RESEARCH ARTICLE
INTERNATIONAL MICROBIOLOGY (2004) 7:27–34
INTERNATIONAL MICROBIOLOGY

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Nucleotide sequence and expression of the *ncr* nickel and cobalt resistance in *Hafnia alvei* 5-5

Received 5 September 2002
Accepted 7 September 2003

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Summary. The structural genes for the nickel and cobalt resistance of the conjugative plasmid pEJH 501 of *Hafnia alvei* 5-5, contained on a *Sal*I-*Eco*RI fragment of 4.8 kb, were cloned and sequenced. The DNA sequence included five genes in the following order: *ncrA, ncrB, ncrC, ncrY*, and *ncrX*. The predicted amino acid sequences of *ncrA* were homologous to the amino acid sequences of *nreB* of *Achromobacter xylosoxidans* 31A. Expression of *ncr* with the T7 RNA polymerase-promoter system allowed *Escherichia coli* BL21 (DE3) to overexpress NcrA, NcrB, and NcrC but not NcrY, and NcrX. The apparent molecular masses of NcrA, NcrB, and NcrC were 30, 33, and 17 kDa, respectively. Primer-extension analysis showed that *ncr* mRNA started at nucleotide position 23 upstream from *ncrA*. The promoter region of the *ncr* operon possessed a strong, putative –35 element of σ^{32}-type promoter sequence, and transcriptional *'lacZ* fusion studies indicated that the –35 element influenced σ^{32}-specific transcription. [**Int Microbiol** 2004; 7(1):27–34]

Key words: plasmid pEJH501 · operon NcrABCYX · σ^{32}-type promoter sequence · nickel-resistant bacteria

Introduction

Nickel is a trace element that serves as an essential constituent of enzymes, such as hydrogenase, CO dehydrogenase, and urease in bacteria [10]. However, excess nickel ions interact with cellular components, such as amino acids and nucleotides, resulting in a disturbance of enzyme activity, DNA replication, transcription, and translation [1,16]. Plasmid-mediated resistance to nickel has been described in *Ralstonia metallidurans* CH34 [7,8,15,17,32–34] and *Achromobacter xylosoxidans* 31A [28–30]. Resistance is due to the action of an inducible, operon-encoded energy-dependent specific efflux system that secretes the cation from the cell thereby lowering the intracellular concentration of the toxic compound [31].

The *cnr* (cobalt-nickel resistance) operon of the *R. metallidurans* CH34 plasmid pMOL28 consists of *cnrYXHCBA*. The genes *cnrCBA* encode a membrane-bound protein complex catalyzing an energy-dependent efflux of cobalt and nickel [5]. The mechanism of action of the CnrCBA complex may be that of a proton/cation antiporter based on the considerable similarity of CnrCBA amino acid sequences to those of CzcCBA (for cadmium-zinc-cobalt resistance). The topological orientation and function in the membrane of CzcCBA have been well-studied in strain CH34 [20,21,23]. The Cnr regulatory genes *cnrYXH* are arranged in a region upstream of the structural genes and are responsible for full transcription of the CnrCBA structural resistance genes. CnrH, a 21-kDa protein, belongs to a sigma factor of the extracytoplasmic function (ECF) family, whose members share a helix-turn-helix motif at the carboxy terminus [18,24,37]. CnrX, which may function as a periplasmic sensor, contains histidine residues that probably bind nickel ions. CnrY is a *trans-*

acting regulatory protein thought to act as a repressor or anti-sigma factor [7].

The *ncc* (nickel-cobalt-cadmium resistance) operon of the *A. xylosoxidans* 31A pTOM9 comprises seven open reading frames (ORFs), *nccYXHCBAN*, and the encoded proteins share strong homology to proteins encoded by *cnrYXHCBA* [30]. The NccCBA complex also shows close similarities to the CzcCBA complex, which seems to be a three-component cation-proton antiporter [30]. NccH probably belongs to a family of ECF σ^{70}-like proteins, as it has the conserved regions of the ECF sigma factors. NccX contains several histidine residues and seems to be capable of binding nickel ions; therefore, the protein may function as a periplasmic sensor. NccY is a *trans*-acting regulatory protein and down-regulates the operon [30].

In addition to the *ncc* locus, *A. xylosoxidans* 31A contains another nickel resistance locus, *nre*, on plasmid pTOM9, which confers a low level of resistance [8,30]. Gene *nreB* is induced by nickel and may be involved in resistance by efflux coupled to a chemiosmotic gradient. The topological orientation of this gradient in the membrane has been elucidated largely by comparison with proteins of the major facilitator superfamily (MFS) transporters [8].

