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SHORT COMMUNICATION

Psychrotolerant *Sphingobacterium kitahiroshimense* LT-2 Isolated from Dhundi Glacier, Himachal Pradesh: Origin Prediction and Future Application

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Abstract A psychrotolerant bacterium, isolated from Dhundi Glacier, Himachal Pradesh (India) was identified as *Sphingobacterium kitahiroshimense* LT-2 on the basis of biochemical, molecular and phylogenetic analysis. *Sphingobacterium kitahiroshimense* was first reported from Japan and was isolated from the city of Kitahiroshima, Hokkaido, Japan. In this report we have discussed about the origin of our strain and predicted that air masses and dust associated microbial cells transportation phenomena may be applicable for the origin of this species in this region. Enzymes and secondary metabolites secreted by the genus *Sphingobacterium* have enormous potentiality regarding their biotechnological application. Preliminary study of our strain based on metabolic profiling through HPLC showed many new metabolites were secreted by the bacterium when grown in presence of different sugar medium at 28 °C. As far as our knowledge this is the first report about *Sphingobacterium* species isolated from this region. This preliminary finding will help to draw an idea about the bacterial population in this Himalayan Glaciers (in HP) as well as biotechnological application of this strain can be explored further.

Keywords Psychrophiles · Psychrotolerant bacteria · *Sphingobacterium* · Secondary metabolites · Cold active enzymes · Dust associated microbial cells transportation

The organisms thriving in extreme ecosystems have prosperous resource of various chemical and novel natural products with interesting bioactivities. Among them, psychrophiles which can be found in Arctic, Antarctic and Himalaya are "cold-loving" microorganisms living at subzero temperatures undergo certain adaptations by using variety of mechanisms to sustain their cell cycle [1]. These include the production of cold-shock and anti freeze proteins, synthesis of cold tolerant enzymes for the regulation of metabolic pathways, alterations in membrane composition to increase membrane flexibility, ample translation and proper protein folding under cold conditions. These adaptations are often accompanied by certain unique modifications to both gene regulation and metabolic pathways, increasing the possibility of finding unique functional metabolites of pharmaceutical importance [2]. The micro organisms dwelling in the cold habitat are also found to be the rich source of secondary metabolites which mainly showed antibacterial and free radical properties. The psychrophilic microorganisms are of two types viz. Stenopyschrophiles (true psychrophiles) that grow at definite low range of temperature (0–15 °C) and cannot grow at higher temperature range and Eupsychrophiles (psychrotolerant or psychrotrophs) which can grow at low temperature conditions and can tolerate up to mesophilic temperature range (20–25 °C) [3]. Very little study has been done till date on the bacterial diversity of the Himalayan glaciers whereas

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this region is considered as a place of one of the most underutilized resources. The psychrophilic and psychrotolerant cultivable bacterial strains are dominant in the Himalayan ecosystem. Approximately 45% of cultivable bacterial strains from this region belonged to the *Proteobacteria* group in which γ-*Proteobacteria* and β-*Proteobacteria* constitutes up to 39 and 31% respectively. The second most predominant class is *Firmicutes* (32%) followed by *Actinobacteria* (16%) and *Bacteroidetes* (6%). The most abundant strains of microorganisms in Himalayan range belonged to the genus *Bacillus* (30%) followed by *Pseudomonas* (24%) and *Arthrobacter* (12%) [4].

