Bioresource Technology 130 (2013) 69–74

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Comparative study on Ni^{2+}-affinity transport of nickel/cobalt permeases (NiCoTs) and the potential of recombinant *Escherichia coli* for Ni^{2+} bioaccumulation

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HIGHLIGHTS

- Two kinds of NiCoTs were comparatively studied on their Ni^{2+} transport ability.
- Expression of NiCoT made recombinant strains more sensitive to the toxicity of Ni^{2+}.
- NixA from *Helocobacter pylori* had a higher Ni^{2+}-affinity than NisA from *Staphylococcus aureus*.
- Recombinant *Escherichia coli* expressing NixA and MT accumulated maximum Ni^{2+} of 83.33 mg g^{-1}.
- Both NiCoT and MT were essential for Ni^{2+} bioaccumulation of recombinant strains.

ARTICLE INFO

Article history:
Received 13 August 2012
Received in revised form 22 November 2012
Accepted 28 November 2012
Available online 8 December 2012

Keywords:
Nickel
Transmembrane protein
Metallothionein
Bioaccumulation
Gene engineering

ABSTRACT

Comparative evaluation on Ni^{2+}-uptake of two nickel-affinity transmembrane proteins (NiCoTs) respectively from *Helocobacter pylori* (NixA) and *Staphylococcus aureus* (NisA) was performed. Expression of NiCoTs alone did not promote Ni^{2+} uptake of the recombinant strains and made the growth susceptible to Ni^{2+}. However, recombinant strains expressing both NiCoTs and Metallothionein (MT) showed enhanced tolerance to Ni^{2+} and Ni^{2+} uptake. The maximum Ni^{2+}-uptake capacity of recombinant strain N1c expressing NixA+MT reached 83.33 mg g^{-1}, higher than 45.45 mg g^{-1} of recombinant strain N1d expressing NisA+MT. N1c exhibited more effective Ni^{2+} accumulation than N1d in the presence of Na^{+}, Co^{2+} and Cd^{2+}. NiCoTs promoted intracellular Ni^{2+} uptake of the recombinant strains. Phosphate groups dominated Ni^{2+} binding of wild type *Escherichia coli*, but carboxyl groups contributed more for N1c and N1d. The result suggested that NixA has a higher specificity in Ni^{2+} binding than NisA, and both NiCoTs and MT are important for Ni^{2+} bioaccumulation.

1. Introduction

As an essential metal for some metalenzymes involved in energy and nitrogen metabolism, nickel can be absorbed by most microorganisms from natural environment, but in trace amounts to keep metal homeostasis inside the cells. However, for the purpose of bioremediation of nickel-polluted environments, this characteristic is not welcome as many metal tolerant microorganisms have been found to have a relatively low Ni-binding capacity (Williams et al., 1998, 2012; Castillo-Zacarias et al., 2011). Ozdemir et al. (2012) and Ferreira et al. (2011) also found that the biosorption of nickel by different strains was less than that of other metal ions.

It was reported that the nickel accumulation capacity of microorganisms could be improved by expressing high-affinity nickel transmembrane proteins (Tiwari et al., 2011; Hebbeln and Eitinger, 2004). For most bacteria and fungi, such transmembrane proteins are designated as nickel–cobalt permeases (NiCoTs), a rapidly growing group of nickel transporters characterized by a seven or eight-helix structure and a number of conserved signatures mainly located in transmembrane domains (Mulrooney and Hausinger, 2003). Recent advances in NiCoTs identification included NhlF from *Rhodococcus rhodochrous* (Komeda et al., 1997), NixA from *Helicobacter pylori* (Wolfram and Bauerfeind, 2002), HoxN from *Ralstonia eutropha* (Degen and Eitinger, 2002), NcrC from *Serratia marcescens* (Marrero et al., 2007), NCT from *Neurospora crassa* (Ramya et al., 2009) and TNC from *N. crassa* (Tiwari et al., 2011). According to the substrate preferences, NiCoTs were classified into three classes (Hebbeln and Eitinger, 2004): Class I, represented by

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0960-8524/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2012.11.133

HoxN and NixA, is a highly selective nickel transporter; Class II (NhlF) transports both nickel and cobalt ion with a preference for cobalt; Class III, including some NiCoTs from *Staphylococcus aureus* and *Klebsiella pneumoniae*, also transports both nickel and cobalt ion but has a higher capacity for nickel. Previous studies showed that by expressing different classes of NiCoT genes the nickel uptake capacity of recombinant strains could be enhanced (Zhang et al., 2007; Ramya et al., 2009; Tiwari et al., 2011). However, due to different host strains, plasmid vectors and experimental conditions used in those studies, it was difficult to evaluate which kind of NiCoTs has a higher nickel uptake capacity, thus hindering the application of NiCoTs in Ni^{2+} bioaccumulation.

In the present study, two kinds of NiCoTs, NixA (Class I) from *H. pylori* and NisA from *S. aureus* (Class III), were expressed in *Escherichia coli* for the comparative evaluation on their capability and specificity for nickel uptake. These two NiCoTs were chosen because they were reported to possess relatively high Ni^{2+} affinity (Hebbeln and Eitinger, 2004). Further, in order to avoid the tight homeostatic metal control in microbial cells which might limit metal uptake or even exclude metals already entering the cells, a metal-binding protein (metallothionein, MT) was also intracellularly overexpressed (Deng et al., 2003).

