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Abstract

The worldwide use of antibiotics has rapidly increased since the discovery of the secondary metabolite penicillin made by Alexander Fleming in 1928. Since then, thousands of useful secondary metabolites of microbial origin have been discovered. About 80 % of these substances originate from Actinomycetes and in particularly *Streptomyces* species. Lately, the search have been focused upon finding rare-actinomycetes where the chance of discover a novel secondary metabolite is larger. The use of antibiotics the past decades has led to a rapid evolution in microorganisms where naturally occurring resistance genes to antibiotics are spreading fast, hence the need for new antibiotics increases. New isolation and screening methods applied on samples from under-explored ecological niches like marine environments and plant endophytes are examined to increase the change of finding new useful antimicrobial metabolites. In the current work, samples were collected from a broad range of ecological niches; five marine samples from the west coast of Sweden, two plant samples and one sample from lichen from the middle of Sweden and one sample of bat guano from the Philippines. The samples were plated onto three different media containing one of five different antibiotics in two concentrations. Two hundred and thirty-five bacterial isolates were collected and 64 isolates were identified by sequencing their 16S rDNA. Using 58 of these isolates, a phylogenetic tree were constructed on the genus and species level. A PCR-screening covering four genes in different enzymatic pathways for secondary metabolite production were done on all samples to evaluate the theoretical ability to produce the most important types of secondary metabolites. Finally to determine production of antibacterial activity a zone-inhibition assay against one species of *Staphylococcus* and one species of *Pseudomonas* were performed on 14 isolates.

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Introduction

Antibiotics – discovery and origin

One of history's most fortunate discoveries occurred by chance in London in 1928. Professor Alexander Fleming inoculated a strain of staphylococci on an agar plate and left it on his workbench. After a longer period of time the plate caught his attention – a mold colony had contaminated it but around the mold colony no bacterial growth could be observed (Fleming, 1929; Sköld, 2006). The substance suppressing bacterial growth was named penicillin and about ten years later its full potential as a drug had been disclosed. The discovery and further development of penicillin provided a cure for many of the so far deadly diseases caused by bacteria. Spurred by the success of penicillin, an extensive search among other microorganisms started for similarly useful compounds (Sköld, 2006). During the following 20 years the most important groups of antibiotics were discovered and these includes e.g. macrolides and tetracycline (Bérdy, 2005). About 70 to 80 % of all antibiotics discovered during this time originate from actinomycetes and especially from various *Streptomyces* species. Actinomycetes are a group of gram-positive bacteria most commonly isolated from soil but also from other environmental niches such as in marine sediments. When compared to other microorganisms they generally carry a large number of genes encoding for many different enzymes involved in the production of secondary metabolites that could be of interest for discovering novel antibiotics (Baltz, 2008; Tiwari & Gupta, 2013). Various environmental samples have been studied extensively over the last 50 years, mostly resulting in the re-discovery of many already known compounds. However, as only a tiny fraction of the available samples have, so far, been collected many actinomycetes taxon's could still be undiscovered (Baltz, 2008).

Antibiotic resistance

Effective antibiotics are essential in order to maintain the high standard of healthcare that nowadays is taken for granted by enabling medical control of bacterial infections. The success of antibiotics has led to their massive consumption and distribution in healthcare for humans and animals but also to abundant use in agriculture and in animal breeding. This overconsumption of antibiotics has led to an enormous selective pressure on the bacteria, which have been forced to adapt to the antibiotics and hence gain resistance or die. The adaption is very rapid and resistance usually occurs one to two years after a new antibiotic is introduced (Sköld, 2006). An additional problem is the occurrence of multi-resistant bacteria, which are resistant against many different antibiotics. A study by Costa *et al.* 2006 shows that

out of 480 different bacterial strains found in soil, two was multi-drug resistant to 15 of 21 drugs tested and every strain in the study was in average multi-drug resistant to seven or eight different antibiotics.

The need for new antibiotics

As the antibiotic resistance is spreading, the need for new antibiotics is increasing. One of the possible ways to increase the chance of finding novel antibiotics is to find new approaches for isolating interesting bacteria and fungi or at least make the existing methods more efficient. One of the more promising techniques is to use the resistance and enhance the self-protection mechanism antibiotic producers need to have, in order to avoid suicide. A study by Thaker *et al.* (2013) showed that using resistance as a trait for selection increases the hit rate of interesting isolates with at least four orders of magnitude as compared to classical screening. The second approach is to search for new sampling sites and to focus on isolation of rare actinomycetes rather than the most common *Streptomyces* species that have been found in soil samples worldwide. Rare actinomycetes are harder to isolate and culture but the possibility that an yet undiscovered secondary metabolite is produced is higher since these species are not so well studied as others actinomycetes (Tiwari & Gupta, 2013).

Microbial diversity and sampling strategy

Studies have shown that unknown species and genetically different strains of actinomycetes are still abundantly present in nature. Paradoxically some of the last places to be explored by mankind for its microbiological diversity are the oceans, which cover more than 70 % of the earth's surface. Studies with promising results have now been made where rare actinomycetes have been isolated from marine environments from e.g. shallow water sediments of the Trondheim fjord (Bull *et al.*, 2000; Bredholt *et al.*, 2007). Some ecosystems are more under-explored than others and therefore more interesting as for example microbial plant endophytes, microbial insect symbionts, lichens and most marine environments. These biological niches will most likely contain unknown actinomycetes communities that hopefully may be connected to novel secondary metabolites (Genilloud *et al.*, 2011).

Isolating microorganisms by screening for antibiotic resistance

In this study microorganisms, including actinomycetes, were isolated from five marine samples from the west coast of Sweden, two plant samples from Sweden and one sample from the feces of bats from a cave in the Philippines. The different samples harbor a broad range of different bacterial species and the possibility to isolate rare actinomycetes from interesting ecological niches might, therefore be higher. Also the samples origin varied from three

gastrointestinal samples of different fish species, one from mussels, one from macroalgea, one from bat guano, one from lichens to samples of both leaves and roots of the traditional medicine plant Celandine.

Portions of the samples were incubated on three different growth media with different nutrient composition as well as five different antibiotics. Based on their morphology, colonies were isolated and identified using sequence analysis. Subsequently a genome-wide PCR-based screening was performed to investigate the potential of the isolated microorganisms to create secondary metabolites. Some of the isolates were also tested for their ability to inhibit the growth of the two non-pathogenic species of bacteria, *Staphylococcus warneri* and *Pseudomonas resinovorans*, which are closely related to the bacterial pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Material and methods

Isolation substrates

Three growth media with different nutrient composition were used in the experiment (Table 1). Five different antibiotics, Spectinomycin (Duchefa, The Netherlands), Rifampicin (Sigma), Erythromycin (Sigma), Lincomycin (Sigma) and Streptomycin (Sigma) were supplemented to the media rendering six different plates of each media type including a control without antibiotics. In isolation experiment A, the concentration of antibiotics in the media was adjusted to be 25 µg/ml. In isolation experiment B, the antibiotic concentration was 50 µg/ml except for Rifampicin where the concentration was 12.5 µg/ml.

Table 1. Media composition of the media used in the experiments.

Media	Composition
IM7	Chitin 2g (Alfa Aesar), K_2HPO 0.5g (Merck) , $FESO_4 \cdot 7\ H_20$ 1mg (Sigma) , Agar 18 g (Difco), Sea Salt 23 g (Foodgrade), H_20 1L
NBC	Nutrient broth 1g (Difco), Casamino acids 1g (Dicfo), Agar 14g (Difco), H_2O 1L
NBC-SW	Nutrient broth 1g (Difco) , Casamino acids 1g (Difco), Agar 14g (Difco), Sea Salt 33g (food grade quality), H_2O 1L

Biological material

Eight samples of different natural origin were collected and treated differently depending on its origin and composition (Table 2). All samples except for the Celandine had been frozen in -80 °C prior the experiments.

Table 2. Sample denotation, content and origin.

Sample	Content	Origin
B	Cuckoo wrasse, *Labrus mixtus*	The Swedish West coast
T	cod, *Gadus morhua*	The Swedish West coast
M	Mackerel, *Scomber scombrus*	The Swedish West coast
E	Blue mussels, *Mytilus edulis*	Mollösund, Skagerak, Sweden
P	Mackroalgea	Torekov, Skälderviken, Sweden
G	Bat guano	The Philippines
SB	Leaves from Celandine, *Chelidonium majus L.*	Uppland, Sweden
SR	Roots from Celandine, Chelidonium majus L.	Uppland, Sweden
L	Lichen	Uppland, Sweden

Isolation of colonies

Microbial colonies were chosen from the plates for further purification and preservation based on colony morphology (shape, size and color) and preferably from plates with relatively large difference in the number of colonies between the control plates and the antibiotic plates. The selected colonies were re-streaked onto the same type of plates and further incubated for about 10 days at 20 °C. For preservation and DNA purification, liquid cultures were started from pure bacterial isolates by inoculating a loop of bacteria in 1 ml of half strength VPB medium (15 g Vegetable Peptone broth, (Oxoid), 1000 ml deionized H_2O) followed by incubation for 2 days at 25 °C on a rotary shaker (135 rpm). Aliquots from the liquid cultures were frozen in 15 % glycerol for storage at -80 °C and a part of the remaining material was used for DNA purification.

DNA analysis

DNA was extracted using the FastDNA™ SPIN Kit for Soil (MP Biomedicals) following the manufacturer's description, except for the speed setting for the Fast DNA instrument that was were set on 6.0 for 2×40 seconds.

PCR amplification of the 16S rDNA was performed using the primers 27F (5'-AGAGTT TGATCMTGGCTCAG-3') to 1492R (5'-CGGTTACCTTGTTACGACTT-3'). Each PCR reaction of 25 µl in total included 12.5 µl DreamTaq DNA polymerase mix (ThermoFisher Scientific), 6.5µl dH_2O, the final concentration 0.4 µM of each primer and 4 µl DNA template. The cycling conditions for the amplification of the 16s rDNA region were as follow: 5 min at 95 °C, 25 cycles at 94 °C for 40 s, at 55 °C for 30 s and 1 min at 72 °C, then followed by a final elongation step for 7 min at 72 °C. When needed, an additional round of PCR for the 16S rDNA were performed using the primers 27F to 765R (5'-CTGTTTGCTCCCCACGCTTTC-3') and 704F (5′-GTAGCGGTGAAATGCGTAGA-3') to 1492R using the same cycling conditions. The amplified PCR-products were purified using the Qiagen PCR-purification kit. The purified PCR-products were mixed with either the primer 27F or 1492R and sent to Macrogen for Sanger sequencing.

