



UNIVERSITI PUTRA MALAYSIA

***BIODEGRADATION POTENTIAL OF PHENOL BY PURE AND DEFINED
MIXED COLD-ADAPTED BACTERIAL CONSORTIA FROM ANTARCTICA***

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MIXED COLD-ADAPTED BACTERIAL CONSORTIA FROM ANTARCTICA**

By

KAVILASNI A/P SUBRAMANIAM

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Philosophy**

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DEDICATION

This thesis is dedicated with love and grateful heart

To amma, naina, anna, ruby and my better half,

To my mentors and friends,

and

To all those good souls who wished well during this journey



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Doctor of Philosophy

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November 2021

Chair : Associate Professor Siti Aqlima Binti Ahmad, PhD
Faculty : Biotechnology and Biomolecular Sciences

The risk of phenol pollution from daily waste discharge and accidental oil spillage is ever-present due to increasing activities in the Antarctic continent, mainly related to the supply and operation of research stations and field expeditions, tourism, marine and air transportation. Increased levels of phenol concentration in the Antarctic environment bring significant risk to both aquatic and terrestrial biota due to its highly toxic properties and persistence. Sustainable human presence and activity in Antarctica require effective remediation technologies to be developed and their rapid application when required. The main purpose of the present study was to isolate new taxa of pure phenol-degrading bacteria from Antarctic soil and, both as a pure isolate and together with previously isolated phenol-degrading bacteria as a consortium, will be capable of rapid degradation of phenol at low temperatures (0-15°C). In addition, this study also focuses on identification of phenol-degrading pathway(s) of the pure culture, conventional and statistical optimisations of phenol degradation by both pure and mixed cultures, and the effect of heavy metals on phenol degradation by pure and mixed cultures. This thesis reports the isolation of a potential phenol-degrading bacterial strain (AQ5-15) from soil from King George Island, South Shetland Islands, Antarctica. This strain was identified as a member of the genus *Arthrobacter* based on 16S rRNA gene sequence analysis. Based on whole genome sequencing (WGS), the strain's nearest identified relative was suggested to be *Paeniglutamicibacter sulfureus* (99.38% similarity). Preliminary screening showed that strain AQ5-15 was capable of completely degrading 0.5 g/L phenol within 108 h at 10°C and it was selected for a detailed study. The genomic analysis identified the presence of genes encoding a complete pathway of aromatic compound metabolism in strain AQ5-15, consistent with the ability of the strain to utilise phenol as the sole carbon source. The genomic analysis was validated using enzyme assays of catechol 1,2-dioxygenase and catechol 2,3-dioxygenase, which confirmed the presence of the enzyme catechol 1,2-dioxygenase, consistent with the genes identified in the WGS. A study of the influence of parameters including nitrogen source, salinity, pH, and temperature

was conducted to optimise the conditions for phenol degradation using one-factor-at-a-time (OFAT) and response surface methodology (RSM). Based on the results from OFAT, strain AQ5-15 showed the highest phenol degradation at 0.5 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.1 g/L NaCl, pH 7 and 20°C, proving that this strain is a psychrotolerant and prefers low salinity and near-neutral conditions. Statistical analysis of the results obtained from RSM showed that the strain could degrade phenol optimally at 0.5 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.13 g/L NaCl, pH 7.25 and 15°C, with pH and temperature identified as significant factors. This strain was mixed with two other previously isolated phenol-degrading strains (AQ5-06 and AQ5-07) in different combinations to further enhance degradation efficiency. The data obtained showed that mixture of strains AQ5-06 and AQ5-15 together could completely degrade 0.5 g/L phenol within 48 h at 10°C while mixture of strains AQ5-06, AQ5-07 and AQ5-15 together could completely degrade 0.5 g/L phenol within 60 h at 10°C. RSM analysis showed that the combination of strains AQ5-06 and AQ5-15 could degrade phenol optimally at 0.4 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.13 g/L NaCl, pH 7.25 and 12.5°C, with only temperature as a significant factor. RSM analysis showed that the combination of strains AQ5-06, AQ5-07 and AQ5-15 can degrade phenol optimally at 0.4 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.13 g/L NaCl, pH 7.25 and 12.5°C, with ammonium sulphate concentration, sodium chloride concentration and temperature being significant factors. The tolerance levels of pure and mixed cultures towards different heavy metals that are widely present in Antarctic soils was studied by exposing strains AQ5-06, AQ5-07 and AQ5-15 individually as well as in consortia to the heavy metals Arsenic (As), Aluminum (Al), Copper (Cu), Zinc (Zn), Lead (Pb), Cobalt (Co), Cadmium (Cd), Chromium (Cr), Nickel (Ni), Silver (Ag) and Mercury (Hg) at an initial concentration of 1.0 ppm. Phenol degradation by strain AQ5-15 was inhibited when exposed to Cd, Ag and Hg while strain AQ5-06 was inhibited when exposed to Ag and Hg, and strain AQ5-07 was inhibited when exposed to Cd and Hg. Consortia containing strains AQ5-06 and AQ5-15 and all three strains were inhibited when exposed to Hg, Cd and Ag. In a nutshell, the attempt to develop highly efficient phenol-degrading bacterial consortia for significant inclusion in cold region bioremediation, specifically Antarctica was successful with a few limitations in the event of the co-occurrence of some heavy metals such as Hg, Cd, and Ag.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

