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Article

Comparative genomic study of polar bacteria having tolerance to abiotic stress and potential for environmental and agricultural implementation

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Abstract: The polar regions are home to a variety of microorganisms. These microorganisms have been reported to have developed adaptive methods for survival in extreme conditions and resist varieties of abiotic stress such as heavy metals (HMs). Despite this, very limited studies have been done in bacteria from polar regions than bacteria from non-polar regions. The main aim of this study is to explore microorganisms from polar regions that could tolerate the abiotic stress of HMs. In this study, microorganisms from polar areas have been isolated and various bioinformatics tools were used for understanding the genomic features, comparison, and analysis. The wet-lab experiments were performed for the validation where the isolated bacteria were exposed to the abiotic stress of HMs. The genome analysis of all the isolated bacteria showed the presence of heavy metals resistance proteins. This study is very helpful in exploring the diversity of abiotic stress resistance microorganisms, monitoring environmental health, and utilizing these potential microorganisms for the betterment of the environment, agriculture, and ultimately humankind.

Keywords: Comparative genomics**,** Heavy metals, *Mesorhizobium* sp., Polar regions, *Sphingomonas* sp.

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1. Introduction

The *Sphingomonas* genus was defined by Yabuuchi et al., in 1990 and is a member of the family *Sphingomonadales*, phylum *Pseudomonadota,* and class Alphaproteobacteria [1]. They have been reported to be isolated from the soil, water, air, and marine environment [2-5]. In addition to that, many strains that exhibit the ability to use the toxins as nutrition have been identified from environments contaminated with hazardous chemicals. Furthermore, *Sphingomonas* are gram-negative, aerobic, rod-shaped, chemoheterotrophic bacteria that produce yellow or off-white pigmented colonies and possess a characteristic compound called sphingolipids. The sphingolipids have a unique sphingoglycolipid with the long-chain base dihydro sphingosin, ubiquinone 10 (Q-10), and 2-hydroxymyristic acid (2-OH C14:0) and the absence of 3-hydroxy fatty acids [6, 7]. Both pathogenic and non-pathogenic *sphingomonas* have been reported. *Sphingomonas elodea* strains (PHP1 and PBAD1) were reported as non-pathogenic [8] and *Sphingomonas meloni,* as phytopathogenic which is also linked to the demise of coral reefs off the coast of Florida [9, 10]. *Sphingomonas* species were well reported to degrade PAHs including fluorene and fluoranthene [11, 12]. They are HMs/metalloid-tolerant, with the potential to be used in bioremediation purposes [13, 14].

The *Mesorhizobium* genus was identified by Jarvis et al., in 1997 [15] is a member of the family *Phyllobacteriaceae,* phylum *Pseudomonadota*, and class Alphaproteobacteria [16, 17], and have been reported to be isolated from the seawater, wastewater treatment system, and groundwater [18–20]. They are also gram-negative, aerobic. Bacteria like *Mesorhizobium* but they are non-spore forming. The broad distribution of this genus and their ability to make a symbiotic relationship to several plant genera makes them an interesting candidate for agronomic and ecological applications [21, 22]. The majority of research on HMS in *Mesorhizobium* has been conducted in polluted and agricultural environment [23, 24]. The majority of *Mesorhizobium* research is focused on symbiotic relationships, plant growth promotion, nitrogen fixation in plants like chickpeas, and resistance ability to various antibiotics [25–29]. Besides that, *Mesorhizobium* species were also reported to have tolerance to heavy metals as well as potential for bioremediation of heavy metals [30, 31]. Even though both *Sphingomonas* and *Mesorhizobium* were reported to have HMs tolerance capacity and are well-known in contaminated and agricultural settings, very little work has been done from the polar areas.

Polar regions, being an isolated environment and having an extremely harsh climate have been reported to be affected by increased human activities including climate change and global warming. Furthermore, the abiotic stress of HMs has been reported in the polar areas. The microorganisms living in these areas have been reported to have developed adaptive strategies to survive in extreme conditions and resist varieties of abiotic stresses [32]. The development of these strategies in polar microorganisms is a fascinating area of research. Despite this fact, very limited studies have been done on bacteria from the polar areas of distant locations and harsh climates. In addition to that, the information regarding the diversity of such microorganisms is very limited.

