

Functioning of Salt Tolerant *Anabaena variabilis* and *Nostoc calcicola* Strains in Salt Stress, Destructors of Hexachlorocyclohexane (HCH) in Saline Conditions

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Abstract

We investigated the degradation of hexachlorocyclohexane (HCH) in soil by two cyanobacteria isolates *Anabaena variabilis* N 21 and *Nostoc calcicola* N 25 under high concentrations of NaCl. After four months of incubation cultures in saline soils with 4% NaCl decreased HCH concentrations considerably from 2 µg/g soil to 14 ng/g soil, representing 1% of the introduced HCH. In pure cultures 1 M NaCl was not lethal but significantly changed the morphology of the cyanobacteria. Enzyme assays for aldehyde oxidase and xanthine oxidase indicated the overexpression of xanthine oxidase in *Nostoc calcicola* N25 under salt-stress. Our results indicate that the strains can be used as biopreparations for bioremediation of salt-affected soils polluted with persistent chlororganic compounds often found in Uzbekistan soils.

Keywords: Cyanobacteria, 1,2,3,4,5,6-hexachlorocyclohexane (γ-HCH), Aldehyde oxidase, Xanthine dehydrogenase, Biotransformation, Salinity

1. Introduction

Lindane, technically 1,2,3,4,5,6-hexachlorocyclohexane (γ-HCH), is a widely distributed organochlorine pesticide in diverse environmental compartments. The production of lindane results in the generation of large quantities of waste HCH isomers (mainly α-, β- and γ-isomers). All these isomers are toxic and have a long-range environmental transport potential. HCH biodegradation by aerobic and anaerobic microorganisms has been documented in various environments. HCH-degrading microorganisms such as fungi, cyanobacteria, and anaerobic and aerobic bacteria have been widely investigated (Phillips *et al.*, 2005). Also, the genes involved in HCH-degradation have been studied. For example, a detailed characterization of the pathway for aerobic HCH

degradation by *Sphingomonas paucimobilis* strain UT26 showed the involvement of *lin* genes encoding the HCH biodegradation pathway (Nagata *et al.*, 1999). These *lin* genes have also been found and studied in several other HCH-degrading bacteria (Kumari *et al.*, 2002; Thomas *et al.*, 1996). However, when applying living cells for the in situ biodegradation of HCH in the field, in most cases, the number of introduced cells decreases shortly after inoculation due to both abiotic and biotic stresses (Alexander, 1999). Abiotic factors controlling the survival of introduced microorganisms include moisture content, temperature, salinity, pH, soil texture, oxygen concentration and nutrient availability (Phillips *et al.*, 2005; van Elsas *et al.*, 1986). Biological factors also play an important role for the survival of introduced cells (Lypez-Gomollin *et al.*, 2009, von Elsas and Heijnen, 1990). An approach to overcome some of the problems associated with microbial survival after inoculation is the use of protective carriers, for example the immobilization of cells by encapsulation (Mertens *et al.*, 2006, van Veen *et al.*, 1997).

In Uzbekistan, a considerably high portion of the arable land is both, contaminated with biocides used for crop protection, such as HCH, and burdened with high salinity as a result of extensive irrigation with river waters during the last century (Forkutsa *et al.*, 2009; Shirokova *et al.*, 2000). In addition, HCH contaminations in Uzbekistan are often diffuse and spread over large areas so that chemical and/or physical remediation methods appear economically unfeasible. We therefore evaluate the application of cyanobacteria for the remediation of HCH contaminated saline soils. The efficiency of remediation of HCH-contaminated and salt-affected soils in Uzbekistan depends to a large extent on the ability of cells to survive under high-salt conditions. It is important to know in advance of remediation efforts how well an HCH degrading strain is adapted to high-salt conditions to predict the probability of survival in the natural habitat. The aim of the current study was to investigate growth and biodegradative capacities of strains *Anabaena variabilis* N 21 and *Nostoc calcicola* N 25 under high-salt conditions. For that, we compared growth in low-salt versus growth in high-salt media. In addition, we studied the expression of aldehyde oxidase and xanthine dehydrogenase, which are synthesized in high concentrations of NaCl.

2. Materials and Methods

2.1 Microorganisms

Two cyanobacterial strains of our culture collection were selected for the present study. The two strains *Anabaena variabilis* N 21 (Uzb1) and *Nostoc calcicola* N 25 (Uzb2) were previously isolated from HCH-containing, salt-affected soils from Kashkadaryo and Syrdaryo Province in Uzbekistan, respectively (Kadirova *et al.*, 2007). Initial classification of the isolates was done by classical methods.