POTENSI PENGURAIAN FENOL OLEH KULTUR BAKTERIA TULEN DAN CAMPURAN YANG SEJUK BERADAPTASI DARI ANTARTIKA

Oleh

KAVILASNI A/P SUBRAMANIAM

November 2021

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Kesan negatif akibat pencemaran fenol daripada pembuangan sampah harian dan tumpahan minyak yang tidak disengajakan selalu terjadi disebabkan oleh peningkatan aktiviti di benua Antartika, khususnya yang berkaitan dengan pembekalan dan operasi stesen penyelidikan dan ekspedisi lapangan, pelancongan dan pengangkutan laut dan udara. Peningkatan tahap kepekatan fenol di persekitaran Antartika membawa risiko yang tinggi kepada biota akuatik dan daratan kerana sifatnya yang sangat toksik dan kekekalannya. Kehadiran dan aktiviti manusia yang berterusan di Antartika memerlukan teknologi pemulihan yang berkesan untuk dikembangkan. Tujuan utama kajian ini adalah untuk mengasingkan taksa baharu bakteria tulen yang dapat menguraikan fenol daripada tanah Antartika dan, kedua-duanya sebagai bakteria tulen dan campuran bakteria pengurai fenol yang diasingkan sebelum, akan mampu menguraikan fenol dalam masa singkat pada suhu rendah (0-15°C). Di samping itu, kajian ini juga memfokuskan pada pengenalpastian laluan jalan lengkap metabolisme fenol bagi kultur tulen, pengoptimuman konvensional dan statistik penguraian fenol oleh kedua-dua kultur tulen dan campuran, dan kesan logam berat ke atas penguraian fenol oleh kultur tulen dan campuran. Tesis ini melaporkan pengasingan strain bakteria yang dapat menguraikan fenol (AQ5-15) dari tanah dari King George Island, South Shetland Islands, Antartika. Strain ini dikenal pasti sebagai genus *Arthrobacter* sp. berdasarkan analisis gen 16S rRNA. Berdasarkan penjujukan keseluruhan genom (WGS), spesies terdekat yang dikenal pasti kepada strain ini dicadangkan sebagai *Paeniglutamicibacter sulfureus* (99.38% kesamaan). Pemeriksaan awal menunjukkan bahawa strain AQ5-15 mampu menguraikan 0.5 g/L fenol sepenuhnya dalam masa 108 jam pada suhu 10°C dan dipilih untuk kajian terperinci. Analisis genomik mengenal pasti adanya gen yang mengekodkan jalan lengkap metabolisme sebatian aromatik dalam strain AQ5-15, selaras dengan kemampuan strain untuk

