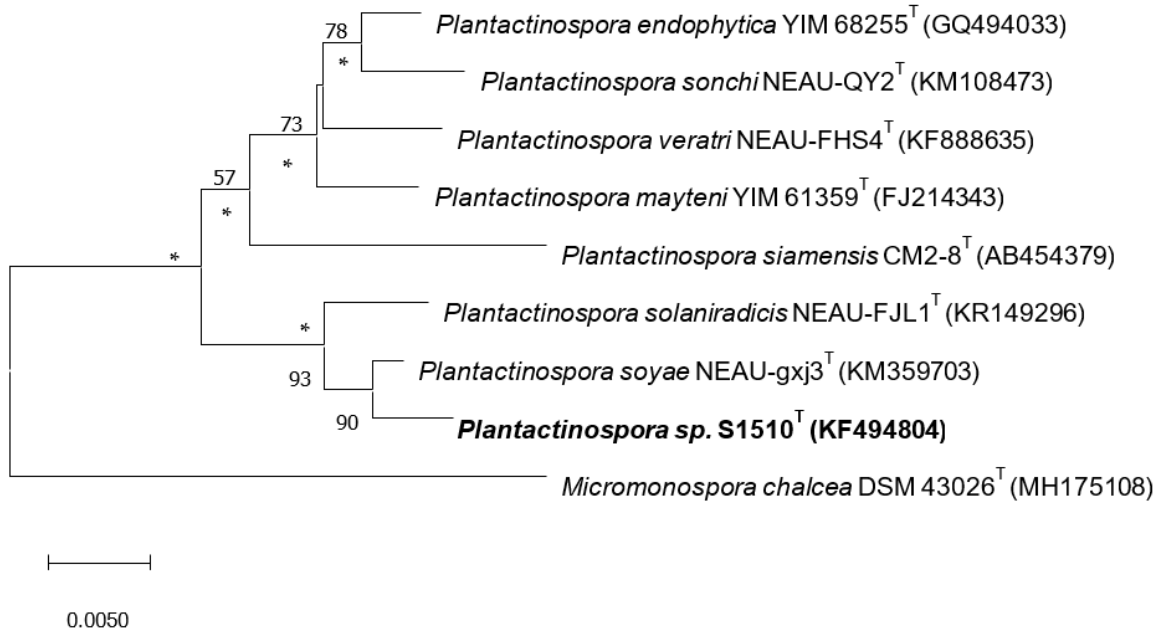

Figure 1. Neighbour-joining phylogenetic tree, based on the 16S rRNA gene sequences of strain S1510^T and members of the genus *Plantactinospora*. Asterisks indicate conserved nodes for the maximum likelihood and the maximum parsimony trees. Bootstrap of 1000 were used and results are indicated at nodes. *Micromonospora chalcea* DSM 43026^T was used as the outgroup.

3.1.3. Genome Sequencing

A draft genome sequence of *Plantactinospora* sp. S1510^T was obtained from a de novo genome assembly of the reads, obtained by Illumina sequencing (accession number JADPUN000000000). The main characteristics of the assembled genome and the closely related type of strain of *P. soyae* are given in Table 1. As like other members of the genus, the genome size is large, with over 9 Mbp, which is considerably higher than the average size of *Micromonospora*, the second closely related genus, which is around 7 Mbp [35]. The genome contains a total of 8545 genes, 8174 of which are proteins, 112 RNA genes, and five rRNA genes (three copies of 5 S, and one copy of 16 S and 23 S). Two CRISPR arrays were also detected. The GC content of *Plantactinospora* sp. S1510^T, according to genome data, is 70.5 mol%.

Table 1. Genome sequence characteristics of *Plantactinospora* sp. S1510^T and a closely related type strain.

	S1510^T	***P. soyae* DSM 46832^T**
Size (bp)	9,219,583	9,715,930
G + C content (%)	70.5	70.8
No. of contigs	441	1
No. of coding sequences	8174	8658
No. of RNAs	112	91
N50 value	31,205	9,715,930

3.1.4. Phylogenomic Analysis

The obtained genome sequence of the strain was used to calculate the dDDH values between *Plantactinospora* sp. S1510^T and the available genomes of the species of the genus *Plantactinospora* (Table S1), which offer a range of values between 28.6 and 41.1, largely below the threshold of 70%, used for the species definition [36]. The phylogenomic reconstruction, based on the genomic information is shown in Figure 2 where, in addition to the *Plantactinospora* strains, the closely related *Micromonospora* type strains were included. This tree is in agreement with the results obtained from the 16S rRNA gene phylogeny, exhibiting a close relationship of the strain under study with the type strain of *Plantactinospora soyae;* nevertheless, the branches length, together with the dDDH results, indicate that *Plantactinospora* sp. S1510^T would represent a novel taxon within the genus *Plantactinospora*.

