Phenylalanine Ammonia-lyase Enzyme as a New Marker for Selection of Cyanobacteria for Absorption of Nickel in the Aquatic Environment

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Received: 2016-09-11 Revised and accept:2017-07-11

Abstract

Industrial activity can cause the release of nickel into aquatic ecosystems with a negative impact on living organisms. Cyanobacteria have an important role in regulating pollution, reducting dissolved nickel in water, and increasing the mineral's sedimentation. Recently the presence of phenylalanine ammonia lyase enzyme has been proved in cyanobacteria. This study aims to investigate the correlation between phenylalanine ammonia lyase activity and the rate of nickel absorption. For this purpose, a number of cyanobacteria *Anabaena* species, endogenous to Iran, were prepared by dry and wet treatment with nickel. The nickel uptake was evaluated by atomic absorption and comparison with a standard curve .The phenylalanine ammonia lyase was extracted from cyanobacteria and the enzyme activity was measured at different pH values. The results showed that some of the samples under study had remarkable ability to absorb nickel and that there were significant differences in the activity of the enzyme phenylalanine ammonia lyase at different pH values. This study established the relationship between the level of activity of this enzyme and the rate of nickel absorption.

Keywords: Absorbtion, Nickle, Cyanobacteria, Ammonia-lyase.

Introduction

Nickel is used for the production of electronic parts, stainless steel, and other metal plating activities and is highly resistant to temperature. It is also used in vehicle parts, processing machinery, manufacturing tools, electrical equipment, household appliances and to mint coins. In industry, nickel compounds are used as catalysts, pigments, and as a component in batteries. These wide-ranging industrial activities inevitably lead to release of nickel into sewage and wastewater networks and affect aquatic ecosystems.

In water, nickel exists as dissolved salts in clay particles or in organic matter (such as bacteria, algae) or it may be found absorbed into organic particles such as humic acids, fulvic acids, and proteins.Nickel deposits are released if the absorption process is reversed.

Marine organisms respond to nickel in different ways depending on their sensitivity to the metal. It is estimated that every year about 30 micrograms per liter are added to the concentration of nickel in seawater. It has been shown that the increase of nickel concentration to 141 micrograms per liter can lead to significant changes in the ability of shrimp to spawn.

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The enzyme that has recently been found in cyanobacteria is phenylalanine ammonia lyase (PAL).

This enzyme catalyzes the non-oxidative deamination of phenylalanine to produce trans-cinnamic acid and ammonia. This is the first step in the biosynthesis of phenylpropanoid (Hahlbrock and Scheel, 1989).

Many secondary metabolites in plants, such as flavonoids, Anthocyanins, plant hormones, lignin, and phytoalexins are phenyl propanoid products (Dixon and Paiva, 1995) (Lu-Sheng et al., 2011).

In cyanobacteria, PAL is responsible for the synthesis of phenyl propanoid where it acts on two substrates. The main substrate in the reaction is "L-phenylalanine" and the second substrate is "L-tyrosine". The main reaction is: L-Phenyalanine↔trans-cinamic Acid + NH_3 followed by the sub-reaction: L-Tyrosin↔Cumaric Acid + NH_3.

This study has been designed to assess the activity of phenylalanine ammonia lyase (PAL) in cyanobacteria and the connection of this activity with the absorption of nickel in cyanobacteria.

Materials and Methods

Sample microalgae studied in this research include *Anabaena* ISC55, *Anabaena* ISC88 and *Anabaena* ISC90. The cultivation of the microalgae had been carried out by the Applied Sciences Research Centre at Shahid Beheshti University and were supplied in pure culture.

Culture and mass proliferation of algae

Cultivation of the microalgae samples was achieved in BG11 medium at 25°C in 1500 to 2000 lux light intensity with aeration. In all experiments, 0.02 gram of cyanobacteria in dry or wet form was used.

