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Metabolic Pathway of Phenol Degradation of a Cold-Adapted Antarctic Bacteria, *Arthrobacter* sp.

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Abstract: Phenol is an important pollutant widely discharged as a component of hydrocarbon fuels, but its degradation in cold regions is challenging due to the harsh environmental conditions. To date, there is little information available concerning the capability for phenol biodegradation by indigenous Antarctic bacteria. In this study, enzyme activities and genes encoding phenol degradative enzymes identified using whole genome sequencing (WGS) were investigated to determine the pathway(s) of phenol degradation of *Arthrobacter* sp. strains AQ5-05 and AQ5-06, originally isolated from Antarctica. Complete phenol degradative genes involved only in the ortho-cleavage were detected in both strains. This was validated using assays of the enzymes catechol 1,2-dioxygenase and catechol 2,3-dioxygenase, which indicated the activity of only catechol 1,2-dioxygenase in both strains, in agreement with the results from the WGS. Both strains were psychrotolerant with the optimum temperature for phenol degradation, being between 10 and 15 °C. This study suggests the potential use of cold-adapted bacteria in the bioremediation of phenol pollution in cold environments.

Keywords: phenol; metabolites; whole genome sequencing; xenobiotics

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1. Introduction

Antarctica has long been considered one of the last pristine and remote wilderness areas on Earth. However, in recent years, pollution by hydrocarbons such as polycyclic aromatic hydrocarbons, chlorinated biphenyls, and phenols has become an increasing concern in Antarctica [1–4]. A previous study on the impacts of lubricant oil on Antarctic infauna reported the presence of not readily biodegradable phenol additives in both "conventional" and "biodegradable" lubricants [5]. Hydrocarbons from oil, diesel, and lubricant spills tend to persist due to the harsh conditions in Antarctica [6–8]. Furthermore, aromatic petroleum components such as phenol and phenolic compounds (PCs) are harmful to Antarctic terrestrial and aquatic ecosystems [9,10]. In addition, persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs), are associated with human activities and are subject to long-range atmospheric transport [11]. The direct discharge of phenol into the environment causes severe damage and threats to terrestrial and aquatic ecosystems and, even at low concentrations, it is extremely toxic to aquatic life [10,12–14].

Phenol is a hazardous pollutant that must be removed from the environment [15–17]. Several methods have been published to treat phenol, including the application of physical, chemical, and biological processes. Many studies have shown that bioremediation is more

effective in degrading toxic compounds compared to physicochemical treatments and leads to the complete degradation of these compounds [16,18]. Among the various microorganisms reported, phenol-degrading bacteria are the most extensively studied [1,10,19]. Several reports have indicated that members of the genera *Pseudomonas*, *Rhodococcus*, *Acinetobacter*, and *Arthrobacter* are capable of degrading phenol [20–22]. In cold environments, the bioremediation of polluted sites requires cold-adapted microorganisms [23–25]. A number of phenol-degrading microorganisms exhibiting cold-adapted features have been isolated from cold regions, such as the alpine areas and Antarctica. Certain bacteria are able to utilize phenol as a carbon and energy source under aerobic or anaerobic conditions [26]. The application of next-generation sequencing (NGS) has catalysed a revolution in biodegradation by predicting the genes involved in biodegradation and other metabolic processes of bacteria [27].

Bioremediation is increasingly viewed as an appropriate remediation technology for cleaning industrial wastewater and contaminated sites [3,28,29]. Aromatic pollutants, such as phenol, are recalcitrant and exhibit anti-microbial properties. The main objectives of this study were to identify the pathway(s) of phenol degradation using whole genome sequencing (WGS) and enzyme assays in two phenol-degrading strains of bacteria originally isolated from Antarctica.

2. Results and Discussion

Detection of Phenol Degradative Genes via WGS

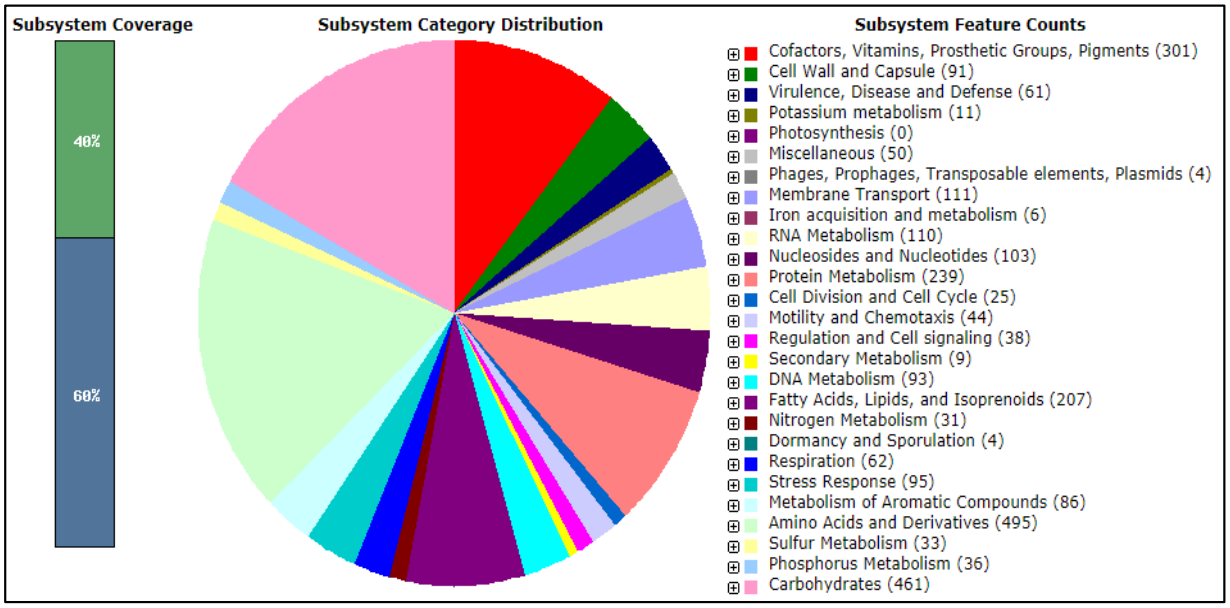
The key structural features of the draft genomes of strains AQ5-05 and AQ5-06 are summarized in Table 1. The draft genome size of strain AQ5-05 was 4647352 base pairs (bp) in 116 contigs with an average G + C content of 65.7%, while strain AQ5-06 was 4,139,264 bp in 70 contigs with an average G + C content of 65.7%. The annotated draft genome of strain AQ5-05 contained a total of 4294 coding sequences (CDSs), 9 rRNAs, and 57 tRNAs and that of strain AQ5-06 contained a total of 3981 CDSs, 3 rRNAs, and 50 tRNAs. In comparison, the total CDSs of completely sequenced cold-adapted *Arthrobacter alpinus* ERGS4:06 and *Arthrobacter* sp. strain FB24 were 3538 and 3279, respectively [30,31]. Members of the genus *Arthrobacter* are known as high-GC-content Actinobacteria, consistent with the findings here and on the previously reported *Arthrobacter* spp. [32–35]. According to Kim et al. [36], members of the genus *Arthrobacter* typically have a DNA GC content of 59 to 66%, in agreement with the genome of strains AQ5-05 and AQ5-06. Similar to the genomic size of strain AQ5-06, a 4.12 Mb draft genome sequence for Arthrobacter sp. strain 7749 isolated from marine sediment samples from the Antarctic Peninsula was reported by Sastre et al. [35].

Table 1. Genomic features of *Arthrobacter* sp. strains AQ5-05 and AQ5-06.

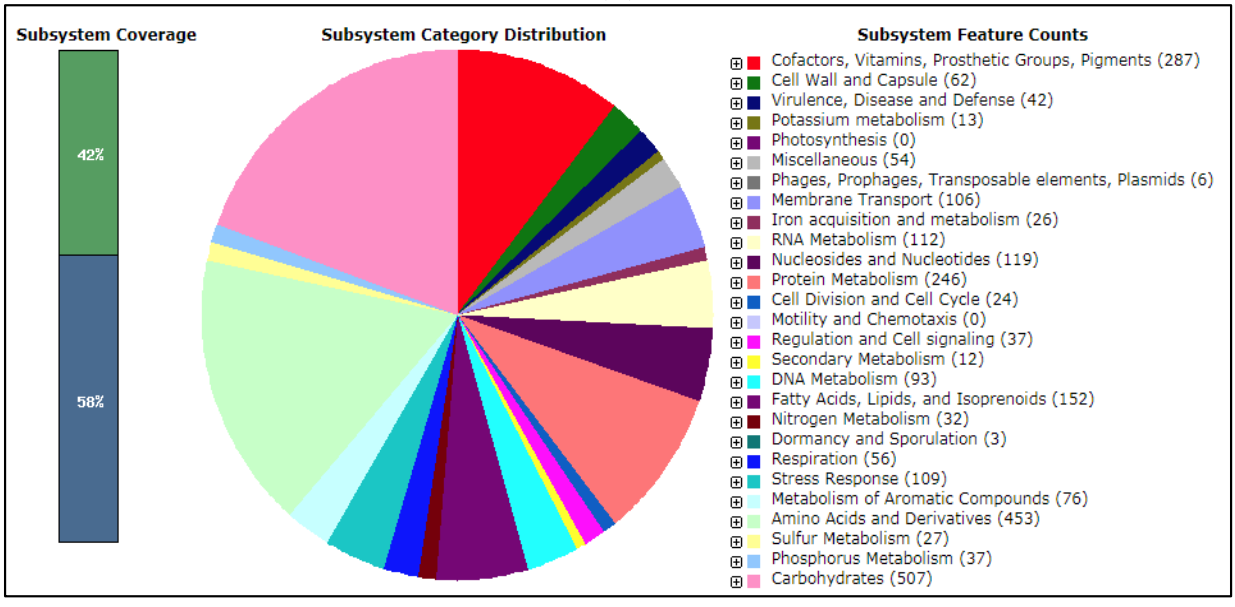
Feature	Count/Value	
	AQ5-05	**AQ5-06**
Genome size (bp)	4,647,352	4,139,264
GC content (%)	65.7	64.9
Number of contigs	116	70
Length of the longest contig (bp)	554,363	406,582
Number of Subsystems	415	411
Number of CDSs	4294	3981
Number of rRNAs	9	3
Number of tRNAs	57	50

