J. Microbiol. Biotechnol. (2002), **12**(1), 114–120

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Characterization of the Nickel Resistance Gene from *Legionella pneumophila*: Attenuation of Nickel Resistance by *ppk* (polyphosphate kinase) Disruption in *Escherichia coli*

HAHM, DAE-HYUN*, MI-JUNG YEOM, WHAE-MIN KO[1], EUNJOO H. LEE, HYE-JUNG LEE, INSOP SHIM, AND HONG-YEOUL KIM[1]

Department of Oriental Medical Science, Graduate School of East-West Medical Science, Kyung Hee University, Youngin-Si, Kyungki-Do 449-701, Korea
[1]Department of Molecular Biology, Institute of Oriental Medicine, College of Oriental Medicine, Kyung Hee University, Seoul 130-701, Korea

Received: August 30, 2001
Accepted: November 23, 2001

Abstract A 1,989-bp genomic region encoding nickel resistance genes was isolated from *Legionella pneumophila*, a pathogen for legionellosis. From a sequencing and computer analysis, the region was found to harbor two structural genes, a *nreB*-like protein gene (1,149 bp) and a *nreA*-like protein gene (270 bp), in a row. Both genes exhibited a significant degree of similarity to the corresponding genes from *Synechocystis* sp. PCC6803 (54% amino acid sequence identity) and *Achromobacter xylosoxidans* 31A (76%). The gene was successfully expressed in *E. coli* MG1655 and conferred a nickel resistance of up to 5 mM in an LB medium and 3 mM in a TMS medium including gluconate as the sole carbon source. *E. coli* harboring the nickel resistance gene also exhibited a substantial resistance to cobalt, yet no resistance to cadmium or zinc. Since the extracellular concentration of nickel remained constant during the whole period of cultivation, it was confirmed that the nickel resistance was provided by an efflux system like the Ni^{2+} permease (*nrsD*) of *Synechocystis* sp. strain PCC6803. Since polyphosphate (poly-P) is known as a global regulator for gene expression as well as a potential virulence factor in *E. coli*, the nickel resistance of a *ppk* mutant of *E. coli* MG1655 harboring the nickel resistance gene from *L. pneumophila* was compared with that of its parental strain. The nickel resistance was significantly attenuated by *ppk* inactivation, which was more pronounced in an LB medium than in a TMS medium.

Key words: Nickel resistance gene, *Legionella pneumophila*, polyphosphate kinase

Nickel, a transition metal, is an essential cofactor for several metallo-enzymes catalyzing the reversible oxidation of carbon monooxide, hydrolysis of urea, methanogenesis, detoxification of superoxide radicals, and production of molecular hydrogen in both prokaryotic and eukaryotic cells [6]. However, like the other essential metals acting as cofactors in enzymatic reactions, nickel ions are also very toxic to living cells at high extracellular concentrations through interfering with the function of intracellular macromolecules and generating toxic free radicals. Therefore, transporters, which are responsible for the uptake or export of nickel ions through the membrane barrier, have evolved in different bacteria to maintain the appropriate intracellular concentration of nickel [1, 4, 7]. With regards bacterial resistance to nickel, two different kinds of energy-dependent specific efflux systems have been reported: one is chemiosmotic cation/proton antiporters, such as the *cnr* of *Ralstonia* sp. (formerly *Alcaligenes eutrophus* strain CH34) and the *ncc* of *Achromobacter xylosoxydans* 31A (formerly *Alcaligenes xylosoxidans*), while the other is the P-type ATPase of *Helicobacter pylori*, which produces Ni^{2+}-containing urease for gastric survival [4, 15, 18]. It has been suggested that the Ni^{2+} efflux pumps of *Helicobacter pylori* may function as safety valves to attenuate an overload of Ni^{2+} by rapid accumulation, while mediated by the high-affinity uptake system, NixA, for the synthesis of active urease in the bacterium [3, 5, 15]. In addition to an *ncc* operon for high-level nickel resistance, *A. xylosoxydans* 31A, isolated from an environment highly polluted with heavy metals, has been shown to have another genetic loci, *nreB*, for low-level nickel resistance on the megaplasmid, pTOM9 [18]. Although the resistance mechanism of the *nreB* of *A. xylosoxydans* 31A has not yet been characterized in detail, it is known that the gene encodes a Ni^{2+} transporter responsible for nickel ion efflux and can confer a nickel resistance of up to 3 mM of $NiCl_2$ to *E. coli* as well as *A. xylosoxydans* 31A [9, 18]. Computer analyses and *in vivo*

