Biotechnology Advances 34 (2016) 859–873

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Bio-recovery of non-essential heavy metals by intra- and extracellular mechanisms in free-living microorganisms

Jorge D. García-García *, Rosina Sánchez-Thomas, Rafael Moreno-Sánchez

Departamento de Bioquímica, Instituto Nacional de Cardiología "Ignacio Chávez", México D.F. 14080, México

ARTICLE INFO

Article history:
Received 27 October 2015
Received in revised form 10 May 2016
Accepted 12 May 2016
Available online 13 May 2016

Keywords:
Recovery of heavy metals
Free-living microorganisms
Euglena gracilis
Cyanogenic bacteria
Heavy metal bioaccumulation
Phytochelatin metabolism
Bioleaching
Cyanide biosynthesis
Gold
Cadmium

ABSTRACT

Free-living microorganisms may become suitable models for recovery of non-essential and essential heavy metals from wastewater bodies and soils by using and enhancing their accumulating and/or leaching abilities. This review analyzes the variety of different mechanisms developed mainly in bacteria, protists and microalgae to accumulate heavy metals, being the most relevant those involving phytochelatin and metallothionein biosyntheses; phosphate/polyphosphate metabolism; compartmentalization of heavy metal-complexes into vacuoles, chloroplasts and mitochondria; and secretion of malate and other organic acids. Cyanide biosynthesis for extra-cellular heavy metal bioleaching is also examined. These metabolic/cellular processes are herein analyzed at the transcriptional, kinetic and metabolic levels to provide mechanistic basis for developing genetically engineered microorganisms with greater capacities and efficiencies for heavy metal recovery, recycling of heavy metals, biosensing of metal ions, and engineering of metalloenzymes.

Contents

Abbreviations: APSR, adenosine 5′-phosphosulfate reductase; CS, cyanide synthase; CPCB, Computer Printed Circuit Boards; dw, dry weight; γ-ECS, γ-glutamylcysteine synthetase; IC_{50}, half-maximal inhibition of growth; PCS, phytochelatin synthase; PCs, phytochelatins; polyPs, poly-phosphates; PMST, plasma membrane sulfate transporters.

* Corresponding author at: Instituto Nacional de Cardiología "Ignacio Chávez", Departamento de Bioquímica, Juan Badiano No. 1, Sección XVI, Tlalpan, México D.F. 14080, México.
E-mail addresses: jorge.garcia@cardiologia.org.mx, jorgedonatogg@gmail.com, jorgedonatogarciagarcia@yahoo.com (J.D. García-García).

http://dx.doi.org/10.1016/j.biotechadv.2016.05.003
0734-9750/

1. Introduction

Bio-recovery of heavy metals is a technology that involves the removal of non-essential and essential heavy metals from water bodies or soils by micro- and macro-organisms (Cui and Zhang, 2008; Lee and Pandey, 2012). The identification and subsequent isolation of these organisms to be further applied for industrial purposes (Das, 2010) is also a promising biotechnological task. Therefore, bio-recovery of heavy metals may become in the near future a suitable and economically viable strategy to clean polluted areas (bioremediation) and/or to recycle metals in controlled industrial systems.

Several plant species from the *Asteraceae* and *Brassicaceae* families have shown high potential for recovery of heavy metals from soils (phytoremediation). For instance, *Senecio* sp. (*Asteraceae* family) collected from a mine spoil accumulates 0.4 and 4.2 mg Pb^{2+}/g_{dw} in roots and shoots, respectively, after at least 1 year exposure to 13 g Pb^{2+}/kg soil (Bech et al., 2012; Radford and Cousens, 2000). Laboratory studies have shown that roots and shoots of *Thlaspi caerulescens* remove 3.5 and 5 mg Cd^{2+}/g_{dw}, respectively, after 3 months exposure to 250 mg Cd^{2+}/kg soil; and roots of *Brassica juncea* seedlings remove up to 227 mg Au^{3+}/g_{dw} after 24 h exposure to 26 mM Au^{3+} (Bali et al., 2010; Pongrac et al., 2009).

Phytoremediation has been considered a "green" and commercially viable technology to clean soils, because its cost is $5–40 USD/ton, which is 5 to 13 times lower than the costs involved when using ecologically aggressive methods such as electrokinetics, chemical treatment, vitrification and landfilling (Lee and Pandey, 2012). However, phytoremediation has some limitations: (i) slow growth rates and low biomass yields of hyperaccumulator plants; (ii) lengthy processes for complete heavy metal removal and soil regeneration; (iii) slow bioavailability due to the tight binding of metal ions to soil components; and (iv) biomagnification, *i.e.* risk of food chain contamination in case of mismanagement and lack of proper care (Ali et al., 2013).

