Study of airborne bacteria and their relation to air pollutants

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Abstract

The atmosphere may not strike people as a very suitable habitat for bacteria, but recent studies indicate it may harbour diverse and active bacterial communities. Although their complexity is similar to those found in soil or marine environments, it is not as well understood. Active bacterial communities in the soil, for example, have not only been observed to become more resistant when exposed to pollutants, but they also developed degradation mechanisms. Can similar processes occur in airborne bacterial communities? This possibility holds great potential for dealing with air pollution in the future. As basic research is limited, we aimed to study how active life in the atmosphere is influenced by airborne stressors. An unusually high fraction of airborne bacteria was observed to be pigmented, presuming to resist UV radiation. Furthermore, the airborne bacteria from three different locations in the region of Antwerp (Belgium), i.e. the centre of the city, the harbour, and a greener suburb were compared for their cadmium resistance. Furthermore, the growth of airborne bacteria exposed to volatile organic compounds (VOCs) was assessed. The results indicate that airborne bacterial communities adapt to the present air pollutants, possibly using VOCs as nutritional substrates. Airborne bacteria are therefore good candidates for future applications in biomonitoring and bioremediation.

Keywords: airborne bacteria, air pollution, bioremediation, heavy metals, pigmentation, volatile organic compounds.

1 Introduction

Air pollution is a global problem and despite stagnating trends for certain pollutants in Europe [1], air pollution and its adverse effects are still increasing on a worldwide scale [2]. This is also illustrated by a recent report of the World Health

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Organization (WHO), stating that 7 million deaths in 2012 were linked to air pollution, which translates to 1/8 of the world mortality [3].

The atmosphere may not strike people as a very suitable habitat for bacteria, but recent studies indicate it harbours diverse bacterial phyla, such as *Firmicutes, Actinobacteria, Proteobacteria*, and *Bacteroidetes*, which may form active bacterial communities [4]. Their complexity is similar to those found in soil or marine environments, but not yet as well understood. Microbial communities in the soil, for example, have not only been observed to become more resistant when exposed to pollutants, but they also developed degradation mechanisms. Can similar processes occur in airborne bacterial communities?

The study of the metabolic activity of airborne microbes is rather limited. In order to find new and improved ways of biological degradation of air pollutants, we aim at developing novel methodologies to address the interactions between airborne microbes and air pollutants. In this paper we focus on the sampling, culturing, and the study of pollution resistance of airborne bacteria.

Micro-organisms in the atmosphere can come into contact with two kinds of pollutants, namely the solid ones, embedded in airborne particulate matter (PM), and gaseous pollutants, such as ozone (O_3) nitrogen oxides (NOx) and volatile organic compounds (VOCs). PM and tropospheric O_3 are considered the most problematic pollutants in Europe [1]. Especially the respirable fraction of PM, PM2.5 (diameter under 2.5 μm), has been related with respiratory and cardiac diseases [5]. Heavy metals are claimed to be one of the main toxic components of PM2.5 because they can be toxic at low concentrations [6]. We therefore considered them highly relevant to study in relation to airborne bacteria, which are known to occur very often in aggregates and associated to other airborne particles [7]. Tropospheric O_3 is a secondary pollutant and one main group of precursors are VOCs [8]. In this study, we specifically studied the effect of the heavy metal cadmium (Cd) and gaseous acetaldehyde and ethylene on the growth of airborne bacteria.

2 Materials and methods

2.1 Sampling

Outdoor air samples were taken at different locations in the region of Antwerp (Belgium), using the Coriolis®μ air sampler (Bertin Technologies). This is a device using centrifugal force to suspend airborne particles of several m^3 of air into only a few mL of collection liquid. Either the Coriolis collection liquid (Peqlab) or phosphate buffered saline (3.8 mM NaH_2PO_4 (Merck), 16.2 mM Na_2HPO_4 (Merck), 150 mM NaCl (VWR), pH 7.0, autoclaved) containing 0,002% Tween®20 (Sigma-Aldrich) was used as a collection liquid. The sampling rate was set at 300 L min^{-1} and after every run of 10 minutes, the collection liquid was replaced by fresh collection liquid. Samples were kept on ice during transportation and were stored with 25% glycerol at -80°C.