For the amplification of the four genes that indicate the genetic potential of each isolate to produce the secondary metabolites: diterpene synthases (DTSs), type 1 polyketide synthases (PKS-I), type II polyketide synthases (PKS-II) and nonribosomal peptide synthetases (NRPSs), a PCR analysis was run using the primers previously described by Ayuso-Sacido & Genilloud, (2005) (PKS-I and NRPSs) and by Xie *et al.*, (2014) (DTSs and PKS-II). The cycling conditions for amplification of the four genes were as above with the exception that 30 cycles were run and the elongation time was set up to 1 min and 20 seconds. All PCR-products were analyzed on a 1 % agarose gel with a GeneRuler™ 1kb Plus DNA ladder (Thermo Scientific) at 70 V for about 30 minutes.

Sequence analysis

Sequences obtained from Macrogen were analyzed using software *MEGA* version 6 (Tamura *et al.*, 2013). The obtained sequences were sequenced by a forward and a reverse primer and therefore an overlapping central part could be found. The two sequences were aligned the software MUSCLE (Edgar, 2004). Low quality regions were excluded and the trimmed sequences were then uploaded to the RDP database (Maidak *et al.*, 2006) and the SILVA database (Quast *et al.*, 2013). The 10 nearest sequences were then selected and aligned before being downloaded to the software MEGA in order to construct a phylogenetic tree. The tree was constructed using the method Maximum Likelihood with the General Time Reversible model and bootstrap testing of 1000. Branches with bootstrap support lower than 70 were

collapsed. The phylogenetic tree based on the SILVA database was compared to the one obtained from the RDP database.

Zone inhibition assay

The two test strains used in the assay were obtained from the Culture Collection, University of Göteborg (CCUG), *Pseudomonas resinovorans* CCUG 2473T (*P. resinovorans)* and *Staphylococcus warneri* CCUG 7325T (*S. warneri*). Strains were spread on VPA plates (10 g VPB (Oxoid), 1000 ml deionized H_2O, 14 cm in Ø). Following concentrations were used: *P. resinovorans* - 2.6×10^4 colony forming units/ml (cfu/ml) and *S. warneri* 1×10^5 cfu/ml. Directly after, 10 µl of overnight cultures of selected bacterial isolates from experiment A, were spotted in even distance from each other. Twelve isolates were spotted for each plate and the plates were then incubated at 25 °C for 3 days. The plates were inspected for occurrence of inhibition zone around colonies and isolates that gave rise to an inhibition zone were further tested. Agar plugs were cut out with the back of a 200 µl pipet-tip from the inhibition zone and placed in 350 µl of methanol. The tubes with plugs were sonicated for 10 minutes and then centrifuged at 14 000 rpm for 5 min. The liquid were then transferred to new tubes and a HPLC-MS, high performance liquid chromatography – mass spectrophotometer, (Agilent 1100 HPLC/Bruher maXis Impact ESI-QTOF MS) was performed by loading 1 µl sample into the column (Thermo Scientific Accucore 3×50 mm with 2.6 µm particels). The eluent used were A: H_2O (0.2 % FA) and B: MeCN (0.2 %FA). A gradient of the eluent was set at 10-95 % B in 4 min, 95 % B for 3 min, 10 % B for 3 min, 0.8 ml/min.

Results

Cultivation and isolation of microorganisms

The number of microbial colonies growing on the plates in experiment A was extremely variable depending on the sample. The assortment of bacterial colonies on the plates varied not only between samples but also between control plates and plates with different antibiotics. Examples of different colony types and the differences between an antibiotic plate (to the left) and a control plate (to the right) are displayed in Figure 1. In total 86 isolated colonies, mostly bacteria but also some fungi from experiment A and 189 colonies from experiment B was selected and cultured (Appendix 1).

The number of colonies was counted on each plate for experiment A (Figure 2). Number of colonies grown on plates from Cod, Mackerel and Cuckoo wrasse was extremely low even on

control plates without antibiotics. These samples were therefore omitted in subsequent experiment. On plates with the other samples, a difference between the control plates and the plates with antibiotics was visible but in many cases not enough pronounced. Also, nearly no colonies grew on the IM7 medium with chitin as a carbon source. This medium was omitted in experiment B. The higher concentration of antibiotics in experiment B led to a more pronounced decrease in the number of colonies growing on the antibiotic plates as compared to the control plates (Figure 3). The difference in the number of colony forming units between the control and antibiotic plates is generally clearly detectable in both the experiments A and B even though differences are clearer in experiment B. The only exceptions to this statement are the samples of Celandine (leaves and roots) and the blue mussels in experiment A as well as the sample of Celandine leaves in experiment B. The number of colonies on the antibiotic plates from these samples was nearly comparable to the number of colonies growing on the control plates.

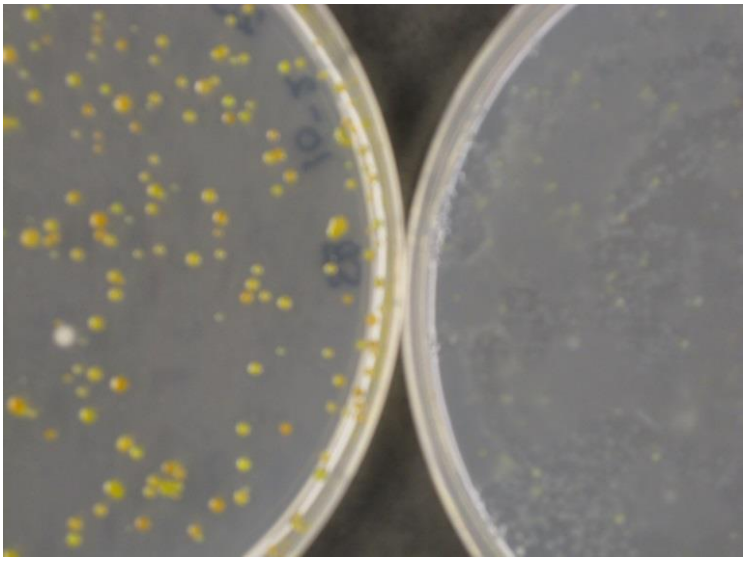

Figure 1. Differences in colony morphology of microorganism growing on antibiotic plate (left) and on control plate (right).

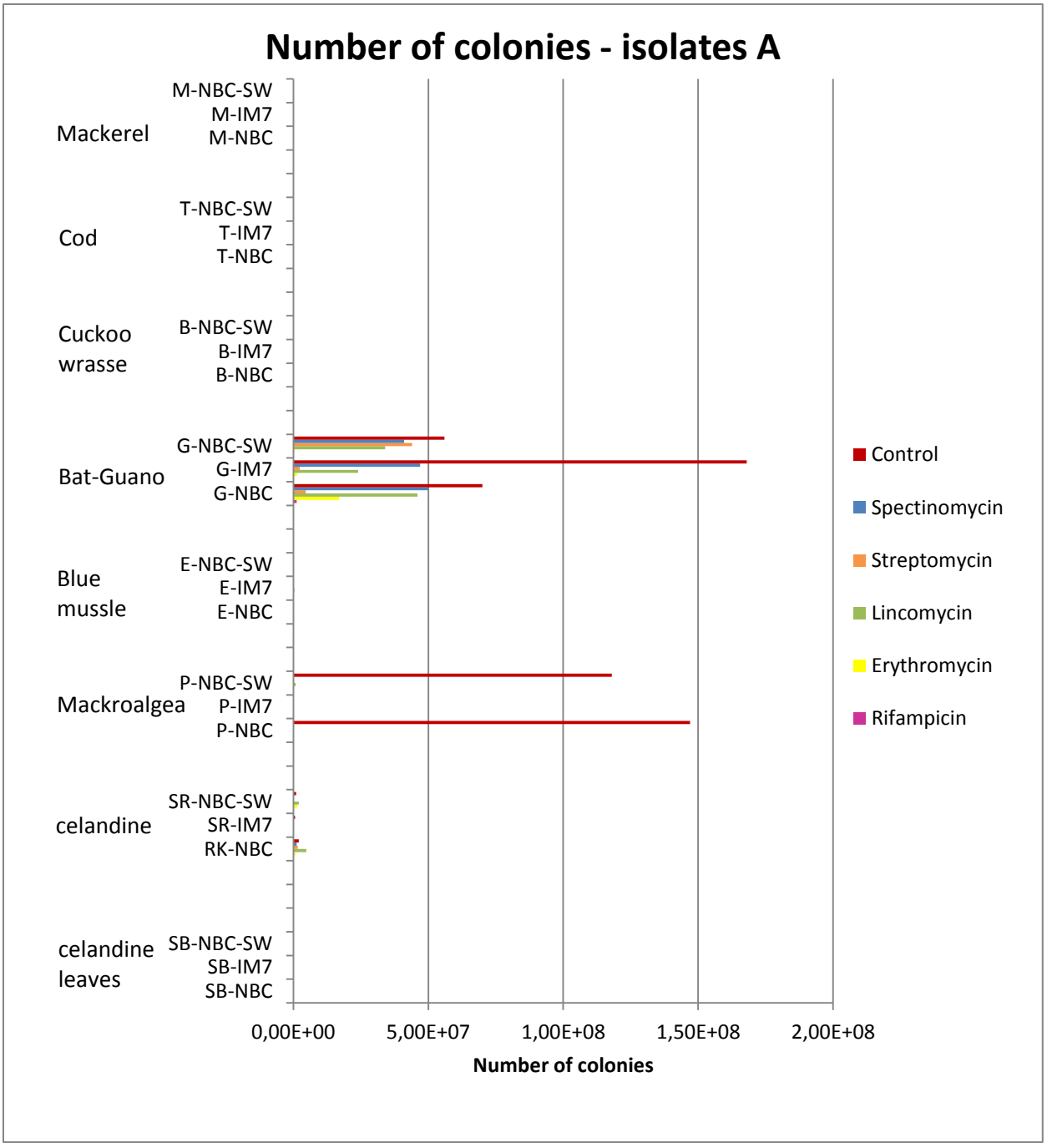

Figure 2. Number of microbial colonies counted in experiment A after incubating the plates for 10 days at 25 °C.