In this work, we have determined the complete nucleotide sequence of the 4.8-kb *Sal*I–*Eco*RI fragment containing the *ncr* (nickel-cobalt resistance) operon from the 70-kb plasmid pEJH501 of *Hafnia alvei* 5-5. Five ORFS for the genes *ncrABCYX* were found. The products of the predicted translation product of *ncrA* and *ncrB* were consistent with those of *nreB* and *nreA* [22].

Materials and methods

Bacterial strains, plasmids, and growth conditions. The bacterial strains and plasmids used in this study are described in Table 1. Growth conditions for *Hafnia alvei* 5-5 were described previously [22]. The bacterial culture was deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Braunschweig, Germany, with the number DSM 15533. *Escherichia coli* strains were grown at 37°C in Tris mineral medium supplemented with 0.3% (w/v) gluconate as carbon source [17]. Amino acids, when necessary, were added at a concentration 20 μg/ml after filtration. For maintenance of plasmid markers, filter-sterilized solutions of antibiotics were added, as appropriate, to the following final concentrations: ampicillin, 100 μg/ml, kanamycin 30 μg/ml, and chloramphenicol 15 μg/ml for *E. coli*. Clones of *E. coli* DH5α harboring recombinant pUC plasmids were identified on LB agar plates containing 100 μg ampicillin/ml, 40 μg 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside/ml, and 0.2 mM isopropyl-β-D-thiogalactopyranoside (IPTG) (Duchepa, Netherlands).

Table 1. Bacterial strains and plasmids used in this study

Bacterial strain and plasmid	Relevant genotype	Reference
Bacterial strains		
Hafnia alvei		
5-5	WT, Ni^r	DSM 15533
ATCC13337	WT, Ni^s	DSM30163
Escherichia coli		
DH5	*sup*E44 *lac*U169(ø80*lac*Z M15)	
	*hsd*R17 *rec*A1 *end*A1 *rel*A1	GIBCO BRL (USA)
BL21 (DE3)	F-*dcm ompT hsdS* (rB- mB-) *galDE3*	Stratagene (USA)
CSR603	*rec*A1 *uvr*A6 *phr*-1	[27]
Plasmids		
pBluescriptII KS (+)	Cloning vector	Stratagene (USA)
pBR322	Cloning vector	Promega (USA)
pUJ9	Cloning vector	[4]
pT7-6	Expression vector	[36]
pEJH501	70 kb from *H. alvei* 5-5	This study
pHF14	4.8 kb, *Sal*I-*Eco*RI fragment of pEJH501 in pBR322	This study
pURS882	0.8 kb, *Sma*I fragment of pHF14 in pUJ9	This study
pRCS208	2.6 kb, *Sal*I-*Cla*I fragment of pHF14 in pBluescriptII KS (+)	This study
pREC203	1.9 kb, *Cla*I-*Eco*RI fragment of pHF14 in pBluescriptII KS (+)	This study
pRSD401	0.8 kb, *Sma*I fragment of pHF14 in pBluescriptII KS (+)	This study
pRSD402	1.1 kb, *Nco*I fragment of pHF14 in pBluescriptII KS (+)	This study
pRSD403	2.8 kb, *Kpn*I-*Sma*I fragment of pHF14 in pBluescriptII KS (+)	This study
pRSD404	0.9 kb, *Cla*I-*Sma*I fragment of pHF14 in pBluescriptII KS (+)	This study
pRSD405	1.3 kb, *Sma*I-*Cla*I fragment of pHF14 in pBluescriptII KS (+)	This study
pRSD406	1.8 kb, *Eco*RV-*Eco*RI fragment of pHF14 in pBluescriptII KS (+)	This study

WT, wild type; s, sensitive; r, resistance

Chemicals, reagents, and enzymes. Analytical grade $NiCl_2 \cdot 6H_2O$ (Sigma, St. Louis, MI, USA) was prepared as 1.0 M stock solution and sterilized by autoclaving. All enzymes were purchased from Promega (Madison, WI, USA), TakaRa (Japan), and New England Biolab (Beverly, MA, USA). [γ-^{32}P] ATP (1000 Ci/mmol) and [α-^{35}S] dATP (1000 Ci/mmol) were purchased from Amersham (UK). β-Galactosidase activity was measured as published before [19] and is reported in Miller units.