2.2 Culturing

Colony forming units (CFUs) were determined by spreading 400–800 μL of sample on R2A agar medium, which was found to be a suitable medium for airborne microbes by Hyvärinen *et al.* [9]. The R2A medium was supplemented with 100 μg L^{-1} cycloheximide (Carl Roth) to prevent fungal growth. The samples were inoculated in duplicate and incubated for 7 days at room temperature before CFUs (and the pigmented colonies) were counted. CFU concentrations per m^3 of air were calculated by taking the sampling rates into account. Pigmentation percentages were determined by dividing the number of non-white and non-transparent colonies by the total amount of colonies per plate.

2.3 Heavy metal resistance

To test heavy metal resistance of the culturable bacteria, R2A-agar plates were enriched with a heavy metal salt. Cadmium sulphate was added to the molten agar to obtain an end concentration of 89 nM. The enriched plates and the control plates (without Cd) were inoculated as described above. Inoculation samples consisted of collection liquid pooled together from different sampling days, representing airborne communities from either the harbour or the city, as shown in Table 1. Total CFU numbers and pigmented colonies were counted after 7 days. The percentage of resistance was determined by the CFU concentrations from the Cd plates divided by the CFU concentrations of the same sample on a control plate. Pure cultures were made from several selected colonies.

Table 1: The sampling time and location of the airborne bacteria used for Cd-resistance testing. The name of a pooled sample refers to the location (C: city; H: harbour) and the season (A: autumn; W: winter) of the air samples it represents.

Date	Time	Location	Pooled sample
07 September 2012	9:47–10:09	City	CA
	10:58–11:22	Harbour	HA
08 October 2012	9:54–10.17	Harbour	HA
	11:01–11:23	City	CA
16 October 2012	5:48–6:11	City	CA
	7:37–7:59	Harbour	HA
05 November 2012	14:17–14:39	City	CA
	16:07–16:28	Harbour	HA
26 November 2012	19:58–20:56	City	CW
	21:40–22:30	Harbour	HW
21 December 2012	6:31–7:19	City	CW
	7:58–8:44	Harbour	HW
01 March 2013	12:50–13:31	City	CW
	11:04–11:57	Harbour	HW
08 March 2013	11:34–12:22	City	CW
	13:05–14:01	Harbour	HW

2.4 VOC resistance

To test VOC resistance of the culturable airborne bacteria, preliminary experiments were set up to expose airborne bacteria to ethylene and acetaldehyde. R2A-agar plates were inoculated with air samples taken in an underground parking lot of a supermarket (Antwerp). The plates were incubated as described above, in an airtight vessel with valves (Schuett-biotec). The vessel was flushed with an air stream containing 500 ppm ethylene. A similar experiment was performed with an outdoor air sample from a gas station and exposed to 500 ppm acetaldehyde. The latter sample was inoculated on a carbon-free mineral salts agar [10], to assess whether gaseous acetaldehyde may also serve as carbon source. During every experiment, two control plates per sample were left unexposed. The resistance was calculated by the ratio of CFU concentrations from the exposed plates and from the control plates. Pure cultures were made from several selected colonies.

2.5 Identification of resistant strains

To identify the bacteria of the pure cultures of the pollution-resistance experiments, their 16S rRNA genes were amplified and sequenced. For polymerase chain reaction (PCR) pure culture cells were transferred from a colony to a PCR tube and microwaved for 3 minutes. High Fidelity PCR Master (Roche) was added together with universal bacterial primers for the 16S rRNA gene: 8F (5'- AGA GTT TGA TCC TGG CTC AG -3') and 1525R (5'- AAG GAG GTG ATC CAG CCG CA -3') [29]. The end concentrations were 0.5 μM of each primer in a final volume of 25 μL. Negative controls for cross contamination and master mix contamination were included. The amplification program was performed with initial denaturation of 5 min at 94°C; followed by 30 cycles of 30 seconds at 94°C, 45 seconds at 54°C and 1 min 45 seconds at 72°C; and a final extension at 72°C for 10 min. The PCR products were loaded on a 1% agarose gel and those that showed clear bands were sent for single read Sanger sequencing with both the 8F and 1525R primer to GATC Biotech. Results were compared to the NCBI database using nucleotide BLAST (http://blast.ncbi.nlm.nih.gov/) to classify the selected isolates at family or genus level.