2.2 Cultivation

The medium used to grow the strains was nitrogen-free "M" medium with following chemical ingredients (g/l): $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ - 0.25; $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$ - 0.0238; $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 5.5 \text{H}_2\text{O}$ - 0.165; K_2HPO_4 - 0.04; trace elements - 1 ml/l: ($\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$ - 0.002; $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$ - 0.222; $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$ - 0.079; $\text{MnCl}_2 \cdot 4 \text{H}_2\text{O}$ - 1.81; $\text{Na}_2\text{MoO}_4 \cdot 2 \text{H}_2\text{O}$ - 0.03; H_3BO_3 - 2.80).

To study HCH destruction 1 mg of HCH was dissolved in 100 ml of hexane. To dose soil with HCH, 50 g soil samples were transferred into Petri plates and the soils flooded with 10 ml of the HCH/hexane solution, resulting in the introduction of 100 μg of HCH corresponding to a concentration of 2 $\mu\text{g/g}$ soil. Then, the Petri plates with the dosed soil samples were dried until all hexane was evaporated. Then, 2 g of NaCl dissolved in water was added to the dried soil to obtain a final salt concentration of 4% (wt/wt). Such plates were then inoculated with 8 ml of cyanobacterial suspensions, mixed and incubated at room temperature. During the incubation time the plates were constantly humidified to maintain growth conditions. In regular time intervals 1 g of soil was taken from each plate for HCH analysis.

As a soil we used sierozem, which is a typical Uzbekistan soil. Before the soil was distributed into 50 g aliquots, it was sterilized by heating for 4 hours at 180°C.

2.3 Chemical analyses

For the detection and quantification of HCH in soil, 1 g samples were extracted with 100 μl of hexane in an ultrasound bath for 15 minutes. After this treatment hexane was recovered, filtered and dried. Two μl of these hexane extracts were then analyzed by injection into a gas chromatograph equipped with electron capture detection. We used a chromatograph with electron capture detector Agilent Technologies 6890N. A capillary column HP5, 30 m length, 0.25 mm inner diameter was used for separation. Temperature of the injector was 250°C, oven temperature was initially 70°C for 2 min, then rising the temperature to 150°C with 25°C/min. After this, temperature was increased to 200°C with 3°C/min, and then to 280°C with 80°C/min. At last the temperature

was hold for 10 min at 280°C. Pressure in the column was 12.21 psi. Carrier gas was nitrogen. As detector we used an μ ECD at a detector temperature of 300°C.

Detection of HCH was done according to its retention time. Quantitative analysis of HCH was performed on the basis of a standard curve with program support of Agilent ChemStation of Agilent Technologies chromatograph 6890N, by external standard (ESTD) and recalculated to 1 g of soil.

2.4 Aldehyde oxidase and xanthine dehydrogenase analysis by native polyacrylamide gel electrophoresis

Aldehyde oxidase (EC 1.2.3.1) activity was assessed in native polyacrylamide gels by activity staining in gels after electrophoresis in 7% acrylamide gels (Laemmli, 1970). Gels were run in the absence of SDS at 4°C. For activity staining gels were immersed in a reaction mixture containing 0.2 M phosphate buffer, pH 7.5, 0.1 M Tris-HCl (pH 7.5), 0.1 mM phenazine methosulfate (PMS), 1 mM 3[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium-bromide (MTT) and 1 mM substrate indole-3-aldehyde for 10 min followed by gentle shaking at room temperature.

Xanthine dehydrogenase (EC 1.1.1.204) activity was detected after native gel-electrophoresis using hypoxanthine as a substrate (Mendel and Møller, 1976), which resulted in the development of specific formazan bands. Aldehyde oxidase and xanthine dehydrogenase activity was estimated by subjecting the strains to salt stress at 300 mM NaCl.

2.5 Molecular methods

PCR and sequencing of 16S rRNA genes were done as described previously (Iteman *et al.*, 2000). For *Anabaena variabilis* N 21 the following primers were chosen: A1 (forward): 5'- GTA TGC TTA CAC ATG CAA GTC GAA CGG -3' and primer A2 (reverse) 5'- TTA CGG CTA GGA CTA CTG GGG TAT CTA -3' (designed from *Anabaena variabilis*, Gene Bank accession number EF488831). For *Nostoc calcicola* N 25 the following primers were used: primer N1 (forward) 5'- TGC TGC CTG AAG ATG AGC TCG CGT CTG -3' and primer N2 (reverse) 5'- GAG TGC CCA ACT TAA TGC TGG CAA CTA -3' (designed from *Nostoc calcicola*, Gene Bank accession number HM573461).