In the present study, a psychrotolerant bacterium identified as *Sphingobacterium kitahiroshimense* LT-2 (accession number MG027863), was isolated from Dhundi Glacier (nearby soil) which is geographically located between 32°21′53.47″N and 77°03′52.77″E in Beas basin in Manali on North of Kullu in Himachal Pradesh (India) situated at an elevation of 4038 m on the eastern slope of Rohtang pass in the western Himalayas. The strain LT-2 was isolated by using the dilution-plating technique on Nutrient Agar Plates incubated at 15 °C and characterized on the basis of biochemical analysis and molecular characterization by Sequencing of 16S rRNA gene. Phylogenetic analysis and multiple sequence alignment of data were done using the software packages MEGA 5 [5] using CLUSTALW [6] respectively. Evolutionary distances and clustering were based on the neighbour joining [7] and maximum-likelihood methods [8]. On the basis of neighbour-joining algorithm the strain LT-2 showed 99.64% similarity with *Sphingobacterium kitahiroshimense* (Fig. 1). The biochemical analysis showed that it is gram negative, rod shaped, non spore forming, catalase and oxidase positive aerobic bacterium. The smooth, circular and creamy white colonies developed on nutrient agar plates after 3–4 days. This bacterium is psychrotolerant and can withstand temperature range in between 4 and 37 °C.

The family *Sphingobacteriaceae* comprises of certain psychrotolerant microorganisms such as members of genus *Sphingobacterium* in which cells comprises of high concentration of sphingophospholipids which helps the microorganisms to adapt in cold conditions [9]. The first discovery of this genus *Sphingobacterium* was proposed by Yabuuchi et al. [10]. The family members of this genus have been isolated from different sources and up to 32 species of *Sphingobacterium* have been identified [11]. The main fatty acid composition of *Sphingobacterium* comprises of iso-$C_{15:0}$, iso-$C_{15:0}$ 2-OH, $C_{16:1}\omega7c$ and $C_{17:0}$ 3-OH and biochemical characteristics includes catalase and oxidase positive and negative for gelatinase and indole production [9].

Only one published data on *Sphingobacterium kitahiroshimense* was reported which was isolated from the city of Kitahiroshima, Hokkaido, Japan [12]. It will be an interesting piece of work with novelty to know the phenomenon of transfer (if any) of this bacterium strain from Japan to Dhundi glacier and vice versa. The microbial communities in troposphere of Himalayan range in Himachal Pradesh are feebly characterized due to the harsh conditions and various difficulties in collecting biomass. A very little study has been done on spatial and temporal variability of these tropospheric microbial groups, mechanism of adaptation of these psychrophiles to low temperature conditions and viability phenomenon of these microbes. The effects of atmospheric processes such as tropical cyclones, hurricanes on microbial groups that can precipitate or aerosolize microbial cells and can cause alteration in composition and functions of microorganisms [13]. In Himalayan range of Himachal Pradesh, there is prevalence of strong winds with higher rate of precipitation during the start of winters. The range of dust concentrations at 5000 m of glacier is in between 8 and 100 μg m^{-3} up to 800 μg m^{-3} with an annual dust fluctuation spanning from 770 to 1030 mg m^{-2} [14]. The microbial groups detected at the middle troposphere altitude (6000 m) get entrapped with the deposition of dust particles constituting dust traps. The strong wind currents, cyclones and hurricanes in the Himalayan range may help in the transfer of dust associated microbes from long distance. Previous studies also supported the fact that air masses and dust associated microbial cells transported with them and generally occurred from lower altitudes and were brought up during the hurricanes/cyclones [13]. Based on these theories, it can be said that transportation of viable microbes can be possible across the continents and same phenomena may also be applicable for the present case. Figure 2 summarises the mechanism of transportation of the microbes across the different continent [15].

The secondary metabolites secreted by psychrophiles/psychrotolerant bacteria have diverse biological activities ranging from cytotoxic, antioxidant, antiviral and antibacterial [16]. It was reported that, *Sphinobacterium* sp. have a set of enzymes to regulate the metabolic pathways in extreme conditions such as lipases, isocitrate dehydrogenase, cellulases, superoxide dismutase, oxidases, pectinase and esterase etc. [17]. Cold active enzymes can withstand extreme range of temperature, pressure and these cold tolerant enzymes have a great importance at commercial levels as these acts as catalyst in various biotechnological industries. The cold tolerant enzymes are used in the field of food industry (pectinase, α-galactosidase), lignin degradation (superoxide dismutase), detergent formulation industries (lipases, cellulases), bioremediation (oxidases), biotransformations (methylases and aminotransferases) and