3 Results

3.1 Airborne bacteria are often pigmented

As a possible adaptation mechanism of airborne bacteria, pigmentation was tested. Pigments are known to protect against UV radiation and are therefore considered more crucial for the survival of airborne bacteria than for bacteria in environments with less UV exposure. The percentage of pigmented bacterial colonies was compared between different environments: outdoor air at different locations, soil, and tap water, fig. 1. Interestingly, between 70–90% of the airborne bacteria were pigmented, while the samples of soil and tap water showed a significantly ($p < 0.05$) lower fraction of pigmented bacteria.

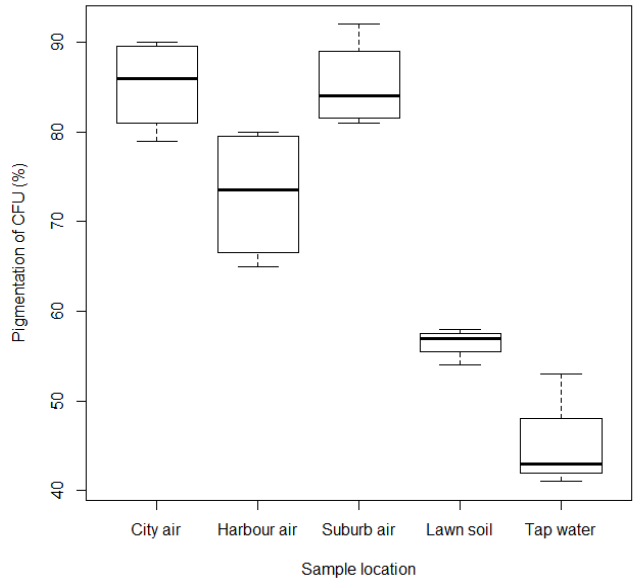

Figure 1: The percentage of pigmentation of the colonies originating from different environments.

3.2 Airborne bacteria from polluted areas show heavy metal resistance

As a second adaptation mechanism, heavy metal resistance was investigated with Cd as an example. The comparison of the numbers of CFUs that survive and grow when exposed to 89 nM Cd are shown in fig. 2. Two way analysis of variance testing in R showed that the survival percentage of the bacteria from the polluted harbour area was significantly higher ($p < 0.05$) than that of the city, without any significant influence of the season.

Subsequently, several Cd-resistant bacteria were identified. One of the isolates belonged to the family of the *Oxalobacteraceae*, which is affiliated to the *Proteobacteria*. Also, *Microbacterium sp.* and *Arthrobacter sp.* of the phylum of *Actinobacteria* were identified. Additionally, a *Rhodococcus sp.* was found to be most closely related (98% identical 16S rRNA gene) to *Rhodococcus fascians*, also of the phylum to the *Actinobacteria*.

3.3 Airborne bacteria from polluted areas show VOC resistance

As a third adaptation mechanism, the resistance of airborne bacteria towards VOCs was investigated. The results for ethylene and acetaldehyde exposure of 500 ppm are shown in fig. 3. Experiments with exposures to lower concentrations are not shown, as they did not show significant differences to that of non-exposed plates. A trend was observed that indicates that acetaldehyde and ethylene induce a selection pressure.

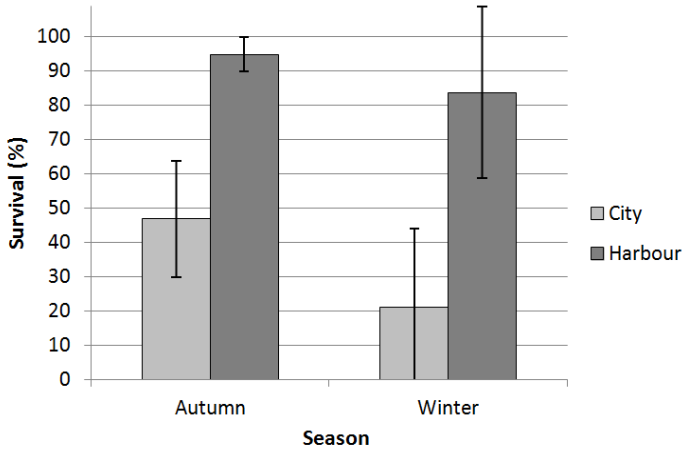

Figure 2: Mean survival percentages of airborne bacteria under Cd stress. Data are shown with standard deviation.