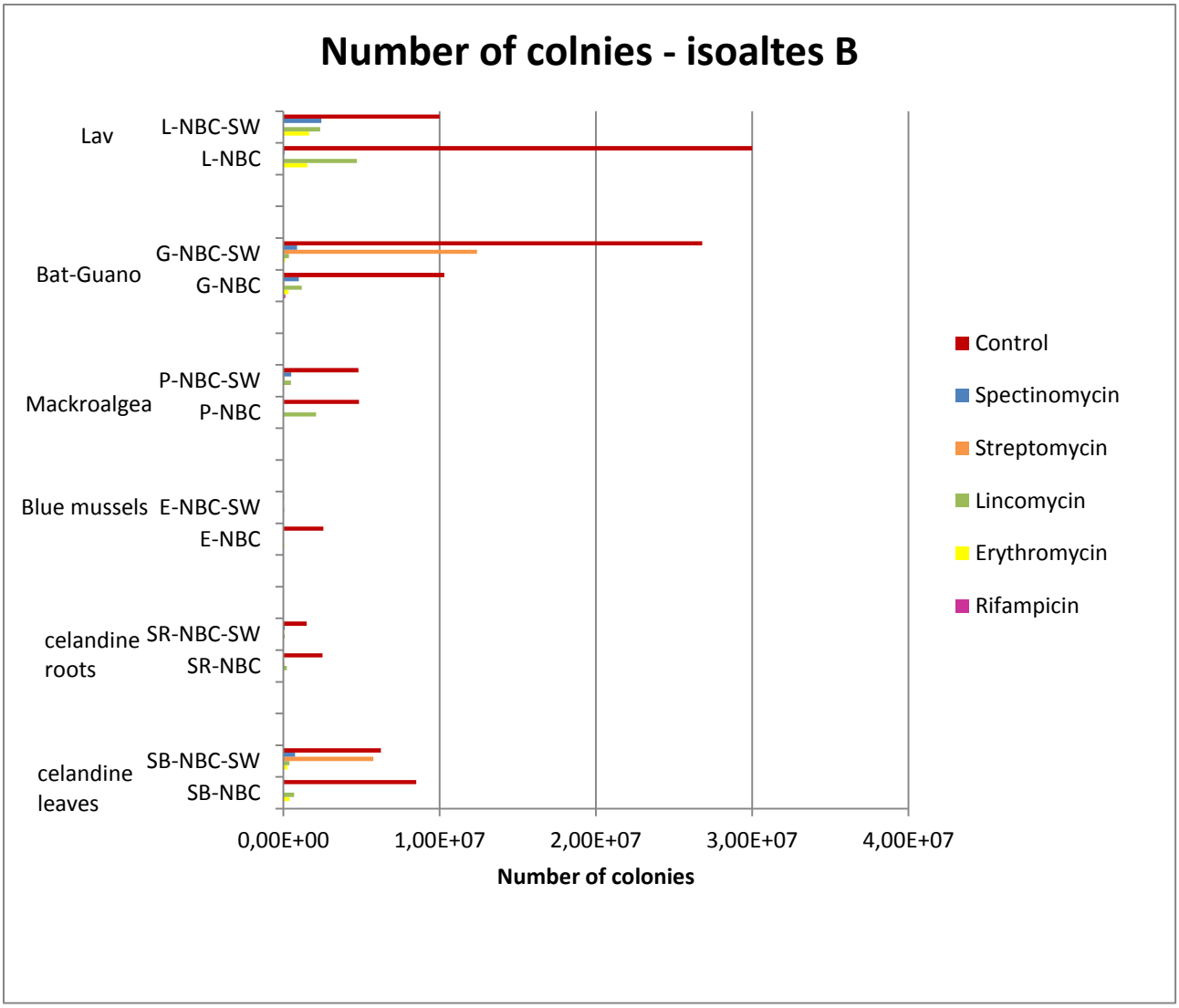

Figure 2. Number of microbial colonies counted in experiment B after incubating the plates for 10 days at 25 °C.

PCR-amplification of 16S rDNA

In order to identify the isolates by 16S rDNA sequencing, a PCR-amplification of the 16S rDNA was done. A positive amplification is seen as a bright band at 1500 bp (Figure 4), which is the expected size of the 16S rRNA gene. An additional set of primes was used to amplify 16S rRNA from isolates that did not amplify at once. The samples that amplified the second time were also included in further sequence analysis.

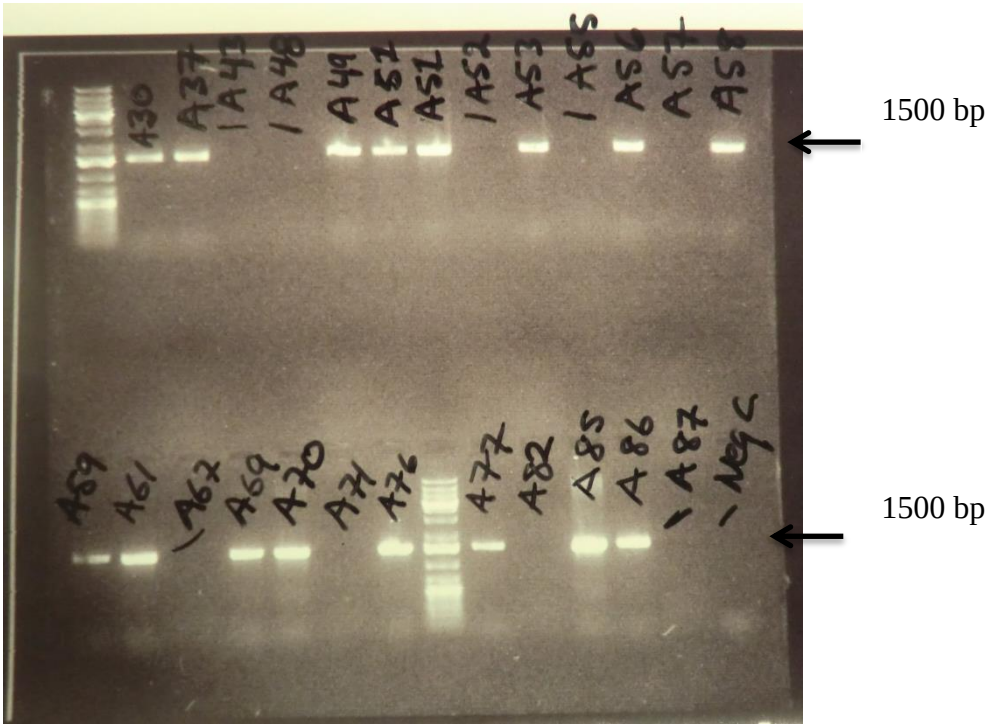

Figure 3. PCR-amplification of rDNA of some isolates from experiment A.

Sequencing

Out of the 64 sequenced isolates, 58 yielded good-quality chromatograms. Analysis performed with help of the RDP database (Figure 5) showed the phylogenetic order of the isolates. With 90 % confidence, 38 out of the 58 sequenced isolates were identified as belonging to the order Actinomycetales. No species could be identified with this level of confidence and therefore the SILVA database was used to find closest relatives to these isolates.

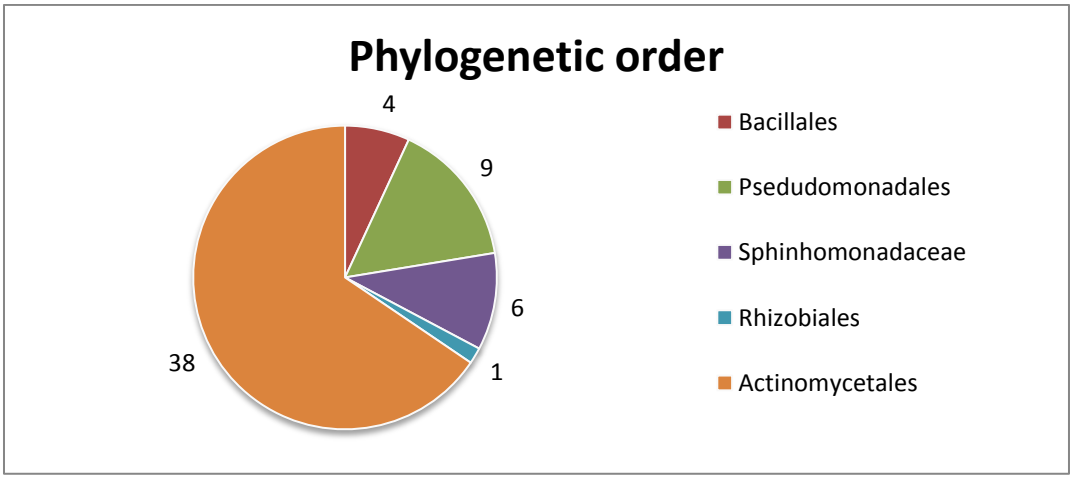

Figure 4. Phylogenetic order of the sequenced isolates.

Phylogeny

The phylogenetic tree (Figure 6) is constructed with the 16S rDNA gene from each of the sequenced isolates as well as with 16S rDNA gene of the one or two closest related species. A broad variety of species were isolated during this experiment (Figure 6), not only Actinomycetales. The 38 isolates belonging to Actinomycetales could be grouped as closest related to the following genera:

- *Streptomyces*
- *Nocardiaceae*
- *Amycolatopsis*
- *Arthrobacter*
- *Kocuria*
- *Kribella*

The majority of the isolates recovered during the experiments as well as the majority of the sequenced sample came from the bat guano sample. However, some of the sequenced samples were from Lichen, Macroalgea and Celandine root. A complete list of the most related species to each of the sequenced isolate as well as isolates origin are found in Appendix 2. The majority of all isolates are closest related to a number of different strains of the same species but sometimes even different species.