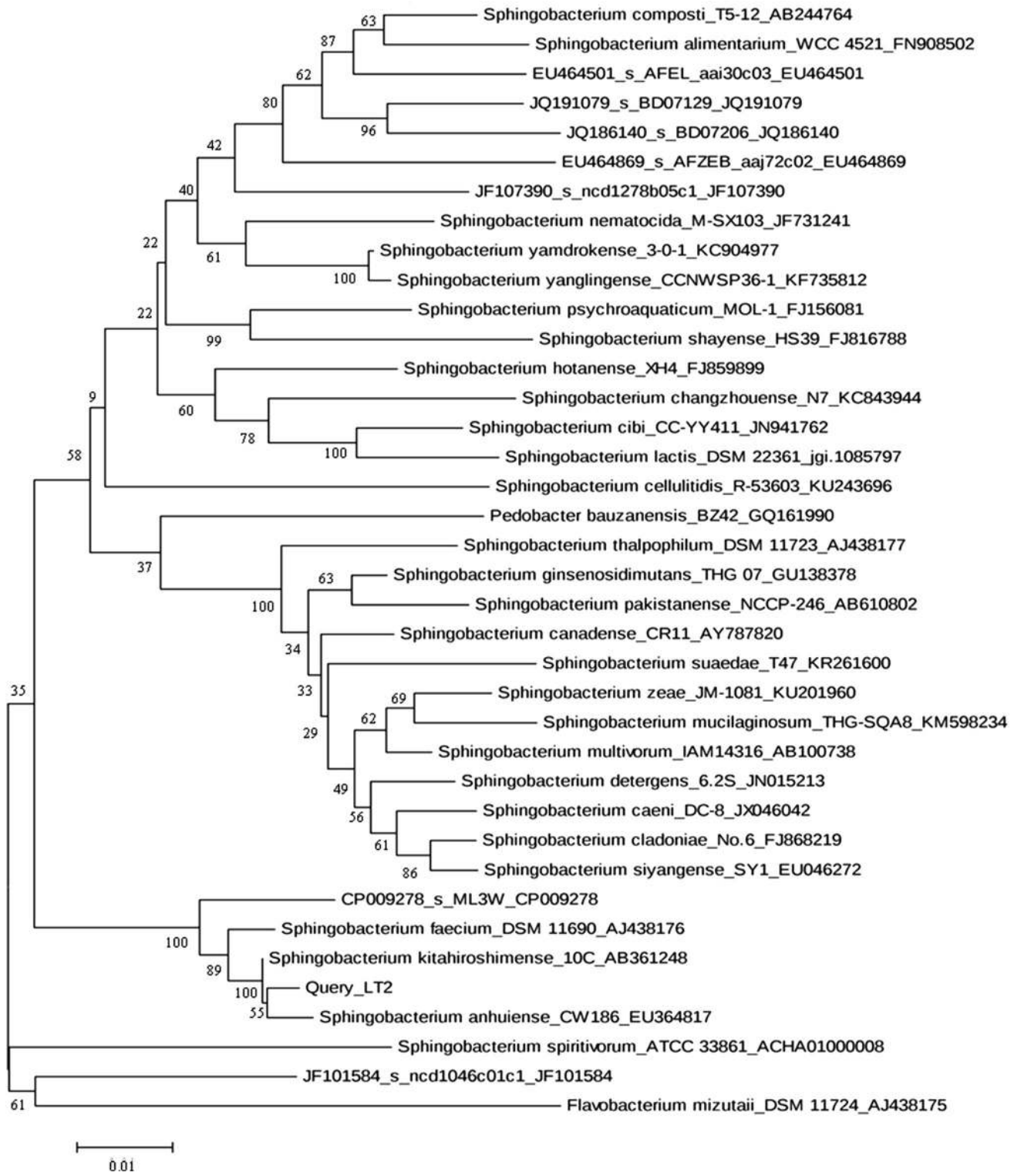

Fig. 1 Phylogenetic tree showing the relationship between *Sphingobacterium kitahiroshimense* LT-2 (Query_LT2) and other related strains, 16S rRNA gene sequences with reference sequences obtained through BLAST analysis. Phylogenetic analysis and multiple sequence alignment of data were done using the software packages MEGA 5 using CLUSTALW respectively. Evolutionary distances and clustering were based on the neighbour joining and maximum-likelihood methods. One hundred bootstrap replicates were performed. Bootstrap values are indicated on the branches