3. Results

Two cyanobacterial strains of our culture collections that were able to transform polychlorinated biphenyls in a previous study (Kadirova *et al.*, 2007) were selected. These strains were isolated from Kashkadaryo and Syrdaryo province in Uzbekistan, respectively. The two strains had initially been identified as *Anabaena* and *Nostoc* species by classical methods. For sequencing the 16S rRNA genes of the two strains, specific primers for each of the two cyanobacteria species were constructed on the basis of published 16S rRNA gene sequences of *Anabaena* and *Nostoc* species. Using these primers we obtained PCR-products from *Anabaena variabilis* N 21 with 725 bp (Figure 1a), and 813 bp from *Nostoc calcicola* N 25 (Figure 1b). PCR-products were then sequenced using primers A1, A2 (for *Anabaena variabilis* N 21) and N1, N2 (for *Nostoc calcicola* N 25)

Partial nucleotide sequence of the 16S rRNA gene of *Anabaena variabilis* N 21:

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GTATGCTTACACATGCAAGTCGAACGGAATCCTTGGGATTTAGTGGCGGACGGGTGAGTAACGCGT
GAGAATCTGGCTTCAGGTCTGGGACAACAGTTGGAACGACTGCTAATACCGGATGTGCCGAGAG
GTGAAAGGCTTGCTGCCTGAAGATGAGCTTGCCTGCTGATTAGCTAGTTGGTGGGGTAAGAGCCTAC
CAAGGGGACGATCAGTAGCTGGTCTCGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCGC
AGACTCCTACGGGAGGCAGCAGTGGGGATTTTCCGCAATGGGCGAAAGCCTGACGGAGCAATAC
CGCGTGAGGGAGGAAGGCTCTTGGGTGTAAACCTCTTTTCTCAGGGAAGAATAAAATGACGGTAC
CTGAGGAATAAGCATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGATGCAAGCGTT
ATCCGGAATGATTGGGCGTAAAGGGTCCGCAGGTGGCTATGTAAGTCTGCTGTAAATAGTCATGC
TCAACATGATCAAGGCAGTGGAACTACAAGGCTAGAGTGCCTTCGGGGCAGAGGGGAATTCTTG
GGTGTAGCGGTGAAATGCGTAGATATCAGGAAGAACCCGGTGGCGATAGCGCTCTGCTAGGCCG
CAACTGACACTGAGGGACGAAAGCTAGGGGAGCGAATGGGATAGATACCCAGTAGTCCTAGCCG
TAAACG
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Partial nucleotide sequence of the 16S rRNA gene of *Nostoc calcicola* N 25:

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CCTAGGCGACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCC
AGACTACCTACGGGAGGCAGCAGTGGGGAATTTTCCGCAATGGGCGAAAGCCTGACGGAGCAATA
CCGCGTGAGGGAGGAAGGCTCTTGGGTTCGTAAACCTCCTCAGGGAAGAACACAATGACGGTACCT
GAGGAATCAGCATCGGCTAACTCCGTGCCAGCAGCCGCGGTATATACGGAGGATAGCAAGCGTTA
TCCGGAATGATTGGGCGTAAAGCGTCCGCAGGTGGCTAATGTAGTCTGCTGTAAAGAGTGAGGCT
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CAACCTCATAAGAGCAGTGGAACTACATGGCTAGAGTGCGCTTCGGGGCAGAGGGAATTCCTGG
TGTAGCGGTGAAATGCGTAGAGATCAGGAAGAACACCGGTGGCGAAAGCGCTCTGCTAGCCGCAT
ACTGACACTCATGGACGAAAGCTAGGGGAGCGAATGGGATTAGATACCCAGTAGTCTAGCCGTA
AACGATGGATCCTAGGCGTGGCTTGTATCGACCCGAGCCGTGCCGTAGCTAACGCGTTATAGTATC
CCGCCTGGGGAGTACGCAGGCAACTGTGAACTCAAAGGAATTGACGGGGGCCCCGACAAGCGGT
GGAGTATCGTGGTTTAATTCGATTGCAACGCGAAGAACCTTACCAAGACTTGACATGTCGCGAAAC
CTTTGTGAAAGCAGAGGGTGCCTTCGGGAGCGCGAACACAGGTGGTGCATGGCTGTCGTCAGCTCG
TGTCGTGAGATGTTGGGTTAAGTCCCGCA

Comparative BLASTN analysis showed that the nucleotide sequence of the 16S rRNA gene of *Anabaena variabilis* N 21 (Uzb1) was 99% identical with an earlier identified strain of *Anabaena variabilis* (Gene Bank accession number EF488831). The strain had sequence identity of 93-98% with other *Anabaena* species. BLASTN analysis of nucleotide sequence of *Nostoc calcicola* N 25 (Uzb2) revealed that this strain had high sequence identity (99%) with an earlier identified organism, *Nostoc calcicola* (Gene Bank accession number AM711529).