Figure 1 displays the number of genes in each subsystem and the subsystem coverage. According to RAST annotation, the genome sequences for strain AQ5-05 are classified into 415 subsystems (Table 1). Based on Figure 1A, 48% of the total CDSs are classified into subsystems, while 68% of the CDSs are not included in the subsystems. The subsystem

of amino acid and its derivatives contained the highest number of coding sequences (495 counts), followed by 461 counts in the subsystem of carbohydrates and 301 counts in the subsystem of the cofactors, vitamins, prosthetic groups, and pigments. Figure 1B presents the overview of each subsystem feature and the subsystem coverage. Forty-two percent of a total of 3981 CDSs were categorised into subsystems, while fifty-eight percent of the CDSs were not categorised into subsystems. The subsystem with the highest number of counts was carbohydrate (507 counts), followed by the subsystem of amino acids and derivatives (453 counts) and 287 counts in the subsystem of cofactors, vitamins, prosthetic groups, and pigments that are essential for maintaining the life of bacterial cells [37].



(**A**)



(**B**)

Figure 1. Subsystem category distribution for *Arthrobacter* sp. Strains AQ5-06 (**A**) and AQ5-06 (**B**) were annotated using the RAST server. The red box marks the genes involved in the metabolism of aromatic compounds.

In order to survive in extremely cold environments, polar bacteria synthesise cold-shock proteins (Csps) in response to rapid temperature declines [38]. In strain AQ5-05,

95 counts of genes were responsible for stress responses with three classified as Csps. Rapid temperature reduction decreases the cell membrane fluidity and enzyme activity, and reduces the efficiency of transcription and translation due to the stabilisation of nucleic acid secondary structures and the inhibition of protein folding and ribosome function [38,39]. The genome of strain AQ5-06 contained 109 counts of the genes responsible for stress responses, including five Csps. Of these, four were classified as cold-shock protein A (CspA) and one as cold-shock protein C (CspC). Many bacteria produce Csps, including psychrophiles, mesophiles, and even thermophiles [38–40]. Dsouza et al. [41] noted that Csps were identified in seven temperate and seven Antarctic *Arthrobacter* genomes examined. Previous studies have noted that the production of CspA is induced by cold shock [38,42]. For instance, the overexpression of CspA was reported in *Psychromonas arctica* KOPRI 22215 isolated from Arctic marine sediment [43]. CspC, responsible for the regulation of the growth and expression of the stress response proteins RpoS and UspA, has also been reported in previous studies [44–46].

Seventy-six counts were categorised in the subsystem for the metabolism of aromatic compounds, with seven counts involved in the central aromatic intermediate metabolism, such as catechol, benzoate, and phenol. Similar findings have been reported in the genomes of several *Arthrobacter* spp., such as *Arthrobacter* sp. Edens01 [32], *Arthrobacter* sp. W1 [47], and *A. antarcticus* strain W2 [48]. Genome analysis of *A. alpinus* R3.8 isolated from a soil sample obtained at Rothera Point, Adelaide Island revealed genes involved in the bioremediation of xenobiotic compounds, including naphthalene [49].

Two phenol biodegradation pathways initiated by the oxygenation of phenol followed by the oxidation of catechol at the 1,2-(ortho) or 2,3-(meta) positions have been reported as being typical aerobic phenol degradation pathways for bacteria [19,50]. According to the gene annotation using RAST, genes encoding phenol degradation genes, including phenol 2-monooxgenase, and enzymes involved in the ortho-cleavage of catechol were present in strain AQ5-05.

The physical maps of the gene clusters for both phenol 2-monooxygenase and catechol 1,2 dioxygenase are shown in Figure 2A, with the genes found in the phenol and catechol degradation gene clusters summarised in Table 2. The results from BLASTP searches revealed that the amino acid sequence of ORF6 shared a 90.1% identity with the phenol 2-monooxygenase of *Arthrobacter* sp. UCD-GKA (accession no. WP_071214692) and 76.9% with the PheA/TfdB family putative FAD monooxygenase from *PaenArthrobacter aurescens* strain TC1 (accession no. WP_011777158). According to Figure 2A, genes encoding the enzymes phosphoribosylformylglycinamidine synthase (ORF1-3), 3-oxoacyl-[acyl-carrier protein] (ORF7), and antibiotic biosynthesis monooxygenase (ORF8) were found within the gene cluster of the phenol 2-monooxygenase of strain AQ5-05. The enzyme of fatty acid biosynthesis, 3-oxoacyl-[acyl-carrier protein] catalyses the first of the two reduction steps in the elongation cycle of fatty acid synthesis, while phosphoribosylformylglycinamidine synthases are involved in the purine biosynthetic pathway [51,52]. The antibiotic biosynthesis monooxygenase of bacteria catalyses oxygen-dependent and cofactor-independent oxidations or monooxygenations involved in the biosynthetic pathways of polyketide antibiotics [53]. Antibiotic coding genes, such as antibiotic biosynthesis monooxygenase, are also present in the genome of *A. crystallopoietes* strain BAB-32, which may have industrial and medical applications [54]. A previous study has reported the presence of antibiotic activities for seven antibiotic-producing *Arthrobacter* spp. isolated from the Arctic Ocean under different culture conditions [55]. However, no other phenol catabolic genes were found within the gene cluster.

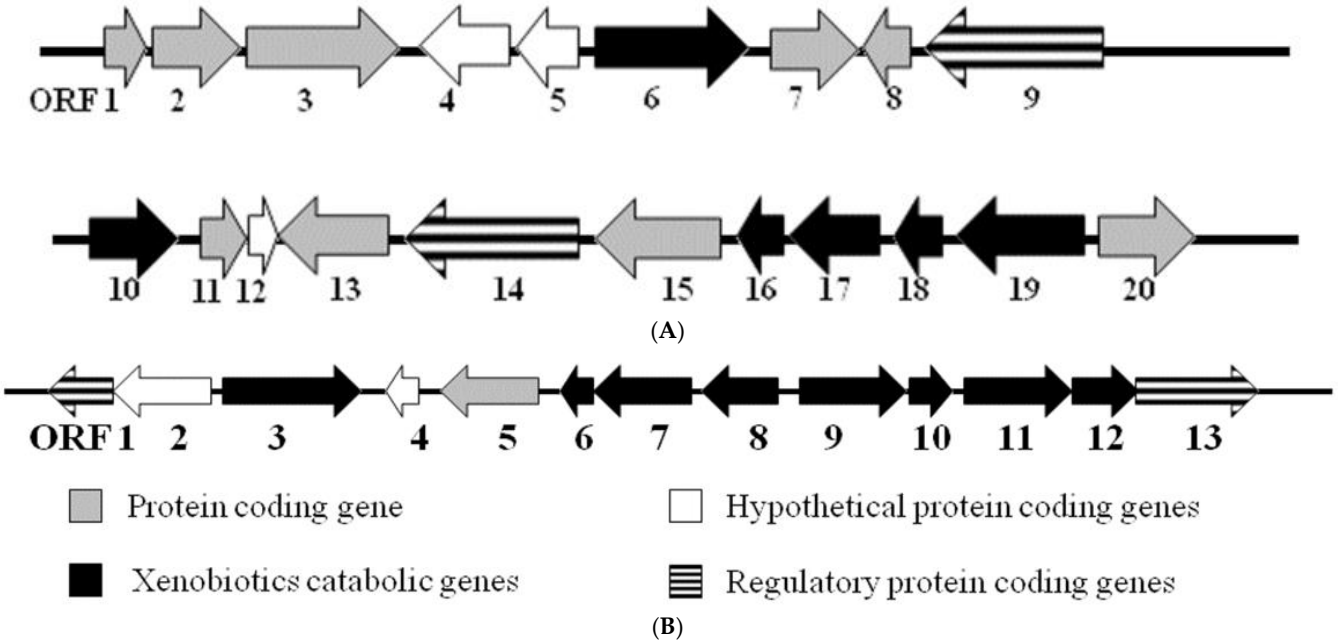


Figure 2. Physical map of gene cluster containing phenol 2-monooxygenase and catechol 1,2-dioxygenase genes from *Arthrobacter* sp. strains AQ5-05 (**A**) and AQ5-06 (**B**).

Table 2. The putative functions of the genes in phenol and catechol degradation gene clusters from *Arthrobacter* sp. strain AQ5-05.