Genetic techniques. Standard molecular genetic techniques were as described by Sambrook et al. [25] or as per the manufacturer's instructions unless mentioned specially. DNA sequences were obtained by the dideoxy-mediated termination method of Sanger using an ABISEQ automatic sequencer. Nucleotide and amino acid sequences were analyzed with the sequence analysis software DNAMAN (Lynnon Biosoft), Clone Manager 5 (Scientific and Educational Software), and Align Plus (Scientific and Educational Software). Homology searches were done using the BLAST search algorithms located at the National Center for Biotechnology Information site (http://www.ncbi.nlm.nih.gov/) on the World Wide Web. The sequence has been submitted to GenBank (accession no. AF322866). Hydropathy profiles were determined using the method of Hopp and Woods [12,14].

Transcript analysis. Total RNA was isolated from uninduced cells or cells exposed to 300 μM $NiCl_2$ for 2 h at 37°C during growth in Tris mineral medium by using the RNeasy total RNA preparation kit (Qiagen, Santa Clara, Calif., USA). The transcription start site was identified by primer extension using primer 5′-ACGCGTCGACTGCAGGAATTCACACTTT-TAATCG-3′, which is complementary to nucleotides +28 to +49 downstream of P1, respectively. The primer was end-labeled using T4 polynucleotide kinase (Promega, Madison, WI, USA) and [γ-^{32}P] ATP following standard protocols. For primer-extension experiments, a modification of the Promega protocol was used. Two pmol of the primers were incubated with 30 μg RNA in 10 μl hybridization buffer (50 mM KCl, 25 mM Tris-HCl, pH 8.3) at 65–75°C for 2 min and allowed to cool. AMV RT (1 U) was used to extend the primer in a reaction mixture containing 50 mM KCl, 10 mM $MgCl_2$, 10 mM dithiothreitol (DTT), 0.5 mM spermidine, and 1 mM deoxynucleotide triphosphate. The reaction was carried out in a total volume of 20 μl at 42°C for 30 min. After phenol-chloroform extraction, the extended product was precipitated by the addition of 0.1 volume of 3 M sodium acetate and three volumes of ethanol. The pellet was washed with 75% ethanol, dried, and dissolved in 10 μl H_2O. The unlabeled primer was used to generate a nucleotide sequence ladder using a Sequenase version 2.0 DNA sequencing kit (Amersham Life Science, Cleveland, Ohio, USA) with [^{35}S] dATP. Primer-extension products were separated in an 8 M urea/6% polyacrylamide gel in parallel with the sequencing reactions in order to map the transcription initiation site.

Protein expression. The *ncr* genes were expressed by the T7 RNA polymerase-promoter system [36]. The genes were cloned into a T7-promoter-containing vector (pT7-6), and the resulting plasmid was transformed into *E. coli* BL21 (DE3) bearing the T7 RNA polymerase gene (λ DE3 lisogen) for expression of target proteins. Cells bearing plasmid were grown at 37°C overnight in Luria Bertani (LB) medium containing 50 μg ampicillin/ml). The culture was diluted 100-fold into 5 ml fresh LB medium, containing ampicillin alone or ampicillin and nickel (250 μM), and was grown at 37°C. When the culture reached the mid-exponential phase, 1.0 mM IPTG was added to induce gene expression. Cultivation was continued for an additional 2 h. Cells were pelleted by centrifugation and suspended in 500 μl of 10 mM Tris-HCl buffer (pH 7.0). Cells were then frozen at –20°C for 16 h and disrupted on ice using an ultrasonicator (Lab Line, USA) at a continuous setting output of 10 times for 10 s. After centrifugation at 9,000 rpm at 4°C for 20 min, the supernatant was added to the same volume of 10 mM Tris-HCl buffer (pH 7.0) and the pellet was dissolved in 20 μl 2× Tricine sample buffer [0.08 M Tris-Cl/SDS (pH 6.8), 24% (v/v) glycerol, 8% (w/v) SDS, 0.2 M DTT, 0.02% (w/v) Coomassie blue G-250]. Proteins were analyzed on a 10% Tris-Tricine/SDS-polyacrylamide gel [30% acrylamide/0.8% bisacrylamide, Tris×HCl/SDS (pH 8.45), glycerol, 10% (w/v) ammonium persulfate, TEMED].

