THE IMPORTANCE OF ACTINOBACTERIA IN ARCTIC SOIL

BACHELOR THESIS IN BIOLOGICAL CHEMISTRY

Dagmara Vašková

Supervisor : Ing. Jiří Bárta, Ph.D.
Consultant : Mgr. Hana Bošková

České Budějovice, Czech republic

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Annotation:

The purpose of my thesis was to investigate and quantify Actinobacteria in the samples of permafrost soil of Siberian Arctic, Vorkuta, and especially to investigate the effect of the temperature (at 4, 12 and 20°C) on the amount of Actinobacteria as well as the influence of the aerobic vs. anaerobic conditions.

Actinobacteria were used because they are widely distributed in the arctic soil and are very important organic matter degraders with the ability of decomposition complex biopolymers, such as cellulose and lignin.

The main methods used in my project were extraction of DNA to obtain the samples from the soil followed by the qPCR method.

I hereby declare that, in accordance with Article 47b of Act No. 111/1998 in the valid wording, I agree with the publication of my Bachelor thesis, in full to be kept in the Faculty of Science archive, in electronic form in publicly accessible part of STAG database operated by the University of South Bohemia in České Budějovice accessible through its web pages. Further, I agree to the electronic publication of the comments of my supervisor and thesis opponents and the record of the proceedings and results of the thesis defence in accordance with aforementioned Act. No. 111/1998. I also agree to the comparison of the text of my thesis with the Thesis.cz thesis database operated by the National Registry of University and a plagiarism detection system.

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In České Budějovice, 16.5.2014

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Table of contents

1. INTRODUCTION

Arctic ecosystems consist of permanently frozen soils with limitations in organisms present and are an important carbon (C) stores. These ecosystems are predicted to be a source of greenhouse gases because of the thawing of permafrost which will increase CO_2 and CH_4 emissions.

The latest studies have estimated that up to 30% of C stocks in permafrost soils is present in cryoturbations (Tarnocai et.al., 2009). Cryoturbations are caused by annual freeze and thaw cycles in the active layer, so the organic matter from the surface is buried down to the soil profile (Schuur et.al., 2008).

Also permafrost peatlands store significant amount of C which consist of peat plateau (PP) with deep organic deposits. Distinct dark spots of bare peat soil on PP, known as peat circles (PC), originated most likely from uplifting of deeper horizons and further wind abrasion. Together with mineral tundra (MT), these three habitats have the major impact on C and nutrient balance of Arctic (Marushchak et.al., 2013).

Bacteria represent dominant forms of life in Arctic soil ecosystems since they have been able to survive in harsh and cold conditions (low temperature, low water activity, low nutrient availability), therefore they undergo very dramatic changes in terrestrial ecosystems (Vishnivetskaya et.al., 2006). To prove their crucial role as organic matter decomposers, the determinantion of abundance and activity is needed.

Actinobacteria are one of the best taxa adapted to the permafrost conditions, especially because of their metabolic activity at low temperatures (Yergeau et al. 2010; Steven et al. 2008; Johnson et al. 2007). They play an important role in recycling of lignin, cellulose and chitin. The most remarkable species are *Streptomyces* and *Arthrobacter*.

New molecular based methods allow us to quantify different groups of bacteria without the need to cultivate them. One of these methods is quantitative PCR (qPCR) during which group specific region of small ribosomal subunit (SSU) is amplified and fluorescently quantified.

The main goals of the thesis were : (1) To quantify Actinobacteria in permafrost soil profile, (2) To determine the influence of temperature on the amount of Actinobacteria and (3) To determine the influence of oxygen (aerobic vs. anaerobic) on the amount of Actinobacteria.

1.1 Arctic soil ecosystems

Arctic is a polar region located at the northern part of the Arctic Cycle (66°33´N) consisting of a vast, ice-covered ocean surrounded by treeless permafrost. The average temperature for the warmest month (July) is below 10°C. Long and very cold winters (-40°C) followed by short and cold summers (0°C) are characteristic for this territory (Fitzpatrick, 1997).

Arctic tundra consists of permanently frozen soils with a limited flora and fauna because of the harsh conditions.

During the summer, the top part of permafrost defrosts but the lower part which is frozen, doesn´t soak the water so it stays on top which is therefore damp and covered by little lake and marshes.

Arctic permafrost soils are significant global C stocks with approx. 1672Gt stored in upper 3m (Tarnocai et.al. 2009), however their biological composition and functioning has still not been fully understood. This is mainly meant for the permafrost regions, the top part of litosphere which has a temperature of 0°C or less. The ability to react on the temperature changes, oxygen, nutrients and other chemical or physical changes is influenced by the genetic potential of microorganisms living in permafrost. Heating the top parts of permafrost due to the global warming can lead to increased microbial activity followed by the organic matter decomposition, CO_2 and CH_4 emission.

Bacteria are still the dominant forms of life in these ecosystems since they can deal with harsh conditions such as very low temperatures, freezing and defrosting of the soil, low or high salinity, pH changes and long lasting winters.