*Corresponding author
Phone: 82-31-201-2198; Fax: 82-31-204-4237;
E-mail: dhhahm@nms.kyunghee.ac.kr

mutational studies have also reported putative homologues of *nreB* in other strains, such as *Klebsiella* sp. and *Synechocystis* sp. (also named *nrsD*) as members of a major facilitator superfamily [8, 16, 18]. *Legionella pneumophila*, a human infectious pathogen, is known to be a causative agent of acute pneumonia (Legionnaires disease) and sometimes Pontiac fever. This pathogen is known to reside in warm and stagnant water, such as that found in water plumbing systems, hot-water tanks, and cooling towers, *etc*. From an investigation of the chemical composition of such habitats in which the concentrations of certain metals, including nickel, are relatively high due to corrosion of the metal container, it has been concluded that several metal ions may be important factors in the survival of *L. pneumophila* [12, 21]. Accordingly, the current study reports on the nickel resistance gene of *L. pneumophila* and its expression in *E. coli* in both a minimal and complex medium. In addition, experimental clues are presented that describe the dependency of the phenotypic expression of the nickel resistance gene of *L. pneumophila* on *ppk*, known as the universal virulence factor in certain pathogenic bacteria.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, Media, and Growth Conditions

The bacterial strains and plasmids used in this study are listed in Table 1. The *ppkx* mutant of *E. coli* MG1655 was constructed by the deletion-insertion inactivation of both *ppk* and *ppx* (exopolyphosphatase) using a kanamycin resistance cassette [14]. Since 98.5% of PPK and 78% of PPX were removed from the *ppkx* mutant, the *ppkx* mutant is referred to as the *ppk* mutant in this work. The virulent strain AA100 of *L. pneumophila*, obtained from the University of Kentucky Chandler Medical Center was used for the isolation of the chromosomal DNA. The vector pBR322 was used for the promoter proving study. The *L. pneumophila* was cultivated in a BCYE (buffered charcoal yeast extract) or BYE broth and the *E. coli* strains were grown on an LB or modified TMS (tris-buffered medium salt) medium. The compositions of the modified TMS were as follows: 6.0 g Tris-Cl l^{-1}, 4.7 g NaCl l^{-1}, 1.5 g KCl l^{-1}, 1.0 g NH_4Cl l^{-1}, 0.4 g Na_2SO_4 l^{-1}, 2.7 g Na_2HPO_4 l^{-1}, 0.025 g $MgSO_4 \cdot 7H_2O$ l^{-1}, 0.15 g $CaCl_2 \cdot 2H_2O$ l^{-1}. Gluconate (10 mM) was used as the sole carbon source and the medium pH was adjusted to 7.0. Most of the liquid cultures were executed at 37°C in a shaking incubator (200 rpm). Ampicillin (50 μg ml^{-1}) and kanamycin (30 μg ml^{-1}) were added as needed for selection. The $NiCl_2$, $CoCl_2$, $CdCl_2$, and $ZnCl_2$ were all purchased from Sigma-Aldrich Co. (MO, U.S.A.).

Construction of Partial Genomic Library of *L. pneumophila*

The CTAB/NaCl method was used for the preparation of the chromosome from *L. pneumophila* [2]. The high molecular chromosomal DNA was partially digested with *Sau*3AI and separated by agarose gel electrophoresis. The DNA fragments with a suitable size (4–12 kb) were eluted from the gel and cloned into the *Bam*HI site of pUC19. *E. coli* DH5α' was transformed with the ligated DNA mixture and plated on LB agar plates supplemented with ampicillin and X-gal (80 μg ml^{-1}) for blue/white screening.

Cloning of Nickel Resistance Gene

The white colonies on the plates were directly transferred to new LB plates including ampicillin and 3 mM of $NiCl_2$. After overnight incubation at 37°C, the colonies exhibiting substantial growth were selected for further study.

Plasmid Preparation and DNA Sequencing

The double-stranded plasmid DNA was purified using a Qiaprep Spin Miniprep Kit (Qiagen Co., Germany) and

Table 1. Bacterial strains and plasmids used in the current study.

Strain or plasmid	Relevant characteristics	Reference
Escherichia coli		
DH5α'	*80d lacZ ΔM15, endA1, recA1, hsdR17(r_k^-, m_k^+), supE44, thi-1, gyrA1, relA1, Δ(lacZYA- argF), U169*	NEBioLabs (Beverly, MA)
MG1655	wild-type K12 strain	[11]
CF5802	*Δppkx::kan* deletion-insertion mutant of MG1655	[13]
Legionella pneumophila		ATCC No.
AA100	virulent strain of *L. pneumophila*	BAA-74
Plasmids		
pBR322	Ap^r, Tc^r, pMB1 ORI	NEBioLabs
pUC19	Ap^r, lacZ', lac promoter	NEBioLabs
pKH1	4.4-kb DNA fragment from *L. pneumophila* AA100 into *Bam*H1 site of pUC19	This study

sequenced in both directions. The sequencing of the DNA fragments in pUC19 was performed with a DNA Analyzer PE 3700 (Perkin Elmer Inc., MA, U.S.A.) using a Big Dye Terminator Cycle sequencing kit. The BLAST network service was used for protein homology searching.