Subsequently, several VOC-resistant bacteria were further identified. One of the isolates from the ethylene-exposed bacteria belonged to the family of the *Rhizobiaceae*, typically found in soil and affiliated to the *Proteobacteria*. Also, *Streptomyces sp.* and *Arthrobacter sp.* of the phylum of *Actinobacteria* were identified. Additionally, *Hymenobacter*, affiliated to the *Bacteroidetes* was found to be an abundant genus in the samples of the gas station air.

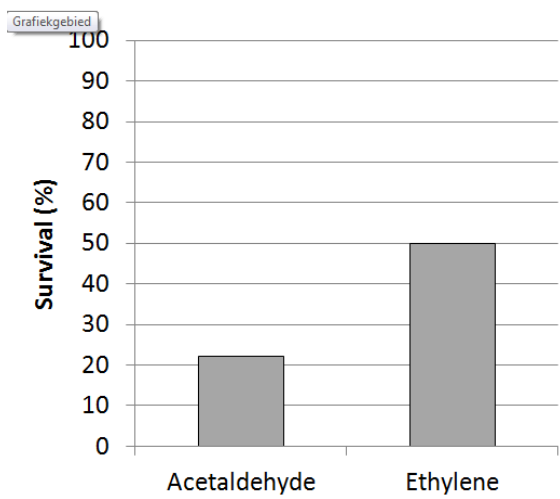

Figure 3: Survival percentage (result of two repeats each) under 500 ppm acetaldehyde and ethylene of bacteria sampled at a gas station and in an underground parking lot, respectively.

4 Discussion

As a first important phenotype, pigmentation was observed in a large fraction of airborne bacteria. Similar high pigmentation percentages of outdoor airborne bacteria have been observed previously by e.g. [11–13]. The colours of atmospheric CFUs usually range from dark red to bright yellow and are attributed to many different carotenoids [14]. Tong and Lighthart [15] observed a correlation between pigmentation and solar radiation and therefore suggested that pigments increase survival chances of airborne micro-organisms by protecting them from cell damage caused by solar radiation.

Additional reasons for high pigmentation percentages of airborne communities were suggested by Vaïtilingom *et al.* [16]. Pigments may, due to their hydrophobic nature, increase the aerosolization of the respective bacteria from a liquid [17]. A carotenoid pigment has also been observed to contribute to survival at low temperatures [18]. Low temperature resistance has been considered as an important trait of airborne microbes, especially for those in clouds or at higher altitudes where long range transport can occur [19, 20].

Subsequently, we investigated resistance of airborne bacteria towards air pollutants. The bacterial communities taken from the city and the harbour showed different resistance capacities to Cd. This could be explained by the great presence of industry in the harbour, which may select for species that are less sensitive to higher heavy metal concentrations. The results of this experiment therefore indicate that the local airborne bacteria may adapt to the pollution they are exposed to. This is a very interesting characteristic that implies great potential for the use of airborne bacteria in biomonitoring of local air pollution and for bioremediation purposes, however, this remains to be further substantiated.

It is known that the composition of airborne bacterial communities varies over seasons [21–23]. This can be attributed to the change of bacterial sources over the season, caused by temperature changes or trees growing and losing leaves [21]. Interestingly, in our study, Cd resistance remained similar over two seasons at the respective locations, which is another indication that the resistance arises from the year-round industrial pollution that marks the harbour.

As exemplar VOCs, acetaldehyde and ethylene also seemed to induce a selection pressure on airborne bacteria, provided that they are used at a high concentration. However, the goal of the acetaldehyde experiment with the carbon-free medium was twofold: it was also used to assess the use of acetaldehyde as a carbon source, which is interesting for future bioremediation efforts. The controls of the acetaldehyde experiment on carbon-free agar showed unexpected growth of colonies, which suggests the use of another atmospheric carbon molecule, such as CO_2 as a carbon source. We even observed more CFUs on the control plates than on the plates that were exposed to acetaldehyde, indicating that 500 ppm of acetaldehyde was more likely to induce a selection pressure, than be used as a carbon source.

Several pollutant-resistant bacteria were further identified. The identification of these isolates showed some interesting links with previous findings. For example, we found a Cd-resistant isolate closely related to *Rhodococcus fascians,*

an economically relevant plant pathogen which has previously been observed of possessing and passing on a plasmid (pD188) that increases Cd resistance [24]. Furthermore, many of the known members of the genus *Hymenobacter* are either isolated from air [25], found to be radiation-resistant [26, 27], or showed resistance to temperatures below 0°C [28], which can be considered characteristics of typical airborne bacteria. However, how these characteristics relate to VOC resistance remains to be further investigated.