A very important factor of micoorganisms occurence is the temperature which influences the reproductive rate, enzymatic activity and metabolism. The lower the temperature, the slower the reproductive rate and other processes, therefore the microbial activity of permafrost is limited.

However, we can find many bacteria which are fully adapted to these specific conditions, since they found mechanisms to keep themselves alive even under very low temperatures. The most important prokaryotes in polar areas are psychrophilic and psychrotollerant bacteria and archae. The minimal temperature required for the growth of psychrophilic microorganisms is 0°C, the optimal is 15°C and the maximal is 20°C (Helmke&Weyland, 2004), whereas in case of psychrotollerant, they are able to grow close to 0°C but the optimal temperature is 20-40°C.

1.2 Cryoturbations

Northern latitude terrestrial ecosystems are the key components in the global carbon (C) cycle (McGuire et.al., 2009).

Cryoturbations are very important processes unique for Arctic permafrost regions (Fig.1) of repeated freeze-thaw cycles leading to the movement of soil organic matter from the surface into the deeper soil layers. A granular structure with many crystalline shapes is formed, producing distinctive patterned ground with different types of soil.

Cryoturbated soils contain more than one third of the arctic soil organic carbon (Tarnocai et.al., 2009). The C stores in this buried horizon are much older than in unburied topsoil horizons, which indicates that decomposition processes are strongly retarded (Kaiser et al.,2007, Hugelius et al., 2010). It is therefore an important mechanism for long-term C storage.

Fig.1 – Part of the pit showing active layers of permafrost with cryoturbations (Gittel et.al., 2014 ISME Journal). O – organic layer. Bg – mineral sub soil. Ajj – cryoturbated organic material.

1.3 Permafrost peatlands

The major components of East-European subarctic tundra are represented by upland tundra on mineral soil – mineral tundra (MT).

The peat plateau (PP) complex has peat deposits that are up to several meters thick and many small themokarst lakes. Most of the landscape around is hilly upland with tundra heath vegetation and occasional patches of spruce forest.

The peat plateaus are often dotted by bare patterned ground features called peat circles (PC) with especially high microbial activity (Repo et al. 2009; Biasi et al. 2014).These are round patches with a diameter of 4-25m and areas from 10-500m^2. The peat circles are flat surfaces, partly covered by a thin moss layer but without any vascular plants. The material here is well decomposed and the surface is irregular and bumpy, assuming it is caused by cryoturbation (Marushchak et.al., 2013). PC are examined mainly because of high N_2O emissions which are subsequently emitted to the atmosphere.

These three habitats have the major impact on C and nutrient balance of the area (Marushchak et al. 2013). As a result of different water regime and historical origin, they are characterized by different availability of C and nutrients and dissimilar C and N transformation pathways in soil, such as respiration and denitrification (Repo et al. 2009; Marushchak et al. 2011; Marushchak et al. 2013; Biasi et al. 2014).

1.4 Microorganisms in permafrost

Significant number of viable microorganisms (10^8 per g of soil) are known to be present within permafrost. Thawing of permafrost stimulates their physiological activity and exposes these microorganisms to modern ecosystems, so it represents a stable and unique physicochemical complex.

The soil ecosystem contains of thousands of microbial species so that it represents the most diverse ecosystem on the Earth (Mongodin et.al., 2006). This diversity of bacteria involves heterotrophs, anaerobic heterotrophs, metanogenes, microorganisms reducing iron, suplhates, nitrification bacteria. Approximately 30 distinct genera were isolated from permafrost (Gilichinsky et.al., 1995) with Actinobacterial genera (*Arthrobacter*, *Streptomyces*, *Nitrobacter*, *Rhodococcus*).

Recent metagenomic analyses showed that the Actinobacteria are highly abundant in these Arctic regions and in cryoturbations represent more than 50% (Gittel et.al., 2014).

1.5 Actinobacteria

1.5.1 Localization and ecology

Actinobacteria represent one of the largest bacterial phyla. They are widely spread in terrestrial and aquatic ecosystems, playing an important role in ecology, medicine, agriculture and industry (McNeill&Brown, 1997). Actinobacteria are the main compositors of Arctic soils and due to their evolved adaptation mechanisms, they can live under harsh and extremely cold conditions. They are crucial for organic matter decomposition and recycling (e.g. cellulose, chitin, lignin).

Common taxonomic feature is their gram- positivity and high GC content (57-75%) (Lo et. al., 2002). Most of actinobacteria are aerobic and their optimal growth is in neutral or slightly alkalic environment (Basilio et. al., 2003).

Among all the known Actinobacteria, *Streptomyces sp* and *Arthrobacter sp*. are the most remarkable genuses.

Streptomyces is the largest genus including aerobic, gram-positive, filamentous bacteria. Most of them produce well-developed spores which colour refers to their nomenclature.