Stock solution of Nickel

To achieve a 1000ppm solution, 0.405g of $NiCl_2.6H_2O$ was dissolved in 1000ml deionized distilled water. The volume of the solution used for measurements was 15ml. A calibration graph was plotted using the following equation to calculate the absorption index (Q) (Cain, 2008)

$$Q = \frac{(C_i - C_f)V}{M}$$

C_i: concentration of Nicke in solution before absorption (mg/l)

C_f: concentration of Nicke in solution after absorption (mg/l)

V: volume of Nicke solution (L)

M: weight of the biomass (g)

(Q): rate of absorption in milligrams per gram of biomass mg/g

The nickel concentration was measured using atomic absorption spectrometry method (AAS). A stock solution was made and standard samples were prepared in various concentrations for use in the atomic absorption spectrophotometer. A calibration curve was then plotted for each sample.

Extraction of PAL

A cyanobacterial sample of 0.02g was centrifuged for 10 min at 6000 rpm and the supernatant was discarded. The bacterial pellet was then re-suspended in 100µl of Tris- HCl buffer at a concentration of 0.16g/l. The samples were centrifuged at 10000 rpm and the supernatants were used to measure the enzymatic activity.

The following solutions were also prepared and their absorption were recorded at 270nm in a spectrophotometer:

a-blank solution: L-phenylalanine 2mm, water 900µl and Tris-HCl buffer 100µl b-test solution: L-phenylalanine 2mM, water 900µl, and 100µl extracted enzyme sample

The measurement was performed continuous spectrophotometric rate determination according to sigma manual (Sigma, 1998) and the data was used to calculate the rate of break down for the substrate phenylalanine in the following formula:

$$\text{Units/ml} = \frac{\Delta A270nm/\min TEST - \Delta A270nm/\min (3)(df)}{(19.73)(0.1)}$$

where:

3 =total volume of the experiment (ml)

df = dilution factor

19.73 = loss factor for trans-cinomate at 270nm

The effect of pH on enzyme activity of PAL

The enzyme activity at different pH, 11 solutions with different pH ranging from 2 to 12 were measured. The extracted enzymes to be used by each solution were adjusted to the solution's corresponding pH in aliquots of 50 µl and the enzymatic activity was measured as described above.

Measurement of the specific activity of the enzyme

To determine the specific activity of the enzymes, the amount of protein in each enzyme solution was assayed by the Bradford method. This was achieved by preparing thirteen dilutions of bovine serum albumin (BSA) with an initial concentration of 5mg/ml. The Bradford solution was then added to each dilution and the spectrophotometric readings were used to plot a standard curve. The concentration of the protein in each extracted enzyme sample was measured and based on the protein content, the specific activity of the enzyme was determined for each sample.

Results

The ability of cyanobacteria to remove nickel A standard curve showing the rate of the removal of nickel was plotted using different dilutions prepared from the stock solution. The ability of each sample, in dry and wet forms, to remove nickel, was then tested.

Nickel reduction compared to the initial value was significant in the wet and dry samples after 30 minutes.

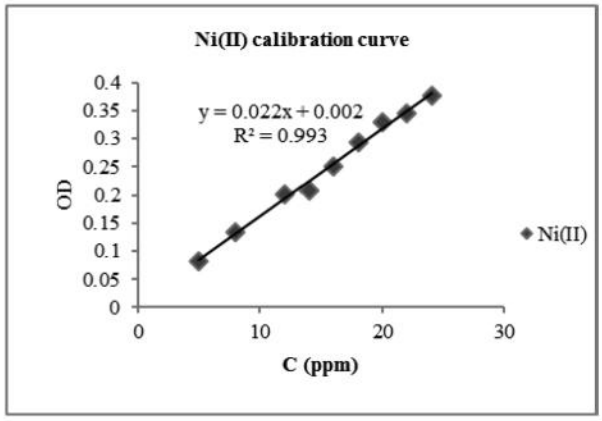

Fig. 1. Nickel standard curve by using atomic absorption spectrometry

Nickel uptake in dried samples was faster than the same sample in the wet form.

