Reviewer 1

Recommendation "accepted as is".

Response: Thanks very much for your time and consideration.

Reviewer 2

The authors did a great job during their response to the reviewers remarks and significantly improved the manuscript entitled "Microbial and Next Generation Sequencing Approach for Bacteria in Snow and Frost Flowers: Selected Identification, Abundance and Freezing nucleation". Nevertheless:

• The manuscript is still highly speculative. For instant, the authors discussed the role of Geobaccter species in uranium utilization (lines 330-336) but I didn't find any support for presence of Geobacter species presented in the dataset (Table 2; Table 3; Table A.1 and Table A.2). Moreover, throughout the manuscript the authors attempt to infer about bacterial origin and physiological characteristics based on 16S gene similarity, however the percentage of similarity presented here range from 87 to 98%. It is well established that even bacteria with 100% of similarity of the 16S gene, may have completely different metabolic capacities. For instance, E. coli strains share 100% similarity however the physiological and metabolic characteristics among them have varied from harmful pathogens to commensal bacteria. Thus I suggest to authors more carefully rewrite the discussion.

Response: Thank you for your comment. As you might have noticed the organization of discussing the data in this article start with analyzing: i) NGS data, ii) cultivable microbial (density, identification, ice nucleation) and, iii) providing evidence of microbial entities and their elemental analysis using high resolution transmission electron microscopy in conjunction with Energy Dispersive x-ray Spectroscopy (EDS).

The presence of Geobacter bacteria and its importance was examined within the first part of discussion (NGS data). This bacteria was only detected using NGS methodology. The reviewer did not find the Geobacter bacteria in Table 2, since this table contain the identified cultivable bacteria using Sanger method. Note Table 3 contains the identified cultivable bacteria with their ice nucleation analysis. Table A.1 shows the relative abundance of bacterial taxa using NGS at phyla (A) and the top four genera with the highest percentage (B) in each Arctic sample. At phylum level, this is named as Proteobacteria. This is the reason that the reviewer could not find it in Table A.1. A. & in Table A.1.B, Geobacter does not belong to the top four genera observed in wind pack snow. Moreover, Table A.2 shows the genus distribution (>1%) of Bacterial community in Arctic samples by NGS. This bacterium was detected at 0.09% level. Please note

that not all NGS data can be given in this paper, as the mega data set is huge. However, we will provide access to NGS data, upon the publication of the manuscript.

As was commented: "*Moreover, throughout the manuscript the authors attempt to infer about bacterial origin and physiological characteristics based on 16S gene similarity, however the percentage of similarity presented here range from 87 to 98%.*"

Response: We did not at all attempt to infer about bacterial origin and physiological characteristics based on 16S gene similarity. Note that in both Tables 2 and 3, the name of identified bacteria is indeed given, along with its percentage of similarity to the known published nucleic acid sequences. The ice nucleation of each cultivable bacteria was examined and is also reported in Table 3. There has been no attempt in our part to compare their ice nucleation activity with other known bacteria with possible similar ice nucleation properties, as the nucleation experiment were performed only with a focus for "cultivable bacteria" of snow that we sampled in the Arctic. The identification of total bacterial pools were done by next generation sequencing. Culture method provides limited genetic identification of bacteria which are cultivable, whereas NGS provides insights into total viable and non-viable poll of bacteria.

"*It is well established that even bacteria with 100% of similarity of the 16S gene, may have completely different metabolic capacities. For instance, E. coli strains share 100% similarity however the physiological and metabolic characteristics among them have varied from harmful pathogens to commensal bacteria. Thus I suggest to authors more carefully rewrite the discussion.*"

Response: Thank you. Please note that even though, this comment might be true for some strains, this observation should not at all undermine the validity of sequencing techniques and questioning its usage for identification. The data presented was not physiologically or metabolically examined or compared with other bacteria. The established molecular biology techniques in determining the nucleic acid sequences were solely used for identification of bacteria in different snow samples. We have provided a series of references to address that the methodology used is quite conventional and widely used and validated. Note also with any techniques, there are advantages and disadvantages, and to our knowledge we are not aware of any techniques without shortcomings in this domain. However, we have modified the section so that we avoid any ambiguity and have added several references.

• *I have serious concerns about the authors' choice for a DNA extraction method. According the extraction methods presented in section 2.4 (lines 209-213) the authors lose most of DNA from*

their samples. The 10 minutes of ultra-sonication will disturb the vast majority of bacteria in the sample, releasing genomic DNA into the aqueous phase. Released DNA will NOT precipitate from this phase during a 15 min centrifugation at 18000 g. For DNA to pellet, the solution needed to contain a sufficiently high concentration of salts. Here, the author's will primarily pellet cell wall fragments, denatured proteins and small proportions of undisturbed cells. The DNA extracted using this method represents a tiny fraction of total DNA, only that from undisturbed cells and DNA that had happened to sorb to cell walls.