Results

Cloning of determinant for nickel and cobalt resistance (ncr). A 4.8-kb *Sal*I–*Eco*RI DNA fragment was cloned in several steps from the 70-kb plasmid pEJH501 of *H. alvei* 5-5 into the *Sal*I–*Eco*RI sites of phagemid vector pBluescript II KS (+), forming plasmid pHF14 [22]. When

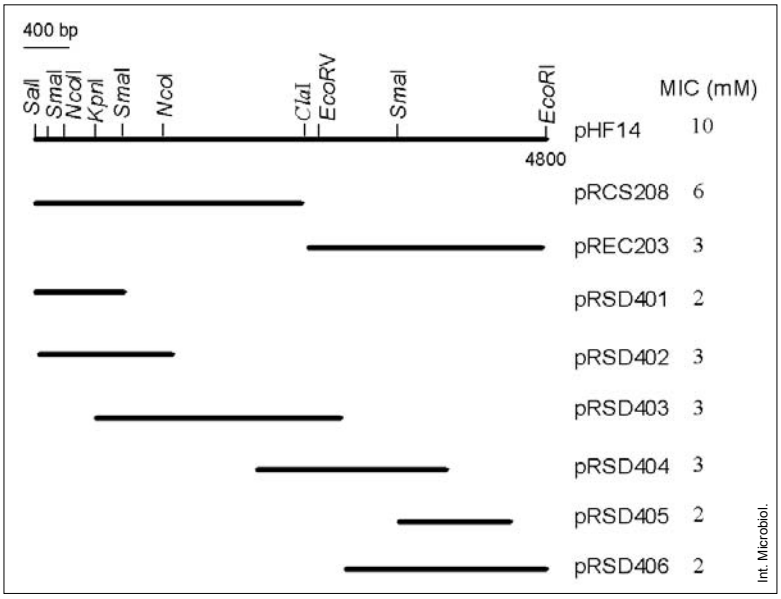

Fig. 1. Restriction map of the 4.8-kb *Sal*I–*Eco*RI fragment of pHF14 and subclones derived therefrom. The minimal inhibitory concentration (MIC) of nickel chloride for *Escherichia coli* DH5a strains bearing these plasmids are shown on the right.

plasmid pHF14 was transformed into *E. coli*, the cells grew in the presence of 10 mM nickel chloride. However, plasmids containing subcloned DNA fragments conferred only weak resistance to nickel, comparable to those with the plasmid pHF14 (Fig. 1). These results indicated that the 4.8-kb *Sal*I–*Eco*RI DNA fragment contains the intact determinant of nickel resistance and functions in *E. coli*. To determine whether a promoter was derived from sequences on the parent plasmid pEJH501, the 822-bp *Sma*I fragment from pHF14 was cloned into the *Sma*I site of plasmid pUJ9 containing the promoterless reporter gene *'lacZ* (Fig. 2). Cells of *E. coli* DH5a carrying the resulting *'lacZ* fusion plasmid pURS882 expressed enhanced β-galactosidase activity, as the concentration of nickel increased, while showing low levels in the absence of inducer. These results indicated that this fragment contains the promoter sequence of the intact nickel resistance determinant of pEJH501, whose expression is induced by nickel chloride.

Nucleotide sequence of the ncr operon. The locus for nickel and cobalt resistance was localized to the 4.8-kb *Sal*I-*Eco*RI fragment of DNA (Fig. 1). The complete nucleotide sequence of the cloned fragment was determined in both strands by sequence walking. Five potential open reading frames (ORFs) for protein-coding regions were identified by computer analysis along with the proposed initiation codons, stop codons, the proposed ribosome-binding sites, and the deduced amino acid sequences. These protein-coding regions were oriented in the same direction, and each ORF contained an AUG translation start codon together with a properly spaced ribosome-binding site (the nucleotide sequence of the 4800-bp DNA fragment of nickel resistance determinants is deposited under the accession no. AF322866).

The first ORF, *ncrA*, starts 403 nucleotides from the beginning of the insert and extends for 831 nucleotides, corresponding to 277 amino acids. *ncrA* has a weak ribosome-binding site, AGC, located ten nucleotides upstream from the initiation ATG codon. The *ncrA* product is a homologue of NreB from *A. xylosoxydans* 31A and of NrsD from *Synechocystis* sp. strain PCC6803 [6,13]. Both the amino acid composition (77% nonpolar amino acids) and the hydropathy profile (data not shown) indicate that the *nreA* gene product should be localized primarily in the membrane.

The second reading frame, *ncrB*, starting at position 1243 and ending at position 2145, encodes a protein of 301 amino acids that is a homologue of NreA of *A. xylosoxydans* 31A [15]. The overall sequence identity between NreA and NcrB was 78 % at the amino acid level. The presumed ribosome-binding site of *ncrB*, GG, is located 6 nucleotides upstream from the initiation ATG codon.

