**Biodegradation of phenol by cold-adapted bacteria from Antarctic soils**

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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 a great challenge due to the harsh environmental conditions. To date, there is little information available concerning the biodegradation of phenol by indigenous Antarctic bacteria. This study addresses the isolation of three phenol-degrading bacterial strains from King George Island, Antarctica. Based on preliminary screening, three isolates (AQ5-05, AQ5-06, and AQ5-07) capable of completely degrading 0.5 g/L phenol within 120 h at 10°C were selected for detailed study. Two were identified as *Arthrobacter* spp.,and one *Rhodococcus* sp.,based on 16S rRNA sequences. All strains were non-motile, Gram-positive, oxidase-negative and catalase-positive. A study on the effects of parameters including temperature, pH, salinity and nitrogen source was conducted to optimise the conditions for phenol degradation. This revealed that the three isolates were psychrotolerant with the optimum temperature for phenol degradation between 10°C and 15°C. This study suggests the potential use of cold-adapted bacteria in the bioremediation of phenol over a wide range of low temperatures.

**Keywords** South Shetland Islands, Bioremediation, Psychrotolerant, One-factor-at-a-time, *Arthrobacter,* *Rhodococcus*

**Introduction**

Antarctica, the Earth’s last largely pristine continent, is facing increasing challenges from anthropogenic impacts on its ecosystem (Tin et al. 2009; Lana et al. 2014; Vodopivez et al. 2015). Even though there is no native human population, and the limited number of settlements are virtually restricted to national scientific research stations, the spatially very limited ice-free areas of Antarctica are particularly sensitive to the effects of soil and water contamination (Lana et al. 2014; Litova et al. 2014). Areas most exposed to the risk of locally-sourced pollution in Antarctica are primarily in the vicinity of the scientific research platforms and their logistic support facilities, nearshore commercial fishing operations, and scientific research or other visitor (tourist) sites that utilise fossil fuels for transportation or on-site energy generation (Prus et al. 2015). Oil spillage is one of the biggest concerns in Antarctica (Luz et al. 2006; Tin et al. 2009). Examples of reported Antarctic pollutants include hydrocarbons (Luz et al. 2006; Polmear et al. 2015), phenol and phenolic compounds (Margesin et al. 2013), inorganic compounds, pesticides residues and radiation contamination (Bharti and Niyogi 2015). Petroleum-derived contaminants in Antarctica tend to be persistent in the environment due to the continent’s chronically low temperatures and often dry conditions, which limit the rates of biological processes as well as abiotic degradation.

Phenol is commonly used in plastic manufacturing and petroleum refining, and is often one of the major pollutants discharged in wastewater. It is also used as an antiseptic agent in the pharmaceutical industry, possessing anti-bacterial effects even at low concentration. Consequently, any natural process of phenol breakdown relying on biological methods faces a particular challenge especially under the harsh conditions of Antarctica. Phenol is a hazardous compound that is classified as one of the major pollutants in the environment (Ahmad et al. 2012). Phenol wastes from sewage and industrial discharge have become a great concern globally due to their toxicity and persistence in environment (Bui et al. 2012). The toxicity of phenol to aquatic life and human health poses a great environmental challenge around the world. Due to this, the World Health Organization (WHO) has recommended limitation of the concentration of phenol in tap water to below 1 - 2 μg/L (Gami et al. 2014).

In recent years, the development of biological remediation processes has become a focus of research interest, surpassing chemical and physical methods, due to their cost effectiveness and potential for application in removing a wide variety of organic pollutants. Nevertheless, some organic compounds are non-biodegradable or toxic to microorganisms. The utilisation of indigenous bacteria has been proposed as the most cost effective and environmentally friendly method for treating phenol-contaminated sites. Several studies have reported the use of indigenous microorganisms to degrade hydrocarbon and phenolic pollutants in Antarctica, taking advantage of their adaptation to the cold and extreme climate (Domenica et al. 2004; Vasileva-Tonkova and Gesheva 2004; Hughes et al. 2007; Gerginova et al. 2013).