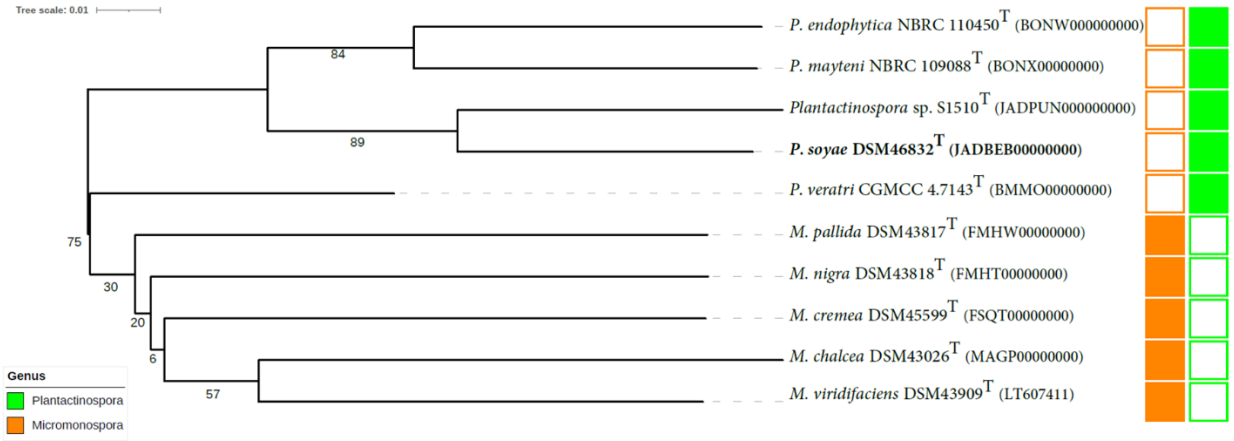

Figure 2. Whole genome phylogenetic tree generated with TYGS of the strain of the study and the closely related strains with available genomes. The tree was inferred with FastME from GBDP distances calculated from the genome sequences. Branch lengths are scaled in terms of the GBDP distance formula d5 and the numbers above the branches indicate the GBDP *pseudo*-bootstrap support values from 100 replications.

3.1.5. Phenotypic Characterization

Plantactinospora sp. S1510^T was able to grow at a pH from 7 to 11, but from all the temperatures tested, only between 28 and 30 °C. No growth was observed over 1% concentration of NaCl (*w*/*v*). *Plantactinospora* sp. S1510^T degraded cellulose, starch, Tween 40, and Tween 80 but not adenine, casein, hypoxanthine, gelatine, guanine, or xanthine. The isolate was also unable to hydrolyse arbutin, allantoin, or urea, and was negative for the reduction of nitrates. Adonitol, L-arabinose, D-fructose, D-galactose, lactose, maltose, L-rhamnose, sucrose, and D-xylose were used as carbon sources by *Plantactinospora* sp. S1510^T, but not D-cellobiose, dextrin, dextran, *myo*-inositol, inuline, D-mannitol, D-mannose, D-ribose, D-sorbitol, L-sorbose, and xylitol. L-alanine, L-asparagine, L-cysteine, L-hydroxyproline, *α-iso*-leucine, L-methionine, L-phenylalanine, L-proline, and L-serine were used by the strain as nitrogen sources, while L-arginine, glycine, L-histidine, L-threonine, L-tyrosine, or L-valine were not. Differences with the closely related strains are indicated in Table S2.

The peptidoglycan of *Plantactinospora* sp. S1510^T included *meso*-diaminopimelic acid and the main sugars detected for this strain were glucose, mannose, ribose, and xylose. The predominant menaquinones were MK-10(H_6) (57%), MK-10(H_8) (29%), MK-9(H_6) (6%), MK-10(H_4) (4%), and MK-9(H_8) (3%). In addition, one unidentified minor component was detected. The polar lipid profile included diphosphatidylglycerol (DPG), phophatidylethanolamine (PE), and phosphatidylinositol (PI) as the main polar lipids, and phosphatidylmethylethanolamine (PEM) and phosphatidylglycerol (PG) as minor components, which corresponds to phospholipid type PII (Figure S2). Major fatty acids (>10%) found for *Plantactinospora* sp. S1510^T included *iso*-$C_{15:0}$ (22.6%), *anteiso*-$C_{15:0}$ (14.4%), *iso*-$C_{16:0}$ (13.3%) and *anteiso*-$C_{17:0}$ (10.0%), other minor components are given at Table S3.