Members of this genus are major contributes to biological buffering of soils (making a mixture of buffered systems containing components having the ability to neutralize acids by bonding H^+ ions as well as bases by the release of hydrogen ions) and play an important role in organic matter decomposition (Debananda et.al., 2009). They have been recognised as producers of bioactive metabolites with broad spectrum of activities which has antibacterial (chloramphenicol),antifugal(nystatin),antibiotic(amoxicilin),herbicide and immunomodulators agents.

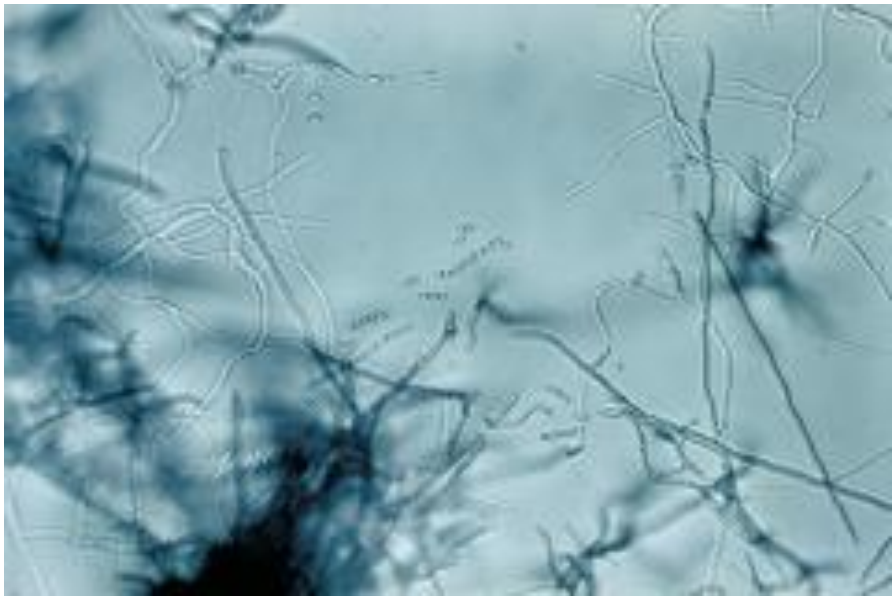

Fig.2 – Slide culture of *Streptomyces* species(Public Health Image Library)

Members of the Arthrobacter genus have been also often isolated from Arctic (Gilichinsky et.al., 1995). All species in this genus are gram-positive facultative anaerobes having rods during exponential growth and cocci in their stationary phase. There are some species which exhibit anaerobic metabolism, though (A.globiformis). Some can reduce hexavalent chromium levels in contaminated soil which can be useful in bioremediation (Megharaj et. al., 2003).

Well known *Arthobacter chlorophenolicus* is capable of degrading high concentrations of 4-chlorophenol (in general ability to degrade xenobiotics) which can cause severe irritations to humans. *Arthobacter chlorophenolicus* may also be used in agriculture for pesticides degradation as an only source of carbon and energy.

The most important fact, though, is that Arthrobacter species are able to live and survive under harsh conditions such as low temperatures and lack of nutrients which make them very important.

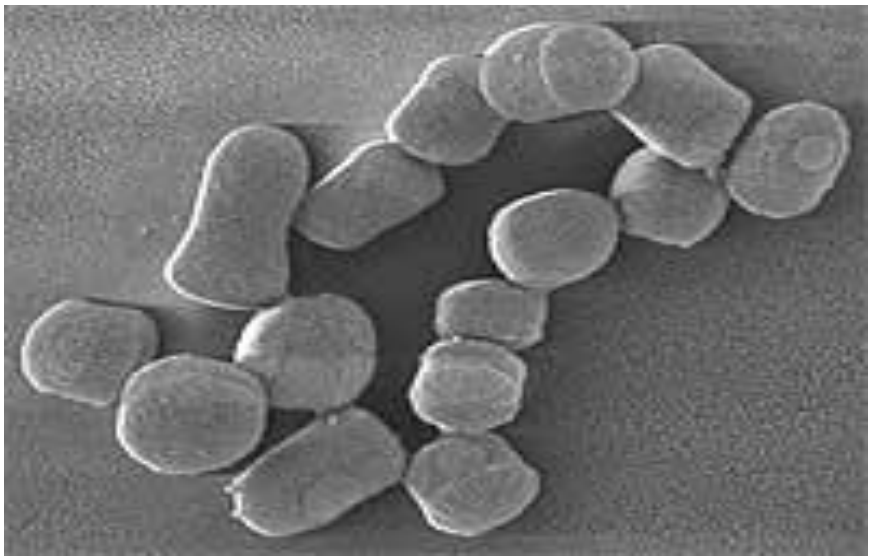

Fig.3 – *Arthrobacter chlorophenolicus*(http://www.jgi.doe.gov/sequencing/why/actinobacteria.jpg)

1.5.2 Metabolism

Actinobacteria possess highly variable physiological and metabolic properties which allow them to live in conditions occuring in permafrost habitats (low temperatures, low water activity, lack of nutrients available, radiation).