2. Methods

2.1. Plasmids and strains

Three compatible plasmids, *pET-HP*, *pET-SA* and *pGPMT* (Deng and Wilson, 2001) were employed in this study for the expression of NixA from *H. pylori*, NisA from *S. aureus* and GST fusion protein of pea MT (GST-MT), respectively. To construct *pET-HP*, plasmid *pUEF202* (Fulkerson et al., 1998) which contained the entire *nixA* gene was digested with the restriction enzymes *Nde I* and *Bam HI*, and then the resultant 1.0 Kb fragment containing *nixA* was inserted and ligated between the *Nde I* and *Bam HI* restriction sites of *pET-28a* (Novagen). To construct *pET-SA*, *S. aureus* GW1.0405, preserved in our lab, was grown overnight in Luria-Bertani broth (LB) at 37 °C, 180 r min^{-1} and genomic DNA was isolated according to BBI Genomic DNA Isolation Protocol and used as template for PCR amplification. Two primers (Forward, 5′-CGCCATATGATGATT AAATATCATGTT-3′; reverse, 5′-CGCGGATCCTTATCTAGACCATTTG TGTTC-3′) with *Nde I* and *Bam HI* sites at 5′ extension ends were designed based on the published sequence of NiCoT gene from *S.aereus* in Genbank (accession number: CP000730.1). The amplified nucleotide sequence (about 1.1 Kb) which contained the entire NiCoT gene (designated to be *nisA* in the study) was digested with *Nde I* and *Bam HI* and the resulting fragment was inserted and ligated into *pET-28a* likewise. Bioinformatics analysis showed that the similarity of gene sequence between *nixA* and *nisA* was 68%. *pET-HP* and *pET-SA* contained Kan^r sequence, and *pGPMT* contained Amp^r sequence. *E. coli* JM109 was used as host strain. All transformations of the compatible plasmids were carried out by electroporating to produce gene recombinant *E. coli* strains harboring *pET-HP* only, *pET-SA* only, *pET-HP* + *pGPMT*, *pET-SA* + *pGPMT* and *pGPMT* only respectively designated as N1a, N1b, N1c, N1d and N1e.

2.2. Tolerance of recombinant strains to Ni^{2+}

E. coli cells harboring various plasmids were grown in LB containing 30 mg L^{-1} kanamycin or 30 mg L^{-1} kanamycin +100 mg L^{-1} ampicillin at 37 °C, 180 r min^{-1}. When OD_{600} reached 0.5, Isopropyl-ß-D-thiogalactoside (IPTG) was added to 1.0 mM and after 4 h incubation the cells were harvested and reinoculated into fresh LB amended with different concentrations of Ni^{2+}. After 18 h incubation at 37 °C with vigorous shaking (180 r min^{-1}), the value of OD_{600} was measured to evaluate the tolerance of recombinant *E. coli* strains to the presence of Ni^{2+}. Original host *E. coli* JM109 was also cultivated likewise as the control, except for the antibiotics adding and IPTG induction.

2.3. Metal accumulation

E. coli strains were cultured in LB broth and induced by IPTG as described above, and pelleted by centrifugation at 8000*g* for 10 min. The harvested cells were washed three times with 10 mM phosphate buffer (pH 7.0) and then resuspended in the solutions with desired Ni^{2+} concentrations. After a 2 h incubation at 37 °C, 180 r min^{-1}, the cells were harvested by centrifugation, dried, and digested overnight with 70% trace-metal grade nitric acid for Ni^{2+} analysis. To determine the specificity of Ni^{2+} transporter, Na^+ (0–1000 mg L^{-1}), Co^{2+} (0–50 mg L^{-1}) or Cd^{2+} (0–50 mg L^{-1}) were added to 10 mg L^{-1} Ni^{2+} solution as competitors. During the experiments, the stirred speed and temperature were kept constant at 37 °C, 180 r min^{-1}. All experiments were repeated thrice.

2.4. Localization of bound Ni^{2+} on the cells

Ni^{2+}-laden *E. coli* cells were pelleted, washed with 10 mM phosphate buffer, and then split into a pair of two samples. One sample was treated with 0.1 M EDTA at 30 °C, 180 r min^{-1} for 30 min, and the cells were separated by centrifugation to obtain supernatant to measure the amount of Ni^{2+} accumulated on the cell surface. The separated pellets were dried and digested with 70% trace-metal grade nitric acid at 30 °C overnight for the determination of intracellular Ni^{2+} uptake. Another sample of the cells was dried and directly digested with 70% trace-metal grade nitric acid to measure the quantity of Ni^{2+} accumulated by the whole cells.

2.5. Participation of functional groups in Ni^{2+} uptake

To understand the role of functional groups in metal ion binding, biomasses were chemically treated in different ways. The purpose of chemical modification was to block a given functional group to prevent its participation in metal uptake and the decrease in uptake capacity of treated cells could be used to evaluate the contribution of blocked functional group in the overall metal uptake process (Chojnacha et al., 2005). The *E. coli* cells were respectively suspended for 30 min in anhydrous methanol and concentrated hydrochloric acid to esterify carboxylic groups, in formaldehyde and formic acid to methylate amine or hydroxyl groups, and in triethyl phosphate and nitromethane to esterify phosphate groups. After chemical treatments, the cells were washed twice, harvested by centrifugation and then exposed to Ni^{2+} solution for bioaccumulation.

2.6. Metal analysis

All metal ion concentrations in the experiments were determined by a sequential inductively coupled plasma optical emission spectrometer (OPTIMA 7000, Perkin Elmer). Metal binding capacities were expressed as mg metals accumulated by gram dry weight of the cells.

3. Results and discussion

3.1. Tolerance of recombinant strains to Ni^{2+}

Unlike Hg^{2+}, Ni^{2+} poses a low toxic effect on bacteria. Although high concentration of Ni^{2+} inhibited the growth of all tested strains,