3.2. Genome Mining

The genome analysis shows a high number of genes related to the resistance to toxic compounds, showing genes implicated with a resistance to heavy metals, including cobalt, zinc, cadmium, and mercury, as well as copper homeostasis (Table 2). In addition, several genes related to the resistance to several antimicrobial compounds have been also detected, including tetracycline, fluoroquinolones, and β-lactamases resistance genes. An important number of genes implicated in the membrane transport were found (117), including the ABC transporter systems and specific metal transporters. The determination of biosynthesis gene clusters (BGCs) within the genome sequence using antiSMASH shows the high potential of the strain to produce a whole range of compounds, such as detected 37 BGCs (Table S4). Among them, the most abundant belong to the nonribosomal peptide-synthetases (NRPS) and combinations of them, but also terpenes, polyketide synthases (PKS), and bacteriocins are present (Figure 3).

Table 2. List of genes related with the heavy metal tolerance or transport localized in *Plantactinospora* sp. S1510^T genome.

Length (bp)	Function	Subsystems	Code	Number
999–1026	Alcohol dehydrogenase, zinc-binding domain protein	-none-	-	2
1026	Alcohol dehydrogenase, zinc-binding domain protein	-none-	-	
1368	Apolipoprotein N-acyltransferase (EC 2.3.1.-)/Copper homeostasis protein CutE	Copper homeostasis: copper tolerance, Lipoprotein Biosynthesis, tRNA-methylthiotransferase containing cluster, tRNA-methylthiotransferase containing cluster	cutE	1
792	ATPase component CbiO of energizing module of cobalt ECF transporter	ECF class transporters, Transport of nickel and cobalt	-	1

Table 2. *Cont.*

Length (bp)	Function	Subsystems	Code	Number
933–999	Bifunctional protein: zinc-containing alcohol dehydrogenase; quinone oxidoreductase (NADPH:quinone reductase) (EC 1.1.1.-); Similar to arginate lyase	-none-	-	6
717	ChlI component of cobalt chelatase involved in B12 biosynthesis/ChlD component of cobalt chelatase involved in B12 biosynthesis	-none-	chlD	1
1587	Cobalt-precorrin-2 C20-methyltransferase (EC 2.1.1.130)/Cobalt-precorrin-3b C17-methyltransferase	Cobalamin synthesis, Cobalamin synthesis	-	1
771	Cobalt-precorrin-4 C11-methyltransferase (EC 2.1.1.133)	Cobalamin synthesis	-	1
753	Cobalt-precorrin-6 x reductase (EC 1.3.1.54)	Cobalamin synthesis	-	1
1227	Cobalt-precorrin-6 y C5-methyltransferase (EC 2.1.1.-)/Cobalt-precorrin-6 y C15-methyltransferase [decarboxylating] (EC 2.1.1.-)	-none-	-	1
630	Cobalt-precorrin-8 x methylmutase (EC 5.4.1.2)	Cobalamin synthesis	-	1
1035–1188	Cobalt-zinc-cadmium resistance protein	Cobalt-zinc-cadmium resistance	czc	2
852–915	Cobalt-zinc-cadmium resistance protein CzcD	Cobalt-zinc-cadmium resistance	czcD	2
3657	CobN component of cobalt chelatase involved in B12 biosynthesis	-none-	cobN	1
606	COG4300: Predicted permease, cadmium resistance protein	-none-	-	1
750–822	Conserved membrane protein in copper uptake, YcnI	Copper Transport System	ycnI	2
1770–4425	Copper binding protein, plastocyanin/azurin family	Bacterial hemoglobins, copper transport and blue copper proteins	-	3
210	Copper chaperone	Copper homeostasis	-	1
519–543	Copper resistance protein CopC	Copper Transport System, copper homeostasis	copC	2
954–2139	Copper resistance protein D	Copper homeostasis	copD	3
744	Cytoplasmic copper homeostasis protein cutC	-none-	cutC	1
2076–2289	Lead, cadmium, zinc and mercury transporting ATPase (EC 3.6.3.3) (EC 3.6.3.5); Copper-translocating P-type ATPase (EC 3.6.3.4)	Copper Transport System, copper homeostasis	-	3
1038–1428	Magnesium and cobalt efflux protein CorC	CBSS-56780.10.peg.1536, copper homeostasis: copper tolerance, Glycyl-tRNA synthetase containing cluster, Magnesium transport, tRNA-methylthiotransferase containing cluster	corC	4
1065–1227	Magnesium and cobalt transport protein CorA	Magnesium transport	corA	2
1245	Membrane-associated zinc metalloprotease	-none-	-	1
411–3825	Multicopper oxidase	Copper homeostasis	-	5
840	Predicted cobalt transporter CbtA	Transport of nickel and cobalt	cbtA	1
903	YpfJ protein, zinc metalloprotease superfamily	Broadly distributed proteins not in subsystems	ypfJ	1