The main objective of this study was to isolate such types of bacteria from various polar areas and perform genomic analysis and wet-lab experiments. In this study, we have isolated three different bacteria from the polar region two bacteria of the same genus but different species and one bacteria of a different genus. Genomic analysis was done by using various bioinformatics tools to understand all the genomic features and wet-lab experiments were performed. All the isolated bacteria might have the potential to be utilized in the future for the betterment of the environment, agriculture, and ultimately humankind.

2. Materials and Methods

2.1. Isolation and genomic DNA extraction of polar bacteria to study their adaptability to tolerate abiotic stress, as well as the study's possible application to future environmental and agricultural aspects

Sphingomonas sp. (1) and *Sphingomonas* sp. (2) were isolated from a rock of an arctic lichen *Umbilicaria* sp. and an arctic lichen *Cetraria* sp. respectively whereas, the strain *Mesorhizobium* sp. was isolated from soil samples from Uganda. All three bacteria were culture for 4-5 passages in an R2A medium. Then the bacterial strains were subculture in R2A agar plate 2-3 times at 15°C on R2A agar until the pure single colony of each strains were obtained. After that, each individual colony of bacterial strains were cultured and genomic DNA from all the bacteria was extracted using the QIAamp DNA Mini Kit, and its quantity and purity were assessed using a spectrophotometer. The extracted DNA was further evaluated for quality using agarose gel electrophoresis and stored at -20°C.

2.2. Genome sequencing and assembly process

The genome sequencing for *Mesohizobium* sp. was carried out using the PacBio RS II single-molecule real-time (SMRT) sequencing technology from Pacific Biosciences (Menlo Park, CA, USA). SMRTbell library inserts of 20 kb were prepared and sequenced using SMRT cells. The raw sequencing data were generated and subjected to de novo assembly utilizing the hierarchical genome assembly process (HGAP) protocol [33] and RS HGAP4 Assembly in SMRT analysis software (ver. 2.3; Pacific Biosciences, SMRT Link 4.0.0) protocols. The annotation of the genome was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). Furthermore, coding DNA sequences (CDSs) were predicted and annotated using the Rapid Annotation using Subsystem Technology (RAST) server [34]. The genome sequencing of *Sphingomonas* sp. (1) and *Sphingomonas* sp. (2) was analyzed by using a combined approach with the 454 GS FLX Titanium system (Roche Diagnostics, Brandford, CT) with an 8-kb paired-end library and the illumina GAIIx system (San diego, CA) with a 500-bp paired-end

library The detailed about these strains were mentioned by Jungeun Lee et al., 2012 [35] and Hyoungseok Lee et al., 2012 [36].

2.3. Functional annotation and comparative genomics analysis

Annotation tools such as RAST server was used, predicted gene sequences were translated and subjected to the National Center for Biotechnology Information (NCBI) non-redundant database, UniProtKB/Swiss-Prot, and Protein Data Bank proteins (PDB). The comprehensive annotation approach allowed us to gather a thorough understanding of the genomic features of all the strain (*Sphingomonas* sp. (1), *Sphingomonas* sp. (2), and *Mesohizobium* sp.

2.4. Bacterial isolation and growth

The *Sphingomonas* sp. (1), *Sphingomonas* sp. (2), and *Mesorhizobium* sp. was isolated using 0.1 x R2A agar (MB cell Ltd., Seoul, Korea). The environmental temperature during the isolation of the strain was 15°C.

2.5. Bacterial tolerance test to heavy metals

The tolerance of all three bacterial strains towards the heavy metals (HMs) was measured at 15°C by using salts of heavy metals such as copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$), cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$), 2 mM, 1 mM, 0.5 mM, 0.25 mM, 0.1 mM, 0.05 mM, and 0.01 mM. Spectrophotometer was used for the measurement of bacterial OD_{600}. All the experiments were performed in triplicates.