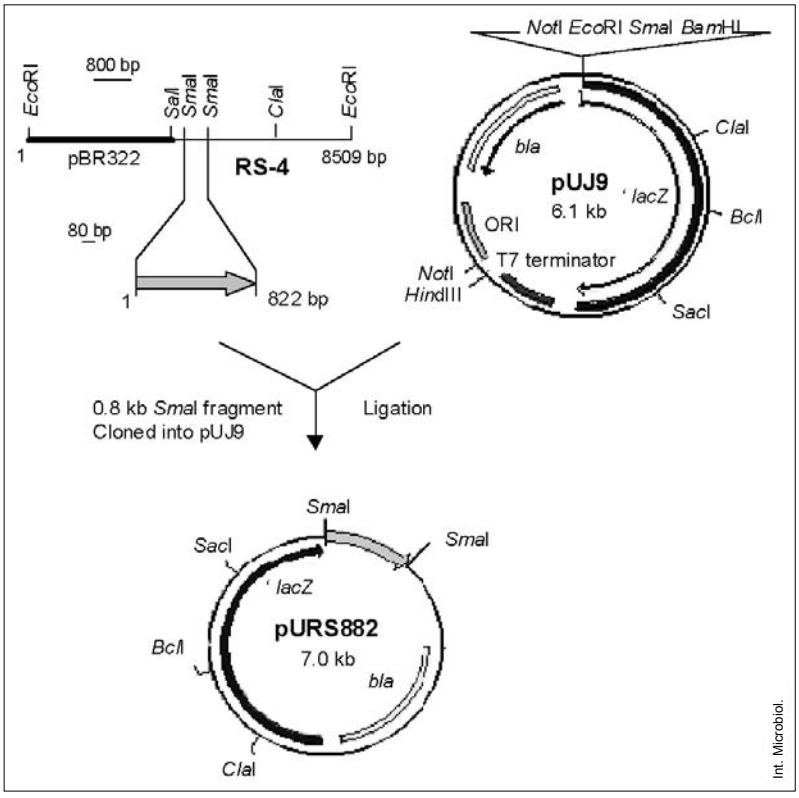

Fig. 2. Construction of recombinant plasmid pURS882, containing the promoter region including the 0.8-kb *Sma*I fragment from plasmid pHF14.