Table 2. *Cont.*

Length (bp)	Function	Subsystems	Code	Number
762	Zinc ABC transporter, ATP-binding protein ZnuC	-none-	znuC	1
1023	Zinc ABC transporter, inner membrane permease protein ZnuB	-none-	znuB	1
975	Zinc ABC transporter, periplasmic-binding protein ZnuA	-none-	znuA	1
741	Zinc D-Ala-D-Ala carboxypeptidase (EC 3.4.17.14)	Metallocarboxypeptidases (EC 3.4.17.-)	-	1
1296–1314	Zinc protease	-none-	-	2
1146	Zinc transport protein ZntB	-none-	zntB	1
459	Zinc uptake regulation protein ZUR	Glycyl-tRNA synthetase containing cluster, Oxidative stress	zur	1
405	Zinc-binding protein of the histidine triad (HIT) family	-none-	-	1

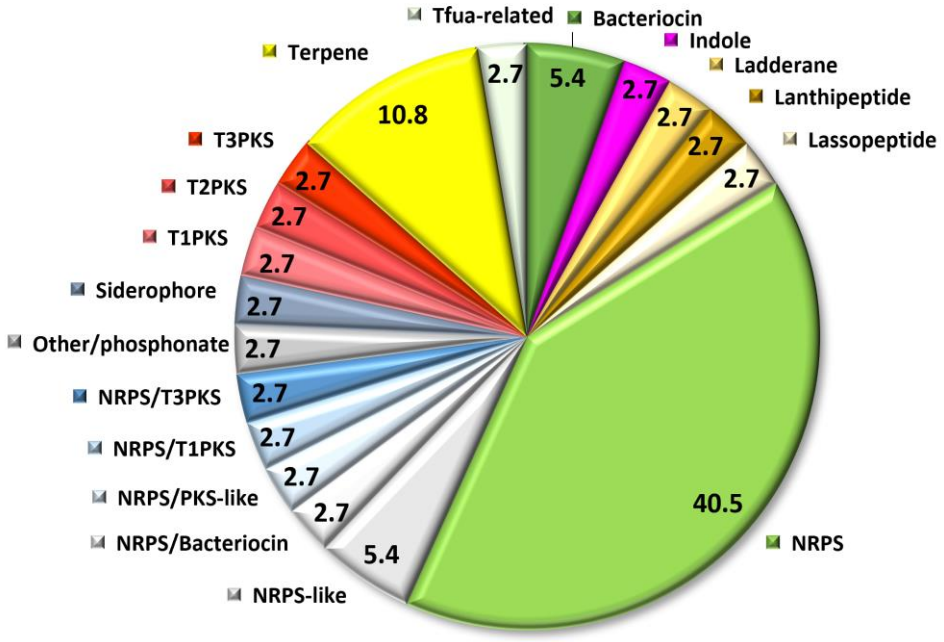

Figure 3. Main groups of biosynthetic gene clusters detected in *Plantactinospora* sp. S1510^{T}. NRPS (Non-ribosomal peptide synthetases); PKS (Poliketyde synthases), T1, T2, and T3 correspond to different types of PKS); tfua-related (Thioamitide ribosomally synthesised and post-translationally modified peptide products (RiPPs)). Values indicate the percentage of genes corresponding to each category.