They have adopted different lifestyles and can be pathogens (Mycobacterium), soil inhabitants (Streptomyces), plant commensals (Leifsonia) or gastrointestinal commensals (Bifidobacterium).

The optimal growth of terrestrial Actinobacteria is in neutral or slightly alkaline environment (Basilio et.al., 2003). There are also bacteria which grow under acidic conditions (pH 4-5), though.

Wide variety of bacteria have been isolated from Artcic soils, such as aerobic (use organic compounds as a source of energy and carbon in an oxygenated environment) and anaerobic heterotrophes (in non oxygentaed environment), methanogenes (producing methane in anoxic conditions), iron and sulphate reducers, nitrifying (grow by consuming inorganic nitrogen compounds) and nitrogen fixing bacteria.

Actinobacteria are helpful in decomposition of complexed substrates, especially cellulose and lignin. Extracellular enzymes recycle organic polymers (by decomposition and humus formation) so the ability of Actinobacteria to produce these enzymes may them be crucial decomposers.

1.5.3 Quantification af Actinobacteria

Despite classical agar cultivation based methods and microscopic methods (i.e. DAPI staining) which have served for the long time for enumeration of different microbial species, a quantitaive polymerase chain reaction (also called real-time polymerase chain reaction) is another laboratory technique of molecular biology. Its advantage is that it does not need cultivation of microorganisms. It uses extracted DNA from any environment to amplify specific region of studied gene(s) and simultaneously quantify a targeted DNA molecule, so it enables both detection and quantification.The quantity can be either an absolute number of copies or a relative amount when normalized to DNA input or additional normalizing genes. The amplified DNA is detected as the reaction progresses in real time, whereas the standard PCR´s product is detected at its end.

Primers (short DNA fragments) containing sequences complementary to the target region with a DNA polymerase are the key components to enable selective and repeated amplification. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified.

Two common methods for the detection of products in qPCR are non-specific fluorescent dyes that intercalate with and double-stranded DNA and a sequence-specific DNA probes consisting of oligonucleotides that are labelled with a fluorescent reporter which permits detection just after hybridization with its complementary sequence.

This method relies on thermal cycling consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA.

2. METHODS AND MATERIALS

2.1 Locality

The samples were collected 70 km southwest of the city of Vorkuta, Komi republic, Russia (67°03`N, 62°57`E, 100m a.s.l.), in the region of permafrost zone of subarctic tundra. The locality is divided into several areas. All examined soils were highly acidic with pH around 3.3 (Diakova,K.,2013, unpublished data).

Sampling site	$N\text{-}NO_3^-$ (µg gdw^{-1})	$N\text{-}NH_4^+$ (µg gdw^{-1})	$P\text{-}PO_4^{3-}$ (µg gdw^{-1})	DOC (µg gdw^{-1})	DOC : DIN
PP	0.08 ± 0.13	0.88 ± 1.53	25.1 ± 22.0	613 ± 152	636
PC	15.9 ± 11.9	4.55 ± 3.29	0.33 ± 0.57	849 ± 234	42
MT	0.00 ± 0.00	1.38 ± 2.39	5.36 ± 1.30	630 ± 357	457

Tab.1 – The content of nitrogen organic forms(DIN) (unpublished data) , phosphates, dissolved organic matter (DOC) and DOC:DIN ratio in tundra soils in July 2011 (concentration values are means with standard errors, n = 3). Abbreviations represent the following: PP – vegetated peat plateau, PC – peat circles, i.e. circular cryogenic formations of bare peat soil, MT – mineral tundra.

Fig.4 – Locality of sampling area. Vorkuta, Russia

2.2 DNA extraction

Nucleic acid extractions were conducted according to a modified bead-beating protocol (Urich et.al., 2008) and further purified using the CleanAll DNA/RNA Clean-up and Concentration Micro Kit (Norgen Biotek Corp., Ontario, Canada). Total DNA was quantified using SybrGreen (Leininger et.al., 2006).

2.2.1 Incubation experiment

Bulk soil from three different tundra microhabitats was collected in August 2012 at experimental site in Komi Republic, Russia: mineral tundra (MT) , peat plateau (PP) and peat circles (PC).

Soil samples were taken from near surface, 2 to 15 cm of depth from all the microhabitats. This was pure peat for peat plateau and peat circles. For mineral tundra, this layer (2-15 cm) encompassed O,- A- and partly also B horizons, depending on the site.

Approximately 70g of soil was put into the 1l incubation bottles and incubated at three different temperatures: 4, 12 and 20°C. Moisture (oxygen status) manipulation was proceeded in two ways of treatment : Aerobic: 80% of soil water holding capacity (WHC), aerobic atmosphere. Anaerobic: 100% WHC, artificial anaerobic atmosphere (initially 5% CO_2 and He, then changed to 1% CO_2 with He in 7^{th} week).

The gases measured were CO_2, CH_4 and N_2O. The 500 ml incubation bottles were used in the experiment.

In total, there were 42 samples in VORKUTA experiment. In the Tab(S1)(see supplementary materials), there is a list of samples used in my experiment.

