Microbial diversity of internal environment of Johann Gregor Mendel Station, Antarctica

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Abstract

During January and February 2011 two sets of microbiological samples were collected inside the main building of Johann Gregor Mendel station located at the James Ross Island near the coast of Antarctica. The aim was to examine the changes of microbial profile of the antarctic station environment. The first set of samples was collected from the station environment before the staff entry, *i.e.* after 10 months of quiescent state when nobody was present at the station. The second set of samples was collected from the same places before the Antarctic expedition staff left the station after 45 days of the stay. The cultivation of samples was focused on mesophilic bacteria. Twenty-three strains were obtained from the Set No. 1 and 27 strains were obtained from the Set No. 2. However, 8 strains from each set were not reliably identified by mass spectrometry. Altogether 13 strains of Gram-positive bacteria were identified in the Set. No. 1, while only 7 in the Set No. 2. Contrastingly, Gram-negative bacteria were much more abundant in the Set No. 2 (12 strains) than in the Set No. 1 (2 strains). *Bacillus* sp. was the most common Gram-positive strain (9 isolations from the first set, 2 isolations from the second set). *Pantoea agglomerans* was the most common Gram-negative strain (2 isolations from the first set, 7 isolations from the second set). The first experience with the microbial profile of the research station showed that we were able to detect mainly bacteria commonly present in the outer environment that could survive under extreme conditions. We did not isolate any microbes related to human colonisation except of enterococci and *Escherichia vulneris*. For further investigation of the station environment, it will be necessary to choose alternative way of collection and storage of samples to ensure survival of all present bacteria.

Key words: bacteria, *Bacillus* sp.**,** *Pantoea agglomerans***,** James Ross Island

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Introduction

James Ross Island is a large island in the Southern Ocean near the coast of Antarctica off the southeast side of the northernmost extremity of Antarctic Peninsula, from which it is separated by Prince Gustav Channel. The island´s area is approximately 2,500 km^2 with very rugged topography and 70-80% of the surface is covered with a glacier. Johann Gregor Mendel Czech Research Antarctic Station was built in the northern deglaciated part of the island in the years 2005 – 2006. The project of station construction was realized by the Masaryk University in Brno. Since 2007, scientists and researchers from different Czech institutions have been visiting Johann Gregor Mendel station each austral summer to carry out their scientific projects there. Typical number of station summer crew is 15 people, it consists of technicians, scientists, climatologists, geologists, ornithologists, paleontologists and botanists. During January and February 2011, a physician took part in the expedition for the first time who among others collected two sets of microbiological samples inside the main building of Johann Gregor Mendel station. These were the first microbiological samples collected at the station. The aim was to examine the changes of microbial profile of the antarctic station environment related to the date of sampling. It was hypothesised that microbial community inside the buiding of Johann Gregor Mendel station would differ when samples are collected before and after Antarctic summer expedition. The first set of samples was collected from the station environment before the staff entry, *i.e.* after 10 months of quiescent state when nobody was present at the station (March-December 2010). During that time, the station was winterized and the lowest inside temperature reached -20ºC (K. Láska, personal communication).

Therefore, only freeze-resistent microbiota were expected in the first set of samples. The second set of samples was collected from the same places before the Antarctic expedition staff left the station after 45 days of the stay. During the station functioning the inside temperature was around 15 - 17 ºC, therefore, some additional microbial taxa that had been delivered by the staff were expected in the sample two. The aim of the presented study was to classify the microbial community at the Johan Gregor Mendel station, evaluate the differences in species diversity and discuss the possible causes of the differences.

Material and methods

In January 2011, two sets of samples, each containing 10 smears, were collected in several rooms from various places. The first set of 10 samples (denoted as Set No. 1 in the following text) was collected before the expedition staff entred the station, *i.e.* the samples represented microbial community surviving antarctic winter. Smears were collected from the surface of windows sills in bedrooms, from the surface of work tables in several rooms, from the kitchen table, wash-basin at the toilet and upper edge of the toilet bowl. Areas of 25 cm^2 at the above mentioned places were wiped with a dry swab. The swabs were placed into Amies transport medium (Copan, Italy) which allowed the survival of microorganisms without their proliferation. The samples were kept at the station at room temperature ranging 15 - 17ºC. Before the departure of staff members, after 45 days