Assay Methods

The bacterial growth was monitored by measuring the increase in optical density at 580 nm using HP8453 (Hewlett-Packard Co., CA, U.S.A.) spectrophotometer. An Inductively Coupled Plasma Atomic Emission Spectrometer IRIS-AP (Thermo Jarrell Ash Co., MA, U.S.A.) was used to determine the residual amount of nickel in the minimal medium. The instrumental operating conditions of the ICP-AES were as follows: Flush pump rate, 1.85 ml min^{-1}; Analysis pump rate, 1.85 ml min^{-1}; Nebulizer pressure, 32 psi; auxiliary gas flow rate, 0.5 liter min^{-1}; Relaxation time, 0 s.

Results and Discussion

Cloning and Sequencing of Nickel Resistance Gene from *L. pneumophila*

Several clones from the genomic library of *L. pneumophila* were recovered after screening for nickel resistance. Among them, a recombinant plasmid, pKH1, harboring the smallest size of DNA insert (about 4.4 kb) was selected for sequencing. The whole sequence of the DNA insert in pKH1 revealed that the nickel resistance determinants consisted of two putative open reading frames at a distal end (Fig. 1). The nucleotide sequence of the nickel resistance gene was submitted to the GenBank under accession number AY030282. From the sequence analysis using the BLAST search engine of NCBI, it was found that the open reading frames exhibited significant homologies with other low-level nickel resistance genes, such as the *nreAB* operon of *A. xylosoxydans* 31A (GenBank accession No. L31491) and *nreB* of *Synechocystis* sp. (GenBank accession No. D64005). However, there was little homology with other representative nickel resistance genes, such as the *cnr* of *Ralstonia* sp. strain CH34 and *ncc* of *A. xylosoxidans* 31A [10, 18]. The sequence also exhibited putative -35 and -10 promoter region, ribosomal binding sites, and translational start and stop signals. To examine the existence of an intrinsic promoter in the cloned gene, the whole region of DNA insert in pKH1 was moved to the *Ssp*I site of pBR322, and then the construct was used to transform *E. coli* cells. Since the *E. coli* transformants still showed a comparable nickel resistance with those harboring pKH1 in both solid and liquid media, the expression of the nickel gene was confirmed to be controlled by its own promoter (data not shown). The alignment of the deduced amino acids of the cloned gene with those of other nickel resistance genes showed 54% amino acid sequence identity with the NreB of *Synechocystis* sp. PCC6803 and 76% with the NreB in the pTOM9 of *A. xylosoxidans* 31A (Fig. 2). In addition, there was also a region exhibiting a 73% amino acid sequence identity with the NreA in the pTOM9 of *A. xylosoxidans* 31A. Unlike the arrangement of the *nreAB* operon of *A. xylosoxydans* 31A, the *nreA*-like protein gene was located behind the *nreB*-like protein gene and 264 bp apart in *L. pneumophila*. There has been no previous report on the resistance mechanism and functions of the *nreA* or *nreB* of *A. xylosoxydans* 31A. Therefore, to examine whether the *nreA*-like protein gene of *L. pneumophila* contributed to the nickel resistance of the *E. coli* transformants, the region of the *nreA*-like protein gene was removed from the DNA insert in pKH1 through the deletion of the DNA fragment from *Nco*I to *Nru*I (Fig. 1). It was found that the *nreB*-like gene was sufficient to produce nickel resistance, irrespective of the existence of the *nreA*-like protein gene in the *E. coli* cells. This means that the *nreA*-like protein gene of *L. pneumophila* was not properly expressed or its contribution to the nickel resistance was not substantial in *E. coli*.

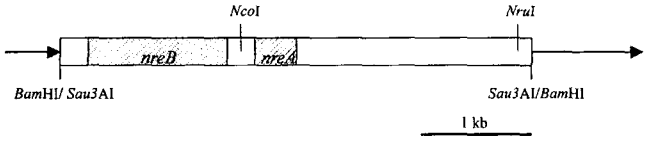

Fig. 1. Restriction map of pKH1.
Double line, insert DNA fragment including *nreA*- and *nreB*-like protein genes; single line, pUC19.

Growth Characteristics of Recombinant *E. coli* Harboring Nickel Resistance Gene

The *E. coli* MG1655 transformed with pKH1 was cultivated in an LB or TMS minimal medium under various concentrations of nickel ions. Figure 3 shows the growth curves of three different strains of *E. coli* MG1655, i.e. a wild-type, a recombinant with pKH1, and a recombinant with pUC19 in LB media including 0, 3, or 5 mM of $NiCl_2$. Although a minimal difference was observed between the three strains in the nickel-free media in terms of the growth rate and final yield, the *E. coli* recombinant cells with pKH1 exhibited outstanding growth in the media containing high concentrations of nickel ions, as shown in Fig. 3. No strain showed any detectable growth with a nickel concentration of over 5 mM. In a TMS medium, the *E. coli* cells with pKH1 exhibited similar patterns of nickel resistance as in the LB medium, except that the upper limit for the nickel concentration at which a considerable cell growth was observed was 3 mM (Fig. 4). This threshold concentration for nickel ions implies that the nickel-resistant gene of *L. pneumophila* in pKH1 included an enzymatic property of low-level nickel resistance as in the *cnr* of *Ralstonia* sp. strain CH34 and *nre* of *A. xylosoxidans* 31A. In general,