ORF	Putative Functions
1	Phosphoribosylformylglycinamidine synthase subunit, purS
2	Phosphoribosylformylglycinamidine synthase subunit, purQ
3	Phosphoribosylformylglycinamidine synthase subunit, purl
4	Hypothetical protein
5	Hypothetical protein
6	Phenol 2-monooxygenase, PheA/TdfB family
7	3-oxoacyl-[acyl-carrier protein] reductase
8	Antibiotic biosynthesis monooxygenase
9	LuxR family transcriptional regulator
10	Catechol 1,2-dioxygenase, catA
11	Flavin reductase
12	Hypothetical protein
13	Pimeloyl-ACP methyl ester carboxylesterase
14	LuxR family transcriptional regulator
15	AAHS family benzoate transporter-like MFS transporter, benK
16	1,6-dihydroxycyclohexa-2,4-diene-1-carboxylate dehydrogenase, benD
17	Benzoate 1,2-dioxygenase reductase subunit, benC
18	Benzoate 1,2-dioxygenase beta subunit, benB
19	Benzoate 1,2-dioxygenase alpha subunit, benA
20	Benzoate membrane transport protein, benE

In the gene-encoding for enzyme C12O, the catA of strain AQ5-05 was shown as ORF10, sharing a 96.5% amino acid sequence identity with the C12O of *Arthrobacter* sp. UCD-GKA (accession no. WP_071214119). Apart from catA, benzoate catabolic genes, benABCD encoding for benzoate 1,2-dioxygenases and dihydroxycyclohexadiene carboxylate dehydrogenase, and benEK encoding for two benzoate transport proteins, were detected. Similar to the findings of previous studies, catA is chromosomally linked with the ben genes in strain AQ5-05 [56–58]. Two steps are required to convert benzoate to catechol. BenABC encodes for benzoate dioxygenase, which is thought to catalyse the oxidation of benzoate to benzoate diol, followed by decarboxylation to catechol by 1,6-dihydroxycyclohexa-2,4-

diene-1-carboxylate dehydrogenase encoded by benD [59]. Genes encoding pimeloyl-ACP methyl ester carboxylesterase and flavin reductase were also detected within the gene cluster.

According to Figure 2A, both genes encoding putative LuxR family transcriptional regulators were detected in the gene clusters of phenol 2-monooxygenase and C12O. Previous studies have shown that LuxR family transcriptional regulators are activators of the expression of genes encoding enzymes associated with ortho-cleavage through the β-ketoadipate pathway in an aromatic-degrading *Corynebacterium glutamicum* [60,61]. Many transcriptional regulators, including GntR, LuxR, CRP, FNR, TetR, and the Fis family transcriptional regulator, have been identified to be potentially involved in phenol biodegradation in the phenol-degrading *Acinetobacter* sp. DW-1 [62].

Based on the gene annotations, genes encoding the phenol 2-monooxygenase and ortho cleavage enzyme for catechol were also detected in the genome of strain AQ5-06. Figure 2B displays the physical map of the gene cluster containing genes coding for phenol 2-monooxygenase, catechol 1,2 dioxygenase, muconolactone isomerase, and muconate cycloisomerase, and the putative functions of the genes are listed in Table 3.

Table 3. The putative functions of the genes in phenol and catechol catabolic gene clusters from *Arthrobacter* sp. strain AQ5-06.

ORF	Predicted Functions
1	GntR family transcriptional regulator
2	Hypothetical protein
3	Phenol 2-monooxygenase, PheA/tdfB family
4	Hypothetical protein
5	Benzoate transport protein, benE
6	Muconolactone isomerase, catC
7	Muconate cycloisomerase, catB
8	Catechol 1,2- dioxygenase, catA
9	Benzoate 1,2-dioxygenase alpha subunit, benA
10	Benzoate 1,2-dioxygenase beta subunit, benB
11	Benzoate 1,2-dioxygenase reductase subunit, benC
12	1,6-dihydroxycyclohexa-2,4-diene-1-carboxylate dehydrogenase, benD
13	LuxR family transcriptional regulator

Genetic annotation predicted that strain AQ5-06 is capable of degrading phenol only through the ortho pathway as genes encoding for meta-cleavage were absent in the genome. According to Figure 2B, a transcriptional regulator GntR family was detected within the gene cluster, shown as ORF1. Previous studies have reported the involvement of the GntR transcriptional regulator family in the degradation pathways of aromatic compounds [62–64]. For instance, Gu et al. [62] claimed that a GntR-type regulator was potentially involved in phenol degradation in *Acinetobacter* sp. DW-1. Moreover, Rucká et al. [65] reported that the GntR regulator is required besides the XylR regulator for efficient regulation of phenol degradation operons. Teramoto et al. [66] and Arai et al. [67] reported the presence of the GntR family of transcriptional regulators in the expression of the multicomponent phenol hydroxylase (mPH) of *Comamonas testosteroni* spp.

The gene encoding phenol 2-monooxygenase is shown as ORF3, located between two hypothetical proteins with unknown functions. According to the result from BLASTP searches, the amino acid sequence of phenol 2-monooxygenase from strain AQ5-06 shared the highest identity (88.1%) with phenol 2-monooxygenase from *Arthrobacter* sp. B6 (accession no. WP_066285143). Phenol 2-monooxygenase serves as the first enzyme in the ring cleavage of phenol by catalysing the ortho-hydroxylation of phenol to catechol [68,69].

The phenol 2-monooxygenase gene cluster of strain AQ5-06 also harbours the genes coding for both the catechol and benzoate pathways. Catechol catabolic genes, catABC, were detected within the gene cluster and are shown as ORF6-8. Previous studies have demonstrated that several bacteria have catABC genes in one operon responsible for the

first three steps of β-ketoadipade, similar to the gene organisation in strain AQ5-06 [70–72]. The amino acid sequence of catA for strain AQ5-06 showed the highest (94.9%) identity with the catA amino acid sequence of *Arthrobacter* sp. AGTC131 (accession no. WP_104138975.1). Contiguous to the strain AQ5-06 cat operon, benzoate catabolic genes, benABCD, and the benzoate transport protein, benE, were detected within the gene cluster, which is responsible for metabolising benzoate to catechol. Several studies have noted that cat genes are chromosomally linked with ben genes forming a cluster in bacteria [56,73,74]. Adjacent to the benABCD operon, a LuxR family transcriptional regulator was detected. Brinkrolf et al. [60] noted that LuxR family transcriptional regulators are the activators of the expression of genes encoding enzymes associated with ortho-cleavage through the β-ketoadipate pathway in an aromatic-degrading *Corynebacterium glutamicum*.

Bioinformatics analysis revealed the presence of genes related to the phenol and catechol degradation pathways in strains AQ5-06 and AQ5-05 (Figure 3). Based on gene annotation (Figure 3A), the phenol degradation pathway of strain AQ5-05 is most likely to be the ortho-pathway. Detailed information, including the gene name, gene products, locus tags, length of amino acid sequence, KEGG, COG, and Enzyme Commission number (EC No.), is given in Table 4 for strain AQ5-05 and in Table 5 for strain AQ5-06. The PheA/tfdB family gene in strains AQ5-05 and AQ5-06 were predicted to encode phenol 2-monooxygenase, containing 634 amino acid residues that catalysed the oxidation of phenol to catechol.

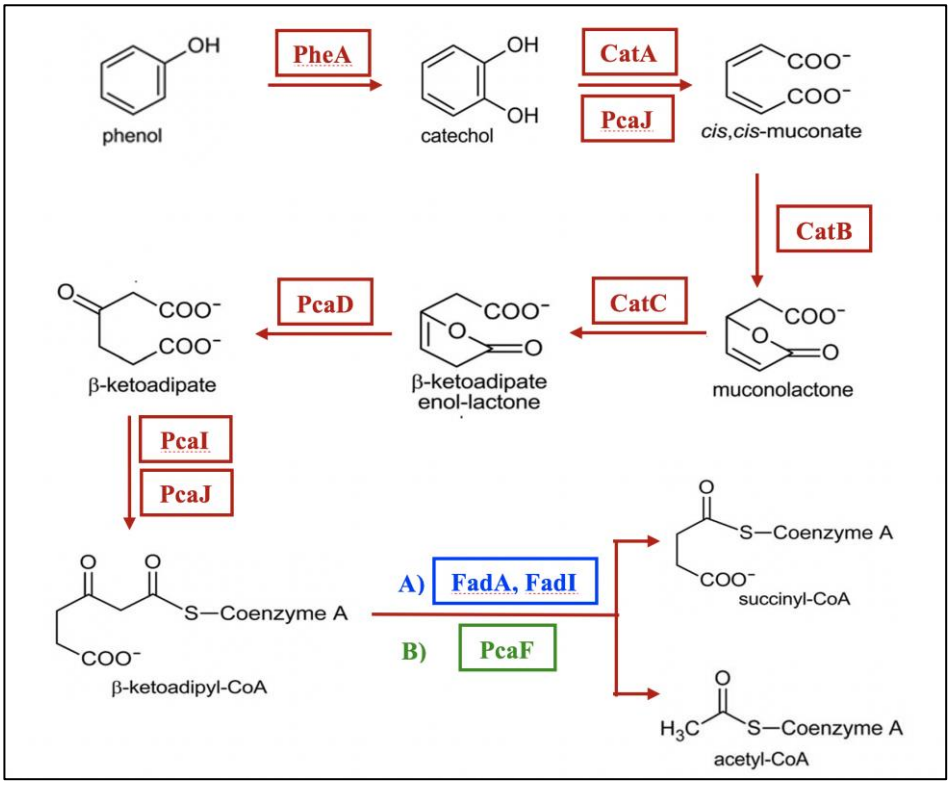


Figure 3. Predicted pathways for the degradation of phenol in *Arthrobacter* sp. strains AQ5-05 (**A**) and AQ5-06 (**B**) based on the presence of gene sequences shown in red fonts.

Table 4. Characteristics of phenol catabolic genes detected in the genome of *Arthrobacter* sp. strain AQ5-05.