We analyzed the influence of high concentrations of NaCl on cell morphology. NaCl concentrations of up to 500 mM did not affect growth or morphological development of the two blue-green algae (Figure 2c and 2d) compared to growth on low-salt medium (Figure 2a and 2b). Specifically, we surveyed the morphological changes of trichomes of the two cyanobacterial strains by light microscopy. Cells of *Anabaena variabilis* N 21 in observed trichomes were morphologically not changed in medium with 500 mM NaCl salinity (Figure 2c) compared to those in medium without additional NaCl (Figure 2a). However, a smaller cell diameter was observed in trichomes in the high-salinity samples. Also the number of heterocyst was decreased compared with cells growing in low-salt medium. Salt concentration up to 500 mM was not critical for normal growth of the strains. In contrast, NaCl concentration of 1 M led to deformation of the *Anabaena variabilis* N 21 cells in the trichomes (Figure 2e), and the periplasmic membrane between the cells in trichomes in *Anabaena variabilis* N 21 disappeared. This resulted in the appearance of a long unique cell in *Anabaena variabilis* N 21 trichomes.

Influence of salinity on the morphology of strain *Nostoc calcicola* N 25 was considerably different than the effect of salt on *Anabaena variabilis* N 21. For example, 500 mM NaCl did not lead to considerable morphological changes of *Nostoc calcicola* N 25 cells (Figure 2b and 2d). At 1 M NaCl *Nostoc* trichomes dissociated into separated single cells and heterocysts completely disappeared (Figure 2f). When cells of both cyanobacteria, that had been subjected to salt stress of 1 M NaCl, were replated on fresh nutrient media without NaCl the complete recovery of both cyanobacteria *Anabaena variabilis* N 21 and *Nostoc calcicola* N 25 was observed.

The two strains *Anabaena variabilis* N 21 and *Nostoc calcicola* N 25 were incubated with HCH at a concentration of 2 µg/g soil with no salt additions as a control or with addition of 4% NaCl. After four months of incubation of the soil samples with cultures of the tested strains of *Anabaena variabilis* N 21 and *Nostoc calcicola* N 25 the concentration of HCH was decreased from 2 µg/g soil to 14.2 ng/g soil and 14.3 ng/g soil, respectively. In contrast, the control with no added microorganisms showed no decrease of the HCH concentration (Figure 3). Therefore, the two cyanobacterial strains removed 99% of the introduced HCH within four months under laboratory conditions. This demonstrated HCH degradation activity in the presence of 4% NaCl.

It is known that aldehyde oxidase (EC 1.2.3.1) and xanthine dehydrogenase (EC 1.1.1.204) play a role in processes that are responsible for adaptation of living organisms to stress conditions. We therefore analyzed aldehyde oxidase (Figure 4) and xanthine oxidase (Figure 5) activities in the *Anabaena variabilis* N 21 and *Nostoc calcicola* N 25 under low-salt and high-salt conditions using a native gel approach. The results show that only one type of aldehyde oxidase was expressed in the cells of *Nostoc calcicola* N 25. No aldehyde oxidase activity was detected in *Anabaena variabilis* N 21. In *Nostoc calcicola* N 25 the activity of aldehyde oxidase was weak under both culturing conditions, without or with 300 mM NaCl (Figure 4). Testing for xanthine dehydrogenase on native gels revealed the presence of one single form of the enzyme both in *Anabaena variabilis* N 21 and *Nostoc calcicola* N 25 (Figure 5). Activity of xanthine dehydrogenase of both strains was much higher than that of aldehyde oxidase. While we could not detect differences in the activity of aldehyde oxidase with or without added salt, the activity of xanthine dehydrogenase was increased under salt stress in the *Nostoc* strain. From our obtained data it can be concluded that, in the cells of *Nostoc calcicola* N 25 xanthine dehydrogenase is synthesized in response to salt stress.

4. Discussion

Cyanobacteria respond very rapidly to salinity in their environment. Salinity induced three prominent types of modification in nitrogen-fixing cyanobacteria (Apte & Bhagwat, 1989; Bhagwat & Apte, 1989). First, the synthesis of several proteins was inhibited, especially in the salt-sensitive strains; second, the synthesis of certain proteins was significantly upregulated; and third, synthesis of a specific set of proteins was induced *de novo* by salinity stress. Proteins which were selectively synthesized or induced *de novo* during salt stress, were tentatively called the salt-stress proteins.