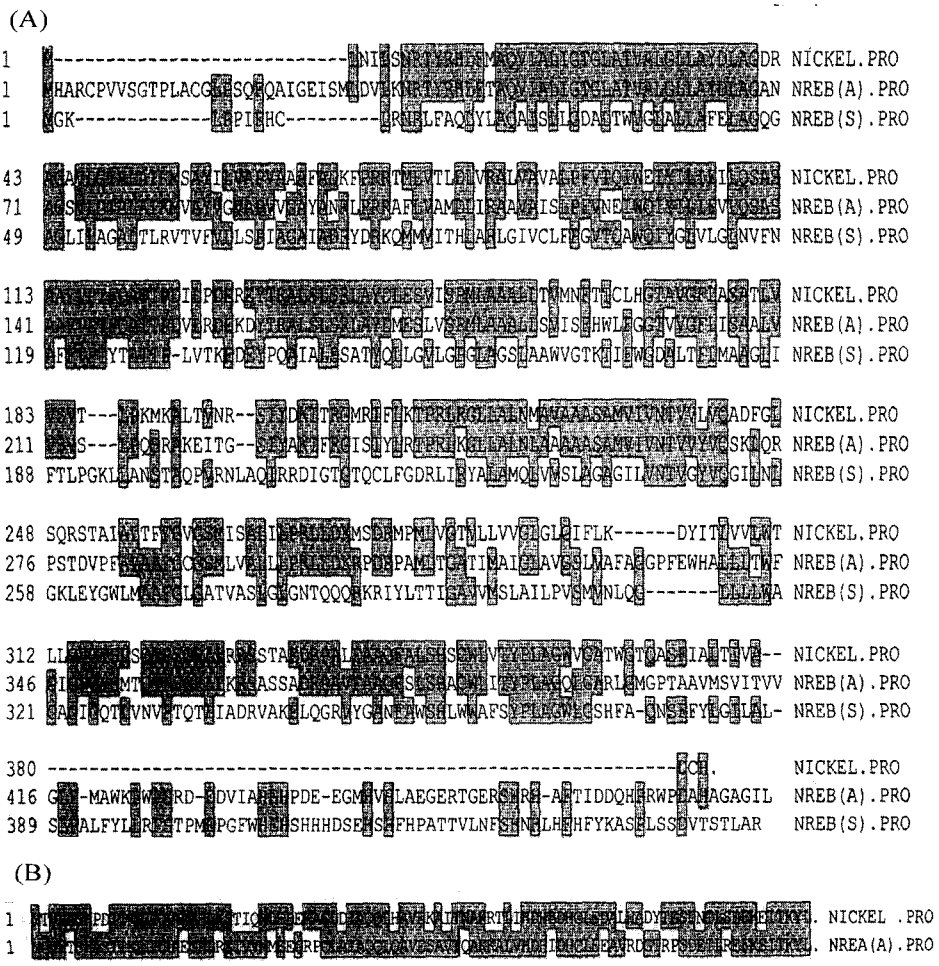

Fig. 2. Sequence similarities of deduced amino acid sequences of nickel resistance gene from *Legionella pneumophila* with those of other nickel resistance genes.

The upper lines of (A) and (B) indicate *nreB*- and *nreA*-like proteins from *Legionella pneumophila*, respectively; the middle line of (A) indicates NREB from *A. xylosoxidans* 31A; the bottom lines of (A) and (B) indicate NREB from *Synechocystis* sp. PCC6803 and NREA from *A. xylosoxidans* 31A, respectively. The identical amino acids are shown in gray boxes.

high-level nickel resistance genes exhibit the resistance with up to 40 mM of nickel ions as in the case of the *ncc* of *A. xylosoxidans* 31A and *A. eutrophus* KT02 [18, 19]. *E. coli* cells are known to have two kinds of nickel transport system: one is *nikABCDE*, an archetypal ABC transporter, which is highly specific and tightly regulated by the external nickel concentration, and the other is *corA*, a low-affinity high capacity Mg^{2+} transport system, which can contribute to a nonspecific and massive Ni^{2+} uptake under physiological conditions [6]. Therefore, at higher nickel concentrations than those of natural environments (5–30 nM), nickel ions are readily taken up by the Mg^{2+} transport system and then are pumped out of the cells by an energy-dependent efflux system. As shown in Fig. 5, the concentration of external nickel ion remained constant throughout the whole period of batch cultivation in a TMS minimal medium. Accordingly, this study would seem to suggest that the nickel ions that readily entered the cells were excluded by an efflux pump encoded by the nickel resistance gene of *L. pneumophila*, although the resistance mechanism was not characterized in detail.