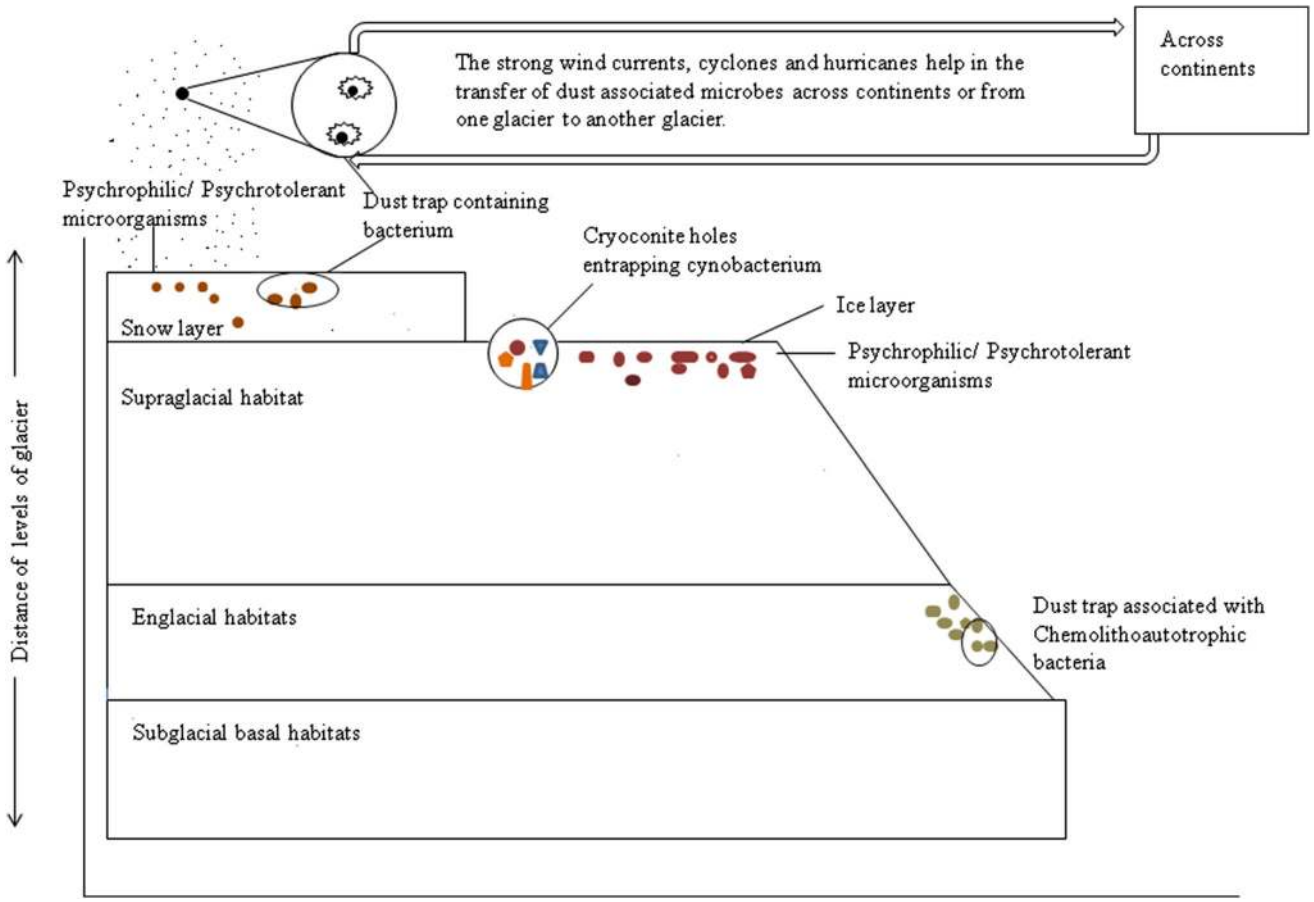

Fig. 2 Mechanism of transportation of the microbes across the different continent