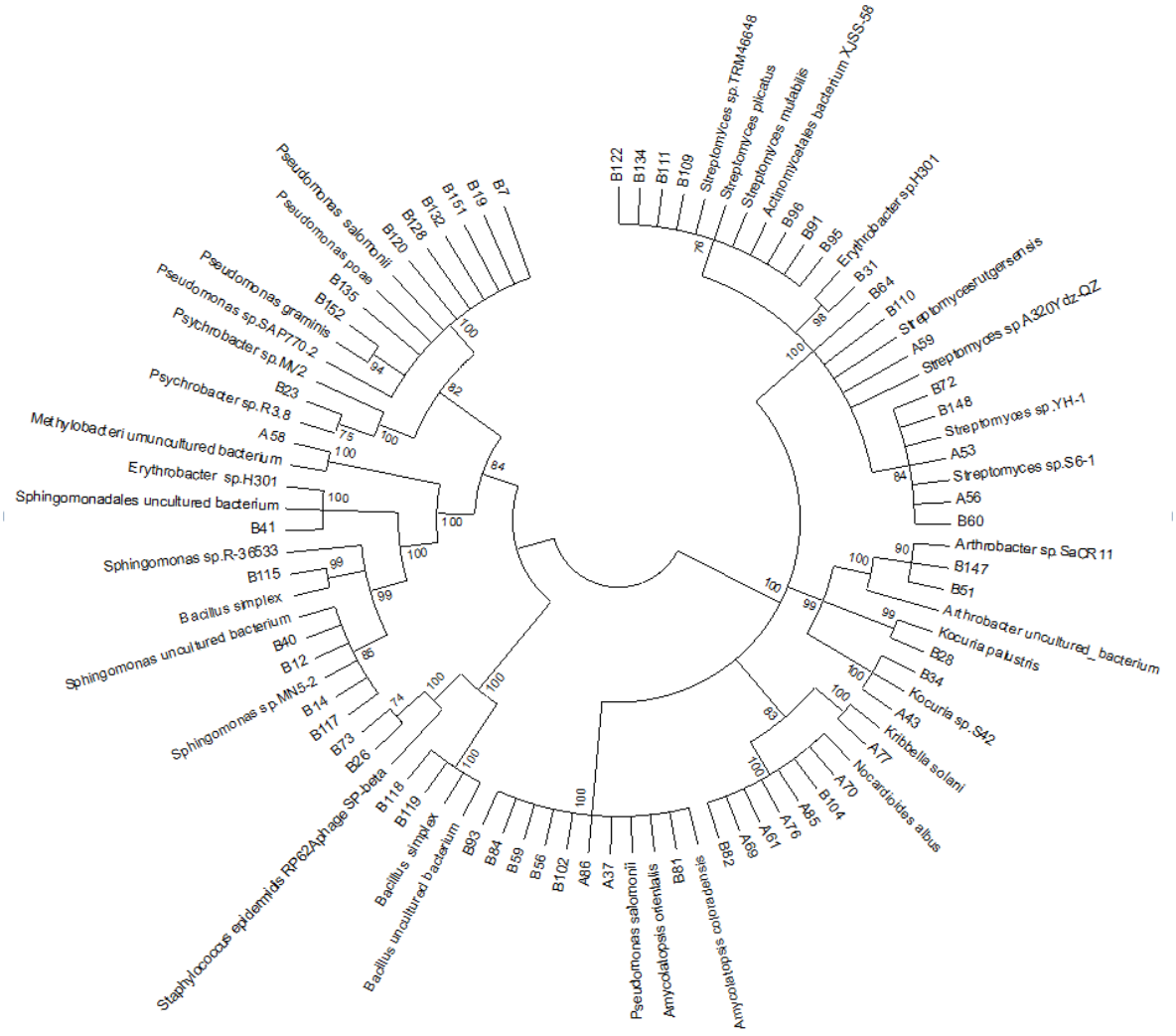

Figure 5. Phylogenetic tree of the 58 isolates recovered in this experiment constructed with Maximum Likelihood with the General Time Reversible model and bootstrap testing of 1000, branches with bootstrap support lower than 70 was collapsed.

Genetic potential

The genetic potential of each isolate for producing secondary metabolites was investigated by PCR-amplification of four different genes known to be involved in secondary metabolite production. Figure 7 shows the bands for the NRPS gene. Three other genes were also amplified, (DTS, PKS-I and PKS-II) (figures not shown). The profiles from the amplification of all four genes were used to grade the isolates in order to create a diagram over their genetic potential. The grading depends on the number of bands each isolate displays – one band gives one point, two bands two points and three bands three points. Figures 8 and 9 shows the genetic potential for each isolate created with this grading system.

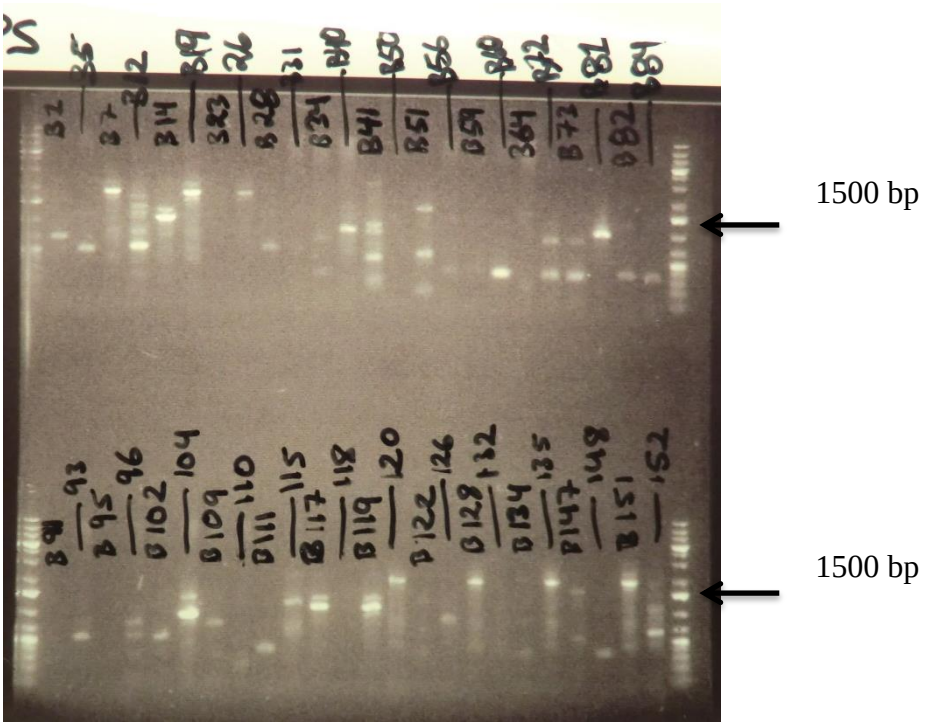

Figure 6. PCR-amplification of the NRPS gene for some isolates in experiment B.

In experiment A (Figure 8), the highest score is 6 for two isolates (A69 and A85) but only one of these two isolates has all four genes (A69). These isolates are considered to be the most interesting with theoretically highest genetic potential to produce secondary metabolites. In this experiment, many of the isolates have only two out of the four genes and three isolates do not have any. Four out of the fifteen samples originated from control plates without antibiotics (A51, A53, A59 and A61), and these had at least one out of four detected genes.

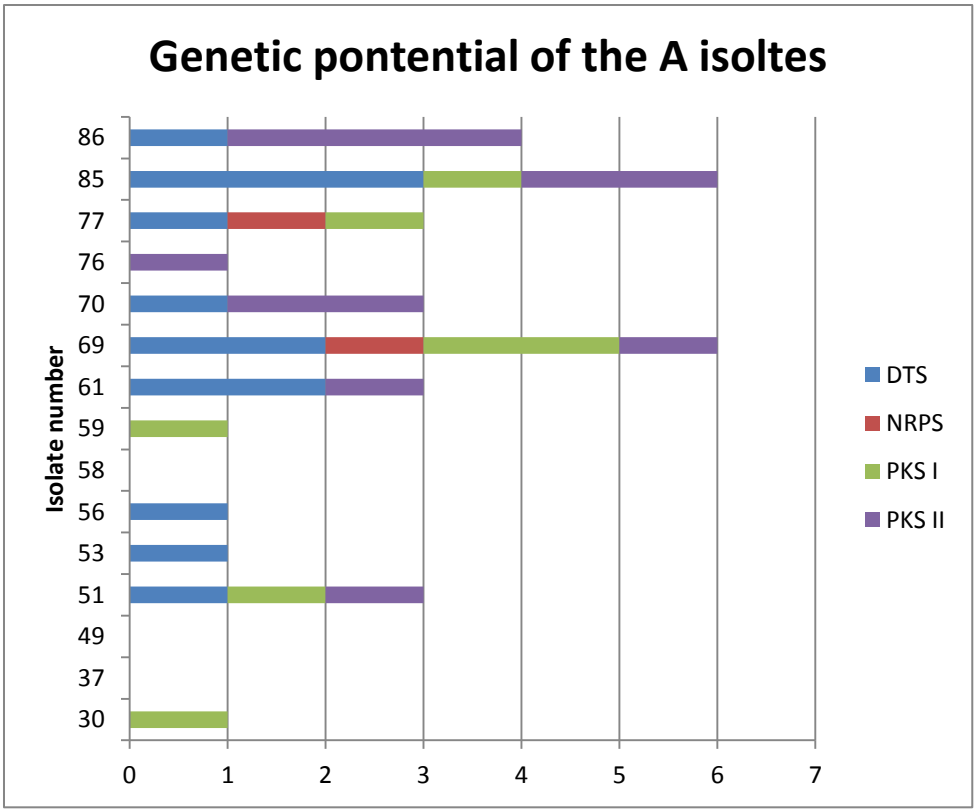

Figure 7. The estimated genetic potential of some of the isolates from experiment A for the four genes diterpene synthases (DTSs), type 1 polyketide synthases (PKS-I) type II polyketide synthases (PKS-II) and nonribosomal peptide synthetases (NRPSs).

The genetic potential of the B isolates is generally a bit higher as compared to the A isolates with an overall higher score and the maximum score of seven compared to six for the A isolates. Also, 14 out of 48 isolates in this experiment have all four genes and five isolates reaches the highest score of seven. Only three isolates were from control plates (B19, B23 and B60) and out of these three isolates the isolate B19 have all four genes whereas isolate B23 does not have any of these genes. This shows that the theoretical ability to create secondary metabolites is high even when the bacteria not are under the selection pressure.