5 Conclusion

Even though bioremediation of soil has proven to be a very useful, cost-efficient technology, the use of airborne microbes for the bioremediation of polluted air has been barely studied. During this study, we developed methods to address this issue and found bacteria that are capable of resisting high concentrations of air pollutants. The micro-organisms of the atmosphere are known to be very diverse, and we therefore expect they possess a wide range of biodegradation mechanisms for many different air pollutants. The preliminary results presented in this paper indicate the potential use of airborne bacteria in bioremediation.

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References

[1] Guerreiro, C., F. de Leeuw, V. Foltescu, J. Schilling, J. van Aardenne*, et al.*, Air quality in Europe – 2012 report. European Environment Agency, 2013.

[2] Air quality deteriorating in many of the world's cities. World Health Organisation (WHO). http://www.who.int/mediacentre/news/releases/2014/air-quality/en/.

[3] Kuehn, B.M., WHO: More Than 7 Million Air Pollution Deaths Each Year. *JAMA*, **311(15)**, pp. 1486-1486, 2014.

[4] Gandolfi, I., V. Bertolini, R. Ambrosini, G. Bestetti, and A. Franzetti, Unravelling the bacterial diversity in the atmosphere. *Applied microbiology and biotechnology*, **97(11)**, pp. 4727-4736, 2013.

[5] Schwartz, J. and L.M. Neas, Fine particles are more strongly associated than coarse particles with acute respiratory health effects in schoolchildren. *Epidemiology*, **11(1)**, pp. 6-10, 2000.

[6] US EPA, Stay of federal water quality criteria for metals; water quality standards; establishment of numeric criteria for priority toxic pollutants;

states' compliance revision of metals criteria; final rules. *Federal Register*, **60(86)**, pp. 22228-22237, US Environmental Protection Agency, Washinton, D.C., 1995.
[7] Maron, P.A., C. Mougel, D.P. H Lejon, E. Carvalho, K. Bizet, *et al.*, Temporal variability of airborne bacterial community structure in an urban area. *Atmospheric Environment*, **40(40)**, pp. 8074-8080, 2006.
[8] Kleinman, L.I., The dependence of tropospheric ozone production rate on ozone precursors. *Atmospheric Environment*, **39(3)**, pp. 575-586, 2005.
[9] Hyvärinen, A., P. Martikainen, and A. Nevalainen, Suitability of poor medium in counting total viable airborne bacteria. *Grana*, **30(2)**, pp. 414-417, 1991.
[10] Kumar, D.J.M., B.A. Magesh, S. Devika, M.D. Balakumaran, and P.T. Kalaichelvan, Biodegradation of polycyclic aromatic hydrocarbons by Pseudomonas sp. PSS6 isolated from municipal wastes sediment. *Der Chemica Sinica*, **3(3)**, pp. 543, 2012.
[11] Griffin, D.W., C. Gonzalez, N. Teigell, T. Petrosky, D.E. Northup, *et al.*, Observations on the use of membrane filtration and liquid impingement to collect airborne microorganisms in various atmospheric environments. *Aerobiologia*, **27(1)**, pp. 25-35, 2011.
[12] Fahlgren, C., Å. Hagström, D. Nilsson, and U.L. Zweifel, Annual variations in the diversity, viability, and origin of airborne bacteria. *Applied and environmental microbiology*, **76(9)**, pp. 3015-3025, 2010.
[13] Zweifel, U.L., Å. Hagström, K. Holmfeldt, R. Thyrhaug, C. Geels, *et al.*, High bacterial 16S rRNA gene diversity above the atmospheric boundary layer. *Aerobiologia*, **28(4)**, pp. 481-498, 2012.
[14] Dybwad, M., P.E. Granum, P. Bruheim, and J.M. Blatny, Characterization of airborne bacteria at an underground subway station. Applied and environmental microbiology, **78(6)**, pp. 1917-1929, 2012.
[15] Tong, Y. and B. Lighthart, Solar radiation is shown to select for pigmented bacteria in the ambient outdoor atmosphere. *Photochemistry and photobiology*, **65(1)**, pp. 103-106, 1997.
[16] Vaïtilingom, M., E. Attard, N. Gaiani, M. Sancelme, L. Deguillaume, *et al.*, Long-term features of cloud microbiology at the puy de Dôme (France). *Atmospheric Environment*, **56**, pp. 88-100, 2012.
[17] Burger, S.R. and J. Bennett, Droplet enrichment factors of pigmented and nonpigmented Serratia marcescens: possible selective function for prodigiosin. *Applied and environmental microbiology*, **50(2)**, pp. 487-490, 1985.
[18] Fong, N., M. Burgess, K. Barrow, and D. Glenn, Carotenoid accumulation in the psychrotrophic bacterium Arthrobacter agilis in response to thermal and salt stress. *Applied microbiology and biotechnology*, **56(5-6)**, pp. 750-756, 2001.
[19] Amato, P., M. Parazols, M. Sancelme, P. Laj, G. Mailhot, *et al.*, Microorganisms isolated from the water phase of tropospheric clouds at the Puy de Dôme: major groups and growth abilities at low temperatures. *FEMS Microbiology Ecology*, **59(2)**, pp. 242-254, 2007.