In my thesis, I used DNA extracts for the determinantion of Actinobacteria.

2.3 qPCR protocol

qPCR assays were conducted in 48-well plates on the StepONE RealTime PCR System (Applied Biosystems), each plate containing the appropriate set of standards (ten-fold dilution of purificated PCR product of Micrococcus luteus) and negative controls.

The first step was to prepare the Master Mix of volume 950µl. Each 20µl reaction contained the following: 7.86 µl water, 10 µl FastStart Universal SYBR Green Master (Roche) – dye unspecifically attaching to double stranded DNA, 0.2 µl of primer Actino234 (5´- CGC GGC CTA TCA GCT TGT TG - 3´) and Eub518 (5´- ATT ACC GCG GCT GCT GG - 3´) (Fierer et al. 2005), both at the concentration of 100µl. The ideal primers are those of: 14-20 bp, 40-60% GC content, annealing temperature 55-65°C. Then 0.24 µl BSA – bovine serum albumine (enhancer), 0.5 µl DMSO – dimethyl sulfoxide (enhancer) and 1 µl of DNA template.

In my experiment, the qPCR programme conditions were : initial step at 95°C for 10 min, followed by - 40 cycles at 95°C for 30s, Annealing step at 60°C for 30s, Elongation step at 72°C for 1 min. After elongation step, the fluorescence was recorded.

To control the quality and quantification , the soil DNA samples were run on a 1% agarose gel (90V, 500mA, 45min; Fig. 5).

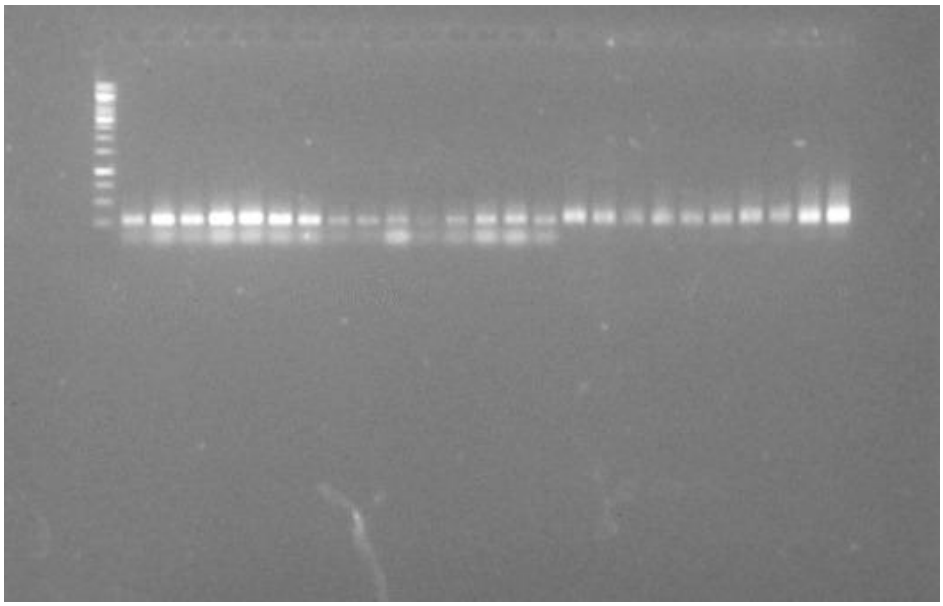

Fig.5 – Results after gel electrophoresis (1.5 % agarose, 45min, 8V/cm). Line 1 – 1kb GeneRuler ladder, Lines 2 to 32 amplicons from in-situ samples. Amplicons were of expected size of 300bp. Some of them showed high amount of primer-dimers.

3. RESULTS AND DISCUSSION

3.1 Abundance of Actinobacteria in Arctic soil

We dont have results from 20°C experiment yet, since there were many samples which didn´t work properly, so this is still the target for future experiments. Therefore, we will focus on 4°C and 12°C, only.