It is known that survival and growth of bacteria depend on the salt concentration of the environment. Influence of salt on bacterial cells is stipulated by osmotic binding of water, leaking of salt into the cytoplasm and/or specific actions of specific ions in the protoplasm. At high salt concentrations water is less available for bacterial cells. In our study, salt concentrations of up to 500 mM did not inhibit growth of the two investigated cyanobacteria, but at NaCl concentration of 1 M deformation of the *Anabaena variabilis* N 21 cells in trichomes was seen. However, because we could recover live cells from 1 M NaCl medium we conclude that 1 M NaCl is not a lethal salinity for the strains but impairs growth and assumingly physiological characteristics. Other studies have shown that several salt tolerant *Azotobacter* and *Azospirillum* strains can survive up to 1.5 M NaCl not losing their nitrogen fixing activity (Rasulov, 2010; Shakirov, 2006). At the same time at 0.8 M NaCl 75-80% acetylene-reductase activity, which is a proxy for nitrogen fixing activity, was preserved. Comparing our results with other studies in which cyanobacteria were analyzed for their salt tolerance (Manickam *et al.*, 2006; Rasulov *et al.*, 2010), *Anabaena variabilis* N 21 and *Nostoc calcicola* N 25 transformed HCH far more effectively in salt stress than other described cyanobacteria, even catabolizing introduced HCH in the presence of 4% NaCl.

Biodegradation of HCH has been studied in soil, slurry and culture media but very little information exists on biodegradation in salt affected environment by bacteria, particularly by cyanobacteria. Monitoring studies by some authors revealed that in some cases industrial areas may be contaminated with all major isomers of HCH. The analytical results confirmed that accumulation depends on the plants species, soil and climate conditions. As is found for most organic compounds, HCH degradation in soil occurs at moderate temperatures, at near neutral pH and at both low and high moisture contents (Phillips *et al.*, 2005). Information on the effects of salinity on biodegradation is scattered and less well investigated in regard to cyanobacteria. In our previous research we tested several mesophylic strains of *Bacillus* to degrade PCBs (polychlorinated biphenyls) in salt-affected soils under aerobic conditions (Rasulov *et al.*, 2010) and found that some strains were able utilize PCBs even in high concentration of NaCl (4%). Several microbes have been reported to degrade HCH (Ito *et al.*, 2007; Lal *et al.*, 2008; Manickam *et al.*, 2006; Nagata *et al.*, 2007; Wu *et al.*, 2007). Also the biodegradation of diverse pesticides, insecticides and other organic compounds by cyanobacteria has been demonstrated. Some cyanobacteria degrade phenylcarbamate herbicides, such as propanil and chlorpropanil, to aniline and chlorine derivatives (Gromov, 1996). Also many other complex hydrocarbons were transformed by cyanobacteria. Cyanobacteria of the genera *Anabaena*, *Phormidium*, or *Oscillatoria* degrade different aromatic compounds (Radwan & Al-Hasan, 2002). *Microcoleus chthonoplastes*, *Phormidium corium*, and *Synechocystis* sp. were found to utilize up to 25% of added n-alkanes within 7-10 days (Safonova *et al.*, 2004). The marine cyanobacterium *Phormidium valderianum* BDU 20041 was able to dwell and grow in the presence of chlorpyrifos (*O,O*-diethyl-*O*-[3,5,6-trichloro-2-pyridyl] phosphorothioate), a phosphorothioate insecticide, at a concentration of 45 ppm (Palanisami *et al.*, 2009).

As it was shown in Figure 3 the strains normally catabolized HCH even in the presence of NaCl. It is worth noting that *Anabaena variabilis* N 21 was more active than *Nostoc calcicola* N 25. After two months of incubation with *Anabaena variabilis* N 21 the concentration of HCH was decreased by 207.5 – 242.3 ng/g soil from an initial amount of 2041.6 ng/g soil introduced. After four months, HCH concentrations were decreased to 12.2 – 14.2 ng/g soil.

Our results allowed us to conclude that the tested cyanobacterial strains either transformed HCH to volatile compounds or completely mineralized them. The amount of HCH introduced into the cyanobacterial cultures was relatively high with 2 µg/g soil. Nevertheless, the strains clearly showed their potential to survive these high concentrations of HCH and that they were able to degrade them at high efficiency even at high salt concentrations of 4% NaCl. Both these characteristics are important for the practical application of cyanobacteria for the bioremediation of soils in Uzbekistan. The high resistance towards HCH would be important for survival in highly contaminated soil, which in addition of HCH also often contains PCBs and DDT in Uzbekistan. Further studies will investigate the survival of cyanobacteria in a mix of different chloroorganics.