Resistance to Other Heavy Metals and Antibiotics

Most nickel-resistant determinants are known to exhibit resistance to other transition metals as well as nickel, for example, the *cnr* of *A. eutrophus* KTO2 (Co^{2+}, Cr^{2+}, and Hg^{2+}), the *ncc* of *A. xylosoxidans* 31A (Co^{2+} and Cd^{2+}), and P-type ATPase of *H. pylori* (Co^{2+}, Cd^{2+}, and Zn^{2+}) [17, 18]. With regards to the *nre* system of *A. xylosoxidans* 31A, little information has been reported about its metal specificity and functional mechanism except that it has evolved separately from *ncc* and is a widespread gene among nickel resistance bacteria [18]. To examine the substrate specificity of the nickel resistance gene of *L. pneumophila*, its resistance to other metal ions, such as Co^{2+}, Cd^{2+}, and Zn^{2+}, was tested in an LB medium. The *E. coli* MG1655

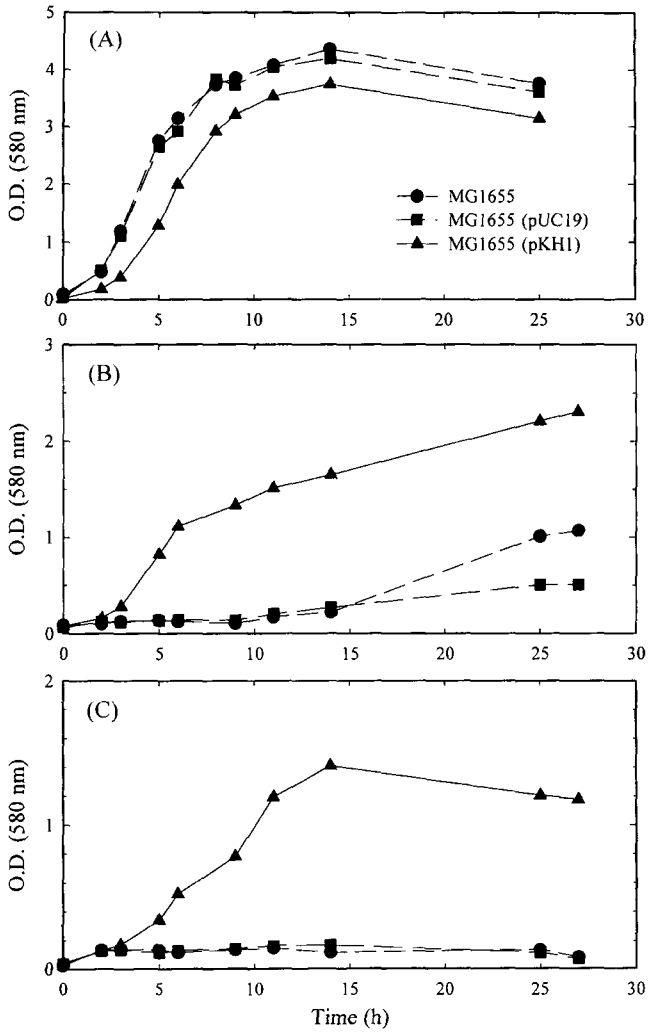

Fig. 3. Time courses of growth curves of MG1655 wild-type, recombinant harboring pUC19, and recombinant harboring pKH1. The LB media including 0 mM (A), 3 mM (B), or 5 mM (C) of $NiCl_2$ were used to cultivate the strains.

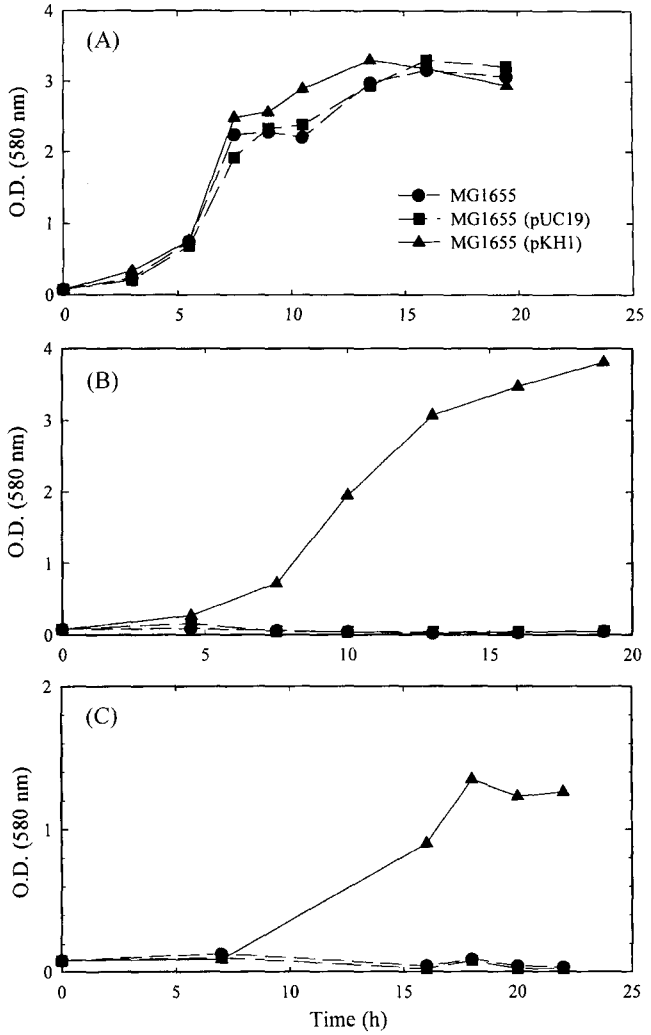

Fig. 4. Time courses of growth curves of MG1655 wild-type, recombinant harboring pUC19, and recombinant harboring pKH1. The modified TMS media including 0 mM (A), 1 mM (B), or 3 mM (C) of $NiCl_2$ were used to cultivate the strains.