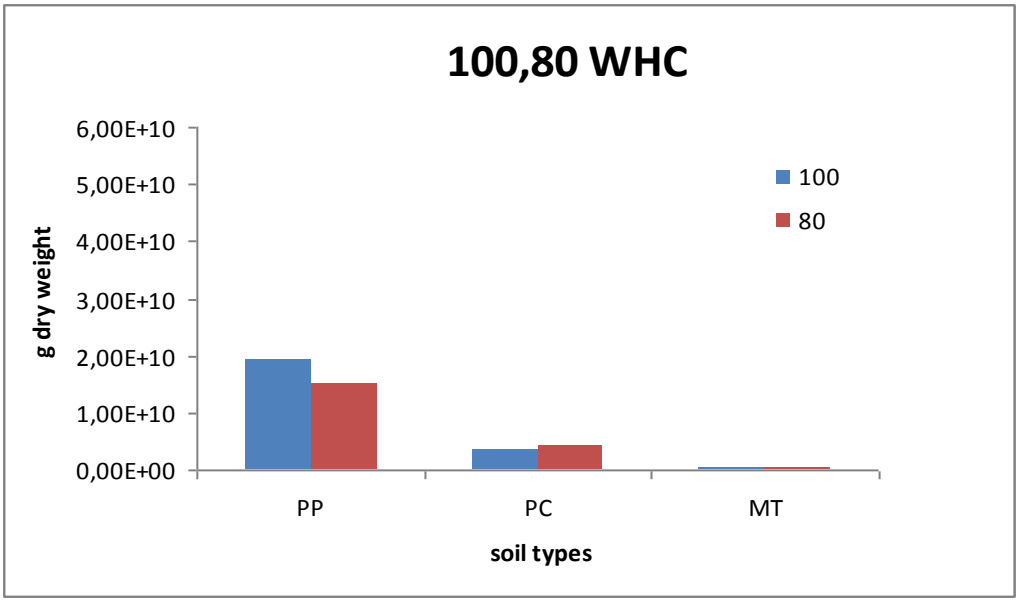

Fig.6 – Initial conditions

The initial amount of Actinobacteria did not differ significantly between the aerobic and anaerobic conditions (because of the same soil).

However, the abundance of Actinobacteria was different in the three habitats. The abundance of Actinobacteria decreased in order: peat plateau (PP), peat circles (PC) and mineral tundra (MT) with $1.91x10^{10}$, $4.02x10^{9}$ and $3.40x10^{8}$ gene copies per g of soil, (Fig.6).

In general, the qPCR does not say anything about the growth of the Actinobacteria. We were quantifying the amount from isolated DNA, which definitely contained DNA from dead, but also dormant Actinobacteria. Therefore, our results show potential amount of Actinobacteria.

The abundance of Actinobacteria at the end of the experiment was determined in three soil types (PP,PC,MT), at two different temperatures (4°C, 12°C) at 80% (aerobic) and 100% (anaerobic) WHC.

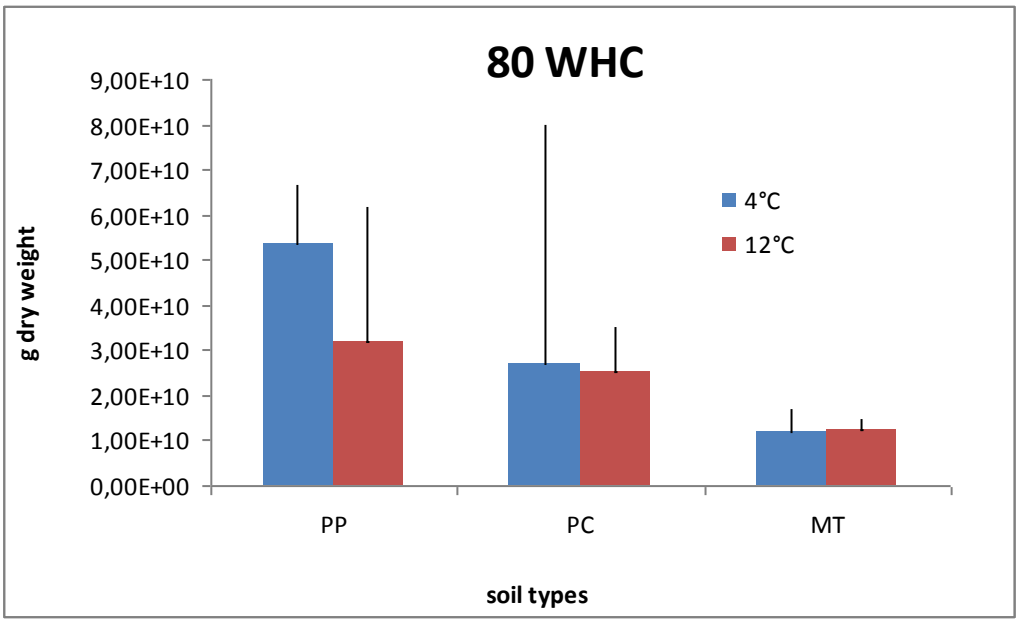

Fig.7 – Graph showing amount of actinobacteria in different soil types, under two different temperatures in 80% WHC environment

The amount of Actinobacteria in 80% WHC was generally higher at of 4°C compared to 12°C (Fig.7).

Looking in more detail, the biggest difference in the amount of Actinobacteria was in case of PP (peat plateau) soil type, where at 12°C the amount was corresponding to ($3.00x10^{10}$ +/- $3.01x10^{10}$) and in case of 4°C, it was almost double amount ($5.37x10^{10}$ +/- $1.33x10^{10}$).

There was not a big difference in the amount of Actinobacteria present in peat circles (PC) and mineral tundra (MT) at 4°C or 12°C, PC showed just slightly higher amount at 4°C and MT at 12°C. MT gave the lowest abundance of Actinobacteria out of all three habitats ($1.19x10^{10}$). This could be due to the very small amount at the beginning of experiment.

