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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.

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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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