transformed with pKH1 exhibited a comparable resistance to cobalt, as shown in Fig. 6. However, no resistance was observed against cadmium or zinc. Whereas most nickel resistance determinants, such as *ncc*, *cnr*, and *czc*, are known to exhibit a broad range of metal specificity, the *nreB* determinant is relatively specific for nickel ions [10, 18, 19]. Recently, it was also reported that the *nre* determinant of *A. xylosoxidans* 31A has a strict metal specificity for only nickel [9]. Since metal resistance is often associated with antibiotic resistance, the resistance to various kinds of commercial antibiotics, such as ampicillin, kanamycin, chloramphenicol, and tetracyclin was also tested. However, no substantial resistance was observed, which means that the nickel resistance of the gene could not be attributed to the function of multi-drug resistance (MDR) (data not shown).

Expression of Nickel Resistance Gene in *ppk* Knock-Out Mutant of *E. coli*

It is known that poly-P exists in almost all kinds of living organisms, from bacteria to animal cells, and has many biochemical and physiological functions, such as a reservoir of phosphate ion (P_i), chelator of metal ions, buffer against alkali, cell capsule, channel for DNA transformation, and global regulator for stress responses and survival in *E. coli* and other bacterial cells [13, 20]. It has also been reported that *ppk*, a gene for poly-P synthesis, is highly conserved among diverse bacterial species, including some of the principal pathogenic bacteria, and is also essential for stationary responses through the control of *rpoS* (a sigma factor for starvation gene expression) in *E. coli* [13]. Based on these observations, the dependence of *ppk* on the virulence of pathogenic bacteria has been argued and *ppk*

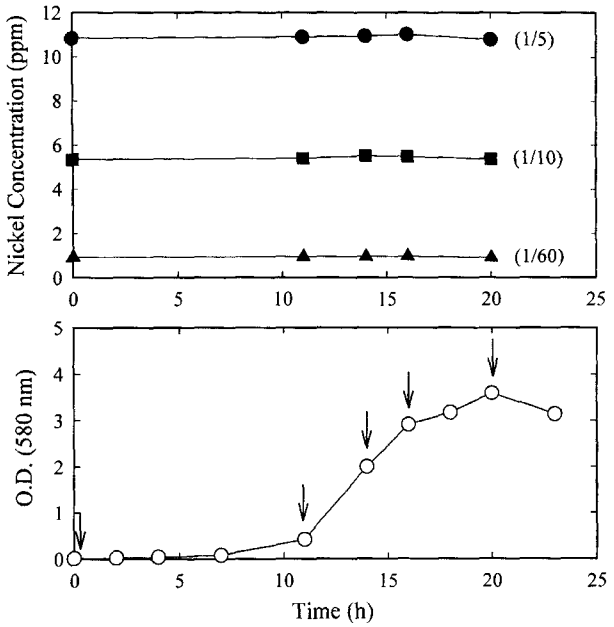

Fig. 5. Time course of residual nickel concentration during cultivation of MG1655 harboring pKH1 in modified TMS medium including 1 mM of $NiCl_2$.
The arrows indicate the points at which the supernatant was sampled and each fraction in the bracket means the dilution factor by which the supernatant was diluted with distilled water.

is now considered as a novel target for the development of new antibacterial drugs. To examine whether the expression of the nickel gene of *L. pneumophila* was affected by PPK in *E. coli*, the growth profiles of *ppk* mutants of *E. coli* harboring pKH1 were compared with those of wild-type strains harboring the same plasmid in the LB and minimal media including nickel ions. As shown in Fig. 7, the *ppk*

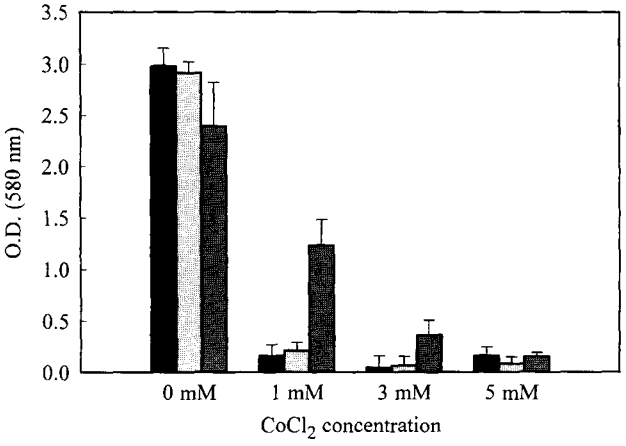

Fig. 6. Comparisons of cobalt resistance of MG1655 wild-type (■), recombinant harboring pUC19 (□), and recombinant harboring pKH1 (▩).
The LB media including 0, 1, 3, and 5 mM of $CoCl_2$ were used to cultivate the strains. The optical density was measured after 20 h of cultivation.