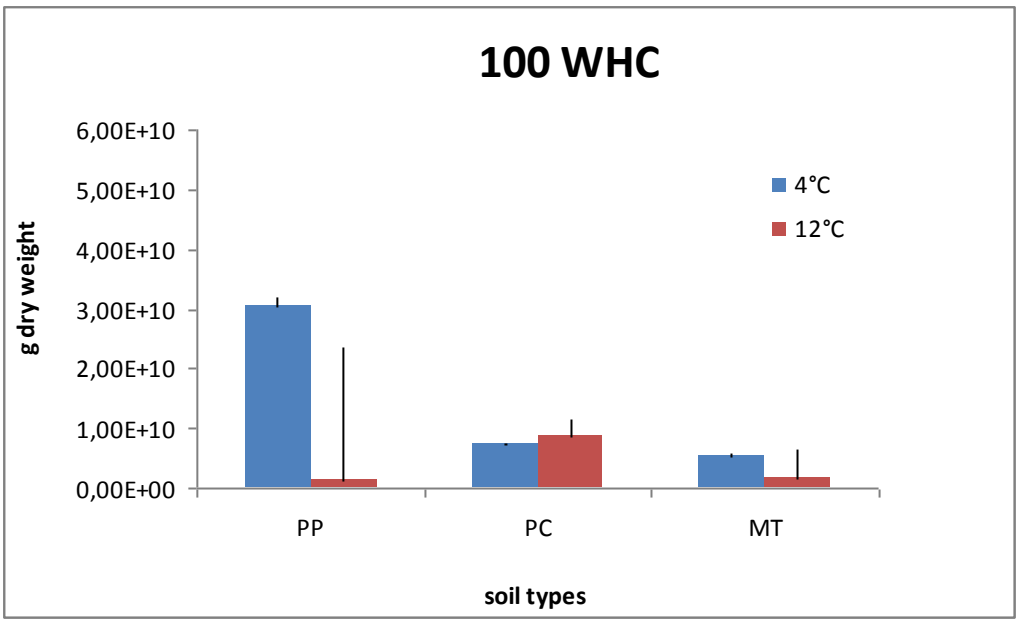

Fig.8 – Graph showing amount of actinobacteria in different soil types, under two different temperatures in 100% WHC environment

In case of anaerobic conditions (100% WHC), there was generally again higher abundance of Actinobacteria in 4°C conditions, especially in PP where again the amount of Actinobacteria was higher at 4°C being $3.04x10^{10}$ gene copies per g of soil than $1.35x10^{9}$ at 12°C. There was more Actinobacteria present in PC (peat circles) under 12°C whereas in case of MT (mineral tundra), 4°C environment showed higher value (Fig.8).

Actinobacteria are one of the most abundant phyla across the Arctic soils (Chu et al. 2010; Kobabe at al. 2004; Liebner et al. 2008; Hansen et al. 2007).

The abundance of Actinobacteria was definitely the highest in peat plateau (PP), followed by peat circle (PC),). In the natural conditions they are localized next to each other while the mineral tundra which showed the lowest amount of Actinobacteria was localized approx 300m from PP and PC. Therefore the differences in abunances of Actionobacteria may be due to spacial distinctness of PC, PP, and MT localities and also with different amount of C, N and P. For example PP contained the highest concentration of PO_4^{3-} and also different general contents of the soil types. PC showed the lowest concentration of PO_4^{3-} but the highest content of DOC (dissolved organic matter, Tab.1).

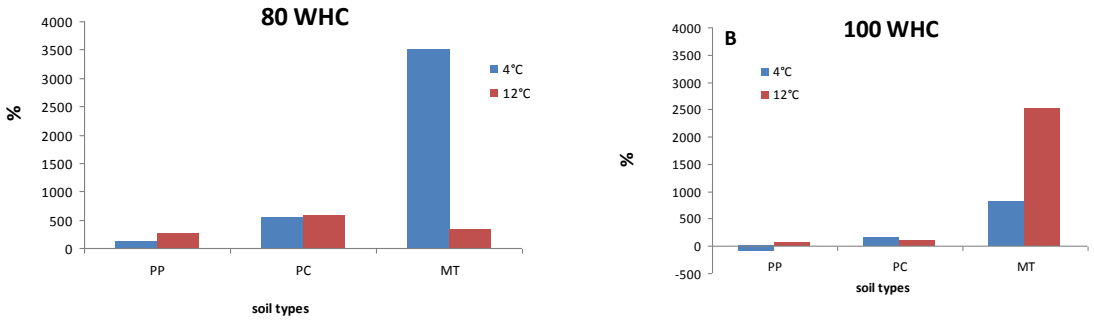

Fig.9 - Percent of change of Actinobacteria after 4 months from the start point for 80%˘WHC (A) and 100% WHC (B) relative to initial amount of Actinobacteria.

The highest increase of Actinobacteria was in mineral tundra at 4°C in aerobic environment and also at 12°C treatment in anaerobic environment.

PC soil types showed very similar relative increase under both temperatures under aerobic conditions. The same trend can be seen in case of anaerobic conditions.