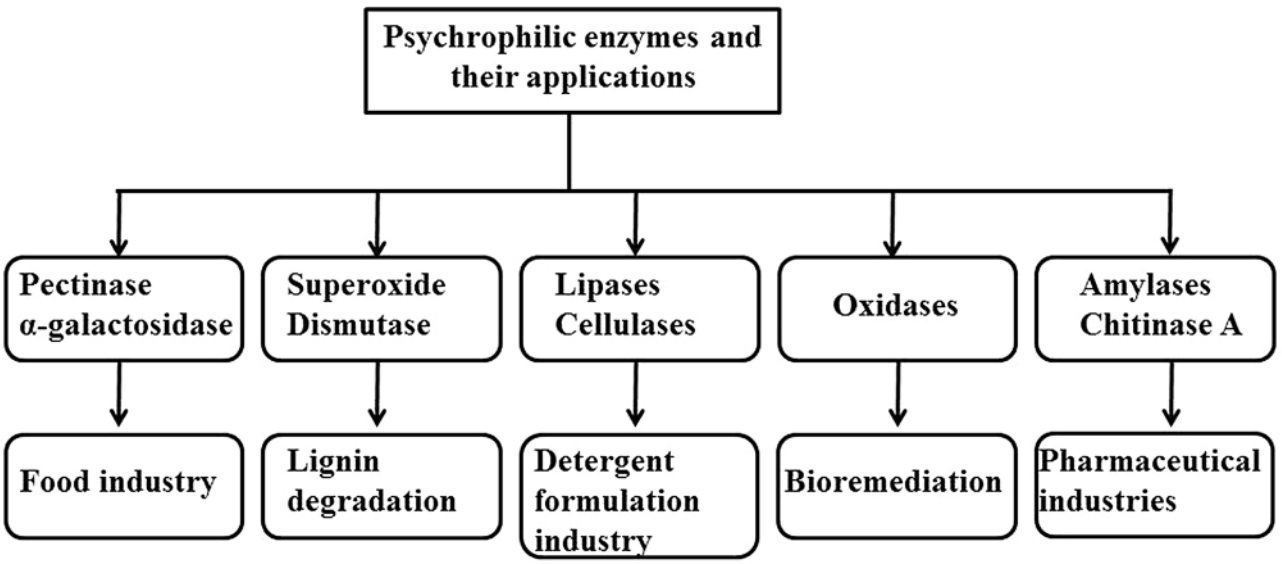

Fig. 3 Various biotechnological applications of the enzymes of *Sphingobacterium* sp.

in pharmaceutical industries (amylases, Chitinase A) [17] (Fig. 3). The bacterium identified in the present study may contribute to the production of novel antibiotics or other biologically active secondary metabolites which can play a potential role in future healthcare system or in biotech industry. A preliminary study has been done based on the

metabolic profiling of the secondary metabolites secreted by this bacterium in various sugars medium at 28 °C. HPLC profiling of these metabolites indicated that few metabolites were exclusively secreted when bacterial culture was grown in these mediums at 28 °C (Fig S1). The characterization of these metabolites and biological activity study are in progress.

As far as our knowledge this is the first report about *Sphingobacterium* species isolated from this region. The findings from this preliminary study are important in a sense that it can give valuable idea about the bacterial population in Himalayan Glaciers (HP) as well as the bacterium isolated from this study can be utilised further for biotechnological application.

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Compliance with Ethical Standards

Conflict of interest Authors declare no conflict of interest.