menggunakan fenol sebagai sumber karbon tunggal. Analisis genomik disahkan menggunakan ujian enzim catechol 1,2-dioxygenase dan catechol 2,3-dioxygenase, yang mengesahkan adanya enzim catechol 1,2-dioxygenase, selaras dengan gen yang dikenal pasti dalam WGS. Kajian mengenai pengaruh parameter termasuk sumber nitrogen, saliniti, pH dan suhu dilakukan untuk mengoptimumkan keadaan penguraian fenol dengan menggunakan satu faktor-pada-satu-waktu (OFAT) dan metodologi permukaan tindak balas (RSM). Berdasarkan hasil dari OFAT, strain AQ5-15 menunjukkan degradasi fenol tertinggi pada 0.5 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.1 g/L NaCl, pH 7 dan 20°C, membuktikan bahawa strain ini boleh tahan sejuk dan lebih suka keadaan kemasinan rendah dan kondisi hampir neutral. Hasil analisis statistik yang diperolehi dari RSM menunjukkan bahawa strain ini dapat menguraikan fenol secara optimum pada 0.5 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.13 g/L NaCl, pH 7.25 dan 15°C, dengan pH dan suhu dikenal pasti sebagai faktor penting. Dalam usaha untuk meningkatkan lagi kecekapan degradasi, strain ini dicampur dengan dua strain pengurai fenol yang terpendek sebelum ini (AQ5-06 dan AQ5-07) dalam kombinasi yang berbeza. Data yang diperolehi menunjukkan bahawa strain AQ5-06 dan AQ5-15 bersama-sama dapat menguraikan 0.5 g/L fenol sepenuhnya dalam masa 48 jam pada suhu 10°C sementara strain AQ5-06, AQ5-07 dan AQ5-15 bersama-sama dapat menguraikan 0.5 g/L fenol sepenuhnya dalam masa 60 jam pada suhu 10°C. Analisis RSM menunjukkan bahawa gabungan strain AQ5-06 dan AQ5-15 dapat menguraikan fenol secara optimum pada 0.4 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.13 g/L NaCl, pH 7.25 dan 12.5°C, dengan hanya suhu sebagai faktor yang signifikan. Analisis RSM menunjukkan bahawa gabungan strain AQ5-06, AQ5-07 dan AQ5-15 dapat menguraikan fenol secara optimum pada kadar 0.4 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.13 g/L NaCl, pH 7.25 dan 12.5°C, dengan kepekatan ammonium sulfat, kepekatan natrium klorida dan suhu menjadi faktor signifikan. Tahap toleransi kultur tulen dan campuran terhadap logam berat yang berlainan yang terdapat secara meluas di tanah Antartika dikaji dengan mendedahkan strain AQ5-06, AQ5-07 dan AQ5-15 secara individu dan juga konsortia kepada logam berat Arsenik (As), Aluminium (Al), Kuprum (Cu), Zink (Zn), Plumbum (Pb), Kobalt (Co), Kadmium (Cd), Kromium (Cr), Nikel (Ni), Perak (Ag) dan Merkuri (Hg) pada kepekatan awal 1 ppm. Degradasi fenol oleh strain AQ5-15 direncat ketika terdedah kepada Cd, Ag dan Hg sementara strain AQ5-06 direncat ketika terdedah kepada Ag dan Hg, dan strain AQ5-07 direncat ketika terdedah kepada Cd dan Hg. Konsortia yang mengandungi strain AQ5-06 dan AQ5-15 dan ketiga-tiga strain direncat apabila terdedah kepada Hg, Cd dan Ag. Ciri-ciri toleransi dan degradasi konsortia campuran menunjukkan bahawa penggunaan kultur campuran mungkin bermanfaat dalam tempat yang tercemar dengan fenol, terutamanya di persekitaran ekstrim seperti Antartika. Secara ringkasnya, usaha untuk membina konsortia bakteria yang menguraikan fenol secara pesat bagi penggunaan dalam proses bioremediasi di kawasan sejuk, khususnya Antartika berjaya dengan beberapa batasan sekiranya terdedah kepada beberapa logam berat seperti Hg, Cd, dan Ag.

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‘God has been there, every step of the way’

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

(NH ₄) ₂ SO ₄	Ammonium sulphate
16S rRNA	16S ribosomal ribonucleic acid
2-HMS	2-hydroxymuconate semialdehyde
4-AAP	4-aminoantipyrine
AFP	Antifreeze protein
Ag	Silver
Al	Aluminium
ANI	Average nucleotide identity
ANOVA	Analysis of variance
AP	Alkylphenol
APE	Alkylphenol ethoxylate
As	Arsenic
ASMA	Antarctic Specially Managed Area
ASPA	Antarctic Specially Protected Area
ATS	Antarctic Treaty System
BDD	Boron-doped diamond
BH	Bushnell–Haas
BLAST	Basic local alignment search tool
BOD	Biochemical oxygen demand
bp	Base pair
BPA	Bisphenol A
C12O	Catechol 1,2-dioxygenase
C23O	Catechol 2,3-dioxygenase