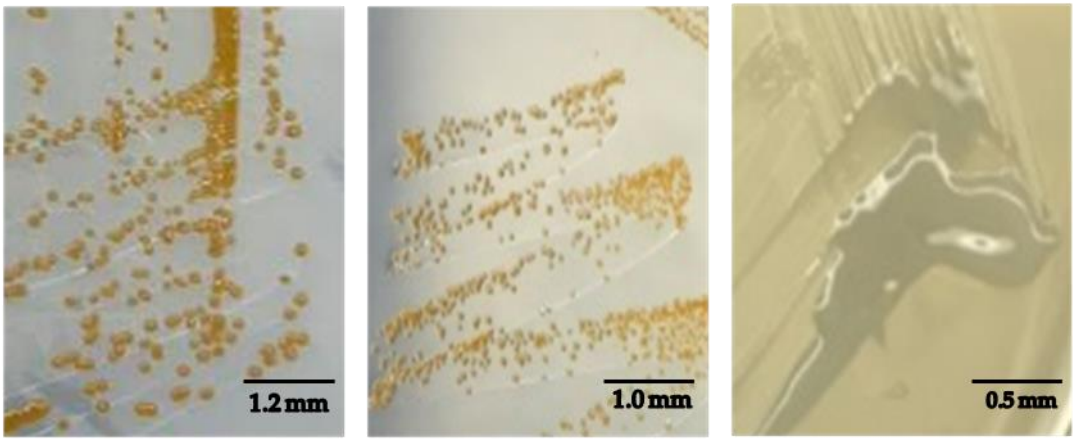
3. Results

3.1. Genomic features and genome analysis

Sphingomonas sp. (1) and *Sphingomonas* sp. (2) are draft genome and *Mesorhizobium* sp. is a complete genome. The genome analysis of *Sphingomonas* sp. (1) and *Sphingomonas* sp. (2) showed the presence of copper resistance protein (CopB, CopC, and CopD) and cobalt-zinc-cadmium resistance protein (CzcC and CzcD). And *Mesorhizobium* sp. showed the presence of copper resistance protein (CopC and CopD) and cobalt-zinc-cadmium resistant protein (CzcD).

3.2. Growth of the bacteria

All bacteria, *Sphingomonas* sp. (1), *Sphingomonas* sp.(2), and *Mesorhizobium* sp. was cultured in R2A agar plates at 15°C (Fig. 1).



(a) (b) (c)

Figure 1. (a) *Sphingomonas* sp. (1); (b) *Sphingomonas* sp. (2); (c) *Mesorhizobium* sp.

3.3. Tolerance of bacteria to different heavy metals (HMs)

Three strains *Sphingomonas* sp. (1), *Sphingomonas* sp. (2), and *Mesorhizobium* sp. showed HMs resistance protein from their genome analysis. Since all the strains showed copper resistant protein and cobalt resistance protein so

all the strains were subjected to HMs resistance toxicity tolerance assay using the salts of copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$), cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$). The concentration of copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) and cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$) used were 2 mM, 1 mM, 0.5 mM, 0.25 mM, 0.1 mM, 0.05 mM, and 0.01 mM. Among the three strains, Sphingomonas sp. (1) showed tolerance to 0.1 mM of $CuSO_4 \cdot 5H_2O$ and 0.25 mM of $CoCl_2 \cdot 6H_2O$, *Sphingomonas* sp. (2) showed tolerance to 0.1 mM of $CuSO_4 \cdot 5H2O$ and $CoCl_2 \cdot 6H_2O$, and *Mesorhizobium* sp. showed tolerance to 1 mM of $CuSO_4 \cdot 5H_2O$ and 2 mM of $CoCl_2 \cdot 6H_2O$ respectively at 15°C. Overall, these results indicate that the three bacterial strains have varying degrees of tolerance to the tested heavy metals at the given temperature. Among the three bacterial strains tested, *Mesorhizobium* has the highest tolerance capacity for both the metals copper as well as cobalt.

Table 1. Table showing HMs toxicity tolerance by *Sphingomonas* sp. (1), *Sphingomonas* sp. (2), and *Mesorhizobium* sp.