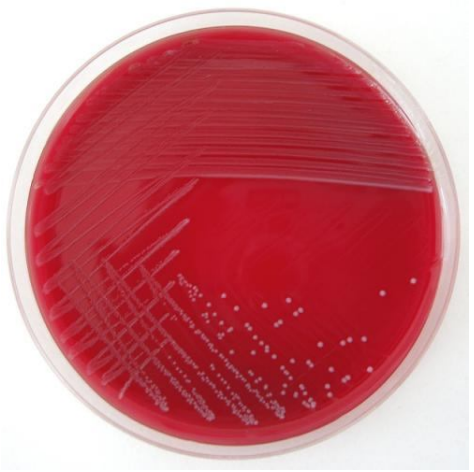

Fig. 1. Culture of *Enterococcus faecalis* isolated from the samples collected inside J.G. Mendel station, and cultivated on agar.

of routine functioning of the station, the samples were collected from the same places in the same manner (denoted as Set No. 2). After the return to the Czech Republic, the swabs were removed from the transport medium and put into liquid broth (Nutrient Broth, HiMedia, India) and after 24-hour cultivation at 37ºC inoculated onto Columbia Agar (Merck, Germany) and MacConkey Agar (Conda, Spain) and again cultivated 24 - 48 hours at 37ºC. In addition to the samples collected inside the station, some samples were taken also from collonized whale vertebra collected on a seashore on neighbouring Vega Island. After the transport to the Microbiological laboratories in Brno, the whale bone samples were put into a broth and after 24-hour cultivation at 37 ºC inoculated on the same solid media. Cultivated colonies were isolated and identified using the mass spectrometry MALDI-TOFF microflex™ LT using the software MALDI Biotyper 2.0 SRI (Bruker Daltonics, Germany).

Fig. 2. General view on the main building of Czech Antarctic station J. G. Mendel located on the Northern seashore of James Ross Island, Antarctica.

Results

Despite of long-term storage of samples in the transport medium it was possible to cultivate a number of bacteria species. Twenty-three strains were obtained from the Set No. 1 and 27 strains were obtained from the Set No. 2. However, 8 strains from each set were not reliably identified by mass spectrometry. Altogether 13 strains of Gram-positive bacteria were identified in the Set. No. 1, while only 7 in the Set No. 2. Contrastingly, Gram-negative bacteria were much more abundant in the Set No. 2 (12 strains) than in the Set No. 1 (2 strains) – *see* Fig. 1).

Bacillus sp. was the most common Gram-positive strain (9 isolations from the first set, 2 isolations from the second set). *Bacillus megaterium* was detected most often (n-5) followed by *Paenibacillus glucanolyticus* (n-3), *Bacillus subtilis* (n-1), *Bacillus cereus* (n-1), *Bacillus pumilus* (n-1). Other Gram-positive bacteria included coagulase-negative staphylococci (n-3), *Microbacterium* sp. (n-2), *Enterococcus* sp. (n-4) which included *E.faecalis* (n-3), *E.durans* (n-1) (Fig. 2).

Enterococci were detected only in the second set of samples, specifically three times from the smears collected at the toilet and once from the work table in one of the staff living room. *Pantoea agglomerans* was the most common Gram-negative strain (2 isolations from the first set, 7 isolations from the second set). Other Gram-negative bacteria included *Acinetobacter radioresistens* (n-1), *Serratia liquefaciens* (n-3), *Escherichia vulneris* (n-1) (Fig. 3).

Pantoea agglomerans, *Serratia liquefaciens* and *Escherichia vulneris* were detected in the samples collected from the toilet environment. *Pantoea agglomerans*, *Serratia liquefaciens* and *Bacillus simplex* were cultivated from the whale bones.