In areas with seasonal temperature fluctuation, the temperature of industrial wastewater can often decrease during the colder seasons, limiting the activity of local phenol-degrading microorganisms (Margesin et al. 2005, 2013; Feller 2007). The poor adaptation of mesophilic phenol-degrading microbiota in cold climates with limited nutrient availability will therefore reduce the rate of degradation in polluted sites (Domenico et al. 2004; Prus et al. 2015). In such situations, the addition of organic nutrients may be required in polluted areas, especially if pollutant concentration is low, since phenol-degrading bacteria require nutrients to grow and to remove pollutants (Aislabie et al. 2000; Domenico et al. 2004). Therefore, the discovery and exploitation of cold-adapted phenol-degrading taxa would provide an essential step forward in treating phenol-contaminated sites, especially if also already adapted to function in nutrient-poor environments. Further environmental variables such as temperature, pH, salinity, and nitrogen source may also have important influences on the biodegradation of phenol (Ahmad et al. 2011; Yaacob et al. 2016; Nawawi et al. 2016). Hence, the optimum conditions for phenol degradation need to be determined in order to achieve the highest rate of phenol degradation. The present study aimed at isolating, screening and identifying cold-adapted phenol-degrading bacteria from Antarctic soils, as well as optimising the conditions for their growth and degradation activity. This research could provide new resources for the bioremediation of phenol-contaminated sites in cold regions.

**Materials and Methods**

**Phenol medium**

Minimal salt medium (MSM) was prepared in 1 L volume by adding 0.4 g KH2PO4, 0.2 g K2HPO4, 0.1 g MgSO4, 0.1 g NaCl, 0.01 g MnSO4.H2O, 0.01 g Fe2(SO4)3.H2O, 0.01 g Na2MoO4.H2O, and 0.4 g (NH4)2SO4 to distilled water. The medium was adjusted to pH 7.2 using NaOH, checked with a pH meter (Mettler Toledo FiveEasy Plus™, Switzerland). The medium was autoclaved for 15 min at 121°C. The sterilised medium was then augmented with 0.5 g of phenol crystal.

**Isolation and screening of phenol-degrading strains**

Strains were isolated from three Antarctic soils obtained on 9th September 2007 by co-author GGR from King George Island, Antarctica (62° 09’ 7.2’’S, 58° 11.4’’W) (Fig. 1). Soil samples (5 g) were suspended in 40 mL of cold sterile minimal salt medium (MSM) containing 0.5 g/L phenol and incubated on a shaking incubator at 150 rpm for 3 d at 4°C. Aliquots of soil suspension were streaked onto mineral medium agar plates supplemented with phenol as carbon source and incubated for 7 d. Isolates exhibiting distinct colonies were sub-cultured on mineral medium agar plates supplemented with the same substrate as used in original enrichment until pure colonies were obtained. A single colony of each of the visibly distinct resulting bacterial colonies was incubated in MSM containing 0.5 g/L phenol on the shaking incubator at 150 rpm and 10°C for 120 h. The incubation temperature of 10°C was selected to represent the maximum summer temperature in King George Island (Carrasco et al. 2012; Araźny et al. 2013; Lee et al. 2016). The phenol degrading activity of each resulting pure colony was monitored at 24 h intervals and bacterial growth was determined by optical density 600 (OD600). Isolates achieving complete 0.5 g/L phenol degradation were selected for further studies.

**Characterisation of phenol-degrading strains**

The physiological and biochemical properties of selected strains were examined with Gram-staining and biochemical tests including oxidase and catalase tests. Scanning electron microscopy (SEM) JSM-7001F (JEOL Co. Ltd., Japan) was used to visualise cell morphological properties. For SEM preparation, cells were fixed with 25% glutaraldehyde and 5% paraformaldehyde in phosphate buffer for 2 h. The fixed samples were then dehydrated by successive treatment with ethanol (30%, 50%, 75%, 95% and 100% v/v) and freeze-dried using t-butyl alcohol. The dehydrated products were placed on copper grids (Cu150P grids Okenshoji Co. Ltd, Japan), dried under vacuum for 30 min, and coated with gold prior to SEM observation.