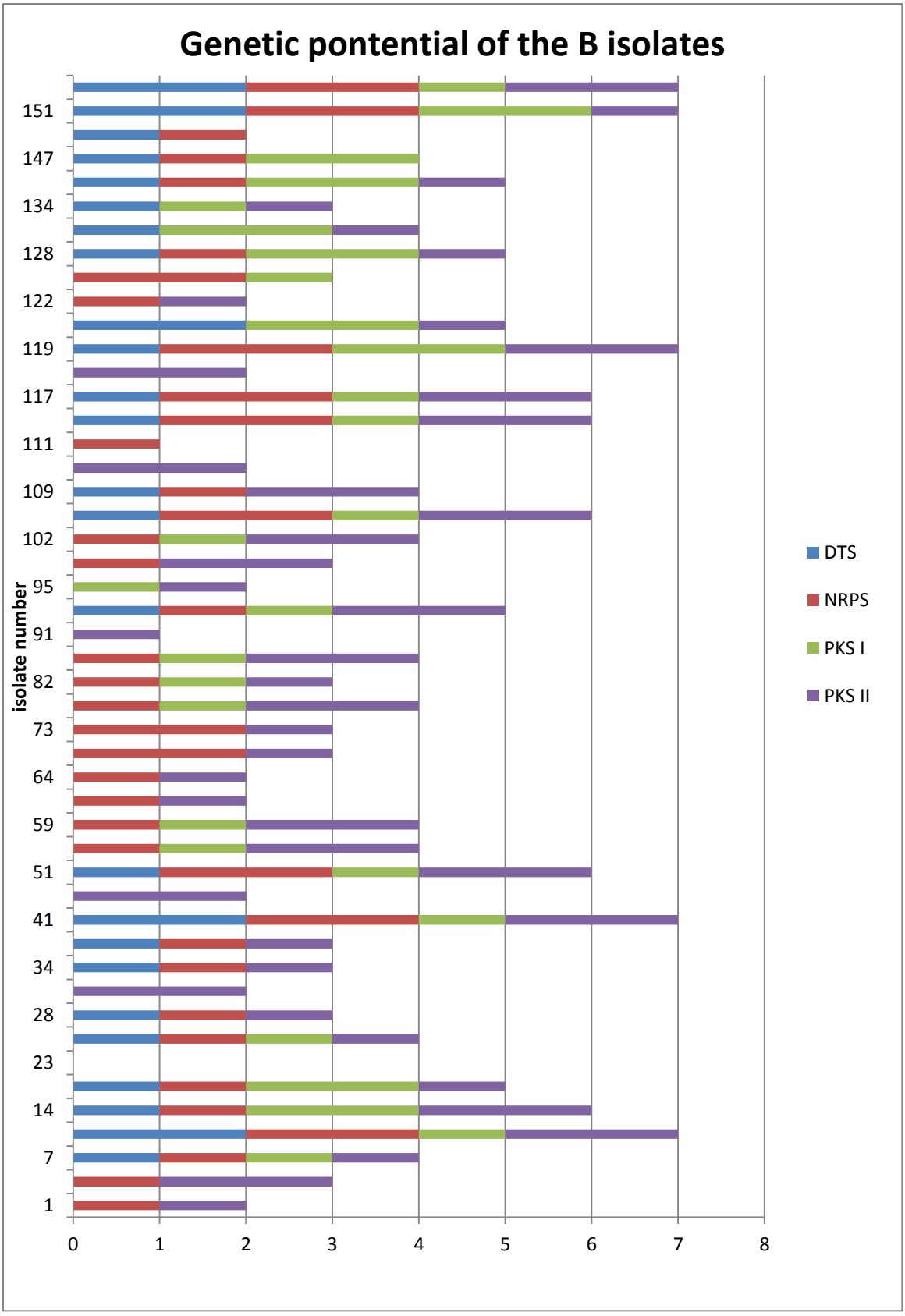

Figure 8. The estimated genetic potential of selected isolates from experiment B for the four genes diterpene synthases (DTSs), type 1 polyketide synthases (PKS-I) type II polyketide synthases (PKS-II) and nonribosomal peptide synthetases (NRPSs).

Zone inhibition assay

Some isolates could inhibit the growth of *S. warneri* and hence inhibition zones could be detected around the colonies, whereas *P. resinovorans* was not inhibited by any of the tested isolates. Figure 10 (A and B) shows the results from the HPLC-MS for bacterial isolate A30 originating from bat guano. This isolate inhibited growth of *S. warneri* (clear inhibition zone around the isolate colony) and did not inhibit the growth of *P. resinovorans*. However, the HPLC-MS chromatograms of the corresponding plug samples (Figure 10A and 10B) did not show anything of interest. The only differences between chromatogram A (*S. warneri)* and B (*P. resinovorans*) are that some peaks are higher or lower than the corresponding peak in the other pictures. Examples are the peaks 7, 8 and 9 in picture A (Figure 10) which are higher than the corresponding peaks in picture B (Figure 10) and that peaks 12 and 13 in picture A are lower than the corresponding peaks in picture B.

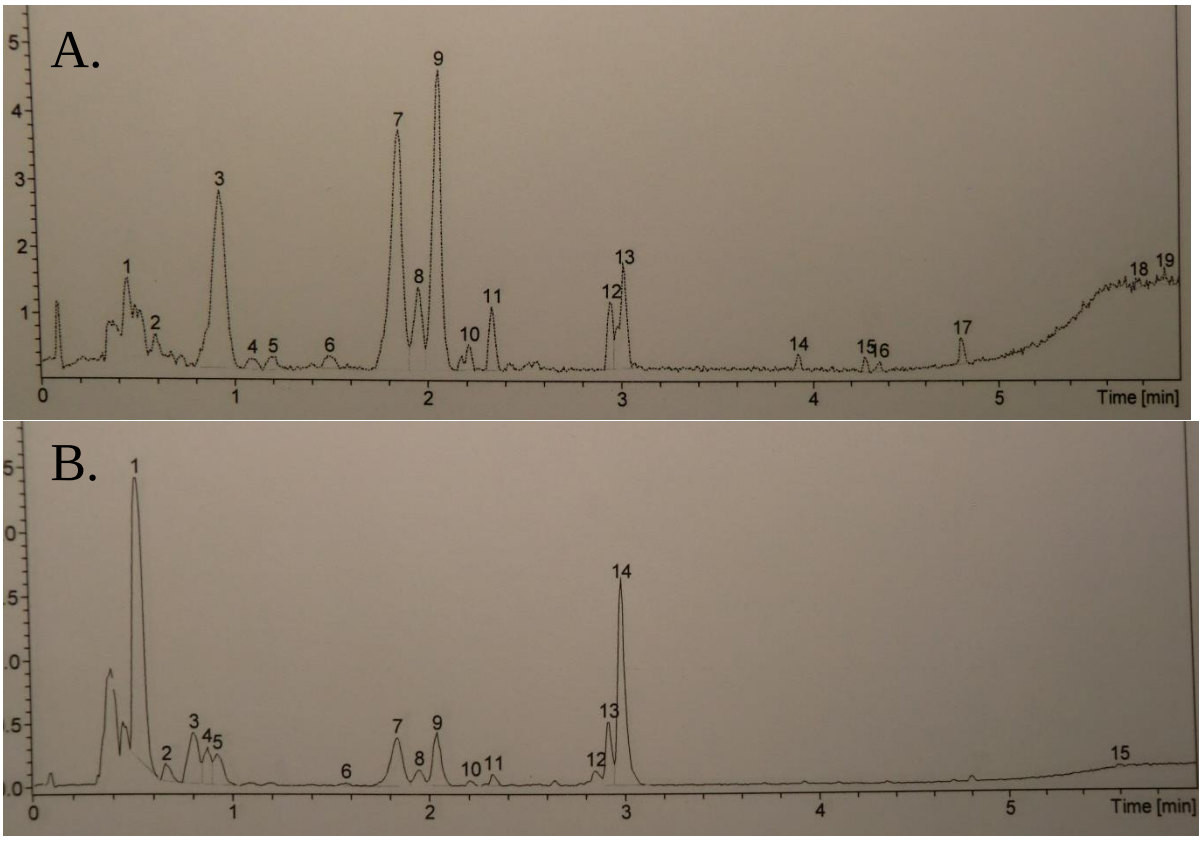

Figure 9. Example of chromatograms for the agar plugs from the zone inhibition assay on the isolates from experiment A, picture A displays *S. warneri* and picture B displays *P. resinovorans.*

Discussion

The aim of this study was to isolate microorganism, in particular rare actinomycetes from interesting ecological niches and preferably with a high genetic potential to produce secondary metabolites. The samples were taken from various environmental niches: the salty waters of the southern west coasts of Sweden, the inland of the middle of Sweden and the tropical Philippines. Moreover, the samples were of very diverse biological nature. Thus many different niches were simultaneously studied as the samples ranged from bat guano to marine environments including different species of fishes, mussels and macroalgea. Furthermore, some samples were of plant origin (both leaves and roots) and finally there was a sample of lichen, a composite organism consisting of fungi and cyanobacteria. The samples were cultivated on different media containing various antibiotics and therefore antibiotic resistant strains were easier selected and separated from non-resistant strains.

All samples did not yield colonies of bacteria under the experimental conditions applied and hence were excluded from the experiments (Cod, Mackerel and Cuckoo wrasse). All the other samples gave rise to colonies in varying amounts. The most successful sample both in colony numbers and morphological diversity was the bat guano sample. Two different antibiotic concentrations were also tested and the higher concentration resulted in a better differentiation between control and antibiotic plates. This shows, as could be expected, that the selection pressure on the bacteria increases with the concentration of antibiotics.

The attempts to amplify the 16S rDNA gene of selected isolates revealed the majority of samples did amplify with the most commonly used primers. A second attempt to amplify the non-amplifying isolates was performed with the use of other primers but no amplification occurred. This could be due to the fact that only universal primer pairs were used during both amplifications. Such universal primers are designed to be complementary to a broad variety of species but obviously not to all species of bacteria and especially not to the unique ones. A different primer pair targeting other groups of microorganism could be useful to try but was not in this study. Another but more unlikely source of error could be that the DNA extraction may have failed or that the DNA has been degraded after the extraction.

The phylogenetic tree based on the 58 obtained 16S rDNA sequences showed that a broad variety of species have been isolated during the experiments. Out of these 58 sequences 38

sequences were from Actinomycetales, many of these isolates are classified as rare actinomycetes i.e. non *Streptomyces* species. These isolates are potential producers of secondary metabolites that may still be unknown and further testing of these isolates is necessary to evaluate their full potential. Moreover, the potential of the 20 isolates of non-Actinomycetales species might also be of interest for further studies.

The genome analysis performed in the experiment proved to be very useful since it could display the genetic potential of each selected isolate before performing e.g. bioassays. The widely spread antibiotic resistance forces us to look after novel antimicrobial compounds that could become useful and therefore antibiotics encoded by novel genes are of the highest interest. The fact that some of the investigated isolates possess multiple genes involved in secondary metabolism does not mean that they actually use them to produce antimicrobial metabolites of interest but they might. Nevertheless, the most promising isolates and especially these that carry all four probed genes should be further investigated to reveal the possible secondary metabolites that could be produced. However, the presence of such a gene does not guaranty the production of a corresponding secondary metabolite. Therefore to evaluate the accuracy of the genetic potential test, the generated data must be combined with future cultivation experiments and the examination of secondary metabolites with promising profiles of antimicrobial activity. Some of the rare actinomycetes isolated during this experiment were from the species of *Arthrobacter* (i.e. B51 and B147) and *Kocuria* (i.e. B34). The genetic potential of these isolates was highest for isolate B51 (4 genes), isolate B147 and B34 had 3 genes each. This high theoretical ability to produce secondary metabolites is very promising and consistent with the theories that rare actinomycetes are an under-explored source for discovery of novel secondary metabolites.

The zone inhibition assay with isolates from experiment A showed a difference in inhibition capacity against *S. warneri* as compared to *P. resinovorans.* Whereas the growth of the *Staphylococcus* could be controlled and inhibited by at least some of the isolates the growth of *Pseudomonas* was not. The assay might be not favorable for tests with actinomycetes as it apparently favors the fast growing *Staphylococcus* and *Pseudomonas*. On the other hand, the applied isolates of actinomycetes usually need more time to grow and thus to produce secondary metabolites. Therefore, the assay should be modified in a way that favors the more slowly growing isolates. For example, older cultures could have been used or the isolates

could have been spread at an earlier time-point compared to the test organisms and hence favor the more slowly growing bacterial isolates.