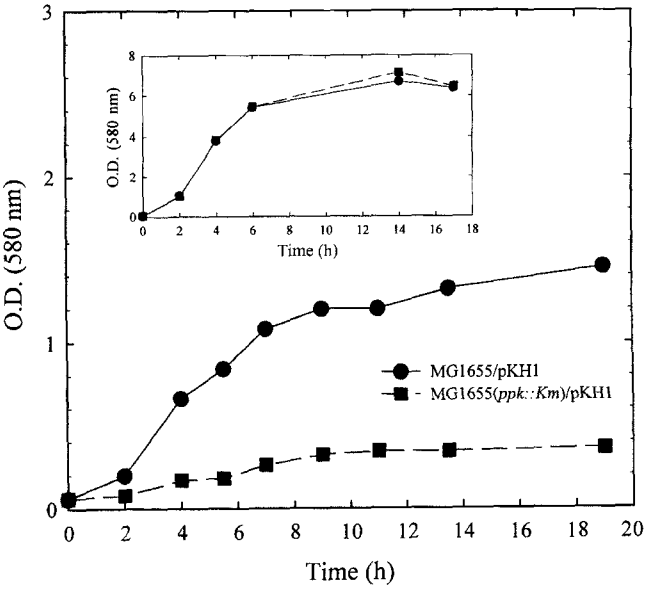

Fig. 7. Time courses of growth curves of MG1655 wild-type and its *ppk* mutant harboring pKH1 in LB medium including 3 mM of $NiCl_2$.
The growth curves of both strains in LB without nickel ions are shown in the inset graph.

mutant with pKH1 was unable to grow in the LB including 3 mM of $NiCl_2$, whereas the wild-type strain could grow under the same conditions. There was little difference in the growth profiles between the *ppk* mutant and the wild-type strain in the LB without nickel ions. This means that PPK positively affected the expression of the nickel resistance gene of *L. pneumophila* either directly or indirectly in *E. coli* cells. However, the attenuation of the growth rate of the *ppk* mutant with pKH1 was not substantial in a TMS minimal medium including 1 mM of $NiCl_2$, despite a longer

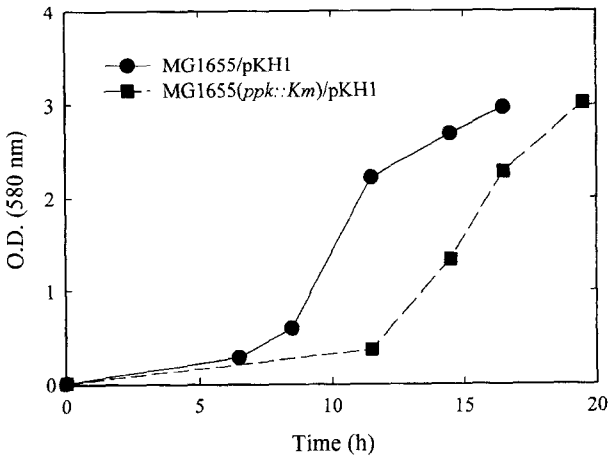

Fig. 8. Time courses of growth curves of MG1655 wild-type and its *ppk* mutant harboring pKH1 in modified TMS medium including 1 mM of $NiCl_2$.

lag at the beginning of the growth (Fig. 8). This might be due to the high concentration of external P_i which was required to support substantial growth as a nutritional component. However, it has not yet been proved that the *ppk* response in *E. coli* is directly affected by the external concentration of P_i in the medium, although putative *pho* boxes has been found in the *ppk* promoter region [13]. It was recently reported that one of the novel functions of *ppk* is to positively regulate the expression of many stress-inducible genes, such as *rpoS*-dependent genes (osmotic stress, oxidative stress, and thermo-tolerance genes, *etc.*) and SOS genes through controlling the intracellular levels of poly-P [20, 22]. The current study was unable to determine whether the expression of the nickel resistance gene was controlled directly by poly-P or indirectly by another global mediator under the control of poly-P. However, it was concluded that the expression of the nickel resistance gene of *L. pneumophila* inevitably required *ppk* expression in the *E. coli* cells and thus may be linked to the survival in their barren habitats.

Acknowledgment

The authors would like to thank Dr. Michael Cashel (Lab. of Molecular Genetics, NICHD, NIH) for providing the *ppkx* mutant of *E. coli*.