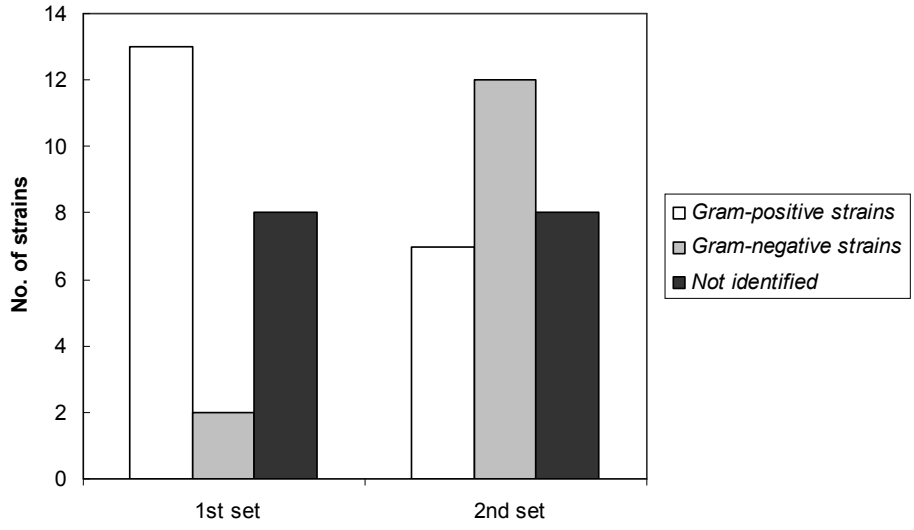

Fig. 3. Number of isolated strains from the first (before the expedition staff first entered J.G.Mendel station, January 2011) and second set (after the expedition, *i.e.* end of February 2011) of microbiological samples collected inside J.G.Mendel station.

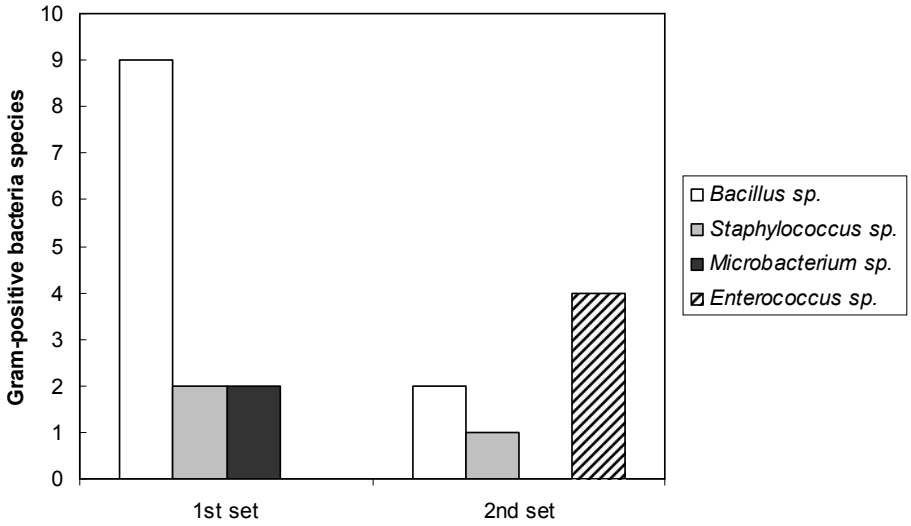

Fig. 4. Number of Gram-positive bacteria species isolated from the first and second set of samples.

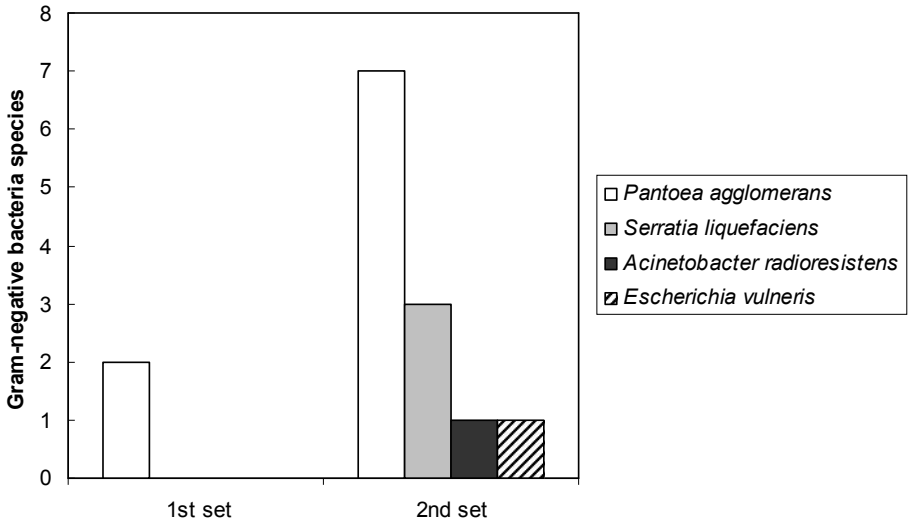

Fig. 5. Number of Gram-negative bacteria species isolated from the first and second set of samples.

Discussion

This is the first attempt to examine the microbial profile of the research station interior environment (a) after 10 months of quiescent state caused by the absence of the staff followed by the study of (b) microbial profile changes due to the presence of the expedition staff during the 2 warmest months of the year. The cultivation of samples was focused on mesophilic bacteria with optimal growth temperature in the range 15 – 40°C. Therefore, the species found may become opportunistic pathogens under suitable conditions.