Gene Name (Locus Tag)	Gene Product	Amino Acid Residues (aa)	COG No.	KEGG No.	EC No.
PheA (STRA2_03637)	Phenol 2-monooxygenase	634	COG2871	K03380	EC:1.14.13.7
CatA (STRA2_03962)	Catechol 1,2-dioxygenase	284	COG3485	K03381	EC:1.13.11.1
CatB (STRA2_03993)	Muconate cycloisomerase	366	COG4948	K01856	EC:5.5.1.1
CatC (STRA2_03992)	Muconolactone isomerase	92	COG4829	K03464	EC:5.3.3.4
PcaD (STRA2_02926)	3-oxoadipate enol-lactonase	271	COG0596	K01055	EC:3.1.1.24
PcaI (STRA2_02962)	3-oxoadipate coa-transferase, alpha subunit	224	COG1788	K01031	EC:2.8.3.6
PcaJ (STRA2_02963)	3-oxoadipate coa-transferase, beta subunit	223	COG2057	K01032	EC:2.8.3.6
FadA, FadI(STRA2_00274)	Acetyl-coa acyltransferase	412	COG0183	K00632	EC:2.3.1.16

Table 5. Characteristics of phenol catabolic genes found in the genome of *Arthrobacter* sp. strain AQ5-06.

Gene Name (Locus Tag)	Gene Products	Amino Acid Residues (aa)	COG No.	KEGG No.	EC No.
PheA (ASC_01925)	Phenol 2-monooxygenase	640	COG0654	K03380	EC:1.14.13.7
CatA (ASC_01930)	Catechol 1,2-dioxygenase	294	COG3485	K03381	EC:1.13.11.1
CatB (ASC_01929)	Muconate cycloisomerase	359	COG4948	K01856	EC:5.5.1.1
CatC (ASC_01928)	Muconolactone isomerase	92	COG4829	K03464	EC: 5.3.3.4
PcaD (ASC_02352)	3-oxoadipate enol-lactonase	272	COG0596	K01055	EC:3.1.1.24
PcaI (ASC_02349)	3-oxoadipate coa-transferase, alpha subunit	219	COG1788	K01031	EC:2.8.3.6
PcaJ (ASC_02348)	3-oxoadipate coa-transferase, beta subunit	226	COG2057	K01032	EC:2.8.3.6
PcaF (ASC_03031)	3-oxoadipyl-CoA thiolase	413	COG0183	K07823	EC:2.3.1.174

Phenol 2-monooxygenase, also known as phenol hydroxylase, is the first enzyme in phenol degradation that catalyses the hydroxylation of phenol to catechol [50]. The presence of genes coding for phenol 2-monooxygenase or phenol hydroxylase in several *Arthrobacter* spp. has been reported in previous studies [32,75,76]. For example, Nordin et al. [75] revealed that the gene-encoding single-component phenol 2-monooxygenase of 4-chlorophenol-degrading *Arthrobacter chlorophenolicus* A6 shared the highest identity with the PheA gene from the *Pseudomonas* sp. strain EST1001. Qu et al. [76] described a phenol hydroxylase gene cluster (4606 bp), containing six components in the order of KLMNOP from *Arthrobacter* sp. W1. The phenol 2-monooxygenase from strains AQ5-05 and AQ5-06 are highly similar to each other, with 490 identical residues and 53 similar residues within 639 aligned residues (83.8% similarity). A domain for FAD-binding was detected within both proteins, between residue 32 and residue 405. This domain is known for binding to flavin-adenine dinucleotide, which is the cofactor for various flavoprotein oxidoreductase enzymes, including aromatic hydrolases such as salicylate hydrolase, *p*-hydroxybenzoate, and 2,4-dichlorophenol hydroxylase [77]. A conserved domain for the phenol hydroxylase

C-terminal dimerization (thioredoxin-like fold) domain and a highly conserved sequence (DGxxSxxR) for identifying the FAD/NAD(P)H-binding flavoprotein [78] were also identified using Uniprot peptide search utility. These findings suggested that both strains employ class A single-component flavoprotein monooxygenase for phenol hydroxylation. Class A single-component flavoprotein monooxygenases contain a FAD cofactor and depend on NAD(P)H as the external electron donor. Unlike the two-component flavoprotein monooxygenase, single-component flavoprotein monooxygenases can utilize NAD(P)H directly as flavin cofactors.

Catechol is often produced as the intermediate from a wide range of aromatic catabolic pathways [50]. Subsequent to phenol hydroxylation, the catechol intermediate formed is cleaved by C12O encoded by the catA gene in the ortho-pathway. C12O (EC 1.13.11.1) is an intradiol-cleaving enzyme with an Fe^{3+} prosthetic group, whereas C23O (EC 1.13.11.2) has a catalytic iron ion (Fe^{2+}) in each of its four identical subunits and performs extradiol cleavage [79]. As shown in Figure 3, C12O is the key enzyme in the catechol ortho-pathway, initiating an intradiol cleavage reaction of catechol to form cis,cis-muconate (CCMA), with further processing as described earlier. Similar to the catechol pathway of strains AQ5-05 and AQ5-06, Hong et al. [80] suggested the ortho-pathway for the degradation of catechol in hydrocarbon-degrading *Achromabacter* sp. HZ01 isolated from oil-polluted seawater in the South China Sea, which was predicted using genome sequencing. Moreover, Li et al. [81] confirmed the predicted ortho-pathway of phenol degradation for *Arthrobacter sulfureus* strain XSH(1) based on proteomic analysis.

To validate the pathway of phenol degradation in strain AQ5-06 experimentally, the two routes for the degradation of phenol via the ortho- and meta-pathways were tested by quantifying the resulting products. Prior to the enzyme assay, the bacterium was cultured in the presence of phenol to induce the production of catechol dioxygenases. The catechol degradation pathways of strains AQ5-05 and AQ5-06 were experimentally confirmed through enzyme assays of C12O and C23O by measuring the amount of CCMA and 2-HMSA produced, using colorimetric methods [82–84]. The amounts of CCMA and 2-HMSA formed were determined spectrophotometrically at 260 nm and 370 nm, respectively (Figure 4). The data obtained revealed that only CCMA was produced in the presence of catechol, consistent with the activity of only the C12O in both strains. The average rates of CCMA production over 1 h were 0.52 and 0.67 µmol/min, with a maximum specific activity of the C120 of 24 and 20 U/mg for strains AQ5-05 and AQ5-06, respectively.

The results from both the bioinformatics analysis and enzyme assays confirmed that both studied strains can degrade phenol via the ortho-pathway. A recent study concluded that C12O was detected in all of the fifteen cold-adapted phenol-degrading strains isolated and examined from cold regions, while only eight strains produced C23O [85]. Previous studies have reported several C12O enzymes produced by members of the genera *Arthrobacter*, *Acinetobacter*, *Pseudomonas*, and *Rhodococcus* [83,86–88].

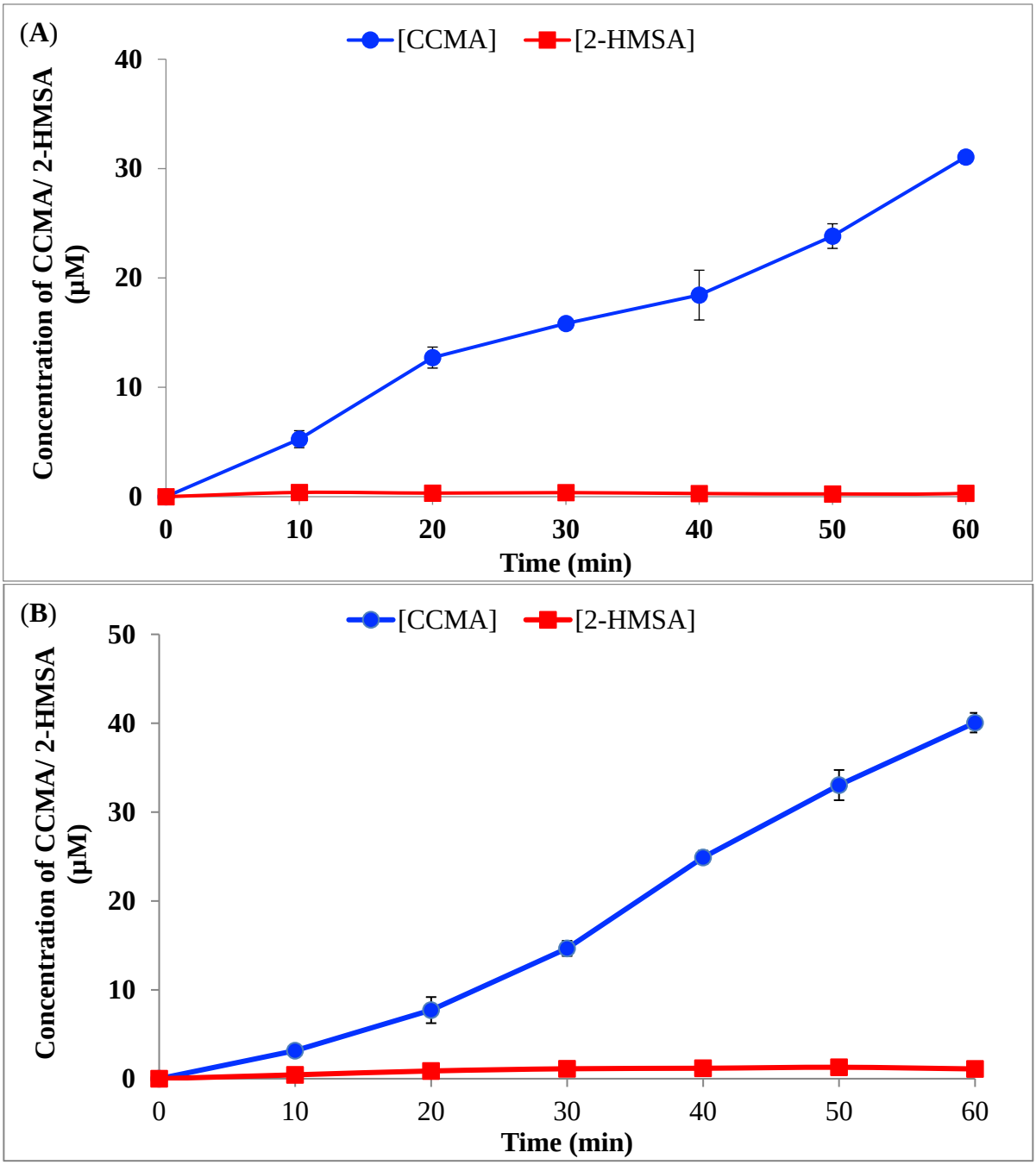


Figure 4. Formation of cis,cis-muconate and 2-hydroxymuconic semialdehyde by catechol dioxygenases of *Arthrobacter* sp. strains AQ5-05 (**A**) and AQ5-06 (**B**). Error bars represent mean ± standard deviation for three replicates.