$C_6N_6FeK_3$	Potassium ferricyanide
CCD	Central composite design
CCMA	<i>cis, cis</i> -muconic acid
ccs	Circular consensus sequencing
Cd	Cadmium
CDS	Protein-coding sequence
Co	Cobalt
COD	Chemical oxygen demand
COG	Clusters of orthologous groups
CP	Chlorophenol
CSP	Cold-shock protein
Cu	Copper
dH ₂ O	Distilled water
DMC	Defined mixed culture
DoE	Design of Experiment
dsDNA	Double-stranded deoxyribonucleic acid
EC	European Community
EC No.	Enzyme commission number
<i>et al.</i>	and others
FAD	Flavin adenine dinucleotide
$Fe_2(SO_4).H_2O$	Iron (II) sulphate monohydrate
Fe_2S_2	Iron sulphide
gDNA	Genomic deoxyribonucleic acid
H ₂ O ₂	Hydrogen peroxide
Hg	Mercury

IC ₅₀	Half maximal inhibitory concentration
IrO ₂	Iridium (IV) oxide
K ₂ HPO ₄	Dipotassium hydrogenphosphate
KH ₂ PO ₄	Potassium dihydrogenphosphate
MEGA	Molecular Evolutionary Genetics Analysis
Mg ₂ SO ₄	Magnesium sulphate
MnSO ₄ .H ₂ O	Manganese sulphate monohydrate
mPH	Multi-component phenol hydroxylase
MSM	Minimal salt medium
NA	Nutrient agar
Na ₂ EDTA	Ethylenediaminetetraacetic acid disodium salt
NaCl	Sodium chloride
NaMoO ₄ .H ₂ O	Sodium molybdate dihydrate
NaOH	Sodium hydroxide
NB	Nutrient broth
NCBI	National Centre for Biotechnology Information
NGS	Next-generation sequencing
NH ₄ Cl	Ammonium chloride
Ni	Nickel
NP	Nonylphenol
OD	Optical density
OFAT	One-factor-at-a-time
OGRI	Overall genome relatedness index
OP	Octylphenol
ORF	Open reading frame

PA	Phenol agar
PacBio	Pacific Biosciences
PAH	Polycyclic aromatic hydrocarbons
PB	Plackett-Burman design
Pb	Lead
PbO ₂	Lead oxide
PCB	Polychlorinated biphenyl
PCR	Polymerase chain reaction
PFAS	Poly/per-fluoroalkyl substances
PGM	Personal Genome Machine
PHA	Polyhydroxyalkanoates
PM	Phenol media
POG	Pairwise orthologous groups
POP	Persistent organic pollutants
RFLP	Restriction fragment length polymorphism
ROS	Reactive oxygen species
rpm	Revolutions per minute
rRNA	Ribosomal ribonucleic acid
RSM	Response surface methodology
SD	Standard deviation
SMRT®	Single-molecule-real-time technology
SnO ₂	Tin (IV) oxide
SOLiD	Sequencing by oligonucleotide ligation and detection
<i>Taq</i>	<i>Thermus aquaticus</i>

TCA	Tricarboxylic acid
TGS	Third-generation sequencing
tRNA	Transfer ribonucleic acid
US EPA	United States Environmental Protection Agency
VCHC	Volatile chlorinated hydrocarbons
WGS	Whole genome sequencing
Zn	Zinc



CHAPTER 1

INTRODUCTION

Phenol is an aromatic hydrocarbon commonly derived from industrial activities. It has been classified as a high-priority toxic compound by the United States Environmental Protection Agency (US EPA) due to its toxicity to living systems and recalcitrant nature. Since the Industrial Revolution, there has been a progressive increase in pollution worldwide (Jacob *et al.*, 2018). Among all pollutants, phenol contamination has been considered one of the most prevalent in the environment, requiring urgent mitigation actions. In accordance, the US EPA and European Community (EC) limited the minimum permissible level of phenol in water bodies intended for drinking to 0.1 µg/L, and to 5 µg/L for washing purposes (Salaudeen *et al.*, 2019). It may cause negative impacts on biological systems as well as directly on humans (Lee *et al.*, 2017; Duan *et al.*, 2018).