Another interesting feature identified by the genome analysis of *Plantactinospora* sp. S1510^{T}, is the presence, between its genes, of the whole pathway for the alka(e)ne synthesis, including the genes: *ole*A, a 3-oxoacyl-ACP synthase III; *ole*B, a haloalkane dehalogenase-like protein; *ole*C, an AMP-dependent synthetase/ligase; and *ole*D, a NAD(P)H steroid dehydrogenase-like protein. These genes appear clustered together and surrounded by a tetracycline resistant gene, another NADH dehydrogenase, and a transcriptional regulator, which could be also implicated in the alkane production (Figure 4). In the same line, the genes related to the metabolism of aromatic compounds seem to be quite abundant in the isolated strain (Table 3). Several genes related to the catechol and protocatechuate pathways were found, in addition to the hydroxybenzoate transporters and hydrolases.

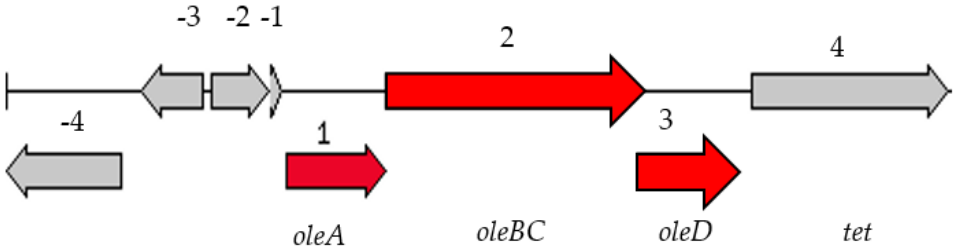

Figure 4. Genome localization and surrounding genes of the alkane synthesis cluster of *Plantactinospora alkalitolerans* S1510^{T}. -4: NADH dehydrogenase (EC 1.6.99.3); -3: Thioredoxin reductase (EC 1.8.1.9); -2: Transcriptional regulator SCO1200, Xre-family with cupin domain; -1: Hypothetical protein; 1: 3-oxoacyl-[ACP] synthase III; 2: Haloalkane dehalogenase-like protein—AMP-dependent synthetase/ligase; 3: NAD(P)H steroid dehydrogenase-like protein; 4: Ribosome protection-type tetracycline resistance. All genes are in contig NODE_88 of the deposited genome sequence.

Table 3. Genes related to the metabolism of aromatic compounds identified in the genome of the *Plantactinospora* strain S1510^{T}.

Genes	Description	No. of Genes Identified
*sal*A	Salicylate hydroxylase (EC 1.14.13.1)	1
*sal*E	Salicylate esterase	1
*qui*B	3-dehydroquinate dehydratase II (EC 4.2.1.10)	1
*bph*C	Biphenyl-2,3-diol 1,2-dioxygenase (EC 1.13.11.39)	1
*pob*A	P-hydroxybenzoate hydroxylase (EC 1.14.13.2)	1
HT	4-hydroxybenzoate transporter	1
HBH	Putative n-hydroxybenzoate hydroxylase	1
GD	Gentisate 1,2-dioxygenase (EC 1.13.11.4)	2
*fah*F	Fumarylacetoacetate hydrolase family protein	2
faa	Fumarylacetoacetase (EC 3.7.1.2)	1
*cat*B	Muconate cycloisomerase (EC 5.5.1.1)	1
*cat*C	Muconolactone isomerase (EC 5.3.3.4)	1
*cat*D	Beta-ketoadipate enol-lactone hydrolase (EC 3.1.1.24)	5
*pca*B	3-carboxy-cis,cis-muconate cycloisomerase (EC 5.5.1.2)	2
*pca*C	4-carboxymuconolactone decarboxylase (EC 4.1.1.44)	1
*pca*D	Beta-ketoadipate enol-lactone hydrolase (EC 3.1.1.24)	5
*pca*G	Protocatechuate 3,4-dioxygenase alpha chain (EC 1.13.11.3)	1
*pca*H	Protocatechuate 3,4-dioxygenase beta chain (EC 1.13.11.3)	1
*pca*I	3-oxoadipate CoA-transferase subunit A (EC 2.8.3.6)	1
*pca*J	3-oxoadipate CoA-transferase subunit B (EC 2.8.3.6)	1
*pca*Q	Pca regulon regulatory protein PcaR	1
HD	Homogentisate 1,2-dioxygenase (EC 1.13.11.5)	1
HPPD	4-hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27)	5
*hmg*R	Transcriptional regulator, IclR family	10

The presence of genes related to the incorporation of alkane compounds from the media was also observed for the *Plantactinospora* sp. S1510^{T} strain, a *ssu*A gene was identified in its genome, an alkanesulfonate ABC transporter substrate-binding protein, as well as a *ssu*D gene, an alkanesulfonate monooxygenase enzyme for its decomposition into an aldehyde. Another gene identified that could have a relationship with the alkanes degradation is the 2-haloalkanoic acid dehalogenase (EC 3.8.1.2), which is implicated in the chloroalkane and chloroalkene degradation pathway. The gene *alm*A, a flavin-binding monooxygenase, related to the oxidation of alkanes C10-C30 [37], was also identified in the genome of *Plantactinospora* sp. S1510^{T}.