Response: Please kindly note that for DNA analysis, bath sonication is a method that has been used to dislodge adherent bacteria in environmental samples (Buesing and Gessner, 2002; Bopp et al., 2011; Joly et al., 2006; Kesberg, and Schleheck, 2013) as well as in medically devised explanted prosthetic instruments studied in hundreds of patients (Piper et al., 2009; Sampedro et al., 2010; Tunney et al., 1999). The dislodge bacteria is viable and can be cultured (Trampuz et al., 2007; Vergidis et al., 2011; Piper et al., 2009; Sampedro et al., 2010; Tunney et al., 1999; Joly et al., 2006; Kesberg, and Schleheck, 2013; Solon et al., 2011). We have modified the manuscript accordingly.

Please note that the action of ultrasound as a potential microbial inactivation method is considered as a complex process and the mechanism of its action and factors influencing its effectiveness are not completely understood. The impact of ultrasound on microbial inactivation is dependent on: i) the type of bacteria being treated, and its growth phase, ii) the composition of sonication tubes/container (e.g. glass is more effective than plastic or softer materials), iii) temperature (35 °C>22 °C), and iv) exposure time (Monsen et al., 2009; Piyasena et al., 2003). Shearing of DNA is usually achieved by directly submerging the disrupter horn, or probe tip inside the bacterial suspension in combination with high temperature (Naughton et al., 2013), mechanical and or chemicals (Fykse et al., 2003; Herceg et al., 2012; joyce et al., 2010).

For removing the viable bacteria from surface by sonication, no major differences were reported for: i) duration of exposure time at 5 or 10 min, and ii) temperatures at 22 °C or 6 °C; though the latter only slightly improved bacterial viability (Monsen et al., 2009). In our experiment, all the above factors were considered for removing the bacteria from filters. The extraction of DNA from snow samples used in materials and methods is similar to what has been done by Moran et al., 2008; Gharaibeh et al., 2009; Gantner et al., 2011; and Medinger et al., 2010.

• All published data must be publicly available!!!The author MUST deposit the generated sequencing data into a public access archive like NCBI and provide their accession numbers both for NGS and isolates.

WITHOUT THESE ACCESSION NUMBERS WE CAN NOT PUBLISH THE MANUSCRIPT.

Response: We have no objection, and the Genome Canada is in the process. It will be done simultaneously upon the publication of this manuscript.

References

Bopp, J. D., Musser, A. K., and Nazarian, J. E.: Legionella, in: Molecular Detection of Human Bacterial Pathogens, CRC Press, 919-928, 2011.

Buesing, N., and Gessner, O. M.: Comparison of detachment procedures for direct counts of bacteria associated with sediment particles, plant litter and epiphytic biofilms, Aquat Microb Ecol, 27, 29–36, 2002.

Fykse, E. M., Olsen, J. S., and Skogan, G.: Application of sonication to release DNA from Bacillus cereus for quantitative detection by real-time PCR, Journal of microbiological methods, 55, 1-10, 2003.

Gantner, S., Andersson, A. F., Alonso-Saez, L., and Bertilsson, S.: Novel primers for 16S rRNA-based archaeal community analyses in environmental samples, Journal of microbiological methods, 84, 12-18, 10.1016/j.mimet.2010.10.001, 2011.

Gharaibeh, D. N., Hasegawa, H., and Hase, C. C.: Development of a quantitative real-time PCR assay for detection of Vibrio tubiashii targeting the metalloprotease gene, Journal of microbiological methods, 76, 262-268, 10.1016/j.mimet.2008.12.001, 2009.

Herceg, Z., Režek, J. A., Lelas, V., and Mededovic, T. S.: The Effect of High Intensity Ultrasound Treatment on the Amount of Staphylococcus aureus and Escherichia coli in Milk, Food Technology & Biotechnology, 50, 46, 2012.

Joly, P., Falconnet, P. A., Andre, J., Weill, N., Reyrolle, M., Vandenesch, F., Maurin, M., Etienne, J., and Jarraud, S.: Quantitative real-time Legionella PCR for environmental water samples: data interpretation, Applied and environmental microbiology, 72, 2801-2808, 10.1128/aem.72.4.2801-2808.2006, 2006.