Anthropogenic pollution is apparent even in the continent that many consider being the most pristine and isolated, Antarctica. Since the mid-Twentieth Century, the construction and operation of over 70 Antarctic research stations, increased tourism activities, shipping and air transportation as well as the rapid growth of industrial activities in distant countries in the Southern and Northern Hemispheres, have resulted in detectable pollution, including by phenol, in Antarctica (Bargagli, 2005, 2008; Mazuki *et al.*, 2019). Antarctica's ecosystems and biota are susceptible to the impacts of pollution. Furthermore, the continent's extremely cold and harsh climate means that the natural processes involved in remediating pollution happen more slowly than elsewhere. As a result, the accumulation of chemical contaminants such as hydrocarbons, heavy metals and other persistent organic pollutants (POPs) has been reported to cause significant damage to Antarctica's simple ecosystems and food webs (Jara-Carrasco *et al.*, 2017). As recognised in the Environmental Protocol, the effects of such pollutants in the Antarctic environment are not considered as either "minor" or "transitory". They will continue to have significant and long-term impacts on the environment unless active remediation is applied along with changes in national operational and tourism management (Tin *et al.*, 2009). The Antarctic Treaty Parties (i.e. the signatory nations to the Antarctic Treaty) and, in particular, those operating stations and other facilities on the continent must take responsibility for achieving effective clean-up of both historically polluted sites and sites subjected to new pollution events (Lee *et al.*, 2018a).

Phenol can be removed from the environment by physical and chemical means (Mohammadi *et al.*, 2014; Víctor-Ortega *et al.*, 2016; Hernández-Francisco *et al.*, 2017; Vaiano *et al.*, 2018). However, biological remediation technology is preferred due to its cost-effectiveness, eco-friendly nature, applicability and usually generating non-toxic end products (Yaacob *et al.*, 2016; Ahmad *et al.*, 2017a; Suárez-García *et al.*, 2019). The Antarctic Treaty prohibits the importation of non-native organisms into Antarctica (Hughes *et al.*, 2015) meaning that, currently, application of bioremediation approaches can only be

achieved using indigenous microorganisms. Several studies have reported the ability of native Antarctic microorganisms, primarily from the genera *Arthrobacter* and *Rhodococcus*, to degrade phenol at low temperature (Lee *et al.*, 2018a; Ahmad *et al.*, 2018; Zakaria *et al.*, 2018; Tengku-Mazuki *et al.*, 2020). However, identifying such microbes is only the first step in the development of a bioremediation process. Environmental factors and nutritional parameters such as temperature, pH, salinity, and the presence and type of nitrogen and carbon sources, exert important influences on the phenol-degrading capability and growth rate of microorganisms (Lee *et al.*, 2018a; Tengku-Mazuki *et al.*, 2020). Consequently, it is crucial to optimise these factors to enhance the bioremediation process at a laboratory scale before applying any process in the field environment.

With advances in statistical analysis and information technology, numerous statistical software packages have been developed as optimisation tools to aid in bioreactor design (Nawawi *et al.*, 2016; Ramasamy *et al.*, 2017; Titah *et al.*, 2018). Amongst the best-known software in the bioremediation field (Nasr, 2018), Design-Expert® (Stat-Ease Inc.) is designed to calculate the statistical values and probability of defining the minimum run of experiments required to recognise significant cause-and-effect relationships between a given number of variables and responses. This software comprises four categories of Design of Experiment (DoE): factorial, response surface, mixture design and crossed-process mixture (Alben, 2002). Response Surface Methodology (RSM) is the most common design used for optimising multiple factors collectively, avoiding the limitations of single factor optimisation approaches like one-factor-at-a-time (OFAT) (Zhou *et al.*, 2011). RSM is a sequential procedure that begins with significant factor screening using a two-level factorial design such as the Plackett-Burman design (PB) to screen for significant parameters, and then modelling and optimising the response with a three-level factorial design like Box-Behnken central composite design (CCD) (Karamba *et al.*, 2016; Tengku-Mazuki *et al.*, 2020).

Generally, environments polluted by aromatic compounds, including phenol, often receive discharges from non-hydrocarbon co-contaminants such as heavy metals (Ahmad *et al.*, 2017a; Duraisamy *et al.*, 2020). Although heavy metals also occur naturally, anthropogenic exposure plays a vital role in elevating concentrations of these metallic elements in the Antarctic environment. For instance, a study of geochemical baseline values on the Fildes Peninsula, King George Island, revealed several heavy metals in surface soils possibly due to anthropogenic sources such as sewage disposal and petroleum spillage (Lu *et al.*, 2012). Similarly, a study in Solorina Valley, James Ross Island, revealed the presence of cadmium (Cd), mercury (Hg) and lead (Pb) in lichen samples, which act as biomonitors (Zvěřina *et al.*, 2018). Chemical analysis of soil samples collected from various locations on King George Island confirmed areas of high concentration of various heavy metals, particularly mercury (Hg), copper (Cu) and zinc (Zn) in petroleum-contaminated soil samples (Romaniuk *et al.*, 2018).