3.3. Alkane Degradation

In order to evaluate the in vitro capacity of the strain to degrade alkanes, several compounds were used as sole carbon sources in the agar plates: decane, tetradecane,

hexadecane, and eicosane, observing the growth on the plates containing tetradecane, hexadecane, and eicosane, with the best growth observed for the hexadecane-containing plates; however, no growth was detected over the decane-containing plates.

3.4. Heavy Metal Resistance

Within the genome analysis, we found that *Plantactinospora* sp. S1510^T presented an important number of genes related to the tolerance to heavy metals, so we have evaluated the capacity of the strain to grow over a general media (M65), supplemented with several concentrations of toxic heavy metals that could be present in the habitat of the strain of study. Following 14 days of incubation, the strain showed the ability to develop in the presence of 1 ppm of mercury, 10 ppm of cadmium, 100 ppm of cobalt, 300 ppm of chromium, 2000 ppm of copper and lead, and 5000 ppm of Zn (Table S5).

4. Discussion

During an actinobacterial diversity study of the Black Sea, the strain *Plantactinospora* sp. S1510^T was isolated from the marine sediment at a depth of 12 m, close to the mouth of the Melet river, in Turkey. The high content of heavy metals and other organic pollutants was previously described at several points of this river, including 0.4 μg/g of Cd, 4.5 μg/g of Co, 5.7 μg/g of Cr, 28.4 μg/g of Cu, 18.1 μg/g of Pb and 84 μg/g of Zn in the sediments [2]. The presence of those compounds is probably inducing the development of microorganisms capable of growing in their presence or to degrade them, including our isolate. The genome analysis of the strain confirmed the presence of a whole array of genes related to heavy metal resistance, including metal-binding proteins, metal transporters, metal chelators, metal reductases, and other metal-resistant genes. In addition, our in vitro tests confirm the ability of the strain to grow in the presence of all metals evaluated at least at 1 ppm. Several of the heavy metal concentrations indicated to be tolerated by *Plantactinospora* sp. S1510^T are very high, including the toxic elements, such as Cu or Pb at a concentration of 2000 ppm, lethal for other microorganisms. A concentration of 800 ppm is already considered high for Cu-tolerance [38], but *Plantactinospora* sp. S1510^T can tolerate more than double this concentration, so an efficient method for control or an active transporter should be used by the strain to reduce the inhibitory effect of those metals.

Considering the isolation area and the contamination described, due to the petroleum plants located fairly nearby of the Melet river [1], another interesting feature of *Plantactinospora* sp. S1510^T is the presence of genes related to the degradation of alka(e)ne compounds. As this microorganism has been isolated in a contaminated area, a natural adaptation to tolerate or even degrade these kinds of compounds could be expected. For verifying the function of the predicted genes, according to the genomic information, the capacity of the strain to use alkanes as a unique carbon source was tested in vitro, and found that the strain was able to use tetradecane, hexadecane and eicosane as a carbon source but not decane, indicating a preference for long alkanes, which would be consistent with the presence of the *alm*A gene in the genome of *Plantactinospora* sp. S1510^T.

Both results indicate that there is a potential for the strain of study to be applied in waste management, its capacity to grow in the presence of high concentrations of heavy metals could be related to the accumulation in specific structures within the cell, similarly as has been previously described in other high-concentration heavy metal-tolerant bacteria [39]. However, further investigation is needed to confirm this ability on *Plantactinospora* sp. S1510^T. In the same line, the capacity of the strain to reduce the concentration of alka(e)nes of contaminated waste should be further evaluated.