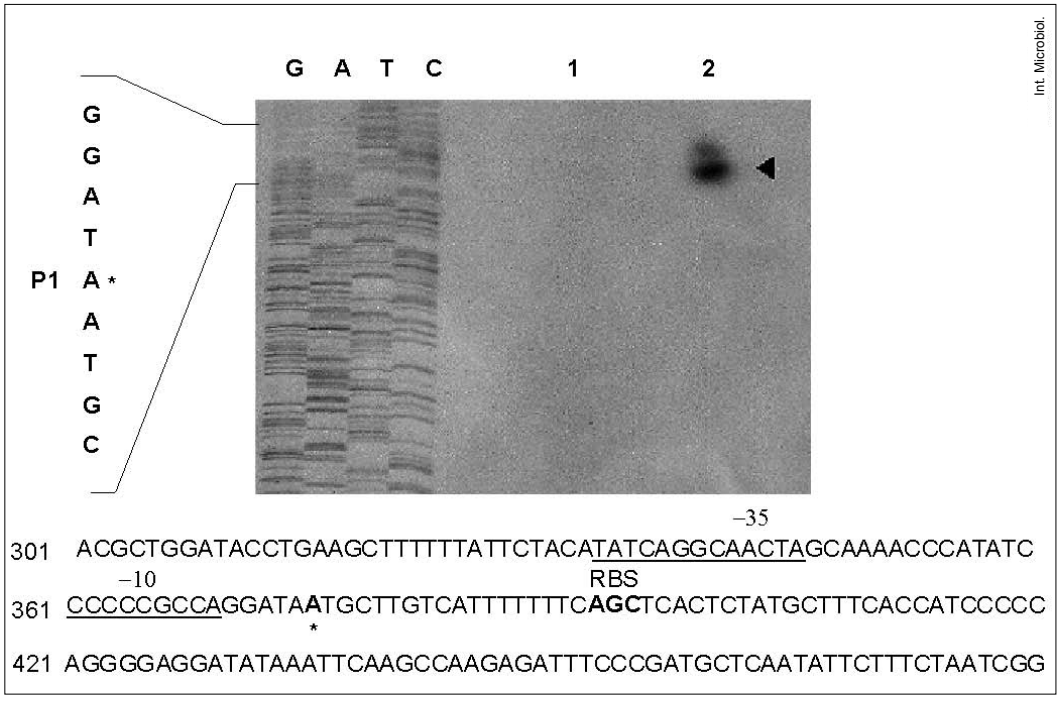

Fig. 3. Primer-extension analysis of the *ncrABC* transcript. RNA was hybridized with a primer complementary to 28–49 bp downstream of P1. Lane 1: negative control without reverse transcriptase; lane 2: extension products from cells induced by nickel chloride; lanes G, A, T, and C: sequence ladder.

The third reading frame, *ncrC*, beginning at position 2260 and ending at position 2715, comprises 456 bp, corresponding to 152 amino acids. The presumed ribosome-binding site, GGA, is located 15 nucleotides upstream from the initiation ATG codon. There was no similarity to known proteins involved in heavy metal resistance. There were no intergenic sequences between *ncrA* and *ncrB*, and no promoter-like sequence was found upstream from *ncrB* and *ncrC*, which suggested that *ncrABC*, conferring nickel resistance, should be read as a single transcript.

The fourth reading frame, *ncrY*, beginning at position 3709 and ending at position 3897, encodes 63 amino acids. The presumed ribosome-binding site, GGA, is located 8 nucleotides upstream from the initiation ATG codon. RT-PCR was used to search for the transcript of *ncrY* in *E. coli* DH5a after cloning into plasmid pHF14. Total RNA was isolated under induced condition and used as the template for RT reactions, using the primer at the 3′ end of *ncrY*. Using cDNA obtained from RT with *ncrY* primer, the PCR product was amplified for *ncrY*. In addition, the putative promoter was identified in the DNA region preceding *ncrY* and highly conserved between *cnrY*p, *cnrC*p, *nccY*p, and *nccC*p promoters. Previous work showed that transposon insertion in this region caused *E. coli* to constitutively express resistance [22]. This established that *ncrY* is expressed and may function as a *trans*-acting regulator.

The fifth reading frame, *ncrX*, starting at position 4354 and ending at position 4434, encodes a protein of 27 amino acids. Previous work showed that transposon insertions in this region also caused the constitutive expression of resistance [22]. Those data also suggested that this gene functions as *trans*-acting regulator.

RT-PCR experiments were done to search for a transcript corresponding to the region between *ncrC* and *ncrY*. There was no PCR product when a primer complementary to the region flanking the 3′ end of *ncrY* and the 5′ end of *ncrC* was used (data not shown). These data indicated that *ncrABC* and *ncrY* were separated by 993 nucleotides that were not transcribed.

Primer extension mapping of the *ncrABC* promoter. To determine the location of the *ncrABC* promoter, the transcriptional start site was mapped by primer extension (Fig. 3). One distinct extension product was obtained, corresponding to the A residue at a position 28 nucleotides upstream from the initiation ATG codon. The deduced start site is therefore 5′-C_{361}CCCCGCCAGGATAATGCTTGTCATTTTTTT-3′ (the +1 nucleotide A375 is underlined). As shown in Fig. 3, the –10 region (CCCCCGCCA) upstream of the transcriptional start is separated from the –35 region (TATCAGGCAACTA) by 14 nucleotides. This is a match to the canonical sequence recognized by σ^{32}-RNA polymerase.

Expression of *ncrABC* in *E. coli*. To determine whether *ncrABC* was transcribed from the intrinsic promoter and then expressed, the 4.8-kb *Sal*I–*Eco*RI fragment was

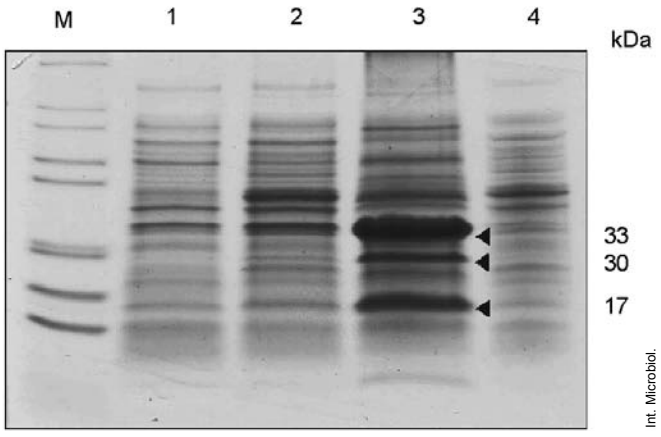

Fig. 4. SDS-PAGE of protein fractions. Proteins were separated on a SDS-12% polyacrylamide gel and stained with Coomassie brilliant blue. Lane M: molecular mass markers; lane l: total protein of *E. coli* BL21 (DE3) harboring pT7-6; lane 2: total protein of *E. coli* BL21 (DE3) harboring pHF14; lane 3: insoluble fraction of *E. coli* BL21 (DE3) harboring pHF14; lane 4: crude extract of *E. coli* BL21 (DE3) harboring pHF14.