	***Sphingomonas* sp. (1)**		***Sphingomonas* sp. (2)**		***Mesorhizobium* sp.**	
(Used concentration of HMs)	**$CuSO_4 \cdot 5H_2O$**	**$CoCl_2 \cdot 6H_2O$**	**$CuSO_4 \cdot 5H_2O$**	**$CoCl_2 \cdot 6H_2O$**	**$CuSO_4 \cdot 5H_2O$**	**$CoCl_2 \cdot 6H_2O$**
2 mM	-	-	-	-	-	+
1 mM	-	-	-	-	+	+
0.5 mM	-	-	-	-	+	+
0.25 mM	-	+	-	-	+	+
0.1 mM	+	+	+	+	+	+
0.05 mM	+	+	+	+	+	+
0.01 mM	+	+	+	+	+	+

4. Discussion

HMs pollution had become an urgent issue worldwide which need rapid solution for future sustainability. Moreover, HMs pollution affected the polar regions too and the community of microbes living there. Microorganisms living there have been reported to have developed adaptive strategies. Furthermore, Microbes were reported to confer various types of resistance mechanism in response to HMs [37]. Copper resistance protein such as (CopA, CopB, CopC and CopD) were reported in various bacteria. These protein were responsible for copper resistance and maintenance of homeostasis [38-40]. Furthermore, multiple proteins such as CzcA, CzcC, and CzcD responsible for resistance of cobalt, zinc, and cadmium were also reported in various bacteria [41, 42]. The comparative study of genome analysis of *Sphingomonas* sp. (1) and *Sphingomonas* sp. (2) showed the presence of copper resistance protein (CopB, CopD) and cobalt-zinc-cadmium resistance protein (CzcC and CzcD) in both the strain. The comparison between *Sphingomonas* sp. (1) and *Sphingomonas* sp. (2) in terms of HMs, the former strain showed tolerance of 0.25 mM of $CoCl_2 \cdot 6H_2O$ and the latter strain showed tolerance of 0.1 mM of $CoCl_2 \cdot 6H_2O$. In the case of $CuSO_4 \cdot 5H_2O$, both strains showed tolerance to 0.1 mM of $CuSO_4 \cdot 5H_2O$ at 15°C. *Mesorhizobium* sp. showed the presence of copper resistance protein (CopC and CopD) and cobalt-zinc-cadmium resistant protein (CzcD). *Mesorhizobium* sp. were able to tolerate up to 1 mM $CuSO_4 \cdot 5H_2O$ and 2 mM of $CoCl_2 \cdot 6H_2O$ at 15 °C. Even though our isolated strain did not showed very high tolerance but showed some tolerance to HMs than the other microorganism from polluted areas, the fact that the polar areas are not exposed to high pollution and considered as pristine [43] should be noted.

5. Conclusions

The polar regions are home to many different microorganisms even being isolated environment with extreme climate. Information regarding the diversity of such microorganisms is valuable. Besides that, these microorganisms have developed various strategies to cope with extreme environment and tolerance to stresses like HMs. In this study, we compare three bacteria isolated from the polar region (*Sphingomonas* sp. (1), *Sphingomonas* sp. (2),

and *Mesorhizobium* sp.) in terms of their genomic features. Furthermore, these strains showed HMs tolerance at low temperature. These strains might have potential for bioremediation in future. This study is very helpful to find the diversity of abiotic stress (HMs) resistant microorganisms, to monitor environmental health, and to utilize these potential microorganisms in future for the betterment of the environment, agriculture, and ultimately humankind.

6. Summary

Three different bacterial strains namely, *Sphingomonas* sp. (1), *Sphingomonas* sp. (2), and *Mesorhizobium* sp. were isolated from polar regions. All three bacterial strains showed HMs resistance protein (copper resistance and cobalt resistance protein) and were able to tolerate HMs at 15°C.

Author Contributions: T.-J.O. designed and supervised the project. A.K. performed the experiments; A.K., S.-R.H., H.L., and T.-J.O. wrote the manuscript. All authors discussed the results, commented on the manuscript, and approved the manuscript.

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Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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