While the degradation of 2 µg HCH per gram soil in this laboratory study was accomplished within 1-4 months, we expect that the removal in the field will take longer. However, a substantial reduction of HCH load over a period of several vegetation periods would be feasible and worth pursuing. According to the results shown in this report, the analyzed cyanobacterial strains can be recommended for their use as biopreparations for the remediation of saline soils contaminated with chloroorganics.

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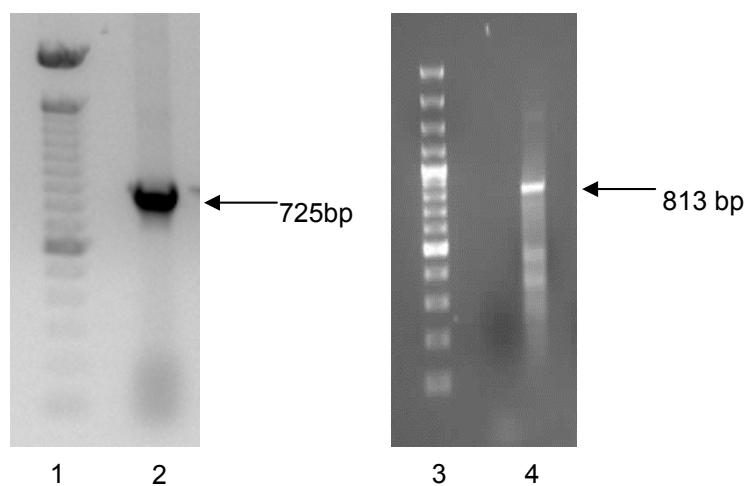


Figure 1. Electrophoretic separation of PCR-products of DNA of *Anabaena variabilis* N 21 and *Nostoc calcicola* N 25 on 1.5% agarose gels. Lanes 1 and 3. Marker DNA ladder 100 bp. Lane 2. PCR-product of *Anabaena variabilis* N 21; Lane 4. PCR-product of *Nostoc calcicola* N 25