In addition to the degradation of alkanes, the four *ole* genes, related to alka(e)ne synthesis in bacteria, were identified in the *Plantactinospora* sp. S1510^T genome. None of these genes have been previously described in closely related strains. A previous heterologous expression of *ole* genes from a *Micrococcus luteus* strain in *Escherichia coli* resulted in the production of long chain alkenes (mainly 27:3 and 29:3; no. carbon atoms: no. C=C bonds) [40]. It is not clear what function alkenes have on bacteria, but they

could be related to the protection against temperature changes or dehydration, as have been described for alkene-producing plants [41]. The alkanes have been also described as chemoattractants [37], and their production could be related to the environmental interaction of the strain of study with other microorganisms in its habitat. For both bacteria and plants, it has been described that the decarboxylation of fatty acids generates alkenes. In the case of *M. luteus* it was proposed that alkenes were obtained from *iso*- and *anteiso*-branched C15 saturated acids [40], both of which have the highest *Plantactinospora* sp. S1510^T strain fatty acids profile. To confirm the functionality of those genes and the ability of *Plantactinospora* sp. S1510^T to produce and degrade these compounds, a further study should be conducted.

The strain *Plantactinospora* sp. S1510^T was analysed as well for the presence of the potential production of secondary metabolites, determining the number of biosynthesis gene clusters (BGCs) identified in its genome sequence. A high diversity of potential compounds was detected, together with the abundance of BGCs identified and the low similarities obtained with known compounds highlight the potential of *Plantactinospora* sp. S1510^T to produce novel compounds of interest. This is the first time that a novel taxon of the genus has been analysed for the presence of BGCs, but there are two genome sequences of strains assigned to the *Plantactinospora* genus, for which BGCs were searched, *Plantactinospora* sp. BB1 and *Plantactinospora* sp. BC1, described by Contreras-Castro et al. [42]. From the compounds indicated to be produced by these strains, only fortimicin and lymphostin were identified for the strain *Plantactinospora* sp. S1510^T, indicating a special richness of this genus to produce secondary metabolites, which should be the focus of future studies.

For a taxonomical characterisation of the isolate of the study, a first morphological evaluation was performed, and the results indicate a relationship with the *Micromonosporaceae* family. To evaluate the taxonomic position of the strain within other members of the family the 16S rRNA gene phylogeny was studied, showing a close relationship of the strain *Plantactinospora* sp. S1510^T with *Plantactinospora soyae* NEAU-gxj3^T (99.8%) and *Plantactinospora solaniradicis* NAEU-FJL1^T (99.1%), two endophytic strains isolated from soybean and tomato roots [43,44]. Other similarities with members of the genera *Plantactinospora* were under the threshold proposed for novel species (<98.5%) [34]. *Plantactinospora* sp. S1510^T was branched together with *P. soyae* NEAU-gxj3^T with a high support value (90), confirming this strain as the most closely related to the isolate *Plantactinospora* sp. S1510^T. The dDDH value of *Plantactinospora* sp. S1510^T and *P. soyae* NEAU-gxj3^T is 41.1%, using the recommended formula 2 of the genome-to-genome distance calculator site, confirming that they do not belong to the same species. The phylogenomic tree is quite limited as several members of the genus have not published genomes yet; however, the strain of study is clustered together with other members of the genus *Plantactinospora* available, grouping again with the *P. soyae* type strain, but distant enough to represent a novel species within the genus *Plantactinospora*. In addition, the chemotaxonomic characteristics shown by *Plantactinospora* sp. S1510^T are typical of a member of the genus *Plantactinospora*. However, some differences were observed with *P. soyae*, such as the presence of PEM, MK-10(H_8), mannose, and ribose, and the absence of MK-10(H_2), MK-9(H_4), and galactose [43]. In addition, *P. soyae* uses L-tyrosine as a nitrogen source and D-sorbitol and D-mannitol as carbon sources, while *Plantactinospora* sp. S1510^T does not, and the opposite is observed for the use of L-arabinose, D-fructose, and sucrose as a carbon source, only used by the strain of study. *Plantactinospora* sp. S1510^T is the first *Plantactinospora* to show good growth at 11 pH, being the maximum for other members of the genus values between 8–10. Moreover, the temperature range was more restricted, and was unable to grow at 20 °C or 37 °C, while other members of the genus presented an ample range of temperature tolerance. Taken together in a polyphasic analysis, all of the results for the characterization of the strain *Plantactinospora* sp. S1510^T indicate that it represents a novel taxon within the *Plantactinospora* genus for which the name *Plantactinospora alkalitolerans* is proposed.

5. Conclusions

All of the results presented in this manuscript show the interest of looking for novel bacteria in extreme habitats, in which particular environmental conditions evolutionarily select special characteristics for the microorganisms living there. The polyphasic taxonomic analysis used here supports the description of a novel species within the genus *Plantactinospora*, showing enough phenotypic and genotypic differences to respect other members of the genus. In addition, other capacities, not previously described for the genus were identified for *Plantactinospora* sp. S1510^{T}, such as the potential to produce and degrade alkanes and other aromatic compounds, the ability to grow in the presence of high levels of heavy metals, and the high tolerance to alkaline environments.