**Identification using 16S rRNA sequencing**

For 16S rRNA sequencing, genomic DNA were extracted using the GeneJET Genomic DNA Extraction Kit (Thermo Scientific, Waltham, MA, USA), followed by PCR amplification performed using two universal primers, 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-TACGGYTACCTTGTTACGACTT-3'. PCR amplification was performed using a T100 Thermal Cycler (Bio-rad Labotories, USA) under the following conditions: pre-denaturation at 94°C for 3 min; 29 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 2 min; and a final extension at 72°C for 10 min. The PCR products were confirmed with gel electrophoresis on 0.8% agarose gel stained with ethidium bromide. Nucleotide sequences obtained from Genomics BioScience and Technology Co., Ltd., Taiwan were compared with the NCBI database to retrieve the 16S rRNA sequences of closely related published species and aligned with ClustalW. Phylogenetic trees were constructed applying a maximum likelihood algorithm based on the Tamura-Nei model using MEGA 6 software (Tamura et al. 2013). Bootstrap analyses were performed using 1000 replications. Nucleotide sequences are deposited in the NCBI database under the accession numbers: *Arthrobacter* sp. strain AQ5-05 (KX946130-KX946131), *Arthrobacter* sp. strain AQ5-06 (KX946127), and *Rhodococcus* sp. strain AQ5-07 (KX946128-KX946129).

**Optimisation of phenol degradation and growth conditions using one-factor-at-a-time**

The three selected strains were cultivated in 50 mL of phenol medium as previously described under conditions of different temperature, salinity, pH and nitrogen source. The temperatures used were 0°C, 5°C, 10°C, 15°C, 20°C and 30°C. The incubation temperature of 10°C, close the upper summer temperature of Antarctic soil was selected in the study of pH, salinity and nitrogen source (Carrasco et al. 2012; Araźny et al. 2013; Lee et al. 2016). The effect of salinity was studied by adding different salinity as a manipulation variable from 0.05 to 0.30 practical salinity unit (psu). Acetate buffer, phosphate buffer and Tris-HCl buffer were used to study the effect of pH on phenol degradation and growth of the three isolates. Adjustment of medium pH was achieved using acetate buffer for pH 5, 5.5, and 6, phosphate buffer for pH 6, 6.5, 7, and 7.5, and Tris-HCl buffer for pH 7, 7.5, 8, 8.5 and 9. Nitrogen sources optimised include: leucine, sodium nitrate, ammonium chloride, glycine, alanine, and ammonium sulphate. The concentration of the selected nitrogen source was varied between 0.1 and 0.8 g/L. Experiments involving nitrogen source, pH and temperature were carried out in triplicate using a one-factor-at-a-time approach. Assessments of bacterial growth and phenol degradation were made after an incubation period of 84 h for AQ5-07 and 108 h for both AQ5-05 and AQ5-06.

**Analytical procedure**

Bacterial growth was determined by Optical Density 600 using a U.V Mini 1240 Shimadzu Spectrophotometer at a wavelength of 600 nm. The determination of phenol concentration was carried out using a UV-vis spectrophotometric method with 4-aminoantipyrine as colorimetric agent following American Public Health Association (2005) at a wavelength of 510 nm. Test samples were centrifuged at 10000 x g for 15 min to aliquot the supernatant, and then buffer solution (NH4Cl) was added until the pH reached 10. The aliquot (1.0 mL) was augmented with 10 µL each of 4-aminoantipyrine solution and potassium ferric cyanide, followed by incubation for 15 min in the dark before absorbance readings were taken at a wavelength of 510 nm. Phenol degradation is defined as the difference of phenol concentration in positive control and the remaining phenol concentration expressed in percentage. The data obtained were analysed using one-way ANOVA.

