Identification Of A Unique Mechanism Of Tolerance Against Nickel In Bacillus Cereus Isolated From Heavy Metal Contaminated Sites.

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Abstract

Bacillus cereus is rod-shaped, Gram-positive, sporulating, aerobe or facultative anaerobe. A Bacillus cereus strain, CMG2K4 was isolated from metal contaminated soil sample. CMG2K4 showed tolerance against nickel chloride up to the concentration of 10 mM. Growth curve pattern has indicated constitutive nature of tolerance and absence of plasmid revealed chromosomally located genes of tolerance. SDS-PAGE of Bacillus cereus cell lysate grown in presence of 1 mM of nickel chloride showed overproduction of ~36kDa protein. Based on N-terminal sequencing and Mass spectrometry fingerprinting this protein was identified as flagellin. Over-expression of flagellin in Bacillus cereus, CMG2K4 has pointed out a role of this protein in nickel tolerance. Potential benefits of flagellin include increased efficiency of nutrient acquirement and prevention of toxic substances to them.

INTRODUCTION

Heavy metals are increasingly found in our environment due to natural and industrial processes (Summers, 1984). These are trace metals with a density at least five times that of water (Morris, 1992). Heavy metals such as copper, nickel, zinc, manganese, cobalt, molybdenum, and vanadium are essential for normal life, although elevated concentrations of both essential and non-essential metals result in growth inhibition by inhibiting the activity of sensitive enzymes (Ahmed et al., 2005).

Most of the essential cell components are potential targets for metal induced damage such as DNA replication, as a result of which cell death can occur (Malakul et al., 1998). Bacteria, being one of the most primitive life forms on earth, naturally developed tolerance to a wide range of toxic heavy metals (Silver and Walderhaug, 1992, Silver and Ji, 1994).

Nickel (Ni) is widely distributed in nature and 8.5% of the earth's crust is nickel (Carson et al., 1986). Nickel is used in the production of stainless steel, as a chemical catalyst in electroplating, ceramics, pigments, batteries and coins (IARC (International Agency for Research on Cancer), 1990). In many bacteria, nickel is required for the activity of enzymes such as urease, dehydrogenase, and hydrogenase (van Valiet et al., 2001), however excess amount is toxic. Nickel binds to proteins and nucleic acids and inhibits enzymatic activities such as DNA replication, transcription, and translation (Grosse et al., 1999). The best known nickel tolerance in bacteria, in Ralstonia eutrophus strain CH34 and related bacteria, is based on nickel efflux driven by a RND transporter. Two systems have been described, a nickel-cobalt resistance Cnr (Liesegang et al., 1993) and a nickel-cobalt-cadmium resistance Ncc (Schmidt and Schlegel, 1994). These systems are closely related to the cobalt-zinc-cadmium resistance system Czc from strain CH34. Flagellin is a component of flagellar filament known to provide motility to the bacteria. The present study describes the involvement of flagellin in nickel tolerance in an indigenous bacterium; B. cereus, CMG2K4.

MATERIALS & METHODS

ISOLATION AND CHARACTERIZATION

The media used were Luria-Bertani (LB) medium (Bopp and Ehrlich, 1988) and Tris minimal media (Mergeay et al., 1985). Soil samples were collected from metal electroplating, auto-batteries and automobile workshops of Gulshan-e-Iqbal, Karachi, Pakistan. Strains were isolated

according to the standard procedure in the tris-minimal medium containing 0.2% gluconate and supplemented with 0.1 mM $NiCl_2$. Ralstonia eutropha CH34 (the kind gift of Dr. D.H. Nies) (Nies and Silver, 1989) was also used as standard.

All the isolated strains were checked for maximum tolerable concentration (MTC) against $NiCl_2$. Nickel accumulation of all the strains was calculated by considering metal concentration both in supernatant and in pellet in parts per million (ppm) using Atomic Absorption Spectrophotometer (Perkin Elmer AAnalyst 200).

CMG2K4 was identified using partial 16S ribosomal RNA gene sequence homology analysis by HPA (Collindale, London UK). Sequence was analyzed using BLAST algorithm (Altschul et al., 1990) (Web ref: http://www.ncbi.nlm.nih.gov/cgi-bin/Blast).

GROWTH CURVES

Growth curves were studied for determining the tolerance mechanism against nickel both through turbidity measurement (OD600) and viable count using overnight cultures grown with and without $NiCl_2$.