inserted into the expression vector pT7-6, which yielded pTF714, in which the *ncr* operon was placed under the control of the T7 promoter. *E. coli* BL21 (DE3), harboring pNRS148, was grown in the presence of inducer, sonicated, and then fractionated into membrane and soluble parts. The inducible proteins were present in large amounts in the membrane fractions and were determined to have apparent molecular masses of 30, 33 and 17 kDa, as predicted for NcrA, NcrB, and NcrC (Fig. 4).

Discussion

The *cnr* system enables *R. metallidurans* CH34 to grow in the presence of 3 mM nickel and 5 mM cobalt ions [31]. The *ncc* system encodes high-level resistance to nickel (40 mM), cobalt (20 mM), and cadmium (1 mM) in *A. xylosoxidans* 31A [29]. The *nre* system also confers the ability to tolerate nickel (3 mM) to *A. xylosoxidans* 31A [30]. *Hafnia alvei* 5-5 was isolated from a soil-litter mixture underneath the canopy of the nickel-hyperaccumulating tree *Sebertia acuminata* in New Caledonia. The bacterium is able to grow in the presence of 30 mM nickel as well as 2 mM cobalt [27,35]. The level of nickel resistance is similar to that produced by *ncc* genes and higher than resistances conferred by the *cnr* and *nre* systems. It was of interest, therefore, to identify the components of the nickel pump in *H. alvei* 5-5 and to compare them with those of the *cnr*, *ncc*, and *nre* systems. As a first step, the genes were sequenced and the amino acid sequences of their potential protein products were deduced. The DNA sequence of a 4.8-kb *Sal*I–*Eco*RI fragment contained five *ncr* genes, *ncrA, ncrB, ncrC, ncrX*, and *ncrY*. The *ncrA* product is a homologue of NreB from *A. xylosoxydans* 31A [15] and NrsD from *Synechocystis* sp. strain PCC6803 [6,13]. Previous work suggested that NreB was responsible for low-level nickel resistance by efflux and closely related to MFS transporters [6]. From the hydropathic profile of the NcrA protein, there were 15 regions of 18 or more amino acid residues in length with a hydropathy index greater than 1.5, which is indicative of possible membrane-spanning α-helices for a transporter. NcrA has a broad range of metal-ion substrates, i.e. nickel, cobalt and zinc, while NreB is more specific for nickel [22].

Although no significant homology was found between NcrB, NcrC and proteins listed in the database, the overall hydropathy profile is similar to that of membrane proteins. Tn*PhoA′-1* mutagenesis has shown that the NcrB and NcrC are necessary for nickel resistance and transport [22]. This implies that NcrB and NcrC may in some way modify the substrate specificity and activity catalyzed by NcrA. Examples of proteins that change the substrate specificity of enzymes include ArsC. This protein changes the substrate specificity catalyzed by ArsA and ArsB thereby allowing recognition of both arsenate and arsenite [3] and σ factors, which alters the recognition site of RNA polymerase during carbon starvation and heat-shock response [9,11]. Based on these data, we propose a model in which NcrA, NcrB, and NcrC form a membrane-bound complex catalyzing cobalt and nickel efflux.

The promoter region of *nreABC* was characterized by primer extension. The major reverse transcript corresponded to a start site that was preceded by a σ^{32}-recognized sequence, indicating that Eσ^{32} would bind to this site with high affinity. Our results support this possibility in that transcriptional fusion of *lacZ* to this promoter produced high levels of β-galactosidase to heat shock response. This was the first instance in which a gene concerned with nickel resistance has been shown to be regulated by a σ^{32} promoter.