The methods to isolate microorganism by screening for antibiotic resistance that were used in this study attempted to isolate a higher proportion of interesting microorganisms i.e. potential producers of secondary metabolites. The result from the two experiments with different antibiotic concentrations seems to point to the conclusion that this selective cultivation method is working since the higher antibiotic concentration renders more isolates with a higher genetic potential to produce secondary metabolites as shown by the genome analysis in Figures 8 and 9. However, further tests with the selected isolates are needed to confirm this conclusion. By using samples from diverse ecological niches in the experiment, the probability of finding rare organisms is further increased and also the probability of finding novel secondary metabolites that possibly could be used as novel antibiotics.

Future aspects

The current need for new antibiotics will always be a relevant problem since the evolution and adaption of the microorganisms will never stop. However, it is not very likely to think that we ever could outsmart evolution and find a secondary metabolite that will kill every pathogenic microorganism in about 80 years. I would say, the best we can hope for is to hold back the spread of antibiotic resistance by changing and restrict the use of antibiotics for example in farming and animal breeding. It is also important to shorten the time between the discovery of useful antimicrobial secondary metabolite and its introduction to the market. Even if we all hope to hold back the microorganisms for a short time, further work in this field is crucial. Infectious diseases are still the biggest cause of death world-wide, even with the health care standards nowadays (Selvameenal *et al.*, 2009) and must be taken care off.

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Appendix

Appendix 1

The table displays a complete list of all isolates recovered during the experiments. The table includes sample origin and a description of their morphology as well as the original media and the media the colonies were transferred to.

Sample	Isolate nr	Medium 1	Temp	Medium 2	Temp	Description
Celandine root	A1	NBC-R	20 °C	ME	20 °C	Fungus - Greenish colony
Celandine root	A2	NBC-R	20 °C	ME	20 °C	Fungus - white and compact
Celandine root	A3	NBC-R	20 °C	ME	20 °C	Fungus - white and compact
Celandine root	A10	NBC-SW-R	20 °C	ME	20 °C	Fungus - white, big
Celandine root	A11	NBC-SW-R	20 °C	ME	20 °C	Fungus - white, small
Celandine root	A12	NBC-E	20 °C	ME	20 °C	Fungus - white
Celandine root	A13	NBC-E	20 °C	ME	20 °C	Fungus - Green
Celandine root	A14	NBC-R	20 °C	ME	20 °C	Fungus - white
Macroalgea	A6	NBC-Sp	20 °C	ME	20 °C	Fungus - green
Macroalgea	A7	NBC	20 °C	ME	20 °C	White Streptomycetes
Macroalgea	A8	NBC-St	20 °C	ME	20 °C	Fungus - white
Macroalgea	A9	NBC-St	20 °C	ME	20 °C	Fungus - dark green
Macroalgea	A56	NBC-L	20 °C	NBC-L	20 °C	White
Macroalgea	A57	NBC-E	20 °C	NBC-E	20 °C	White
Macroalgea	A58	VPA-S	20 °C	VPA-S	20 °C	Yellow-white, topped
Blue mussel	A4	NBC-R	20 °C	NBC-R	20 °C	Fungus - white
Blue mussel	A5	NBC-Sp	20 °C	NBC-R	20 °C	Fungus - white
Celandine leaves	A59	NBC	20 °C	NBC	20 °C	White
Mackerel	A15	NBC-R	20 °C	ME	20 °C	Fungus - dark green
Guano	A16	NBC-R	20 °C	ME	20 °C	Fungus - green, probably Penicilium/Aspergillus
Guano	A17	NBC-R	20 °C	ME	20 °C	Fungus - brownish
Guano	A18	---	20 °C	ME	20 °C	Brown
Guano	A19	---	20 °C	ME	20 °C	Light green

Sample	Isolate nr	Medium 1	Temp	Medium 2	Temp	Description
Guano	A20	---	20 °C	ME	20 °C	Grey
Guano	A21	---	20 °C	ME	20 °C	white
Guano	A22	NBC	20 °C	NBC	20 °C	Grey
Guano	A23	NBC	20 °C	NBC	20 °C	White
Guano	A24	NBC	20 °C	NBC	20 °C	---
Guano	A25	NBC	20 °C	NBC	20 °C	---
guano	A26	NBC-Sp	20 °C	NBC-Sp	20 °C	White, fluffy
Guano	A27	NBC-Sp	20 °C	NBC-Sp	20 °C	Grey, flowery shaped
Guano	A28	NBC-Sp	20 °C	NBC-Sp	20 °C	White
Guano	A29	NBC-L	20 °C	NBC-L	20 °C	White-brown
Guano	A30	NBC-L	20 °C	NBC-L	20 °C	Grey, topped
Guano	A31	NBC-L	20 °C	NBC-L	20 °C	Grey, flowery shaped
Guano	A32	NBC-St	20 °C	NBC-St	20 °C	White
Guano	A33	NBC-St	20 °C	NBC-St	20 °C	White-brown
Guano	A34	NBC-E	20 °C	NBC-E	20 °C	Grey, topped
Guano	A35	NBC-E	20 °C	NBC-E	20 °C	Grey, flowery shape
Guano	A36	NBC-E	20 °C	NBC-E	20 °C	Grey, topped
Guano	A37	NBC-E	20 °C	NBC-E	20 °C	White
Guano	A38	NBC-E	20 °C	NBC-E	20 °C	Grey
Guano	A39	NBC-E	20 °C	NBC-E	20 °C	Dark grey
Guano	A40	IM7	20 °C	IM7	20 °C	Pink
Guano	A41	NBC	20 °C	NBC	20 °C	Grey
Guano	A42	NBC	20 °C	NBC	20 °C	Grey
Guano	A43	NBC	20 °C	NBC	20 °C	White
Guano	A44	NBC-SW-E	20 °C	NBC-SW-E	20 °C	Grey
Guano	A45	NBC-SW-E	20 °C	NBC-SW-E	20 °C	Grey
Guano	A46	NBC-SW-E	20 °C	NBC-SW-E	20 °C	Grey
Guano	A47	NBC-SW-E	20 °C	NBC-SW-E	20 °C	White
Guano	A48	NBC-SW-E	20 °C	NBC-SW-E	20 °C	White, topped

Sample	Isolate nr	Medium 1	Temp	Medium 2	Temp	Description
Guano	A49	NBC-SW-E	20 °C	NBC-SW-E	20 °C	White
Guano	A50	NBC-SW-E	20 °C	NBC-SW-E	20 °C	Grey
Guano	A51	NBC-SW	20 °C	NBC-SW	20 °C	Pink-white with grey circles
Guano	A52	NBC-SW	20 °C	NBC-SW	20 °C	Pink-white in circles
Guano	A53	NBC-SW	20 °C	NBC-SW	20 °C	Yellow grey in circles
Guano	A54	NBC-SW	20 °C	NBC-SW	20 °C	Pink-white in circles
Guano	A55	NBC-SW	20 °C	NBC-SW	20 °C	Yellow-white
Guano	A60	NBC-Sp	20 °C	NBC-Sp	20 °C	White
Guano	A61	NBC	20 °C	NBC	20 °C	White
Guano	A62	NBC	20 °C	NBC	20 °C	White, small
Guano	A63	NBC-SW-L	20 °C	NBC-SW-L	20 °C	White with red middle
Guano	A64	NBC-SW-L	20 °C	NBC-SW-L	20 °C	White with red middle
Guano	A65	NBC-SW-L	20 °C	NBC-SW-L	20 °C	Red
Guano	A66	NBC-SW-L	20 °C	NBC-SW-L	20 °C	Yellow with red middle
Guano	A67	NBC-SW-L	20 °C	NBC-SW-L	20 °C	With a yellow circle
Guano	A68	NBC-SW-L	20 °C	NBC-SW-L	20 °C	White
Guano	A69	NBC-SW-L	20 °C	NBC-SW-L	20 °C	White
Guano	A70	NBC-SW-L	20 °C	NBC-SW-L	20 °C	Yellow
Guano	A71	NBC-SW-L	20 °C	NBC-SW-L	20 °C	Yellow-white
Guano	A72	NBC-SW-L	20 °C	NBC-SW-L	20 °C	Yellow
Guano	A73	NBC-SW-L	20 °C	NBC-SW-L	20 °C	Red
Guano	A74	NBC-SW-L	20 °C	NBC-SW-L	20 °C	White with a red middle
Guano	A75	NBC-SW-L	20 °C	NBC-SW-L	20 °C	White
Guano	A76	NBC-R	20 °C	NBC-R	20 °C	Brown
Guano	A77	NBC-E	20 °C	NBC-E	20 °C	Grey
Guano	A78	NBC-SW-E	20 °C	NBC-SW-E	20 °C	Yellow
Guano	A79	NBC-SW-E	20 °C	NBC-SW-E	20 °C	Yellow
Guano	A80	NBC-SW-E	20 °C	NBC-SW-E	20 °C	Brown
Guano	A81	NBC-SW-E	20 °C	NBC-SW-E	20 °C	White

Sample	Isolate nr	Medium 1	Temp	Medium 2	Temp	Description
Guano	A82	NBC-SW-E	20 °C	NBC-SW-E	20 °C	White
Guano	A83	NBC-SW-Sp	20 °C	VPA-Sp	20 °C	Red
Guano	A84	NBC-SW-Sp	20 °C	VPA-Sp	20 °C	Grey
Guano	A85	NBC-SW-St	20 °C	VPA-St	20 °C	---
Guano	A86	NBC-SW-St	20 °C	VPA-St	20 °C	---
Celandine root	B42	NBC-L	20 °C	NBC-L	20 °C	Yellow, topped
Celandine root	B43	NBC-E	20 °C	NBC-E	20 °C	White-brown, topped
Celandine root	B112	NBC	20 °C	NBC	20 °C	Grey
Celandine root	B114	NBC-L	20 °C	NBC-L	20 °C	Yellow-brown, small
Celandine root	B115	NBC-L	20 °C	NBC-L	20 °C	Orange-Yellow, large
Celandine root	B116	NBC-L	20 °C	NBC-L	20 °C	Orange-brown, large and watery
Celandine root	B117	NBC-L	20 °C	NBC-L	20 °C	Yellow
Celandine root	B118	NBC-L	20 °C	NBC-L	20 °C	Transparent, inhibiting another bacteria
Celandine root	B119	NBC-L	20 °C	NBC-L	20 °C	White with a yellow and irregular edge
Celandine root	B125	NBC-Sp	20 °C	NBC-Sp	20 °C	Yellow-white
Celandine root	B126	NBC-Sp	20 °C	NBC-Sp	20 °C	White
Celandine root	B130	NBC-St	20 °C	NBC-St	20 °C	Orange
Celandine root	B132	NBC-E	20 °C	NBC-E	20 °C	Beige, watery
Celandine root	B136	NBC-SW-Sp	20 °C	NBC-SW-Sp	20 °C	Hyphae
Celandine root	B138	NBC-SW	20 °C	NBC-SW	20 °C	Yellow with a brown middle
Celandine root	B147	NBC-SW-St	20 °C	NBC-SW-St	20 °C	White with circles
Macroalgea	B32	NBC-SW	20 °C	NBC-SW	20 °C	Yellow
Macroalgea	B33	NBC-Sp	20 °C	NBC-Sp	20 °C	Pink
Macroalgea	B34	NBC-Sp	20 °C	NBC-Sp	20 °C	Pink