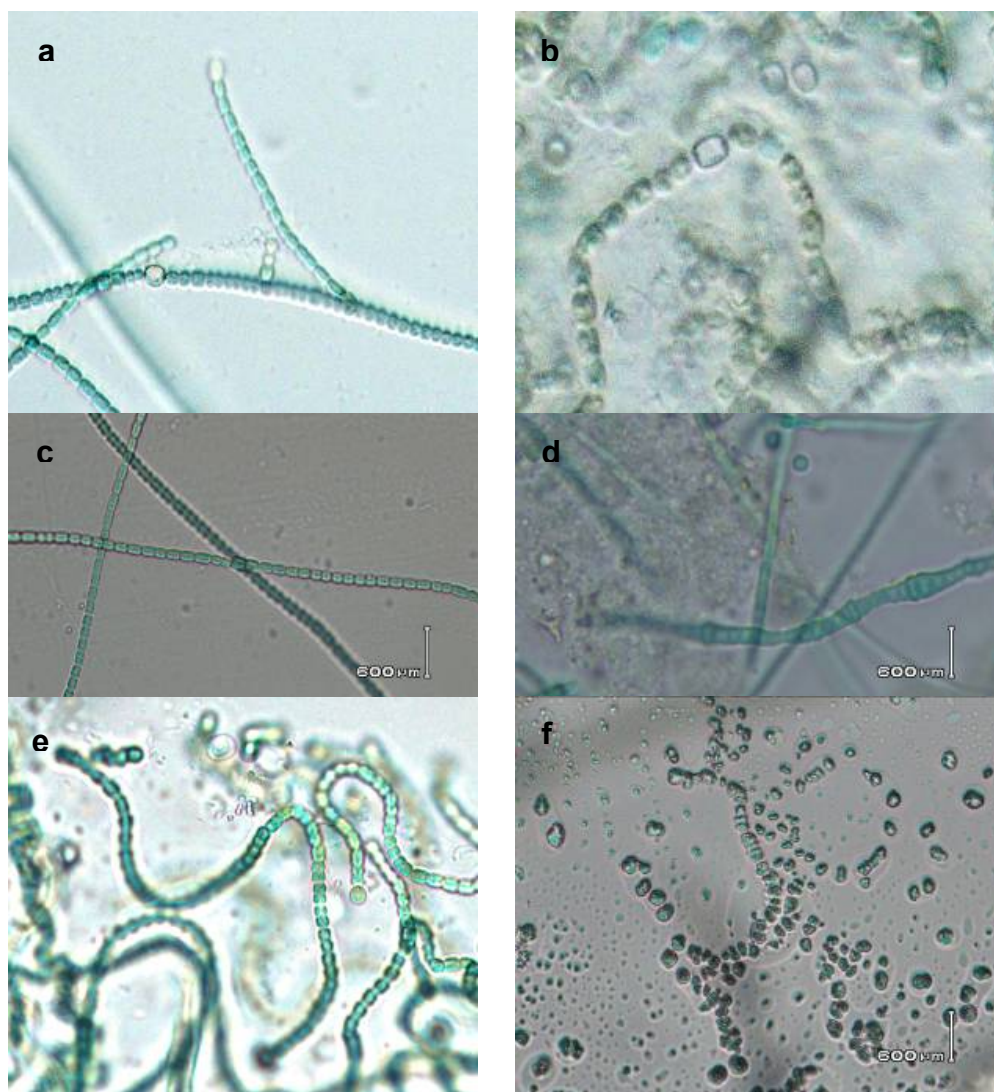
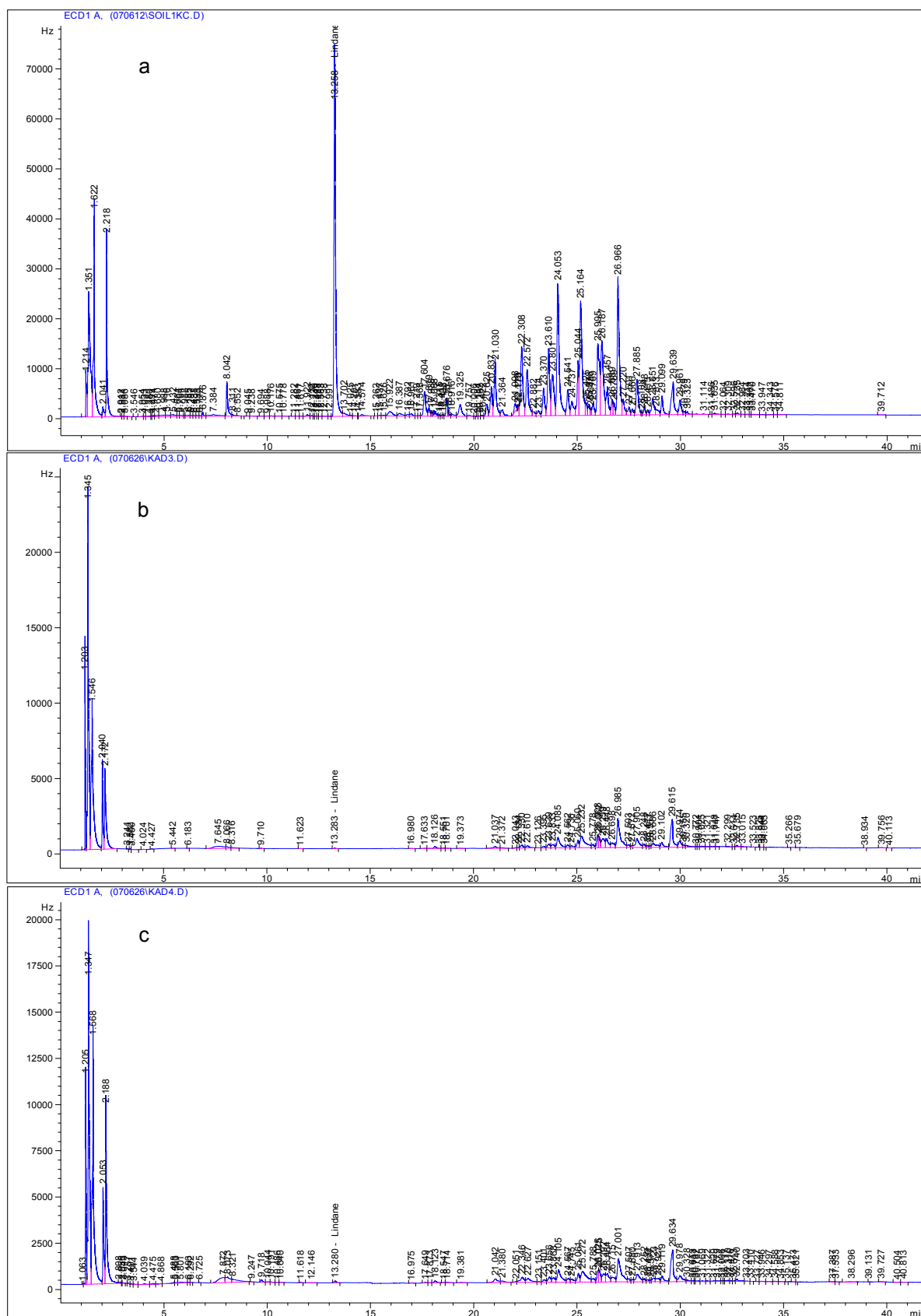