**Results**

Bacteria capable of utilising phenol as carbon source were isolated from soil samples collected at King George Island. Overall, 17 pure colonies were isolated based on the difference in their morphological characteristics on phenol agar. However, only three strains (AQ5-05, AQ5-06, and AQ5-07) showed complete degradation of 0.5 g/L phenol at 10°C within the 120 h incubation period (Fig. 2). Strain AQ5-07 showed the most rapid phenol degradation activity compared to strains AQ5-05 and AQ5-06, with complete degradation within 96 h. These three phenol-degrading strains were selected for further studies.

Colonies of strain AQ5-05 were yellow and translucent, whereas those of strains AQ5-06 and AQ5-07 were white and opaque on the phenol agar. All three selected isolates are aerobic, Gram-positive, catalase-positive, oxidase-negative, non-motile, and non-spore forming bacteria. Fig. 3 (a-c) shows the morphologies of the three strains under SEM after 48 h incubation at 10°C. Strain AQ5-07 appeared as a short rod while both strains AQ5-05 and AQ5-06 had a rod-coccus growth cycle as reported previously in other *Arthrobacter* species (Zhang et al. 2012; Arora and Jain 2013). On nutrient agar, strains AQ5-05 and AQ5-06 displayed the ability to grow at temperatures up to 25°C while strain AQ5-07 could grow at up to 30°C over 7 d of incubation. No growth was observed for all strains at 37°C, suggesting a facultative psychrophile classification following Morita (1975).

Based on 16S rRNA sequences, strains AQ5-05 and AQ5-06 were identified as *Arthrobacter* spp. The phylogenetic tree shown in Online Resource 1 displays the affiliation of strains AQ5-05 and AQ5-06 with 20 closely related types of the genus *Arthrobacter*, with *Pseudomonas putida* used as outgroup. Analysis of 16S rRNA sequences revealed that strain AQ5-05 shared the highest similarity (99%) with *Arthrobacter psychrophenolicus* (99.1%), *Arthrobacter sulfureus* (98.7%) and *Arthrobacter kerguelensis* (98.4%). Sequence comparison of AQ5-06 displayed the highest identity with *Arthrobacter oxydans* (99.1%), *Arthrobacter phenanthrenivorans* (98.9%),and *Arthrobacter chlorophenolicuss* (98.1 %). However, the low bootstrap values obtained were insufficient to classify both strains to species level with confidence. Therefore, the strains are here referred to as *Arthrobacter* sp. AQ5-05 and *Arthrobacter* sp. AQ5-06, respectively. Strain AQ5-07 was classified as *Rhodococcus* sp. (Online Resource 2), and shared highest sequence identity (99.0%) with *Rhodococcus jialingiae* and *Rhodococcus qingshengii*. Three isolated strains were deposited in Microbial Culture Collection Unit (UNiCC) of Universiti Putra Malaysia under accession numbers: *Arthrobacter* sp. strain AQ5-05 (UPMC 1200), *Arthrobacter* sp. strain AQ5-06 (UPMC 1201), and *Rhodococcus* sp. strain AQ5-07 (UPMC 1202).

For the study on the effects of different imposed environmental parameters, assessment of bacterial growth and phenol degradation was made after an incubation period of 84 h for AQ5-07 and 108 h for both AQ5-05 and AQ5-06. All three strains showed growth and phenol degradation activities at temperatures between 0°C and 30°C (Fig. 4), and were classified as facultative pschrophiles as growth continued above 20°C, following Morita (1975). Maximum growth rates were observed between 10°C and 15°C for both AQ5-06 and AQ5-07, while AQ5-05 showed a slightly higher optimum growth temperature, between 15°C and 20°C. Highest phenol degradation activities were observed at 15°C for AQ5-05, 10°C for AQ5-06, and 10°C to 15°C for AQ5-07. AQ5-07 achieved high rates of degradation across a wider temperature range than the other two strains (Fig. 4).