Sample	Isolate nr	Medium 1	Temp	Medium 2	Temp	Description
Macroalgea	B35	NBC-Sp	20 °C	NBC-Sp	20 °C	Yellow
Macroalgea	B36	NBC-Sp	20 °C	NBC-Sp	20 °C	White
Macroalgea	B37	NBC-SW-R	20 °C	NBC-SW-R	20 °C	Small
Macroalgea	B38	NBC-SW-St	20 °C	NBC-SW-St	20 °C	Yellow
Macroalgea	B39	NBC-SW-St	20 °C	NBC-SW-St	20 °C	white
Macroalgea	B40	NBC-St	20 °C	NBC-St	20 °C	Ping-yellow
Macroalgea	B41	NBC-St	20 °C	NBC-St	20 °C	Yellow
Blue mussel	B21	NBC	20 °C	NBC	20 °C	Whitish
Blue mussel	B22	NBC-E	20 °C	NBC-E	20 °C	Whitish, large
Blue mussel	B23	NBC-SW	20 °C	NBC-SW	20 °C	Yellow-white, irregular edge
Blue mussel	B24	NBC-SW	20 °C	NBC-SW	20 °C	Small
Blue mussel	B25	NBC-Sp	20 °C	NBC-Sp	20 °C	White
Blue mussel	B26	NBC-Sp	20 °C	NBC-Sp	20 °C	Grey-white
Blue mussel	B27	NBC-Sp	20 °C	NBC-Sp	20 °C	Yellow-orange
Blue mussel	B28	NBC-Sp	20 °C	NBC-Sp	20 °C	Light Yellow
Blue mussel	B29	NBC-Sp	20 °C	NBC-Sp	20 °C	White
Blue mussel	B30	NBC-Sp	20 °C	NBC-Sp	20 °C	Orange
Blue mussel	B31	NBC-E	20 °C	NBC-E	20 °C	White, topped
Blue mussel	B120	NBC-L	20 °C	NBC-L	20 °C	Yellow-transparent, large
Blue mussel	B121	NBC-L	20 °C	NBC-L	20 °C	Beige, small
Blue mussel	B133	NBC-E	20 °C	NBC-E	20 °C	Brown-beige, large
Blue mussel	B139	NBC-SW	20 °C	NBC-SW	20 °C	Brown with a yellow middle
Blue mussel	B140	NBC-SW	20 °C	NBC-SW	20 °C	Red with a grey middle
Blue mussel	B150	NBC-SW-L	20 °C	NBC-SW-L	20 °C	yellow white, small
Blue mussel	B151	NBC-SW-L	20 °C	NBC-SW-L	20 °C	White
Celandine leaves	B1	NBC-R	20 °C	NBC-R	20 °C	Beige
Celandine leaves	B2	NBC-Sp	20 °C	NBC-Sp	20 °C	White with a beige middle
Celandine leaves	B3	NBC-Sp	20 °C	NBC-Sp	20 °C	White with a beige middle
Celandine leaves	B4	NBC-Sp	20 °C	NBC-Sp	20 °C	White with a beige middle

Sample	Isolate nr	Medium 1	Temp	Medium 2	Temp	Description
Celandine leaves	B5	NBC-Sp	20 °C	NBC-Sp	20 °C	White with a beige middle
Celandine leaves	B6	NBC-Sp	20 °C	NBC-Sp	20 °C	White-beige, small
Celandine leaves	B7	NBC-E	20 °C	NBC-E	20 °C	Beige-brown, mucous consistent
Celandine leaves	B8	NBC-St	20 °C	NBC-St	20 °C	Beige, small
Celandine leaves	B9	NBC-St	20 °C	NBC-St	20 °C	Yellow
Celandine leaves	B10	NBC-St	20 °C	NBC-St	20 °C	Orange
Celandine leaves	B11	NBC-St	20 °C	NBC-St	20 °C	Beige, large
Celandine leaves	B12	NBC-St	20 °C	NBC-St	20 °C	Orange
Celandine leaves	B13	NBC-St	20 °C	NBC-St	20 °C	Yellow
Celandine leaves	B14	NBC-L	20 °C	NBC-L	20 °C	Yellow
Celandine leaves	B15	NBC-L	20 °C	NBC-L	20 °C	Whitish, big
Celandine leaves	B16	NBC-L	20 °C	NBC-L	20 °C	Beige, small, mucous consistent
Celandine leaves	B17	NBC	20 °C	NBC	20 °C	Whitish, irregular edges
Celandine leaves	B18	NBC	20 °C	NBC	20 °C	Whitish, round
Celandine leaves	B19	NBC	20 °C	NBC	20 °C	Whitish, large
Celandine leaves	B20	NBC	20 °C	NBC	20 °C	Whitish, small
Celandine leaves	B129	NBC-St	20 °C	NBC-St	20 °C	Yellow-white, topped
Lichen	B44	NBC-St	20 °C	NBC-St	20 °C	Yellow
Lichen	B45	NBC-St	20 °C	NBC-St	20 °C	Yellowish with a white dot in the middle, topped
Lichen	B46	NBC-St	20 °C	NBC-St	20 °C	Yellowish with a white dot in the middle, topped
Lichen	B47	NBC-St	20 °C	NBC-St	20 °C	Yellowish with a white dot in the middle, topped
Lichen	B48	NBC-St	20 °C	NBC-St	20 °C	White
Lichen	B49	NBC-St	20 °C	NBC-St	20 °C	Yellowish with a white dot in the middle, topped
Lichen	B50	NBC-St	20 °C	NBC-St	20 °C	Yellowish with a white dot in the middle, topped

Sample	Isolate nr	Medium 1	Temp	Medium 2	Temp	Description
Lichen	B51	NBC-St	20 °C	NBC-St	20 °C	Yellow
Lichen	B52	NBC-St	20 °C	NBC-St	20 °C	Yellow-white
Lichen	B53	NBC-Sp	20 °C	NBC-Sp	20 °C	White, topped
Lichen	B54	NBC-Sp	20 °C	NBC-Sp	20 °C	White in circles
Lichen	B55	NBC-Sp	20 °C	NBC-Sp	20 °C	White, topped
Lichen	B56	NBC-Sp	20 °C	NBC-Sp	20 °C	White, topped
Lichen	B57	NBC-Sp	20 °C	NBC-Sp	20 °C	White, topped
Lichen	B58	NBC-Sp	20 °C	NBC-Sp	20 °C	White-beige in circles
Lichen	B59	NBC-Sp	20 °C	NBC-Sp	20 °C	White-beige in circles
Lichen	B127	NBC-Sp	20 °C	NBC-Sp	20 °C	Yellow-white
Lichen	B128	NBC-Sp	20 °C	NBC-Sp	20 °C	Beige, watery
Lichen	B131	NBC-E	20 °C	NBC-E	20 °C	Beige, watery
Lichen	B135	NBC-SW-Sp	20 °C	NBC-SW-Sp	20 °C	White
Lichen	B145	NBC-SW-E	20 °C	NBC-SW-E	20 °C	Beige-brown, irregular edge
Lichen	B152	NBC-SW-L	20 °C	NBC-SW-L	20 °C	Brown
Lichen	B153	NBC-SW-St	20 °C	NBC-SW-St	20 °C	---
Lichen	B154	NBC-SW-St	20 °C	NBC-SW-St	20 °C	---
Guano	B60	NBC-SW	20 °C	NBC-SW	20 °C	White-pink in circles
Guano	B61	NBC-SW	20 °C	NBC-SW	20 °C	Yellow-white in circles
Guano	B62	NBC-SW	20 °C	NBC-SW	20 °C	Red with a white middle
Guano	B63	NBC-SW-L	20 °C	NBC-SW-L	20 °C	Beige in circles
Guano	B64	NBC-SW-L	20 °C	NBC-SW-L	20 °C	Beige in circles
Guano	B65	NBC-SW-L	20 °C	NBC-SW-L	20 °C	Yellow-brown in circles
Guano	B66	NBC-SW-L	20 °C	NBC-SW-L	20 °C	Yellow in circles
Guano	B67	NBC-SW-Sp	20 °C	NBC-SW-Sp	20 °C	Yellow-brown
Guano	B68	NBC-SW-E	20 °C	NBC-SW-E	20 °C	Beige-white, topped
Guano	B69	NBC-SW-E	20 °C	NBC-SW-E	20 °C	Beige-white in circles
Guano	B70	NBC-SW-E	20 °C	NBC-SW-E	20 °C	Yellow-white in circles