References

1. Tian Y, Li YL, Zhao FC (2017) Secondary metabolites from polar organisms. Mar Drugs 15:28. https://doi.org/10.3390/md15030028
2. Amico SD, Collins T, Marx JC, Feller G, Gerday C (2006) Psychrophilic microorganisms: challenges for life. EMBO Rep 4:385–389. https://doi.org/10.1038/sj.embor.7400662
3. Maayer PD, Anderson D, Cary C, Cowan DA (2014) Some like it cold: understanding the survival strategies of psychrophiles. EMBO Rep 15:508–517. https://doi.org/10.1002/embr.201338170
4. Gangwar P, Alam SI, Bansod S, Singh L (2005) Bacterial diversity of soil samples from the western Himalayas, India. Can J Microbiol 55:564–577. https://doi.org/10.1139/w09-011
5. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739. https://doi.org/10.1093/molbev/msr121
6. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4888. https://doi.org/10.1093/nar/25.24.4876
7. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454
8. Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376. https://doi.org/10.1007/bf01734359
9. Liu R, Liu H, Zhang CX, Yang SY, Liu XH, Zhang KY, Lai R (2008) *Sphingobacterium siyangense* sp. nov., isolated from farm soil. Int J Syst Evol Microbiol 58:1458–1462. https://doi.org/10.1099/ijs.0.65696-0
10. Yabuuchi E, Kaneko T, Yano I, Moss CW, Miyoshi N (1983) *Sphingobacterium* gen. nov., *Sphingobacterium spiritivorum* comb. nov., *Sphingobacterium multivorum* comb. nov., *Sphingobacterium mizutae* sp. nov., and *Flavobacterium indologenes* sp. nov.: glucose non-fermenting Gram-negative rods in CDC Groups IIK-2 and IIb. Int J Syst Bacteriol 33:580–598. https://doi.org/10.1099/00207713-33-3-580
11. Long X, Liu B, Zhang S, Zhang Y, Zeng Z, Tian Y (2016) *Sphingobacterium griseoflavu*m sp. nov., isolated from the insect *Teleogryllus occipitalis* living in deserted cropland. Int J Syst Evol Microbiol 66:1956–1961. https://doi.org/10.1099/ijsem.0.000970
12. Hidetoshi M, Hiromichi K, Takahumi O, Atsushi S, Kazuyoshi K, Isao Y (2008) *Sphingobacterium kitahiroshimense* sp. nov., isolated from soil. Int J Syst Evol Microbiol 58:1576–1579. https://doi.org/10.1099/ijs.0.65791-0
13. DeLeon-Rodriguez N, Lathem TL, Rodriguez-R LM, Barazesh JM, Anderson BE, Beyersdorf AJ, Ziemba LD, Bergin M, Nenes A, Konstantinidis KT (2013) Microbiome of the upper troposphere: species composition and prevalence, effects of tropical storms, and atmospheric implications. Proc Natl Acad Sci USA 110:2575–2580. https://doi.org/10.1073/pnas.1212089110
14. Stres B, Sul WJ, Murovec B, Tiedje JM (2013) Recently deglaciated high-altitude soils of the Himalaya: diverse environments, heterogenous bacterial communities and long-range dust inputs from the upper troposphere. PLoS ONE 8:e76440. https://doi.org/10.1371/journal.pone.0076440
15. Boetius A, Anesio AM, Deming JW, Mikucki JA, Rapp JZ (2015) Microbial ecology of the cryosphere: sea ice and glacial habitats. Nat Rev Microbiol 13:677–690. https://doi.org/10.1038/nrmicro3522
16. Berdy J (2005) Bioactive microbial metabolites. J Antibiot 58:1–26. https://doi.org/10.1038/ja.2005.1
17. Choi HA, Lee SS (2012) *Sphingobacterium kyonggiense* sp. nov., isolated from chloroethene-contaminated soil, and emended descriptions of *Sphingobacterium daejeonense* and *Sphingobacterium mizutaii*. Int J Syst Evol Microbiol 62:2559–2564. https://doi.org/10.1099/ijs.0.024737-0