[20] Deguillaume, L., M. Leriche, P. Amato, P. Ariya, A.-M. Delort, *et al.*, Microbiology and atmospheric processes: chemical interactions of primary biological aerosols. *Biogeosciences Discussions*, **5(1)**, pp. 841-870, 2008.
[21] Bowers, R.M., N. Clements, J.B. Emerson, C. Wiedinmyer, M.P. Hannigan, *et al.*, Seasonal variability in bacterial and fungal diversity of the near-surface atmosphere. *Environmental science & technology*, **47(21)**, pp. 12097-12106, 2013.
[22] Bertolini, V., I. Gandolfi, R. Ambrosini, G. Bestetti, E. Innocente, *et al.*, Temporal variability and effect of environmental variables on airborne bacterial communities in an urban area of Northern Italy. *Applied microbiology and biotechnology*, pp. 1-10, 2012.
[23] Franzetti, A., I. Gandolfi, E. Gaspari, R. Ambrosini, and G. Bestetti, Seasonal variability of bacteria in fine and coarse urban air particulate matter. *Applied microbiology and biotechnology*, **90(2)**, pp. 745-753, 2011.
[24] Desomer, J., P. Dhaese, and M. Van Montagu, Conjugative transfer of cadmium resistance plasmids in Rhodococcus fascians strains. *Journal of bacteriology*, **170(5)**, pp. 2401-2405, 1988.
[25] Buczolits, S., E.B. Denner, P. Kämpfer, and H.-J. Busse, Proposal of Hymenobacter norwichensis sp. nov., classification of 'Taxeobacter ocellatus','Taxeobacter gelupurpurascens' and 'Taxeobacter chitinovorans' as Hymenobacter ocellatus sp. nov., Hymenobacter gelipurpurascens sp. nov. and Hymenobacter chitinivorans sp. nov., respectively, and emended description of the genus Hymenobacter Hirsch *et al*. 1999. *International journal of systematic and evolutionary microbiology*, **56(9)**, pp. 2071-2078, 2006.
[26] Zhang, Q., C. Liu, Y. Tang, G. Zhou, P. Shen, *et al.*, Hymenobacter xinjiangensis sp. nov., a radiation-resistant bacterium isolated from the desert of Xinjiang, China. *International journal of systematic and evolutionary microbiology*, **57(8)**, pp. 1752-1756, 2007.
[27] Dai, J., Y. Wang, L. Zhang, Y. Tang, X. Luo, *et al.*, Hymenobacter tibetensis sp. nov., a UV-resistant bacterium isolated from Qinghai–Tibet plateau. *Systematic and applied microbiology*, **32(8)**, pp. 543-548, 2009.
[28] Zhang, G., F. Niu, H.-J. Busse, X. Ma, W. Liu, *et al.*, Hymenobacter psychrotolerans sp. nov., isolated from the Qinghai--Tibet Plateau permafrost region. *International journal of systematic and evolutionary microbiology*, **58(5)**, pp. 1215-1220, 2008.
[29] Weisburg, W.G., S.M. Barns, D.A. Pelletier, and D.J. Lane, 16S ribosomal DNA amplification for phylogenetic study. Journal of bacteriology, **173(2)**, pp. 697-703, 1991.