In the set of samples collected before the expedition participants entry (Set No. 1), Gram-positive bacteria prevailed. High viability of Gram-positive bacteria is evident, especially in the strains *Bacillus* sp. and *Paenibacillus* sp. that may produce endospores which enable bacteria to survive in extreme conditions [1]. The most often isolated species *Bacillus megaterium* is a rod shaped bacterium and one of the largest eubacteria found in soil as common soil saprophyt. According to literature, its high viability enables to survive in extreme conditions such as desert environments [2]. We conclude that *B. megaterium* can survive also in extremely low temperatures reaching -20 °C in the Antarctic research station. Other detected *Bacillus* strains, *B. subtilis* and *B. pumilis* also belong to soil saprophytes [3]. *Paenibacillus* sp., originally included within the genus *Bacillus*, was reclassified as a separate genus in 1993. Bacteria belonging to this genus have been detected in a variety of environments such as soil and water [4]. Isolation of *Bacillus cereus*, bacterial growth of which may result in the production of enterotoxins causing foodborne illness, was an unexpected finding [5]. *Microbacterium* sp. and *Staphylococcus xylosus* were also isolated from the first set of samples. These Gram-positive cocci are found as common skin saprophytes or in the environment and remarkably they survived winter in the uninhabited research station under unfavourable conditions. Regarding Gram-negative bacteria, *Pantoea agglomerans* was able to survive the winter period. This strain belongs among *Enterobacteriaceae* that inhabits plants, soil and water. Its production of various metabolites plays an important role in biological protection of plants against bacterial illnesses. Rarely, it can play a role as an opportunistic pathogen in humans (Koubová 2008, Unsall et al. 2008).

Pantoea agglomerans was the most often isolated species from the Set No. 2 samples collected after 45 days of routine functioning. It was also isolated from the whale bones from the external environment. Other Gram-negative bacteria were also isolated. *Serratia liquefaciens* belongs among *Enterobacteriaceae* as well but is not a common part of intestinal flora. Its presence is limited to external environment, however, it can play a role as an opportunistic pathogen rarely, mainly in immunocompromised patients in hospital care (Grohskopf et al. 2001). *Acinetobacter radioresistens* belongs among Gram-negative nonfermentative rods and its presence is generally related to external environment. Strains of this species were isolated from samples of cotton and soil (Nishimura et al. 1988). Surprisingly, with only two exceptions, we did not find any microorganisms related to human colonisation. The exceptions were enterococci isolated from the toilet environment that are a common part of human intestinal flora and one strain of *Escherichia vulneris* that can be isolated from human samples, for instance in leg injury, but also as a part of intestinal flora (Bannerman et Peacock 1999). We did not isolate any other intestinal bacteria, for example the most common *Escherichia coli*. Since the most often isolated Gram-

negative bacteria *Pantoea agglomerans* and *Serratia liquefaciens* from the Set No. 2 samples were isolated also from the outside environment (whale bones), we can assume that they were transferred to the station from the surrounding of the station and were not related to human presence.

It was not possible to identify reliably some strains by means of mass spectrometry. This method is based on comparison of mass spectra of isolated strains with the database of well known microorganisms. Mass spectra of strains that were not identified did not correlate with any known strain in the database which included approximately 3,500 reference strains. We are aware of the fact that we might not have detected all present microorganisms. The transport medium enables the survival of fastidious microorganisms but only for a limited period that can differ in individual bacteria. Number of bacterial species identified in our samples was smaller if compared with the isolations from air samples collected in the Concordia research station during the year 2005 (Timmery et al. 2011). It may be caused by smaller number of collected samples, different storage and transport conditions or different temperature of cultivation (Van Houdt et al. 2009, Xiao et al. 2007). Moreover, the cultivation at 37°C does not enable the detection of psychrophilic bacteria (Sedláček et al. 2011).

The first experience with the microbial profile of the research station showed that we were able to detect mainly bacteria commonly present in the outer environment that could survive under extreme conditions. Concerning the bacteria that are connected with human presence, only enterococci were detected that are also able to survive in the outer environment. For further investigation of the station environment, it will be necessary to choose alternative way of collection and storage of samples to ensure survival of all present bacteria and provide better comparability of samples collected before staff entry following winter period and after the researchers stay.

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Web resources

[1] http://microbewiki.kenyon.edu/index.php/Bacillus
[2] http://en.citizendium.org/wiki/bacillus_megaterium
[3] http://textbookofbacteriology.net/Bacillus.html
[4] http://en.wikipedia.org/wiki/Paenibacillus
[5] http://en.wikipedia.org/wiki/Bacillus_cereus