3. Materials and Methods

3.1. Bacterial Culture and Medium

Phenol-degrading *Arthrobacter* sp. strains AQ5-05 and AQ5-06 were originally isolated from soil obtained on King George Island, South Shetland Islands (maritime Antarctic) [89]. A phenol medium (0.5 g/L) was prepared in 1 L of volume by adding 0.4 g KH_2PO_4, 0.2 g K_2HPO_4, 0.1 g $MgSO_4$, 0.1 g NaCl, 0.01 g $MnSO_4.H_2O$, 0.01 g $Fe_2(SO_4)_3.H_2O$, 0.01 g $Na_2MoO_4.H_2O$, and 0.4 g $(NH_4)_2SO_4$ to distilled water at pH 7.2. The medium was autoclaved for 15 min at 121 °C. The sterilised medium was then augmented with 0.5 g of crystalline phenol.

3.2. Whole Genome Sequencing

3.2.1. Extraction of Genomic DNA

Strains AQ5-05 and AQ5-06 were each cultured in 50 mL of NB in a shaking incubator at 15 °C for 48 h. The cultured broth (1.5 mL) was transferred to an Eppendorf tube and centrifuged at 15,000× *g* for 2 min in order to remove the supernatant. Genomic DNA was extracted using a GeneJET Genomic DNA Extraction Kit (Thermo Scientific, Waltham, MA, USA) and following the manufacturer's protocol. The extracted DNA was examined using 1.0% (*w*/*v*) agarose gel electrophoresis stained with 0.5 µg/mL ethidium bromide (Vivantis, Oceanside, CA, USA) at 100 V for 40 min. Gel electrophoresis was performed using a Lambda/Hind III marker (Vivantis, Shah Alam, Selangor, Malaysia) as the ladder to examine the size of the extracted genomic DNA prior to observation of the gel under UV light. Subsequently, the purity and concentration of extracted genomic DNA were assessed using a Nanodrop spectrophotometer (Bio-Rad, Hercules, CA, USA). The extracted genomic DNA of both strains was sent to Japan's Shizuoka University for whole genome sequencing.

3.2.2. Genome Sequencing and Assembly

The whole genomes of both strains were sequenced using an Illumina MiSeq platform (301 base paired-end reads) at the Instrumental Research Support Office, Research Institute of Green Science and Technology, Shizuoka University, Japan, and the high-quality reads were then assembled using SPAdes version 3.6.2 [90].

3.2.3. Gene Prediction and Annotation

Gene predictions of the genomes of both strains were performed using Prokka 1.12-beta software [91] and the Expert Review version of the Integrated Microbial Genomes (IMG/ER) [92]. Interspersed repetitive sequences were predicted using RepeatMasker [93]. Tandem repeats were analysed by a TRF (Tandem repeats finder) [94]. Transfer RNA (tRNA) genes were predicted by tRNAscan-SE [95]. Ribosomal RNA (rRNA) genes were analysed by rRNAmmer [96]. Small nuclear RNAs (snRNA) were predicted by BLAST against the Rfam database [97]. Annotations of the whole genome sequences were performed using the automated web-based tool, Rapid Annotations using subsystems Technology (RAST) server, with the SEED database [98]. To screen for genes involved in phenol degradation, the amino acid sequences were searched against the protein sequence database from the Basic Local Alignment Search Tool (BLAST) [99], Uniprot [100], the Kyoto Encyclopedia of Genes and Genomes (KEGG) [101], and Clusters of Orthologous Groups (COG) databases [102].

3.3. Preparation of Cell Extracts

Phenol-degrading bacterial strains were cultured in 10 mL of MSM containing 0.7 g/L phenol on a shaking incubator at 150 rpm at 15 °C for 5 d. Cells (2 mL) were then harvested by centrifugation at 4500× *g* for 15 min. This was followed by washing with a 50 mM phosphate buffer at pH 7.5 and resuspension in 2 mL of the same buffer. Eventually, cells were disrupted by sonication treatment with 30-s intervals of sonication and 30-s intervals of interruption for a total of 6 min in an ice-cooled bath [83]. Pellets were removed by centrifugation at 9000× *g* for 30 min at 4 °C. The collected supernatants were used in the subsequent enzyme assays [82,86].

3.4. Enzyme Assay of Catechol 1,2 Dioxygenase (C12O) and Catechol 2,3 Dioxygenase (C23O)

Enzyme activity of C12O was determined by adding the cell-free extract (20 µL) to a 50 mM phosphate buffer (pH 7.5) containing 20 mM Na_2EDTA and 50 mM catechol to give a final volume of 1 mL, according to the protocols previously reported [82–84]. The enzyme activity of C23O was determined spectrophotometrically at 370 nm, based on the formation of 2-hydroxymuconic semialdehyde (2-HMS). Product formation was measured spectrophotometrically for 1 h at 10-min intervals in an ice bath.

4. Conclusions

Bioinformatics analysis of WGS data was used to predict the pathways of phenol degradation based on the gene-associated functions in the phenol degradation of two Antarctic strains of *Arthrobacter* sp. The studied strains originated from Antarctic soils and showed phenol degradation activity in cultures at low temperatures, supporting the potential use of such bacterial strains in future bioremediation protocols for phenol-contaminated sites in cold regions. Based on genome annotation, a number of genes involved in the metabolism of aromatic hydrocarbons were predicted in both strains, consistent with the results of enzymatic analyses which confirmed the presence only of enzymes in the ortho-pathway of the phenol degradation. Genes encoding cold-shock proteins and antifreeze proteins were also detected in the genome annotation.

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References

1. Mazuki, T.A.T.; Shukor, M.Y.; Ahmad, S.A. Bioremediation of phenol in Antarctic: A mini review. *Malays. J. Biochem. Mol. Biol.* **2019**, *22*, 1–6.
2. Chang, Y.-C.; Fuzisawa, S.; Reddy, M.V.; Kobayashi, H.; Yoshida, E.; Yajima, Y.; Hoshino, T.; Choi, D. Degradation of Toxic Compounds at Low and Medium Temperature Conditions Using Isolated Fungus. *CLEAN Soil Air Water* **2016**, *44*, 992–1000. [CrossRef]
3. De Jesus, H.E.; Peixoto, R.S.; Cury, J.; Van Elsas, J.D.; Rosado, A.S. Evaluation of soil bioremediation techniques in an aged diesel spill at the Antarctic Peninsula. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 10815–10827. [CrossRef] [PubMed]
4. Roslee, A.F.A.; Zakaria, N.N.; Convey, P.; Zulkharnain, A.; Lee, G.L.Y.; Gomez-Fuentes, C.; Ahmad, S.A. Statistical optimisation of growth conditions and diesel degradation by the Antarctic bacterium, *Rhodococcus* sp. strain AQ5-07. *Extremophiles* **2019**, *24*, 277–291. [CrossRef]
5. Thompson, B.A.W.; Goldsworthy, P.M.; Riddle, M.J.; Snape, I.; Stark, J.S. Contamination effects by a 'conventional' and a 'biodegradable' lubricant oil on infaunal recruitment to Antarctic sediments: A field experiment. *J. Exp. Mar. Biol. Ecol.* **2007**, *340*, 213–226. [CrossRef]
6. Zakaria, N.N.; Man, Z.; Zulkharnain, A.; Ahmad, S.A. Psychrotolerant biosurfactant-producing bacteria for hydrocarbon degradation: A mini review. *Malays. J. Biochem. Mol. Biol.* **2019**, *21*, 52–59.
7. Powell, S.M.; Harvey, P.M.; Stark, J.S.; Snape, I.; Riddle, M.J. Biodegradation of petroleum products in experimental plots in Antarctic marine sediments is location dependent. *Mar. Pollut. Bull.* **2007**, *54*, 434–440. [CrossRef]
8. Abdulrasheed, M.; Zakaria, N.N.; Roslee, A.F.A.; Shukor, M.Y.; Zulkharnain, A.; Napis, S.; Convey, P.; Alias, S.A.; Gonzalez-Rocha, G.; Ahmad, S.A. Biodegradation of diesel oil by cold-adapted bacterial strains of *Arthrobacter* spp. from Antarctica. *Antarct. Sci.* **2020**, *32*, 341–353. [CrossRef]
9. Lana, N.B.; Berton, P.; Covaci, A.; Ciocco, N.F.; Barrera-Oro, E.; Atencio, A.; Altamirano, J.C. Fingerprint of persistent organic pollutants in tissues of Antarctic notothenioid fish. *Sci. Total Environ.* **2014**, *499*, 89–98. [CrossRef]