Description of *Plantactinospora alkalitolerans* sp., nov.

Plantactinospora alkalitolerans (al.ka.li.to′le.rans. N.L. n. *alkali* alkali; L. part. adj. *tolerans* tolerating; N.L. part. adj., *alkalitolerans* alkali-tolerating).

Gram positive aerobic actinobacterium with a filamentous growth. Single non-motile spores at the end of the hyphal tips. Colonies are folded and raised, and present a whole range of orange colours, from light to vivid orange, depending on the media used. Chemoorganotroph and aerobic. Good growth on ISP2, ISP3, ISP4, and ISP7 agars, intermediate on Bennett's, ISP6, Nutrient, and TSA agars, and poor on Czapek's and ISP5 agars. Grows from 7 to 11 pH (optimum 7–9), at 28–30 °C and at 1% of NaCl (*w*/*v*). Degrades cellulose, starch, Tween 40, and Tween 80 but not adenine, casein, hypoxanthine, gelatine, guanine or xanthine. Catalase positive. Negative for the hydrolysation of arbutin, allantoin, or urea, and reduction of nitrates. Adonitol, L-arabinose, eicosane, D-fructose, D-galactose, hexadecane, lactose, maltose, L-rhamnose, sucrose, tetradecane, and D-xylose are used as carbon sources, but not D-cellobiose, decane, dextrin, dextran, *myo*-inositol, inuline, D-mannitol, D-mannose, D-ribose, D-sorbitol, L-sorbose, and xylitol. L-Alanine, L-asparagine, L-cysteine, L-hydroxyproline, *α-iso*-leucine, L-methionine, L-phenylalanine, L-proline, and L-serine are used as nitrogen sources, but not L-arginine, glycine, L-histidine, L-threonine, L-tyrosine or L-valine. Presents *meso*-diaminopimelic acid, and glucose, mannose, ribose, and xylose in the cell wall. Predominant menaquinones are MK-10(H_6), MK-10(H_8), MK-9(H_6), MK-10(H_4), and MK-9(H_8), and main polar lipids are DPG, PE, and PI. Major fatty acids are *iso*-$C_{15:0}$, *anteiso*-$C_{15:0}$, *iso*-$C_{16:0}$, and *anteiso*-$C_{17:0}$.

The type strain, S1510^{T} (DSM 45923^{T}; KCTC 29205^{T}), was isolated from the sediment at 12 m of the Black Sea, offshore of the Melet river (40°59.650′ N; 37°58.953′ E). The DNA G+C content of the strain is 70.5%. The accession numbers of the 16S rRNA gene and genome sequences are KF494804 and JADPUN000000000, respectively.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d14110947/s1, Figure S1: Cultural characteristics of the strain S1510^{T} after growth on different media; Figure S2: Polar lipid profile of strain S1510^{T} after spraying with molybdatophosphoric acid. DPG: diphosphatidylgycerol; PE: phosphatidylethanolamine; PEM: phosphatidylmehtylethanolamine; PG: phophatidylglycerol; PI: phosphatidylinositol; Table S1: dDDH values of strain S1510^{T} and type strains used in the phylogenomic reconstruction of *Plantactinospora* and *Micromonospora* genera. Values in brackets indicate the confidence intervals obtained using formula d4 in TYGS server (equivalent to GGDC formula 2); Table S2: Differential characteristics of strain *Plantactinospora* sp. S1510^{T} and close related type strains. * Data from Guo et al. 2016 [43] and Li et al. 2018 [44]; Table S3: Fatty acids profile of strain S1510^{T}; Table S4: Biosynthetic gene clusters detected with antiSMASH 5.1.2 tool for strain S1510^{T}; Table S5: Capacity of S1510^{T} to grow in the presence of several concentrations of different metals after 14 days on M65 medium. + good growth (similar to the positive control); w weak growth; - no growth.

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Abbreviations

dDDH: digital DNA-DNA hybridization; DPG: diphosphatidylglycerol; EB: elution buffer; HS: high sensitive; ISCC-NBS: Inter-Society Color Council—National Bureau of Standards; PG: phosphatidylglycerol; PE: phosphatidylethanolamine; PEM: phosphatidylmethylethanolamine; PI: phosphatidylinositol; SDS: sodium dodecyl sulfate; SPRI: solid phase reversible immobilization.

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