Various heavy metals are considered essential in living organisms' biochemical and physiological functions when present in trace concentrations; however, excessive exposure can lead to damage at cellular and tissue levels (Tchounwou *et al.*, 2012). These metals exhibit adverse effects on bacterial cells, particularly on cellular functions and metabolism and affect cell membrane integrity and can lead to changes in function and diversity of the soil microbial community (Gao *et al.*, 2010, Jiang *et al.*, 2019). The presence of phenol and co-contaminant heavy metals influences intricate chemical and biological processes since their co-occurrence can trigger competitive inhibition that inhibits microbial metabolism, lowering the phenol-degrading efficiency of the microorganisms (Jiang *et al.*, 2019). Therefore, it is necessary to study the phenol degrading potential of bacteria in the presence of toxic heavy metals to improve relevance to their potential for application in bioremediation processes.

In recent years, studies of mixed microbial cultures as distinct from single species have attracted research interest due to their advantages of being more effective, qualitatively, and quantitatively, than pure cultures. Synergistic interactions amongst the different taxa present result in greater metabolic capacity (Bull, 1984; Cerqueira *et al.*, 2011). Consortia may also have the ability to respond more effectively to variation in local environmental conditions (Arnosti *et al.*, 2016), making them better candidates to be applied in biodegradation. Phenol-degrading mixed cultures can be prepared by combining several microorganisms to form a defined mixed culture (DMC) with improved biodegradation capabilities. Attempts to evaluate the interactions between bacterial taxa that can degrade phenol have been carried out successfully (Dey and Mukherjee, 2010; Senthilvelan *et al.*, 2014; Zeng *et al.*, 2014; Bera *et al.*, 2017; Poi *et al.*, 2017; Youssef *et al.*, 2019). However, phenol bioremediation using Antarctic bacterial consortia has yet to receive detailed attention.

The ability of microbes to utilise toxic pollutants as sole carbon and energy sources, breaking down the complex chemical compounds to simpler substrates that can enter the tricarboxylic acid (TCA) cycle through a series of metabolic pathways, depends on the presence of critical microbial enzymes (Sridevi *et al.*, 2012; Arora and Bae, 2014). The development of high-throughput sequencing methodologies contributed to revealing novel knowledge of biochemical pathways of biogeochemical and phylogenetic significance and revealing the functional diversity of microorganisms capable of phenol degradation (Hong *et al.*, 2017). The genomic studies reveal information about the entire genome of the target microorganism, allowing specific genes to be explored, encoding the complete set of enzymes involved in phenol catabolic pathways (Ma and Zhai, 2012). Genomic analyses undoubtedly pave the way towards better understanding of the molecular mechanisms involved in pollutant remediation by native Antarctic bacteria, making a crucial contribution to the knowledge of microbial biodiversity and function in Antarctica. They also aid in the development of more effective remediation strategies.

Considering the hazardous effect of phenol towards the simple ecosystem of Antarctica and the challenging climate that limits natural biodegradation of this

persistent pollutant, this study focuses on developing a reliable tool to mitigate phenol pollution without causing further damage to this continent. The central premise of this study was that new taxa of pure phenol-degrading bacteria can be successfully isolated from Antarctic soil and, both as a pure isolate and together with previously isolated phenol-degrading bacteria as a consortium, will be capable of significant degradation of phenol at low temperatures (0-15°C) in a short time. This study will help explore the native microbial biodiversity of Antarctica and advance the utilisation of cold-adapted bacteria in remediating phenol-polluted sites, thereby contributing to environmental sustainability.

The main objectives of this study are:

1. To isolate, screen and identify cold-adapted phenol-degrading bacteria from Antarctic soil.
2. To identify the phenol-degrading pathway(s), phenol-degrading enzymes and their respective genes involved through bacterial whole genome sequencing and enzyme assays.
3. To determine the optimum conditions for phenol degradation by pure culture using OFAT and RSM.
4. To screen and optimise the degradation of phenol by DMCs of phenol-degrading bacteria using RSM approach.
5. To determine the effects of heavy metals on bacterial growth and phenol degradation for pure and mixed cultures.

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