10. Subramaniam, K.; Ahmad, S.A.; Shaharuddin, N.A. Mini review on phenol biodegradation in Antarctica using native microorganisms. *Asia Pac. J. Mol. Biol. Biotechnol.* **2020**, *28*, 77–89. [CrossRef]
11. Vecchiato, M.; Zambon, S.; Argiriadis, E.; Barbante, C.; Gambaro, A.; Piazza, R. Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in Antarctic ice-free areas: Influence of local sources on lakes and soils. *Microchem. J.* **2015**, *120*, 26–33. [CrossRef]
12. Hill, J.; Nelson, E.; Tilman, D.; Polasky, S.; Tiffany, D. Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 11206–11210. [CrossRef] [PubMed]
13. Koshy, J.; Chandran, N.; Nambisan, P. Biodegradation of phenol using spent substrate of *Pleurotus* sp. *World J. Pharm. Pharm. Sci.* **2012**, *1*, 656–661.
14. Jame, S.A.; Alam, A.R.; Alam, M.K.; Fakhruddin, A. Isolation and Identification of Phenol and Monochlorophenols-Degrading Bacteria: Pseudomonas and Aeromonas Species. *Bangladesh J. Microbiol.* **1970**, *25*, 41–44. [CrossRef]
15. Ahmad, S.A.; Syed, M.A.; Arif, N.M.; Shukor, M.Y.A.; Shamaan, N.A. Isolation, identification and characterization of elevated phenol degrading *Acinetobacter* sp. strain AQ5NOL 1. *Aust. J. Basic Appl. Sci.* **2011**, *5*, 1035–1045.
16. Villegas, L.G.C.; Mashhadi, N.; Chen, M.; Mukherjee, D.; Taylor, K.E.; Biswas, N. A Short Review of Techniques for Phenol Removal from Wastewater. *Curr. Pollut. Rep.* **2016**, *2*, 157–167. [CrossRef]
17. Subramaniam, K.; Shaharuddin, N.A.; Tengku-Mazuki, T.A.; Zulkharnain, A.; Khalil, K.A.; Convey, P.; Ahmad, S.A.; Survey, N.B.A. Statistical optimisation for enhancement of phenol biodegradation by the Antarctic soil bacterium *Arthrobacter* sp. strain AQ5-15 using response surface methodology. *J. Environ. Biol.* **2020**, *41*, 1560–1569. [CrossRef]
18. O'Neill, T.A.; Aislabie, J.; Balks, M.R. Human Impacts on Soils. In *The Soils of Antarctica*; Bockheim, J.G., Ed.; Springer International Publishing: Cham, Switzerland, 2015; pp. 281–303. [CrossRef]
19. Basha, K.M.; Rajendran, A.; Thangavelu, V. Recent advances in the biodegradation of phenol: A review. *Asian J. Exp. Biol. Sci.* **2010**, *1*, 219–234.
20. Zakaria, N.N.; Ahmad, S.A.; Yin, G.L.L.; Yasid, N.A.; Manogaran, M.; Nawawi, N.M.; Shukor, M.Y. Biodegradation of phenol by Antarctic bacterium *Rhodococcus baikonurensis* strain AQ5-001 in the presence of heavy metals. *Malays. J. Biochem. Mol. Biol.* **2018**, *21*, 29–36.
21. Liu, Z.; Xie, W.; Li, D.; Peng, Y.; Li, Z.; Liu, S. Biodegradation of Phenol by Bacteria Strain *Acinetobacter calcoaceticus* PA Isolated from Phenolic Wastewater. *Int. J. Environ. Res. Public Health* **2016**, *13*, 300. [CrossRef]
22. Ahmad, S.A.; Asokan, G.; Yasid, N.A.; Nawawi, N.M.; Subramaniam, K.; Zakaria, N.N.; Shukor, M.Y. Effect of heavy metals on biodegradation of phenol by Antarctic bacterium: *Arthrobacter bambusae* strain AQ5-003. *Malays. J. Biochem. Mol. Biol.* **2018**, *21*, 47–51.
23. Fernández, P.M.; Martorell, M.M.; Blaser, M.G.; Ruberto, L.A.M.; de Figueroa, L.I.C.; Mac Cormack, W.P. Phenol degradation and heavy metal tolerance of Antarctic yeasts. *Extremophiles* **2017**, *21*, 445–457. [CrossRef] [PubMed]
24. Abdulrasheed, M.; Zulkharnain, A.; Zakaria, N.N.; Roslee, A.; Khalil, K.A.; Napis, S.; Convey, P.; Gomez-Fuentes, C.; Ahmad, S. Response Surface Methodology Optimization and Kinetics of Diesel Degradation by a Cold-Adapted Antarctic Bacterium, *Arthrobacter* sp. Strain AQ5-05. *Sustainability* **2020**, *12*, 6966. [CrossRef]
25. Tengku-Mazuki, T.A.; Subramaniam, K.; Zakaria, N.N.; Convey, P.; Khalil, K.A.; Lee, G.L.Y.; Zulkharnain, A.; Shaharuddin, N.A.; Ahmad, S.A. Optimization of phenol degradation by Antarctic bacterium *Rhodococcus* sp. *Antarct. Sci.* **2020**, *32*, 486–495. [CrossRef]
26. Shibata, A.; Inoue, Y.; Katayama, A. Aerobic and anaerobic biodegradation of phenol derivatives in various paddy soils. *Sci. Total Environ.* **2006**, *367*, 979–987. [CrossRef]
27. Arora, P.K.; Bae, H. Integration of bioinformatics to biodegradation. *Biol. Proced. Online* **2014**, *16*, 8. [CrossRef]
28. Darham, S.; Gomez-Fuentes, C.; Zulkharnain, A.; Sabri, S.; Calisto-Ulloa, N.; Ramírez-Moreno, N.; Ahmad, S.A. Isolation and identification of molybdenum-reducing cold-adapted marine bacteria isolated from Bernardo O'Higgins Riquelme Base Station, Antarctica. *Malay. J. Biochem. Mol. Biol.* **2019**, *22*, 16–19.
29. Ibrahim, S.; Zahri, K.N.M.; Convey, P.; Khalil, K.A.; Gomez-Fuentes, C.; Zulkarnain, A.; Alias, S.A.; González-Rocha, G.; Ahmad, S.A. Optimisation of biodegradation conditions for waste canola oil by cold-adapted *Rhodococcus* sp. AQ5-07 from Antarctica. *Electron. J. Biotechnol.* **2020**, *48*, 1–12. [CrossRef]
30. Kumar, R.; Singh, D.; Swarnkar, M.K.; Singh, A.K.; Kumar, S. Complete genome sequence of *Arthrobacter alpinus* ERGS4:06, a yellow pigmented bacterium tolerant to cold and radiations isolated from Sikkim Himalaya. *J. Biotechnol.* **2016**, *220*, 86–87. [CrossRef]
31. Nakatsu, C.H.; Barabote, R.; Thompson, S.; Bruce, D.; Detter, C.; Brettin, T.; Han, C.; Beasley, F.; Chen, W.; Konopka, A.; et al. Complete genome sequence of *Arthrobacter* sp. strain FB24. *Stand. Genom. Sci.* **2013**, *9*, 106–116. [CrossRef]
32. Couger, M.B.; Hanafy, R.A.; Edens, C.; Budd, C.; French, D.P.; Hoff, W.D.; Elshahed, M.S.; Youssef, N.H. Draft Genome of the *Arthrobacter* sp. Strain Edens01. *Genome Announc.* **2015**, *3*, e01475-15. [CrossRef] [PubMed]
33. Kincheloe, G.N.; Eisen, J.A.; Coil, D.A. Draft Genome Sequence of *Arthrobacter* sp. Strain UCD-GKA (Phylum *Actinobacteria*). *Genome Announc.* **2017**, *5*, e01599-16. [CrossRef] [PubMed]
34. Manzanera, M.; Santa-Cruz-Calvo, L.; Vílchez, J.I.; García-Fontana, C.; Silva-Castro, G.A.; Calvo, C.; González-López, J. Genome Sequence of *Arthrobacter siccitolerans* 4J27, a Xeroprotectant-Producing Desiccation-Tolerant Microorganism. *Genome Announc.* **2014**, *2*, e00526-14. [CrossRef] [PubMed]