The peat plateau showed a different response to temperature, more Actinobacteria were present after 12°C treatment in aerobic conditions, whereas the anaerobic environment under 4°C decreased the amount of Actinobacteria.

Interestingly, the percentual change in an abundance of Actinobacteria after 4 months from the start point showed the highest increase in mineral tundra (MT) soil type which, however, gave the lowest amount of Actinobacteria at the end of the experiment under both oxygen environments as mentioned in the previous results. Therefore even if the Actinobacteria were very low in MT copared to PP and PC they showed the highest relative change during the incubation experiment.

Aerobic conditions suited generally better to Actinobacteria at 4°C while anaerobic environment gave a better result at 12°C treatment.

Looking at the results from the point of the influence of oxygen on the amount of Actinobacteria, we can conclude, that at the end of the experiment, the abundance of Actinobacteria ($5.37x10^{10}$ gene copies per g of soil) in aerobic environment was higher comparing to the anaerobic conditions ($3.04x10^{10}$ gene copies per g of soil). The same trend was observed in relative change in the amount of Actinobacteria after 4 months incubation, where aerobic conditions showed the biggest change and increase in growth of Actinobacteria.

These results suppose, that Actinobacteria grow better in presence of oxygen rather than in absence of it, providing them better condition to decompose organic matter and gain more energy.

Temperature is an important and selective factor for dynamics in the structure and biomass of the actinobacterial community (Steger et al. 2006). The lower the temperature, the lower the reproduction rate and therefore microbial activity is limited.

The most suitable temperature seems to be generally 4°C because there was the biggest abundance of Actinobacteria in permafrost soil profile at the end of the experiment. A different trend was shown in percentual change in the amount of Actinobacteria, where the best temperature was 4°C in an aerobic environment and 12°C in an anaerobic environment.

A different response to higher temperature might be also related to a different Actinobacterial community composition.

The decreasing amount of Actinobacteria with higher temperature may also indicate that eventhough Actinobacteria in Arctic are able to live under extreme climate conditions, their tolerance to low temperatures and freezing could be under narrow range of conditions. The previous investigations by Wallenstein et.al., (2006) proved that Actinobacteria were consistently much more abundant in the prefreeze samples, so it is possible that they were physically disrupted by soil freezing and thawing.

4. CONCLUSION

We found that in case of aerobic environment (80 % WHC) the 4°C conditions were better for PP and PC whereas MT showed higher abundance under 12°C. Generally, the PP horizon seemed as the most suitable one for the Actinobacterial growth. To conclude, the lower the temperature, the higher the amount of Actinobacteria in aerobic environment.

The anaerobic conditions (100 % WHC) showed the same trend for PP as in case of aerobic conditions (4°C treatment was better), however PC and MT gave an opposite trend comparing to an aerobic one; Actinobacteria grew better in PC under 12°C and in MT under 4°C. To summarize these results, the best conditions for Actinobacterial growth in an anaerobic environment seem to be 4°C for PP and MT and 12°C for PC. The smallest abundance of Actinobacteria was measured in mineral tundra (MT) soil horizon.

The relative change in the amount of Actinobacteria after 4 months incubation showed an opposite dependency manner. Aerobic conditions were better for PP and PC under 12°C and MT under 4°C (which also gave surprisingly the highest abundance). Anaerobic conditions proved the highest amount of Actinobacteria in MT, too, however under 12°C treatment. PC supported the growth under 4°C and PP under 12°C(under 4°C it showed a decrease in the amount). As a result, Actinobacteria in mineral tundra showed the highest response to temperature, especially under 4°C treatment in oxygen present environment.

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Internet resources:

http://en.wikipedia.org/wiki/File:Streptomyces_sp_01.png (Public Health Image Library)

http://www.jgi.doe.gov/sequencing/why/actinobacteria.jpg

SUPPLEMENTARY MATERIALS

No.	Soil	Temp.(°C)	WHC(%)	Replicate
1.	PP		100	start point
2.	PC		100	start point
3.	MT		100	start point
4.	PP		80	start point
5.	PC		80	start point
6.	MT		80	start point
7.	PP	4	100	1
8.	PP	4	100	2
9.	PC	4	100	1
10.	PC	4	100	2
11.	MT	4	100	1
12.	MT	4	100	2
13.	PP	12	100	1
14.	PP	12	100	2
15.	PC	12	100	1
16.	PC	12	100	2
17.	MT	12	100	1
18.	MT	12	100	2
20.	PP	20	100	2
22.	PC	20	100	2
23.	MT	20	100	1
24.	MT	20	100	2
25.	PP	4	80	1
26.	PP	4	80	2
27.	PC	4	80	1
28.	PC	4	80	2
29.	MT	4	80	1
30.	MT	4	80	2
31.	PP	12	80	1
32.	PP	12	80	2
33.	PC	12	80	1
34.	PC	12	80	2
35.	MT	12	80	1
36.	MT	12	80	2
37.	PP	20	80	1
39.	PC	20	80	1

Tab.S1 – List of samples used in my experiment