References

1. Agranoff, D. D. and S. Krishna. 1998. Metal ion homeostasis and intracellular parasitism. *Mol. Microbiol.* **28:** 403–412.
2. Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl. 1991. *Current Protocols in Molecular Biology*, 2nd ed. John Wiley & Sons, New York, U.S.A.
3. Bauerfeind, P., R. M. Garner, and H. L. Mobley. 1996. Allelic exchange mutagenesis of nixA in *Helicobacter pylori* results in reduced nickel transport and urease activity. *Infec. Immun.* **64:** 2877–2880.
4. Bruins, M. R., S. Kapil, and F. W. Oehme. 2000. Microbial resistance to metals in the environment. *Ecotoxicol. Environ. Safety* **45:** 198–207.
5. Burner, A. B. and Y.-Y. M. Chen. 2000. Bacterial urease in infectious diseases. *Microbial Infec.* **2:** 533–542.
6. Eitinger, T. and M.-A. Mandrand-Berthelot. 2000. Nickel transport system in microorganisms. *Arch. Microbiol.* **173:** 1–9.
7. Francis, M. S. and C. J. Thomas. 1997. Mutants in the CtpA copper transporting P-type ATPase reduce virulence of *Listeria monocytogenes*. *Microb. Pathogenesis* **22:** 67–78.
8. Garcia-Dominguez, M., L. Lopez-Maury, F. J. Florencio, and J. C. Reyes. 2000. A gene cluster involved in metal homeostasis in the cyanobacterium *Synechocystis* sp. strain PCC6803. *J. Bacteriol.* **182:** 1507–1514.
9. Grass, G., B. Fan, B. P. Rosen, K. Lemke, H.-G. Schlegel, and C. Rensing. 2001. NreB from *Achromobacter xylosoxidans* 31A is a nickel-induced transporter conferring nickel resistance. *J. Bacteriol.* **183:** 2803–2807.
10. Grass, G., C. Grobe, and D. H. Nies. 2000. Regulation of the *cnr* cobalt and nickel resistance determinant from *Ralstonia* sp. strain CH34. *J. Bacteriol.* **182:** 1390–1398.
11. Jensen, K. F. 1993. The *Escherichia coli* K-12 "wild types" W3110 and MG1655 have an rph frameshift mutation that leads to pyrimidine starvation due to low pyrE expression levels. *J. Bacteriol.* **175:** 3401–3407.
12. Kang, B. C., J. H. Park, Y. S. Lee, J.-W. Suh, J.-H. Chang, and C.-H. Lee. 1999. Effect of AL072, a novel anti-*Legionella* antibiotic, on growth and cell morphology of *Legionella pneumophila*. *J. Microbiol. Biotechnol.* **9:** 371–375.
13. Kornberg, A., N. N. Rao, and D. Ault-Riche. 1999. Inorganic polyphosphate: A molecule of many functions. *Annu. Rev. Biochem.* **68:** 89–125.
14. Kuroda, A., H. Murphy, M. Cashel, and A. Kornberg. 1997. Guanosine tetra- and pentaphosphate promote accumulation of inorganic polyphosphate in *E. coli*. *J. Biol. Chem.* **272:** 21240–21243.
15. Melchers, K., L. Herrmann, F. Mauch, D. Bayle, D. Heuermann, T. Weitzenegger, A. Schuhmacher, G. Aschs, R. Haas, G. Bode, K. Bensch, and K. P. Schafer. 1998. Properties and functions of the P-type ion pumps cloned from *Helicobacter pylori*. *Acta Physiol. Scand. Suppl.* **163:** 123–135.
16. Pao, S. S., I. T. Paulsen, and M. H. Saier, Jr. 1998. Major facilitator superfamily. *Microbiol. Mol. Biol. Rev.* **62:** 1–34.
17. Silver, S. 1996. Bacterial resistances to toxic metal ions - a review. *Gene* **179:** 9–19.
18. Schmidt, T. and H. G. Schlegel. 1994. Combined nickel-cobalt-cadmium resistance encoded by the *ncc* locus of *Alcaligenes xylosoxidans* 31A. *J. Bacteriol.* **176:** 7045–7054.
19. Schmidt, T., R.-D. Stoppel, and H. G. Schlegel. 1991. High-level nickel resistance in *Alcaligenes xylosoxydans* 31A and *Alcaligenes eutrophus* KTO2. *Appl. Environ. Microbiol.* **57:** 3301–3309.
20. Shiba, T., K. Tsutsumi, K. Ishige, and T. Noguchi. 2000. Inorganic polyphosphate kinase: Their novel biological functions and applications. *Biochemistry (Moscow)* **65:** 315–323.
21. States, S. J., L. F. Conley, M. Ceraso, T. E. Stephenson, R. S. Wolford, R. M. Wadowsky, and R. B. Yee. 1985. Effects of metals on *Legionella pneumophila* growth in drinking water plumbing systems. *Appl. Environ. Microbiol.* **50:** 1149–1154.
22. Tsutsumi, K., M. Munekata, and T. Shiba. 2000. Involvement of inorganic polyphosphate in expression of SOS genes. *Biochim. Biophys. Acta* **1493:** 73–81.