Sample	Isolate nr	Medium 1	Temp	Medium 2	Temp	Description
Guano	B71	NBC-SW-E	20 °C	NBC-SW-E	20 °C	Beige-white in circles
Guano	B72	NBC-SW-E	20 °C	NBC-SW-E	20 °C	Beige-white in circles
Guano	B73	NBC-SW-E	20 °C	NBC-SW-E	20 °C	White-beige, irregular circles
Guano	B74	NBC-SW-E	20 °C	NBC-SW-E	20 °C	white
Guano	B75	NBC	20 °C	NBC	20 °C	Grey
Guano	B76	NBC	20 °C	NBC	20 °C	Grey-white
Guano	B78	NBC	20 °C	NBC	20 °C	Grey-white
Guano	B79	NBC	20 °C	NBC	20 °C	Grey-white
Guano	B80	NBC	20 °C	NBC	20 °C	Brown in circles
Guano	B81	NBC-Sp	20 °C	NBC-Sp	20 °C	Yellow-white
Guano	B82	NBC-Sp	20 °C	NBC-Sp	20 °C	White, topped
Guano	B83	NBC-Sp	20 °C	NBC-Sp	20 °C	White, topped
Guano	B84	NBC-Sp	20 °C	NBC-Sp	20 °C	Yellow-brown in circles
Guano	B85	NBC-Sp	20 °C	NBC-Sp	20 °C	Yellow-brown in circles
Guano	B86	NBC-Sp	20 °C	NBC-Sp	20 °C	White, topped
Guano	B87	NBC-Sp	20 °C	NBC-Sp	20 °C	White, topped
Guano	B88	NBC-R	20 °C	NBC-R	20 °C	White
Guano	B89	NBC-R	20 °C	NBC-R	20 °C	Brown-white
Guano	B90	NBC-R	20 °C	NBC-R	20 °C	Grey-white
Guano	B91	NBC-R	20 °C	NBC-R	20 °C	Brown
Guano	B92	NBC-St	20 °C	NBC-St	20 °C	Yellow-beige
Guano	B93	NBC-St	20 °C	NBC-St	20 °C	Yellow-beige
Guano	B94	NBC-St	20 °C	NBC-St	20 °C	Yellow-beige
Guano	B95	NBC-E	20 °C	NBC-E	20 °C	Grey
Guano	B96	NBC-E	20 °C	NBC-E	20 °C	Grey-white
Guano	B97	NBC-E	20 °C	NBC-E	20 °C	Yellow-white
Guano	B98	NBC-E	20 °C	NBC-E	20 °C	Brown-white
Guano	B99	NBC-E	20 °C	NBC-E	20 °C	Brown-white
Guano	B100	NBC-E	20 °C	NBC-E	20 °C	---

Sample	Isolate nr	Medium 1	Temp	Medium 2	Temp	Description
Guano	B101	NBC-E	20 °C	NBC-E	20 °C	Yellow-brown
Guano	B102	NBC-E	20 °C	NBC-E	20 °C	Brown-beige
Guano	B103	NBC-E	20 °C	NBC-E	20 °C	Brown-beige
Guano	B104	NBC-E	20 °C	NBC-E	20 °C	White, topped
Guano	B105	NBC-L	20 °C	NBC-L	20 °C	Yellow with a brown middle
Guano	B106	NBC-L	20 °C	NBC-L	20 °C	Beige
Guano	B107	NBC-L	20 °C	NBC-L	20 °C	Yellow-beige
Guano	B108	NBC-L	20 °C	NBC-L	20 °C	Brown-beige
Guano	B109	NBC-L	20 °C	NBC-L	20 °C	Grey-brown, topped
Guano	B110	NBC-L	20 °C	NBC-L	20 °C	Yellow with a brown middle
Guano	B111	NBC-R	20 °C	NBC-R	20 °C	Beige-white in circles
Guano	B113	NBC	20 °C	NBC	20 °C	Beige
Guano	B122	NBC-L	20 °C	NBC-L	20 °C	Grey
Guano	B123	NBC-L	20 °C	NBC-L	20 °C	Grey
Guano	B124	NBC-Sp	20 °C	NBC-Sp	20 °C	Beige
Guano	B134	NBC-E	20 °C	NBC-E	20 °C	Yellow
Guano	B137	NBC-SW-Sp	20 °C	NBC-SW-Sp	20 °C	Beige with irregular edge
Guano	B141	NBC-SW	20 °C	NBC-SW	20 °C	Yellow with a brown middle
Guano	B142	NBC-SW	20 °C	NBC-SW	20 °C	Yellow with a brown middle
Guano	B143	NBC-SW	20 °C	NBC-SW	20 °C	White irregular edge, watery
Guano	B144	NBC-SW-E	20 °C	NBC-SW-E	20 °C	Beige-brown
Guano	B146	NBC-SW-St	20 °C	NBC-SW-St	20 °C	Pink-red-yellow
Guano	B148	NBC-SW-L	20 °C	NBC-SW-L	20 °C	Beige, irregular edge
Guano	B149	NBC-SW-L	20 °C	NBC-SW-L	20 °C	Yellow-white, large

Appendix 2

The table displays a complete list of the sequenced isolates with the closest related sequences retrieved from the SILVA database.

Isolate nr.	**Sample**	**Media**	**Closest related species**
A37	Guano	NBC-E	*Amycolatopsis orientalis, Amycolatopsis coloradensis, Pseudomonas salomonii*
A43	Guano	NBC	*Kocuria sp.S42*
A53	Guano	NBC-SW	*Streptomyces sp.YH-1, Streptomyces sp.S6-1*
A56	Guano	NBC-SW	*Streptomyces sp.YH-1, Streptomyces sp.S6-1*
A58	Macroalgea	NBC-E	*Methylobacteri umuncultured bacterium*
A59	Macroalgea	VPA-S	*Streptomyces rutgersensis, Streptomyces sp.A320Ydz-QZ*
A61	Guano	NBC-Sp	*Nocardioides albus*
A69	Guano	NBC-SW-L	*Nocardioides albus*
A70	Guano	NBC-SW-L	*Nocardioides albus*
A76	Guano	NBC-SW-L	*Nocardioides albus*
A77	Guano	NBC-R	*Kribbella solani*
A85	Guano	NBC-SW-Sp	*Nocardioides albus*
A86	Guano	NBC-SW-Sp	*Amycolatopsis orientalis, Amycolatopsis coloradensis, Pseudomonas salomonii*
B7	Celandine leaves	NBC-E	*Pseudomonas poae, Pseudomonas salomonii*
B12	Celandine leaves	NBC-St	*Sphingomonas sp.MN5-2, Sphingomonas uncultured bacterium*
B14	Celandine leaves	NBC-L	*Sphingomonas sp.MN5-2, Sphingomonas uncultured bacterium*
B19	Celandine leaves	NBC	*Pseudomonas poae, Pseudomonas salomonii*
B23	Blue mussels	NBC-SW	*Psychrobacter sp.R3.8*
B26	Blue mussels	NBC-SW-Sp	*Staphylococcus epidermidis RP62Aphage SP-beta*

Isolate nr.	**Sample**	**Media**	**Closest related species**
B28	Blue mussels	NBC-SW-Sp	*Kocuria palustris*
B31	Blue mussels	NBC-E	*Erythrobacter sp.H301*
B34	Macroalgea	NBC-Sp	*Kocuria sp.S42*
B40	Macroalgea	NBC-St	*Sphingomonas sp.MN5-2, Sphingomonas uncultured bacterium*
B41	Macroalgea	NBC-St	*Sphingomonadales uncultured bacterium, Erythrobacter sp.H301*
B51	Lichen	NBC-Sp	*Arthrobacter sp.SaCR11*
B56	Lichen	NBC-Sp	*Amycolatopsis orientalis, Amycolatopsis coloradensis, Pseudomonas salomonii*
B59	Lichen	NBC-Sp	*Amycolatopsis orientalis, Amycolatopsis coloradensis, Pseudomonas salomonii*
Isolate nr.	Sample	Media	*Closest related species*
B60	Guano	NBC-SW	*Streptomyces sp.YH-1, Streptomyces sp.S6-1*
B64	Guano	NBC-SW-L	*Streptomyces rutgersensis, Streptomyces sp.A320Ydz-QZ*
B72	Guano	NBC-SW-E	*Streptomyces sp.YH-1, Streptomyces sp.S6-1*
B73	Guano	NBC-SW-E	*Staphylococcus epidermidis RP62Aphage SP-beta*
B81	Guano	NBC-Sp	*Amycolatopsis orientalis, Amycolatopsis coloradensis, Pseudomonas salomonii*
B82	Guano	NBC-Sp	*Nocardioides albus*
B84	Guano	NBC-Sp	*Amycolatopsis orientalis, Amycolatopsis coloradensis, Pseudomonas salomonii*
B91	Guano	NBC-R	*Streptomyces mutabilis,Streptomyces plicatus, Streptomyces sp.TRM46648, Actinomycetales bacterium XJSS-58*
B93	Guano	NBC-St	*Amycolatopsis orientalis, Amycolatopsis coloradensis, Pseudomonas salomonii*
B95	Guano	NBC-E	*Streptomyces mutabilis, Streptomyces plicatus,*

			Streptomyces sp.TRM46648, Actinomycetales bacterium XJSS-58
Isolate nr.	**Sample**	**Media**	**Closest related species**
B96	Guano	NBC-E	*Streptomyces mutabilis, Streptomyces plicatus, Streptomyces sp.TRM46648, Actinomycetales bacterium XJSS-58*
B102	Guano	NBC-E	*Amycolatopsis orientalis, Amycolatopsis coloradensis, Pseudomonas salomonii*
B104	Guano	NBC-E	*Nocardioides albus*
B109	Guano	NBC-L	*Streptomyces mutabilis, Streptomyces plicatus, Streptomyces sp.TRM46648, Actinomycetales bacterium XJSS-58*
B110	Guano	NBC-L	*Streptomyces rutgersensis, Streptomyces sp.A320Ydz-QZ*
B111	Guano	NBC-R	*Streptomyces mutabilis,Streptomyces plicatus, Streptomyces sp.TRM46648, Actinomycetales bacterium XJSS-58*
B115	Celandine root	NBC-L	*Bacillus simplex*
B117	Celandine root	NBC-L	*Sphingomonas sp.MN5-2, Sphingomonas uncultured bacterium*
B118	Celandine root	NBC-L	*Bacillus simplex, Bacillus uncultured bacterium*
B119	Celandine root	NBC-L	*Bacillus simplex, Bacillus uncultured bacterium*
B120	Blue mussels	NBC-L	*Pseudomonas poae, Pseudomonas salomonii*
B122	Guano	NBC-L	*Streptomyces mutabilis, Streptomyces plicatus, Streptomyces sp.TRM46648, Actinomycetales bacterium XJSS-58*
B128	Lichen	NBC-Sp	*Pseudomonas poae, Pseudomonas salomonii*
Isolate nr.	Sample	Media	*Closest related species*

B132	Celandine root	NBC-E	*Pseudomonas poae, Pseudomonas salomonii*
Isolate nr.	**Sample**	**Media**	**Closest related species**
B134	Guano	NBC-E	*Streptomyces mutabilis, Streptomyces plicatus, Streptomyces sp.TRM46648, Actinomycetales bacterium XJSS-58*
B135	Lichen	NBC-SW-Sp	*Pseudomonas poae, Pseudomonas salomonii*
B147	Celandine root	NBC-SW-St	*Arthrobacter sp.SaCR11*
B148	Guano	NBC-SW-L	*Streptomyces sp.YH-1, Streptomyces sp.S6-1*
B151	Blue mussels	NBC-SW-L	*Pseudomonas poae, Pseudomonas salomonii*
B152	Lichen	NBC-SW-L	*Pseudomonas graminis*