The level of nickel resistance by NcrABC was higher than that produced by NreA. One possibility is that the NcrABC complex provides a more effective mechanism of nickel resistance than NreA. This mechanism has been observed for the *ars* operon of the R-factor R773 from *E. coli* [2]. Cells expressing *arsB* exhibited an intermediate level of arsenite resistance compared with cells expressing both *arsA* and *arsB*. Arsenite exclusion by ArsB was coupled to electrochemical energy, while transport by the ArsA-ArsB complex was coupled to ATP hydrolysis. Another possibility is that the NcrABC complex is highly expressed, at levels comparable to those producing a higher level of resistance. The promoter

region of *nreABC* was shown to be regulated by σ^{32}, which also directs core RNA polymerase to transcribe heat-shock promoters. The transient increase in expression of heat-shock genes after temperature up-shift results from increased transcription initiation at heat-shock promoters, which is mediated by a transient 20-fold increase in the amount of σ^{32} per cell [9]. Higher expression of NcrABC would be a more effective mechanism of providing resistance to nickel ions present in heavily contaminated environments, resulting in a selective pressure for its evolution.

Acknowledgements. The authors wish to acknowledge the financial support of the Korea Research Foundation made in the program year of 1998 (015-D00216). We are also thankful for the financial support of the Alexander von Humboldt-Stiftung, Germany.

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Secuencia nucleotídica y expresión del determinante *ncr* de resistencia a níquel y cobalto en *Hafnia alvei* 5-5

Resumen. Los genes estructurales de la resistencia a níquel y cobalto del plásmido conjugativo pEJH 501 de *Hafnia alvei* 5-5, contenido en un fragmento *Sal*I-*Eco*RI de 4,8 kb, fueron clonados y secuenciados. La secuencia de DNA incluye cinco genes en el siguiente orden: *ncrA*, *ncrB*, *ncrC*, *ncrY*, y *ncrX*. Las secuencias de aminoácidos equivalentes a *ncrA* fueron homólogas a las secuencias de aminoácidos codificadas por *nreB* en *Achromobacter xylosoxidans* 31A. La expresión de los genes *ncr* mediante el sistema promotor de la RNA polimerasa T7 permite a *Escherichia coli* BL21 (DE3) sobreexpresar NcrA, NcrB, y NcrC, pero no NcrY ni NcrX. Los pesos moleculares aparentes de NcrA, NcrB y NcrC fueron 30, 33, y 17 kDa, respectivamente. El análisis de extensión de los cebadores mostró que el mRNA de *ncr* se iniciaba a una distancia de 23 nucleótidos corriente arriba del *ncrA*. La región promotora del operón *ncr* posee una fuerte secuencia promotora de tipo σ^{32} en la posición –35, y estudios transcripcionales de fusión con *´lacZ* indicaron que el elemento situado en –35 influye sobre la transcripción específica de σ^{32}. [**Int Microbiol** 2004; 7(1):27–34]

Palabras clave: plásmido pEJH501 · operón NcrABCYX · secuencia promotora de tipo σ^{32} · bacterias resistentes a níquel

Sequência nucleotídica e expressão do determinante *ncr* de resistência à niquel e cobalto em *Hafnia alvei* 5-5

Resumo. Os genes estruturais de resistência à níquel e cobalto do plasmídeo conjugativo pEJH 501 de *Hafnia alvei* 5-5, contido em um fragmento *Sal*I-*Eco*RI de 4800 pares de bases (pb), foram clonados e sequenciados. A sequência de DNA inclue cinco genes na seguinte ordem: *ncrA*, *ncrB*, *ncrC*, *ncrY*, e *ncrX*. As sequências de aminoácidos equivalentes à *ncrA* foram homólogas às sequências de aminoácidos codificadas para *nreB* em *Achromobacter xylosoxidans* 31A. A expressão dos genes de *ncr* mediante o sistema promotor da RNA polimerase T7 permite a *Escherichia coli* BL21 (DE3) supra-expressar NcrA, NcrB, e NcrC, porém não os genes NcrY e NcrX. Os pesos moleculares aparentes de NcrA, NcrB e NcrC foram 30, 33, e 17 kDa, respectivamente. A análise de extensão de iniciadores mostrou que o mRNA de *ncr* era iniciado a uma distância de 23 nucleotídeos antes de *ncrA*. A região promotora do operon de *ncr* possue uma sequência promotora putativa forte do tipo σ^{32} na posição –35, e estudos transcricionais de fusão com *´lacZ* indicaram que o elemento situado na posição –35 tem influência na transcrição específica σ^{32}. [**Int Microbiol** 2004; 7(1):27–34]

Palavras chave: plasmídeo pEJH501 · operon NcrABCYX · sequência promotora do tipo σ^{32} · bactérias resistentes ao níquel