Different NaCl concentrations measured in psu were used to study the effect of salinity on bacterial growth and phenol degradation activities at 10°C. AQ5-06 showed the lowest optimum salinity among the three strains, with optimum growth and phenol degradation observed between 0.05 to 0.10 psu (Fig. 5). When the concentration of NaCl increased above 0.20 psu, phenol degradation activity of AQ5-06 decreased and was lowest at 0.3 psu. AQ5-06 was more tolerant of low salinity compared to strains AQ5-05 and AQ5-07. Optimum phenol degradation by AQ5-07 was slightly higher than AQ5-05 and AQ5-06, with also a slightly higher optimum salinity (Fig. 5).

The optimum pH for both growth and phenol degradation at 10°C was pH 7.5 for AQ5-05 and AQ5-06 and pH 7 for AQ5-07 (Fig. 6). These three bacterial strains clearly prefer growth at near-neutral pH.

The effects of different nitrogen sources, including alanine, ammonium sulphate, histidine, leucine, sodium nitrate, glycine and ammonium chloride, being added to the phenol medium are shown in Fig. 7. All three phenol-degrading strains exhibited maximum growth when ammonium chloride or ammonium sulphate was provided as the nitrogen source. All three strains exhibited maximum degradation rates with ammonium sulphate as nitrogen source, with one achieving similar rates with ammonium chloride, and two slightly lower rates with sodium nitrate. Hence, ammonium sulphate was selected as the experimental nitrogen source, and the result for the optimisation of ammonium sulphate concentration is shown in Fig. 8. Optimum growth and phenol degradation activities were observed at 0.4 g/L ammonium sulphate for AQ5-05. Maximum phenol degradation was achieved by AQ5-06 and AQ5-07 when the concentration of ammonium sulphate was between 0.3 and 0.4 g/L. Minimal phenol degradation was observed in the absence of a nitrogen source, confirming the importance of nitrogen for bacterial growth and phenol degradation (Fig. 8).

**Discussion**

King George Island is the largest island in the South Shetland archipelago, and hosts a high density of scientific stations operated by different national operators within the Antarctic Treaty System (ATS) (Harris 1991; Simões et al. 1999; Amaro et al. 2015). It is also a focus of cruise-based tourism activity, and the crushed rock runway at Frei station services both aeroplanes and logistic personnel transfer movements of multiple national operators, and tourist flights with DAP (Antarctic Airways) from Punta Arenas in southern Chile. Tourists may arrive by air for either day or overnight stays on the island, or for the purpose of passenger exchange with cruise vessels (Pfeiffer et al. 2007; Tin et al. 2009; Prus et al. 2015). As human activities on the island continue to increase, the use of hydrocarbon fuel and the associated potential of fuel spills inevitably also increase (Hughes et al. 2007; Prus et al. 2015).

Considerable anthropogenic pollution, including chronic presence of hydrocarbon spills, are already present on King George Island, in particular in the vicinity of the multiple research stations clustered near the Fildes Peninsula, speaking to a poor history of environmental management and compliance with existing requirements under the ATS (Braun et al. 2012, 2014; Peter et al. 2013). Thus, a major challenge now faces the Antarctic Treaty Parties, and in particular the nations operating stations and other facilities on the continent, in achieving clean-up of such historically polluted sites, as well as of sites subject to new pollution events. Biological means of degradation are therefore worthy of investigation, and in particular the potential of native degraders as the introduction and release of non-native biota (including microorganisms) is prohibited under the ATS (Stallwood et al. 2005; McWatters et al. 2016).

A number of reports of Antarctic bacteria and fungi capable of degrading hydrocarbons are available (Hughes et al. 2007; Gerginova et al. 2014; Litova et al. 2014). Bacterial reports generally belong to the Gram-negative genera *Pseudomonas*, *Acinetobacter*, *Sphingomonas* and Gram-positive *Rhodococcus* and *Arthrobacter* (Shukor et al. 2009; Panicker et al. 2010; Jurelevicius et al. 2012). The phenol-degrading fungus, *Aspergillus*, has been reported by Gerginova et al. (2013) from Antarctica. Several phenol-degrading bacteria have also been isolated from oil-contaminated sites, assumed to be utilising the phenol contained in the hydrocarbon fuel (Mohite et al. 2010; Margesin et al. 2013; Saxena et al. 2013).