On the other hand, several macroalgae have shown high efficiency for recovery of heavy metals from water bodies, in a process mediated mainly by adsorption to the external cell layers. Indeed, the alga cell wall has a surplus of electronegative groups such as carboxylic, sulfonic, phosphoryl, amino and hydroxyl groups, which enable it to establish strong bonds with heavy metals. These electronegative groups are mainly in peptidoglycans and lipopolysaccharides, which represent up to 40% of dry weight in green algae (Jones and Harwood, 1993; Mehta and Gaur, 2005). The brown alga cell wall is also rich in alginate (which consists of a backbone of up to 1500 units of 1,4-linked α-L-guluronic acid and β-D-mannuronic acid) and fucoidan (conformed by a backbone of 1-3-linked α-L-fucopyranose and sulfate or acetate molecules as radicals), which further increases the cell wall electronegativity and affinity for heavy metals (Ale and Meyer, 2013; Andrade et al., 2010; Paul et al., 2012). Consequently, a number of diverse macroalgae can recover by extracellular adsorption up to 21 and 4 times more Cd^{2+} and Pb^{2+}, respectively, and at a faster rate than plants (Table 1). As an exception, seedlings of *B. juncea* show a higher recovery capacity for gold than macroalgae.

Alginates and fucoidan are much larger extracellular macromolecules than intracellular chelating polymers such as phytochelatins (PCs) and poly-Phosphates (polyPs) in protists and microalgae (Fig. 1), which may help to ensure binding of heavy metals in the extracellular milieu. The binding equilibrium constants for Cd^{2+} and PCs ($Keq = 5.1 \times 10^9\ M^{-1}$) and for Cd^{2+} and alginate ($1.4\ mM^{-1}$)

Table 1
Bio-recovery of non-essential and essential heavy metals by extra- and intra-cellular mechanisms in protists, microalgae and macroalgae.
Values shown in bold and (*italic*) types are expressed in mg/g_{dw} and nmol of $metal/g_{dw}$, respectively. Extracellular adsorption was calculated by using the equation $q_t = [C_0 - C_t] / B$; where q_t is the metal adsorbed, C_0 is the initial metal concentration in the solution, C_t is the metal concentration at time t in the solution, and B is the biomass concentration. Intracellular accumulation was determined in cells washed with [a] 120 mM KCl, 20 mM 3-*N*-morpholino propane sulfonic acid (MOPS), 2 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) buffer pH 7.2; [b] 5 mM ethylenediaminetetraacetic acid (EDTA) pH 2; [c] 50 mM EDTA pH 5; or [d] 0.01 and [e] 5 mM EDTA without pH value indicated. * value in bleached *E. gracilis* cells pretreated by ≈60 generations with 1.5 μM $HgCl_2$ and reported as mg $Cd^{2+}/g_{protein}$; §value in photosynthetic *E. gracilis* cells.

Organism	Time, days	Au^{3+}	Cd^{2+}	Pb^{2+}	Ni^{2+}	Zn^{2+}	Cu^{2+}	References
Extracellular adsorption								
Scenedesmus quadricauda (green microalga)	0.08		**76** (0.7)		**26** (0.4)	**61** (0.9)	**73** (1.1)	Bayramoğlu and Arica, 2009; Bayramoglu and Arica, 2011
Chlorella sp. (green microalga)					**0.6** (0.01)	**33** (0.5)	**14** (0.2)	Maznah et al., 2012; Wong et al., 2000
Fucus vesiculosus (brown macroalga)	0.33	**74** (0.4)	**31** (0.3)	**58** (0.3)			**76** (1.2)	Mata et al., 2009a, 2009b
Padina gymnospora (brown macroalga)	0.12		**67** (0.6)	**43** (0.2)				Andrade et al., 2010
Turbinaria conoides (brown macroalga)	0.04-0.2	**35** (0.2)	**107** (0.9)					Vijayaraghavan et al., 2011; Vijayaraghavan et al., 2012
Galaxaura oblongata (red macroalga)	0.08		**85** (0.7)	**89** (0.4)				Ibrahim, 2011
Ulva lactuca (green macroalga)	0.2		**49** (0.4)	**290** (1.4)		**19** (0.3)	**38** (0.6)	Areco et al., 2012
Intracellular accumulation								
E. gracilis[a] (Protist)	4		**12.2*** (0.11)*			**3.3**§ (0.05)§		*Avilés et al., 2003; §Sánchez-Thomas et al., in preparation
Chlamydomonas sp.[b] (green microalga)	3		**3.4** (0.03)					Aguilera and Amils, 2005
Chlamydomonas reinhardtii C-9[c]	4		**0.001** ($8x10^{-6}$)					Nishikawa et al., 2006
Chlamydomonas acidophila[c]	4		**0.05** ($4x10^{-4}$)					Nishikawa et al., 2006
Chlorococcum hemicolum[d] (green microalga)	10				**13** (0.2)			Harish et al., 2008