Anabaena ISC55 had the highest uptake of nickel, and then *Anabaena* ISC88 and *Anabaena* ISC90 respectively had the next ranks in the absorption of nickel (Figures 2 and 3).

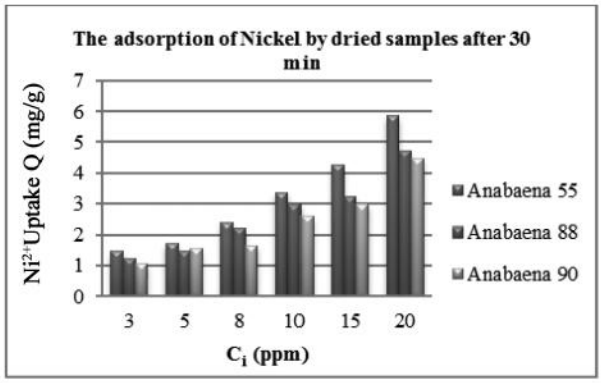

Fig. 2. Rate of nickel absorption in dried samples after 30 minutes

As seen in graphs 2 and 3, *Anabaena* ISC55 shows the highest intake of nickel followed by *Anabaena* ISC88 and *Anabaena* ISC90.

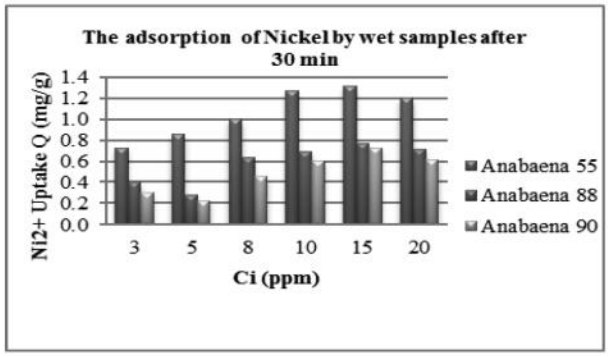

Fig. 3. Rate of nickel absorption in wet samples after 30 minutes

Comparison of PAL enzyme activity in cyanobacterial samples at different pH Activity of phenylalanine ammonia lyase on phenylalanine was studied using10 different pH values for each of the samples extracted from cyanobacteria.

Maximum activity was observed in *Anabaena* ISC55 PAL (Figure 4). The enzymatic activity of this sample at acidic pH values of 3 and 5 and alkaline pH values of 9 and 11 was significantly higher than those in other samples. The second highest activity was observed in *Anabaena* ISC88 at acidic pH values of 2 and 5 and alkaline pH value of 9. The least enzymatic activity was observed in *Anabaena* ISC90.

Discussion

Nickel and its alloys are used in many industries. Humans are exposed to this heavy metal through contaminated food, water, and air. Nickel can cause a variety of cancers including lung, larynx and prostate cancer. It is therefore important to collect the wastewater from industries that use nickel or its alloys with the aim of removing the nickel and preventing it from getting into fresh water. This strategy has considerable implications in ensuring health and safety of the communities that might otherwise be exposed to nickel contamination. One of the important and cost-effective ways to remove nickel from the effluent of the factories is bioremediation.

To reduce nickel contamination, the effluent is collected in special treatment ponds where an appropriate biologic sample is added. The biologic sample then sinks to the bottom of the pond taking the suspended nickel with it. The clean water is then drained into underground water systems.