Joyce, E., Al-Hashimi, A., and Mason, T. J.: Assessing the effect of different ultrasonic frequencies on bacterial viability using flow cytometry, Journal of applied microbiology, 110, 862-870, 10.1111/j.1365-2672.2011.04923.x, 2011.

Kesberg, A. I., and Schleheck, D.: Improved protocol for recovery of bacterial DNA from water filters: Sonication and backflushing of commercial syringe filters, Journal of microbiological methods, 93, 55-57, 10.1016/j.mimet.2013.02.001, 2013.

Medinger, R., Nolte, V., Pandey, R. V., Jost, S., Ottenwalder, B., Schlotterer, C., and Boenigk, J.: Diversity in a hidden world: potential and limitation of next-generation sequencing for

surveys of molecular diversity of eukaryotic microorganisms, Molecular ecology, 19 Suppl 1, 32-40, 10.1111/j.1365-294X.2009.04478.x, 2010.

Monsen, T., Lovgren, E., Widerstrom, M., and Wallinder, L.: In vitro effect of ultrasound on bacteria and suggested protocol for sonication and diagnosis of prosthetic infections, Journal of clinical microbiology, 47, 2496-2501, 10.1128/jcm.02316-08, 2009.

Moran, A. C., Hengst, M. B., De La Iglesia, R., Andrade, S., Correa, J. A., and Gonzalez, B.: Changes in bacterial community structure associated with coastal copper enrichment, Environmental toxicology and chemistry / SETAC, 27, 2239-2245, 10.1897/08-112.1, 2008.

Piper, K. E., Jacobson, M. J., Cofield, R. H., Sperling, J. W., Sanchez-Sotelo, J., Osmon, D. R., McDowell, A., Patrick, S., Steckelberg, J. M., Mandrekar, J. N., Fernandez Sampedro, M., and Patel, R.: Microbiologic diagnosis of prosthetic shoulder infection by use of implant sonication, Journal of clinical microbiology, 47, 1878-1884, 10.1128/jcm.01686-08, 2009.

Piyasena, P., Mohareb, E., and McKellar, R. C.: Inactivation of microbes using ultrasound: a review, International journal of food microbiology, 87, 207-216, 2003.

Sampedro, M. F., Huddleston, P. M., Piper, K. E., Karau, M. J., Dekutoski, M. B., Yaszemski, M. J., Currier, B. L., Mandrekar, J. N., Osmon, D. R., McDowell, A., Patrick, S., Steckelberg, J. M., and Patel, R.: A biofilm approach to detect bacteria on removed spinal implants, Spine, 35, 1218-1224, 10.1097/BRS.0b013e3181c3b2f3, 2010.

Solon, I., Gurian, P. L., and Perez, H.: The Extraction of a Bacillus anthracis Surrogate from HVAC Filters, Indoor and Built Environment, 10.1177/1420326x11420307, 2011.

Trampuz, A., Piper, K. E., Jacobson, M. J., Hanssen, A. D., Unni, K. K., Osmon, D. R., Mandrekar, J. N., Cockerill, F. R., Steckelberg, J. M., Greenleaf, J. F., and Patel, R.: Sonication of removed hip and knee prostheses for diagnosis of infection, The New England journal of medicine, 357, 654-663, 10.1056/NEJMoa061588, 2007.

Tunney, M. M., Patrick, S., Curran, M. D., Ramage, G., Hanna, D., Nixon, J. R., Gorman, S. P., Davis, R. I., and Anderson, N.: Detection of prosthetic hip infection at revision arthroplasty by immunofluorescence microscopy and PCR amplification of the bacterial 16S rRNA gene, Journal of clinical microbiology, 37, 3281-3290, 1999.

Vergidis, P., Greenwood-Quaintance, K. E., Sanchez-Sotelo, J., Morrey, B. F., Steinmann, S. P., Karau, M. J., Osmon, D. R., Mandrekar, J. N., Steckelberg, J. M., and Patel, R.: Implant sonication for the diagnosis of prosthetic elbow infection, Journal of shoulder and elbow surgery / American Shoulder and Elbow Surgeons ... [et al.], 20, 1275-1281, 10.1016/j.jse.2011.06.016, 2011.

Naughton, C., Avlonitis, N., Corless, S., Prendergast, J. G., Mati, I. K., Eijk, P. P., Cockroft, S. L., Bradley, M., Ylstra, B., and Gilbert, N.: Transcription forms and remodels supercoiling

domains unfolding large-scale chromatin structures, Nature structural & molecular biology, 20, 387-395, 10.1038/nsmb.2509, 2013.