35. Sastre, D.E.; Santos, L.P.; Kagohara, E.; Andrade, L.H. Draft Whole-Genome Sequence of Psychrotrophic *Arthrobacter* sp. Strain 7749, Isolated from Antarctic Marine Sediments with Applications in Enantioselective Alcohol Oxidation. *Genome Announc.* **2017**, *5*, e01197-17. [CrossRef]
36. Kim, J.; Kim, S.J.; Kim, S.H.; Moon, Y.-J.; Park, S.-J.; Kim, S.I.; Kahng, H.-Y.; Chung, Y.-H. Genome Sequence of *Arthrobacter* sp. MWB30, Isolated from a Crude Oil-Contaminated Seashore. *Genome Announc.* **2015**, *3*, e00013-15. [CrossRef]
37. Lau, Y.Y.; Yin, W.-F.; Chan, K.-G. *Enterobacter asburiae* Strain L1: Complete Genome and Whole Genome Optical Mapping Analysis of a Quorum Sensing Bacterium. *Sensors* **2014**, *14*, 13913–13924. [CrossRef]
38. Keto-Timonen, R.; Hietala, N.; Palonen, E.; Hakakorpi, A.; Lindström, M.; Korkeala, H. Cold Shock Proteins: A Minireview with Special Emphasis on Csp-family of Enteropathogenic Yersinia. *Front. Microbiol.* **2016**, *7*, 1151. [CrossRef]
39. Kim, M.-J.; Lee, Y.K.; Lee, H.K.; Im, H. Characterization of Cold-Shock Protein A of Antarctic *Streptomyces* sp. AA8321. *J. Protein Chem.* **2007**, *26*, 51–59. [CrossRef]
40. Uh, J.-H.; Jung, Y.H.; Lee, Y.K.; Lee, H.K.; Im, H. Rescue of a cold-sensitive mutant at low temperatures by cold shock proteins from *Polaribacter irgensii* KOPRI 22228. *J. Microbiol.* **2010**, *48*, 798–802. [CrossRef]
41. Dsouza, M.; Taylor, M.W.; Turner, S.J.; Aislabie, J. Genomic and phenotypic insights into the ecology of Arthrobacter from Antarctic soils. *BMC Genom.* **2015**, *16*, 36. [CrossRef]
42. Uppal, S.; Akkipeddi, V.S.R.; Jawali, N. Posttranscriptional regulation of *cspE* in *Escherichia coli*: Involvement of the short 5′-untranslated region. *FEMS Microbiol. Lett.* **2008**, *279*, 83–91. [CrossRef] [PubMed]
43. Jung, Y.H.; Yi, J.-Y.; Jung, H.J.; Lee, Y.K.; Lee, H.K.; Naicker, M.C.; Uh, J.-H.; Jo, I.S.; Jung, E.J.; Im, H. Overexpression of Cold Shock Protein A of *Psychromonas arctica* KOPRI 22215 Confers Cold-Resistance. *J. Protein Chem.* **2010**, *29*, 136–142. [CrossRef]
44. Cohen-Or, I.; Shenhar, Y.; Biran, D.; Ron, E.Z. CspC regulates rpoS transcript levels and complements hfq deletions. *Res. Microbiol.* **2010**, *161*, 694–700. [CrossRef] [PubMed]
45. Phadtare, S.; Inouye, M. Role of CspC and CspE in Regulation of Expression of RpoS and UspA, the Stress Response Proteins in *Escherichia coli*. *J. Bacteriol.* **2001**, *183*, 1205–1214. [CrossRef] [PubMed]
46. Shenhar, Y.; Biran, D.; Ron, E.Z. Resistance to environmental stress requires the RNA chaperones CspC and CspE. *Environ. Microbiol. Rep.* **2012**, *4*, 532–539. [CrossRef] [PubMed]
47. Jiang, Y.; Qu, Y.; Xu, P.; Tang, H. Genome Sequence of a Versatile Aromatic Hydrocarbon-Degrading Bacterium, *Arthrobacter* sp. W1. *Genome Announc.* **2015**, *3*, e00387-15. [CrossRef] [PubMed]
48. Herschend, J.; Raghupathi, P.K.; Røder, H.L.; Sørensen, S.J.; Burmølle, M. Genome Sequence of *Arthrobacter antarcticus* Strain W2, Isolated from a Slaughterhouse. *Genome Announc.* **2016**, *4*, e00073-16. [CrossRef]
49. See-Too, W.-S.; Ee, R.; Lim, Y.-L.; Convey, P.; Pearce, D.A.; Mohidin, T.B.M.; Yin, W.-F.; Chan, K.G. Complete genome of *Arthrobacter alpinus* strain R3.8, bioremediation potential unraveled with genomic analysis. *Stand. Genom. Sci.* **2017**, *12*, 52. [CrossRef]
50. Nair, C.I.; Jayachandran, K.; Shashidhar, S. Biodegradation of phenol. *Afr. J. Biotechnol.* **2008**, *7*, 4951–4958.
51. Batra, R.; Christendat, D.; Edwards, A.; Arrowsmith, C.; Tong, L. Crystal structure of MTH169, a crucial component of phosphoribosylformylglycinamidine synthetase. *Proteins Struct. Funct. Bioinform.* **2002**, *49*, 285–288. [CrossRef]
52. Lai, C.-Y.; Cronan, J.E. Isolation and Characterization of β-Ketoacyl-Acyl Carrier Protein Reductase (*fabG*) Mutants of *Escherichia coli* and *Salmonella enterica* Serovar Typhimurium. *J. Bacteriol.* **2004**, *186*, 1869–1878. [CrossRef] [PubMed]
53. Machovina, M.M.; Usselman, R.J.; DuBois, J.L. Monooxygenase Substrates Mimic Flavin to Catalyze Cofactorless Oxygenations. *J. Biol. Chem.* **2016**, *291*, 17816–17828. [CrossRef] [PubMed]
54. Joshi, M.N.; Pandit, A.S.; Sharma, A.; Pandya, R.V.; Desai, S.M.; Saxena, A.K.; Bagatharia, S.B. Draft Genome Sequence of *Arthrobacter crystallopoietes* Strain BAB-32, Revealing Genes for Bioremediation. *Genome Announc.* **2013**, *1*, e00452-13. [CrossRef]
55. Wietz, M.; Månsson, M.; Bowman, J.S.; Blom, N.S.; Ng, Y.; Gram, L. Wide Distribution of Closely Related, Antibiotic-Producing Arthrobacter Strains throughout the Arctic Ocean. *Appl. Environ. Microbiol.* **2012**, *78*, 2039–2042. [CrossRef] [PubMed]
56. Li, D.; Yan, Y.; Ping, S.; Chen, M.; Zhang, W.; Li, L.; Lin, W.; Geng, L.; Liu, W.; Lin, M. Genome-wide investigation and functional characterization of the β-ketoadipate pathway in the nitrogen-fixing and root-associated bacterium *Pseudomonas stutzeri*A1501. *BMC Microbiol.* **2010**, *10*, 36. [CrossRef]
57. Moreno, M.D.L.; Sânchez-Porro, C.; Piubeli, F.; Frias, L.; García, M.T.; Mellado, E. Cloning, Characterization and Analysis of cat and ben Genes from the Phenol Degrading Halophilic Bacterium *Halomonas organivorans*. *PLoS ONE* **2011**, *6*, e21049. [CrossRef]
58. Suzuki, K.; Ichimura, A.; Ogawa, N.; Hasebe, A.; Miyashita, K. Differential Expression of Two Catechol 1,2-Dioxygenases in *Burkholderia* sp. Strain TH2. *J. Bacteriol.* **2002**, *184*, 5714–5722. [CrossRef]
59. Haußmann, U.; Qi, S.-W.; Wolters, D.; Rögner, M.; Liu, S.-J.; Poetsch, A. Physiological adaptation of *Corynebacterium glutamicum* to benzoate as alternative carbon source—A membrane proteome-centric view. *Proteomics* **2009**, *9*, 3635–3651. [CrossRef]
60. Brinkrolf, K.; Brune, I.; Tauch, A. Transcriptional regulation of catabolic pathways for aromatic compounds in *Corynebacterium glutamicum*. *Genet. Mol. Res.* **2006**, *5*, 773–789.
61. Cramer, A.; Gerstmeir, R.; Schaffer, S.; Bott, M.; Eikmanns, B.J. Identification of RamA, a Novel LuxR-Type Transcriptional Regulator of Genes Involved in Acetate Metabolism of *Corynebacterium glutamicum*. *J. Bacteriol.* **2006**, *188*, 2554–2567. [CrossRef]
62. Gu, Q.; Wu, Q.; Zhang, J.; Guo, W.; Wu, H.; Sun, M. *Acinetobacter* sp. DW-1 immobilized on polyhedron hollow polypropylene balls and analysis of transcriptome and proteome of the bacterium during phenol biodegradation process. *Sci. Rep.* **2017**, *7*, 4863. [CrossRef] [PubMed]

