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RESEARCH ARTICLE

BIOREMEDIATION OF METAL CONTAMINATED SITES BY NATURALLY GROWING LICHENS FOUND IN HILLY AREAS OF HIMACHAL PRADESH

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ABSTRACT

Lichen has a remarkable property of absorbing inorganic cations from the natural substrates on which they grow in amounts which are in excess of any expected requirements. We examined 20 sexicolous lichens which are known to fix metal ions found in Hilly areas of Himachal Pradesh of India for their metal fixation property. The mechanism by which Lichens take up cations is of considerable interest. Touminen (1967) proposed that alkali and alkaline metal ion uptake involved a simple ion-exchange process, which can occur inside the thallus cells of lichen by Intracellular ion-exchange mechanism of the proteins metallothioneins. Out of 20 protein samples 16 samples were found to have bound metal ions in considerable amount. These results enabled us to conclude about a similar mechanism of metal fixation in nearly all lichenic species possessing this property.

INTRODUCTION

Lichens are currently used as biomonitors of atmospheric and soil pollution. Lichens can absorb inorganic cations from the natural substrates on which they grow. During the period of atmospheric nuclear bomb testing, lichens were also found with high levels of various radioactive elements. Recent studies showed that these plants can accumulate high metal contents from industrial fallout while growing in such urban environments. In our study we considered a major mechanism of metal fixation by lichens that are intracellular complexation with metallothioneins. This mechanism is mainly found in terricolous and saxicolous lichens. We examined whether the same mechanism works for various lichens collected from different places and what amount of a particular metal could be found. This study covers the data obtained from biomonitoring study conducted in different parts of Himachal Pradesh, India between 2010- 2011. Metallothioneins is a family of cysteine rich proteins of low molecular weight (500-14000 Da). They are localized to the membrane of Golgi bodies. MTs have the capacity to bind both physiological (Zn, Cu, Se) and xenobiotic (Cd, Hg, Ag, As) ions through thiol group of its cysteine residues, which represents nearly 30% of their amino acid content.

MATERIALS AND METHODS

Sample collection and handling

20 Lichen specimens were collected from isolated places in Himachal Pradesh with application of a stout flat edged chisel to collect lichens growing on rocks and initially stored in polythene packets. The Collected specimens were only of saxicolous lichens from different sites. The lichen thallus together with soil and other waste was floated in deionized water in a beaker. Debris and soil particles settled down. After separation, the samples were placed in folds of filter papers and air-dried in clean glass box approximately for a day at room temperature. The dried samples were kept in the polyethylene bags washed by acids.

Instrumentation and optimization

Chromatography

The separated samples are now subjected to affinity chromatography column with following arrangements: Fractogel EMD chelate is packed into a column with a column dimension of 50 x10mm, 50 x16 mm or 50 x26 mm with a bed height of about 5 cm. Then the column has to be equilibrated with 20-50mM phosphate buffer/ 0.1-1M NaCl at pH 7.5. A subsequent washing step with 2 column volumes 0.1-1M NaCl solution is performed to completely remove the phosphate. The stationary phases have different affinity for different metal ions at definite pH ranges. The 20 samples were loaded one by one and column was regenerated each time after run of a single sample and bound proteins were eluted from the column using a descending pH-gradient. A phosphate buffer (0.1 M phosphate, 0.5-1 NaCl; pH 3.0) or an acetate buffer (0.1 M acetate, 0.5 -1M NaCl; pH 6.0 or pH 4.0) can be used. The declining pH-gradient can be run as step gradient and/or as linear gradient. For the regeneration of the metal chelate columns approximately. 1 bed volume of a 0.1-1M HCl solution at a flow rate of 1.5 cm/min has to be pumped

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through the column. This removes all metal ions; rinse with 2 column volume of a 0.1-1M NaCl solution and then column was equilibrated using 20 mM phosphate buffer. But as the resin is also stable against alkali treatment, thus the column can be cleaned with 0.5M NaOH.

Recovering metal ions collected by thallus surface and fungal cell wall

In order to assess the quantity of uptake and distribution of the ions by the thallus surface, cell wall and the protoplast, varied extraction methods were used, i.e. (successively) distilled water, strontium nitrate, boiling distilled water, 1M nitric acid, and 65% nitric acid. Rinsing with distilled water allows the extraction of unbound metals from the surface of the thallus. Ions of Sr^{2+}, on the basis of ion exchange, remove the metals immobilized in the cell wall. Boiling water can be used to extract the elements accumulated in cell cytoplasm, and 1M HNO_3 can be used to detect metals located in the cell wall, but not undergoing the exchange with strontium ions.

RESULTS AND DISCUSSIONS

Elements	Concentration (g/l)	Elements	Concentration (g/l)
Copper	1.2±0.6	Nickel	0.24±0.2
Zinc	1.3±0.6	Cadmium	0.3±0.1
Lead	0.6±0.2	Aluminium	0.18±0.1

Average concentration of metallothionein bound metal ions is given in the adjoining table. The separated samples of proteins obtained were analyzed for detecting concentration of particular metal bound to metallothionein using UV spectroscopy after making proper dilutions and in 16 out of 20 lichenic samples, metal content was considerable. Average contents of heavy metals in thalli followed the series: Zn>Cu>Pb>Cd>Ni>Al except in 4 of 16 samples where the series was as follows: Zn>Cu>Pb>Cd>Al>Ni.

Conclusion

- A pH decrease of the thallus caused increases of toxic element uptake by the lichens,
- The metal fixation by the lichen by any means either extra-cellular (by cell wall substrates) of intra-cellular is a kind of ion exchange process.
- The Metallothioneins bound to different metal ions can be easily separated by affinity chromatography.

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