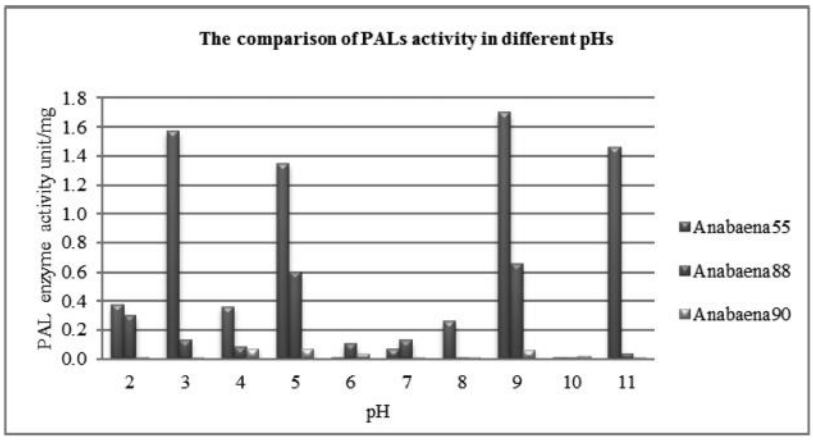

Fig. 4. comparison of PAL specific activity in samples under study at pH values of 2 to 11. The highest activity is seen in *Anabaena* ISC55.

Selecting the appropriate strain for bioremediation of environmental pollution is always the first step in environmental biotechnology. There are several ways to determine the right strain, but using specific markers can be a cost and time effective method to achieve the proper strain. It is important to consider various conditions before choosing a suitable strain.

Microalgae play an important role in reducing the level of nickel contamination. Nickel and cobalt are two essential transition metals for microalgae that are present in small quantities but play an important role in cellular physiology of cyanobacteria (Dupont et al., 2012). Bivalent Nickel is either directly associated with proteins or exists inside the Tetrapyrol coenzyme F430 ring (Ragsdale, 2009). This is despite the fact that cobalt (II) mainly exists as part of vitamin B_{12}. (Kobayashi and Shimizu, 1999). In cyanobacteria, the major enzymes that bind onto nickel are urease and hydrogenase. Urease breaks down urea as a nitrogen source and may be required for the catabolism of arginine (Quintero, 2000; Collier, 1999). Urea is also the main source of nitrogen in the seas where marine and freshwater cyanobacteria break it down to acquire nitrogen.

The second type of nickel-containing enzymes that metabolize hydrogen or produce hydrogen is hydrogenase enzymes. Two different types of hydrogenase enzymes exist in cyanobacteria which are bidirectional hydrogenase and absorbed hydrogenase (McIntosh et al., 2011; Carrieri et al., 2011; Eckert et al., 2012).

The third enzyme, phenylalanine ammonia lyase, is introduced in this study for the first time which seems to have a very important role in nickel uptake.

This study showed that cyanobacterial samples have an extraordinary ability to absorb nickel and under optimal conditions are able to absorb 1.6mg of nickel per gram of wet weight. Cyanobacteria are also able to absorb 5.8mg of nickel per gram dry weight in dry conditions. There is a direct correlation between PAL enzyme activity in cyanobacteria and nickel absorption. Although it was not possible to understand the role of PAL in the absorption of nickel, a significant relationship was observed between the absorption of nickel and PAL's enzyme activity indicating the existence of a molecular link between the enzyme and nickel uptake. Cyanobacteria undoubtedly play an invaluable role in reducing the level of dissolved nickel in water and sedimenting the metal and as a result they can be used in bioremediation of contaminated natural sources. This study showed a higher PAL activity at acidic and alkaline pH values than the neutral ones; therefore, these cyanobacteria can be used as a very convenient bioremediation option in contaminated aquatic environment with an acidic or alkaline pH.

It is hoped that this research can help find other suitable biological samples for bioremediation of nic,kelas. The results showed that it is possible to measure PAL activity as a biological marker to find other cyanobacterial species with higher abilities to absorb nickel.

Acknowledgment

This study is part of an MSc thesis in Microbiology at Islamic Azad University of Karaj. We would like to express our appreciation for the contributions made by Mrs. Ashrafi. We must also acknowledge the support and assistance of the Institute of Applied Sciences

of Shahid Beheshti University throughout this project.

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