63. Ferrández, A.; Miñambres, B.; García, B.; Olivera, E.R.; Luengo, J.M.; García, J.L.; Diaz, E. Catabolism of Phenylacetic Acid in *Escherichia coli* characterization of a new aerobic hybrid pathway. *J. Biol. Chem.* **1998**, *273*, 25974–25986. [CrossRef] [PubMed]
64. Tropel, D.; van der Meer, J.R. Bacterial Transcriptional Regulators for Degradation Pathways of Aromatic Compounds. *Microbiol. Mol. Biol. Rev.* **2004**, *68*, 474–500. [CrossRef]
65. Rucká, L.; Nešvera, J.; Pátek, M. Biodegradation of phenol and its derivatives by engineered bacteria: Current knowledge and perspectives. *World J. Microbiol. Biotechnol.* **2017**, *33*, 174. [CrossRef] [PubMed]
66. Teramoto, M.; Harayama, S.; Watanabe, K. PhcS Represses Gratuitous Expression of Phenol-Metabolizing Enzymes in *Comamonas testosteroni* R5. *J. Bacteriol.* **2001**, *183*, 4227–4234. [CrossRef]
67. Arai, H.; Akahira, S.; Ohishi, T.; Kudo, T. Adaptation of *Comamonas testosteroni* TA441 to utilization of phenol by spontaneous mutation of the gene for a trans-acting factor. *Mol. Microbiol.* **2002**, *33*, 1132–1140. [CrossRef]
68. Al-Khalid, T.; El-Naas, M. Aerobic Biodegradation of Phenols: A Comprehensive Review. *Crit. Rev. Environ. Sci. Technol.* **2012**, *42*, 1631–1690. [CrossRef]
69. Kirchner, U.; Westphal, A.H.; Müller, R.; van Berkel, W.J.H. Phenol Hydroxylase from *Bacillus thermoglucosidasius* A7, a Two-protein Component Monooxygenase with a Dual Role for FAD. *J. Biol. Chem.* **2003**, *278*, 47545–47553. [CrossRef]
70. Eulberg, D.; Schlömann, M. The putative regulator of catechol catabolism in *Rhodococcus opacus* 1CP–an IclR-type, not a LysR-type transcriptional regulator. *Antonie Leeuwenhoek* **1998**, *74*, 71–82. [CrossRef]
71. Kim, S.I.; Ha, K.-S.; Leem, S.-H. Differential organization and transcription of the cat2 gene cluster in aniline-assimilating *Acinetobacter lwoffii* K24. *J. Biosci. Bioeng.* **1999**, *88*, 250–257. [CrossRef]
72. Matsumura, E.; Sakai, M.; Hayashi, K.; Murakami, S.; Takenaka, S.; Aoki, K. Constitutive expression of *catABC* genes in the aniline-assimilating bacterium *Rhodococcus* species AN-22: Production, purification, characterization and gene analysis of CatA, CatB and CatC. *Biochem. J.* **2005**, *393*, 219–226. [CrossRef] [PubMed]
73. Kim, D.; Kim, S.W.; Choi, K.Y.; Lee, J.S.; Kim, E. Molecular cloning and functional characterization of â€ƒthe genes encoding benzoate and p-hydroxybenzoate degradation by the halophilic *Chromohalobacter* sp. strain HS-2. *FEMS Microbiol. Lett.* **2008**, *280*, 235–241. [CrossRef] [PubMed]
74. Patrauchan, M.A.; Florizone, C.; Dosanjh, M.; Mohn, W.W.; Davies, J.; Eltis, L.D. Catabolism of Benzoate and Phthalate in *Rhodococcus* sp. Strain RHA1: Redundancies and Convergence. *J. Bacteriol.* **2005**, *187*, 4050–4063. [CrossRef] [PubMed]
75. Nordin, K.; Unell, M.; Jansson, J.K. Novel 4-Chlorophenol Degradation Gene Cluster and Degradation Route via Hydroxyquinol in *Arthrobacter chlorophenolicus* A6. *Appl. Environ. Microbiol.* **2005**, *71*, 6538–6544. [CrossRef]
76. Qu, Y.; Shi, S.; Zhou, H.; Ma, Q.; Li, X.; Zhang, X.; Zhou, J. Characterization of a Novel Phenol Hydroxylase in Indoles Biotranformation from a Strain *Arthrobacter* sp. W1. *PLoS ONE* **2012**, *7*, e44313. [CrossRef]
77. Kälin, M.; Neujahr, H.Y.; Weissmahr, R.N.; Sejlitz, T.; Jöhl, R.; Fiechter, A.; Reiser, J. Phenol hydroxylase from *Trichosporon cutaneum*: Gene cloning, sequence analysis, and functional expression in *Escherichia coli*. *J. Bacteriol.* **1992**, *174*, 7112–7120. [CrossRef]
78. Eppink, M.H.M.; Van Berkel, W.J.H.; Schreuder, H.A. Identification of a novel conserved sequence motif in flavoprotein hydroxylases with a putative dual function in FAD/NAD(P)H binding. *Protein Sci.* **2008**, *6*, 2454–2458. [CrossRef]
79. Silva, A.S.; Camargo, F.; Andreazza, R.; Jacques, R.J.S.; Baldoni, D.B.; Bento, F.M. Enzymatic activity of catechol 1,2-dioxygenase and catechol 2,3-dioxygenase produced by *Gordonia polyisoprenivorans*. *Química Nova* **2012**, *35*, 1587–1592. [CrossRef]
80. Hong, Y.-H.; Ye, C.-C.; Zhou, Q.-Z.; Wu, X.-Y.; Yuan, J.-P.; Peng, J.; Deng, H.; Wang, J.-H. Genome Sequencing Reveals the Potential of *Achromobacter* sp. HZ01 for Bioremediation. *Front. Microbiol.* **2017**, *8*, 1507. [CrossRef]
81. Li, F.; Song, W.; Wei, J.; Liu, C.; Yu, C. Comparative proteomic analysis of phenol degradation process by Arthrobacter. *Int. Biodeterior. Biodegrad.* **2016**, *110*, 189–198. [CrossRef]
82. Ahmad, S.A.; Shamaan, N.A.; Syed, M.A.; Khalid, A.; Ab Rahman, N.A.; Khalil, K.A.; Dahalan, F.A.; Shukor, M.Y. Meta-cleavage pathway of phenol degradation by *Acinetobacter* sp. strain AQ5NOL 1. *Rendiconti Lincei* **2016**, *28*, 1–9. [CrossRef]
83. Margesin, R.; Bergauer, P.; Gander, S. Degradation of phenol and toxicity of phenolic compounds: A comparison of cold-tolerant *Arthrobacter* sp. and mesophilic Pseudomonas putida. *Extremophiles* **2004**, *8*, 201–207. [CrossRef]
84. Tavakoli, A.; Hamzah, A. Characterization and evaluation of catechol oxygenases by twelve bacteria, isolated from oil contaminated soils in Malaysia. *Biol. J. Microorg.* **2017**, *5*, 71–80.
85. Margesin, R.; Gander, S.; Zacke, G.; Gounot, A.M.; Schinner, F. Hydrocarbon degradation and enzyme activities of cold-adapted bacteria and yeasts. *Extremophiles* **2003**, *7*, 451–458. [CrossRef] [PubMed]
86. Guzik, U.; Hupert-Kocurek, K.; Sitnik, M.; Wojcieszyńska, D. High activity catechol 1,2-dioxygenase from *Stenotrophomonas maltophilia* strain KB2 as a useful tool in cis,cis-muconic acid production. *Antonie Leeuwenhoek* **2013**, *103*, 1297–1307. [CrossRef]
87. Briganti, F.; Pessione, E.; Giunta, C.; Mazzoli, R.; Scozzafava, A. Purification and catalytic properties of two catechol 1,2-dioxygenase isozymes from benzoate-grown cells of *Acinetobacter radioresistens*. *J. Protein Chem.* **2000**, *19*, 709–716. [CrossRef]
88. Wang, Z.; Sun, Y.; Shi, Y.; Song, W.; Zhang, C. Cloning, Expression and Characterization of a Mesophilic Catechol 1,2-dioxygenase from *Rhodococcus ruber* OA1. *Biotechnol.* **2016**, *16*, 10–18. [CrossRef]
89. Lee, G.L.Y.; Ahmad, S.A.; Yasid, N.A.; Zulkharnain, A.; Convey, P.; Johari, W.L.W.; Alias, S.A.; Gonzalez-Rocha, G.; Shukor, M.Y. Biodegradation of phenol by cold-adapted bacteria from Antarctic soils. *Polar Biol.* **2017**, *41*, 553–562. [CrossRef]
90. Bankevich, A.; Nurk, S.; Antipov, D.; Gurevich, A.A.; Dvorkin, M.; Kulikov, A.S.; Lesin, V.M.; Nikolenko, S.I.; Pham, S.; Prjibelski, A.D.; et al. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* **2012**, *19*, 455–477. [CrossRef]

91. Seemann, T. Prokka: Rapid Prokaryotic Genome Annotation. *Bioinformatics* **2014**, *30*, 2068–2069. [CrossRef]
92. Markowitz, V.M.; Mavrommatis, K.; Ivanova, N.; Chen, I.-M.A.; Chu, K.; Kyrpides, N. IMG ER: A system for microbial genome annotation expert review and curation. *Bioinformatics* **2009**, *25*, 2271–2278. [CrossRef]
93. Bedell, J.A.; Korf, I.; Gish, W. MaskerAid: A performance enhancement to RepeatMasker. *Bioinformatics* **2000**, *16*, 1040–1041. [CrossRef] [PubMed]
94. Benson, G. Tandem repeats finder: A program to analyze DNA sequences. *Nucleic Acids Res.* **1999**, *27*, 573–580. [CrossRef] [PubMed]
95. Lowe, T.M.; Eddy, S. tRNAscan-SE: A Program for Improved Detection of Transfer RNA Genes in Genomic Sequence. *Nucleic Acids Res.* **1997**, *25*, 955–964. [CrossRef] [PubMed]
96. Lagesen, K.; Hallin, P.; Rødland, E.A.; Staerfeldt, H.-H.; Rognes, T.; Ussery, D.W. RNAmmer: Consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res.* **2007**, *35*, 3100–3108. [CrossRef]
97. Griffiths-Jones, S.; Moxon, S.; Marshall, M.; Khanna, A.; Eddy, S.R.; Bateman, A. Rfam: Annotating non-coding RNAs in complete genomes. *Nucleic Acids Res.* **2004**, *33*, D121–D124. [CrossRef]
98. Overbeek, R.; Olson, R.; Pusch, G.D.; Olsen, G.J.; Davis, J.J.; Disz, T.; Edwards, R.A.; Gerdes, S.; Parrello, B.; Shukla, M.; et al. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res.* **2014**, *42*, D206–D214. [CrossRef]
99. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic local alignment search tool. *J. Mol. Biol.* **1990**, *215*, 403–410. [CrossRef]
100. Apweiler, R.; Bairoch, A.; Wu, C.H.; Barker, W.C.; Boeckmann, B.; Ferro, S. UniProt: The universal protein knowledgebase. *Nucleic Acids Res.* **2004**, *32*, D115–D119. [CrossRef]
101. Kanehisa, M.; Goto, S. KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res.* **2000**, *28*, 27–30. [CrossRef]
102. Tatusov, R.L.; Galperin, M.Y.; Natale, D.A.; Koonin, E.V. The COG database: A tool for genome-scale analysis of protein functions and evolution. *Nucleic Acids Res.* **2000**, *28*, 33–36. [CrossRef] [PubMed]