Several studies have identified psychrophilic or psychrotolerant members of *Arthrobacter* as dominant components of bacterial communities in Antarctic soils (Zdanowski et al. 2013; Orlandini et al. 2014; Dsouza et al. 2015). Karigar et al. (2006) and Margesin et al. (2013) also reported the phenol-degrading ability of *Arthrobacter*. Several phenol-degrading *Arthrobacter* spp. are also capable of degrading phenol and phenolic compounds under low temperatures (Unell et al. 2008; Margesin et al. 2013).

Members of the genus *Rhodococcus* are Gram-positive rods and acid-fast bacteria that are commonly recognised for their high ability in degrading phenol (Arif et al. 2013; Suhaila et al. 2013; Norazah et al. 2015). Members of this genus are also often found in hydrocarbon-contaminated sites in cold climate regions such as the Arctic and Antarctica (Margesin et al. 2003; Ruberto et al. 2005; Gesheva et al. 2010; Jesus et al. 2015). For instance, Margesin et al. (2012) reported that cold-adapted *Rhodococcus* spp. were able to degrade petroleum hydrocarbons, including n-alkanes, phenol, anthracene and pyrene, at 15°C.

Understanding the influence of temperature in phenol biodegradation is crucial as it is an important factor for both bacterial growth and the activity of phenol-degrading enzymes. Most Antarctic bacteria are psychrotolerant rather than strictly psychrophilic, which is appropriate given the high temperature fluctuations typical of terrestrial habitats on the continent (Helmke and Weyland 2004; Peck et al. 2006; Hoover and Pikuta 2010; Sandle and Skinner 2012; Ahmad et al. 2013). Previous studies of hydrocarbon-degrading psychrotolerant bacteria have reported that the optimum temperature for degradation activity is also between 10°C and 15°C (Shukor et al. 2009; Margesin et al. 2013), similar to the optimum temperature ranges identified for the three strains studied here. Polar environments are highly seasonal. In the High Arctic and maritime Antarctic (the region of the Antarctic Peninsula and Scotia Arc) mean monthly air temperatures only reach low positive temperatures for 1-4 months during the summer, and in the more extreme environments of the continental Antarctic they are never positive (Convey 2013). Terrestrial habitat microenvironmental temperatures often exceed those of the air, and are considerably more variable, through the absorbtion of solar radiation, although long term averages remain in low single figures (Davey et al. 1991; Coulson et al. 1995; Peck et al. 2006; Convey et al. 2014). Prolonged winter sub-zero habitat temperatures in these regions mean than biological activity is minimal to undetectable and, hence, that any bioremediation approaches will only have effect during the short polar summer period.

Other environmental factors such as salinity can influence bacterial growth (Suhaila et al. 2013). High salinity that beyond the tolerance level can inhibit metabolic activity as the result of increased osmotic stress on the microorganisms and altered solubility or sorption of toxic or essential ions (Qin et al. 2012). According to Navas et al. (2008), salt content and composition in Antarctic soils is known to vary, most obviously with proximity to the coast or to dense colonies of marine vertebrates, but also with soil age in polar desert soils such as those of Victoria Land (Convey et al. 2014). Several phenol-degrading bacteria have been reported to show optimum phenol degradation activities at low salinity when phenol was used as sole carbon source, in agreement with the data obtained in the current study (Ahmad et al. 2011; Suhaila et al. 2013)

Our data also indicate that the bacterial strains studied are very sensitive to pH, requiring near-neutral pH for optimal growth and phenol degradation activity. Marrot et al. (2006), Ahmad et al. (2012) and Arif et al. (2013) also reported that substrate decomposition rate and phenol degradation were affected by changes in pH and, when pH deviated from neutral conditions, degradation rates slowed.