Figure 2. Microscopic pictures of *Anabaena variabilis* N 21 (a, c and e) and *Nostoc calcicola* N 25 (b, d, and f)

in different NaCl concentrations. a and b: low salt-concentration, c and d: 500 mM NaCl; e and f: 1 M NaCl



Soil inoculated with *Anabaena variabilis* N 21; c) Soil inoculated with *Nostoc calcicola* N 25

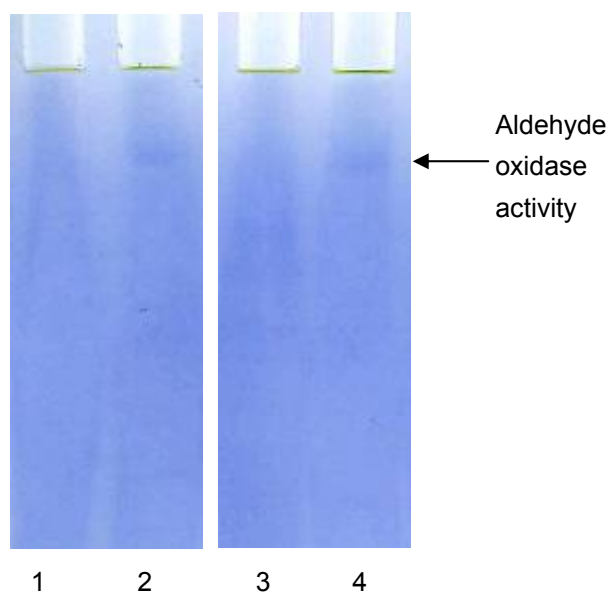


Figure 4. Aldehyde oxidase activity of *Anabaena variabilis* N 21 and *Nostoc calcicola* N 25 in medium with or without high-salt concentration: Lane 1: *Anabaena variabilis* N 21 without NaCl; lane 2: *Nostoc calcicola* N 25 without NaCl; lane 3: *Anabaena variabilis* N 21 with 300 mM NaCl; lane 4: *Nostoc calcicola* N 25 with 300 mM NaCl

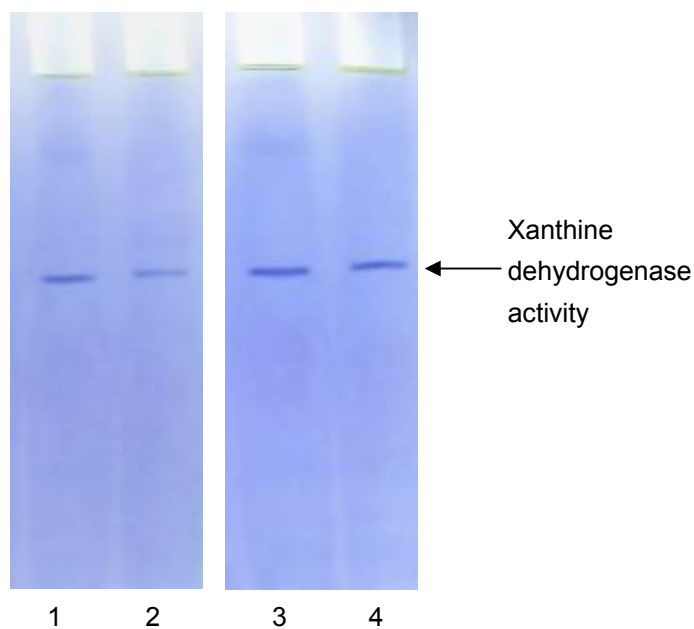


Figure 5. Xanthine dehydrogenase activity of *Anabaena variabilis* N 21 and *Nostoc calcicola* N 25, in low-salt vs. high-salt conditions. Lane 1: *Anabaena variabilis* N 21 without NaCl; lane 2: *Nostoc calcicola* N 25 without NaCl; lane 3: *Anabaena variabilis* N 21, 300 mM NaCl; lane 4: *Nostoc calcicola* N 25, 300 mM NaCl