The availability of an appropriate nitrogen source is an important controlling factor for bioremediation (Boopathy 2000; Calvo et al. 2009; Das and Chandran 2010). However, high concentrations of sources such as ammonium sulphate can also inhibit growth. Ammonium sulphate is widely available in the environment and is commonly utilised by bacteria as a nitrogen source (Shukor et al. 2009). In the context of practical bioremediation it is also affordable in instances where fertilization of nutrient-poor habitats is required (Arif et al. 2013). Several phenol-degrading bacteria have been reported to degrade phenol using ammonium sulphate as their nitrogen source (Ahmad et al. 2011; Arif et al. 2013; Suhaila et al. 2013), to which can be added the three strains identified in the current study.

In summary,three cold-adapted phenol-degrading bacterial strains isolated from Antarctic soils were identified to be capable of degrading phenol up to 0.5 g/L within a 120 h incubation period at 10°C. Optimal growth temperatures were in the range 10-20°C (10-15°C for two of the strains), with growth continuing above 20°C, confirming their psychrotolerant status. Optimized physicochemical parameters, including temperature, salinity, pH and nitrogen source, were found to strongly enhance phenol degradation activity in these bacteria. Optimal phenol degradation was observed between 10-15°C, indicating that summer in Antarctica provides suitable soil habitat conditions for bioremediation of phenol by these strains. Ongoing research is being conducted to identify the pathway for phenol degradation and the underlying genomic processes.

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**Figures caption**

**Fig. 1** Map of study area. **a** and **b** Location of the South Shetland Islands in relation to the southern South America and Antarctic Peninsula, and **c** King George Island within the South Shetland Islands, including the study sampling location and the permanent research stations on the island.

**Fig. 2** Degradation of 0.5 g/L phenol by strains AQ5-05 (), AQ5-06 () and AQ5-07 ( ) at 10°C. The error bars represent the mean ± standard deviation for three replicates

**Fig. 3** Morphological study under SEM. **a** *Arthrobacter* sp. strain AQ5-05, **b** *Arthrobacter* sp*.* strain AQ5-06, **c** *Rhodococcus* sp. strain AQ5-07. Scale bars represent 1 μm

**Fig. 4** Effects of temperature on the growth of strains AQ5-05 (), AQ5-06 () and AQ5-07 () in phenol medium and phenol degradation by strains AQ5-05 (), AQ5-06 () and AQ5-07 (). The error bars represent the mean ± standard deviation for three replicates

**Fig. 5** Effects of salinity on the growth of strains AQ5-05 (), AQ5-06 () and AQ5-07 () in phenol medium and phenol degradation by strains AQ5-05 (), AQ5-06 () and AQ5-07 (). The error bars represent the mean ± standard deviation for three replicates

**Fig. 6** Effects of pH on the **a.** growth and **b**. phenol degradation of strains AQ5-05 (), AQ5-06 () and AQ5-07 () using an overlapping buffer system consisting of acetate () phosphate () and Tris-HCl () system in phenol medium. The error bars represent the mean ± standard deviation for three replicates

**Fig. 7** Effects of different nitrogen sources on the **a.** growth and **b.** phenol degradation of strains AQ5-05 (), AQ5-06 () and AQ5-07 (). The error bars represent the mean ± standard deviation for three replicates

**Fig. 8** Effects of ammonium sulphate concentration on the growth of strains AQ5-05 (), AQ5-06 () and AQ5-07 () in phenol medium and phenol degradation by strains AQ5-05 (), AQ5-06 () and AQ5-07 (). The error bars represent the mean ± standard deviation for three replicates

**Electronic Supplementary Material caption**

**Online Resource 1** Maximum likelihood derived phylogenetic tree of strains AQ5-05 and AQ5-06 with type strains of *Arthrobacter* species. The percentage of trees (bootstrap scores > 50%) in which the associated taxa clustered together is shown next to the branches. Numbers in brackets are the accession numbers of the sequence with the superscript T indicates the type strains of each species. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site

**Online Resource 2** Maximum likelihood derived phylogenetic tree of *Rhodococcus* sp. AQ5-07 conducted with MEGA 6 based on Tamura-Nei model. The percentage of trees (bootstrap scores > 50%) in which the associated taxa clustered together is shown next to the branches. Numbers in brackets are the accession numbers of the sequence with the superscript T indicates the type strains of each species. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site