DNA ANALYSIS

Genomic DNA was isolated by using Wizard Genomic DNA Purification Kit Cat No A1125 from Promega (USA) and Puregene DNA isolation kit of Gentra (cat no. D5500A). Plasmid isolation was carried out by QIAprep Spin Miniprep Kit (Cat No 27104 of QIAGEN).

Oligonucleotide primers were designed by using primer designing programme [Primer 3 Output (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3_cgi v 0.2)].

PROTEIN ANALYSIS

For protein analysis SDS-PAGE was employed. 8% Resolving gel and 4% Stacking gel was prepared. SDS-PAGE was performed as described by Baines (2000). Standard protein used in SDS-PAGE was See Blue® plus 2 pre-stained standard (1X) cat no. LC5925 of Invitrogen, which had 10 pre-stained protein bands of 4-250 kDa range. Vertical electrophoresis system of Hoefer miniVE was used with Hoefer EPS 2A200 power pack.

Protein samples for (SDS)-poly acrylamide gel electrophoresis (PAGE) were prepared as described by Cumming and Iceton (2001). Total protein concentration of the sample was quantified by the method of Bradford (1976) as described by Ausbel (1994). Bradford Reagent (Protein dye reagent) Sigma B-6916 was used with IgG (Biorad) as a standard.

Protein sequencing of band of interest was conducted commercially by amino acid sequencing Facility, School of Biochemistry and Microbiology, LIGHT Laboratories, University of Leeds, Leeds, UK.

Resultant sequence was searched for homology in databases, NCBInr at www.ncbi.nih.gov and Uni-Prot at www.ebi.ac.uk, using Blast (version 2.2.13) and MPsrch (version 1.3) options respectively.

Multiple sequence alignment of amino acid sequences of flagellin protein of different strains of Bacillus cereus was conducted through ClustalW. (1.83) in EBI, Uni-Prot database at http://www.ebi.ac.uk/clustalw (Higgins et al., 1994, Lopez, 1997).

Peptide Mass Fingerprinting (PMF) was conducted commercially at MALDI-Mass Spectrometry Facility, School of Biochemistry and Microbiology, LIGHT Laboratories, University of Leeds, where the dried plate was transferred to a M@LDI L/R mass spectrometer (Waters) each was analyzed and combined to produce a raw spectrum.

The set of monoisotopic peptide masses produced through trypsin digest was used to search the databases in National Centre for Biotechnology Information (NCBI) at www.ncbi.nih.gov and European Bioinformatics Institute, a part of European Molecular Biology Laboratories (EMBL-EBI) at www.ebi.ac.uk using the Mascot search engine (Matrix Science) in order to identify the parent protein. Peptide Mass Fingerprinting data was characterized and identified in Swiss-Prot database at www.expasy.ch using Proteomics Server through FindMod tool (Wilkins et al., 1999, Gasteiger, 2005).

RESULTS

ISOLATION AND CHARACTERIZATION

43 bacterial strains were isolated and purified initially. Maximum tolerable concentrations (MTC) of all the strains against nickel have shown that few strains such as CMG2K4, CMG2K6, CMG2K7, CMG2K8 tolerated high concentrations of nickel, which was as high as 10, 13, 17, and 9 mM respectively. Highest accumulation was found in CMG2K4 which accumulated 50% of the total amount of

$NiCl_2$ added. Other high nickel tolerant strains did not show high rate of accumulation, on the basis of tube results CMG2K4 was selected for further studies.

CMG2K4 was identified through 16S rRNA gene DNA sequencing as Bacillus cereus. Sequences were submitted in the gene bank and the accession number obtained is DQ211695.

GROWTH CURVES

Results of growth of CMG2K4 in presence of nickel, both through turbidity measurement and viable count, have suggested that nickel tolerance is constitutive in CMG2K4 (Figure 1a and 1b).

DNA ANALYSIS

Plasmid isolation indicated that CMG2K4 harbor no plasmid. PCR results did not indicate presence of ncc and cnr operons in CMG2K4.

PROTEIN ANALYSIS

As a result of SDS-PAGE of CMG2K4 a single band of protein having molecular weight of ~36 kDa (approximately 327 amino acids) was identified from the crude cell extract of culture grown in the presence of 1mM nickel chloride overnight, whereas rest of the bands found in control were all diminished (Figure 2). As a result of N-terminal protein sequencing 10 amino acid long sequence was obtained. As a result of Blast through MPsrch in EMBL-EBI database (UniProt) this 10 amino acid long sequence showed 100% homology with flagellin protein of Bacillus cereus. The sequence has been submitted to UniProt Knowledgebase, accession number is P85307.

Multiple sequence alignment of selected sequences which had the length close to the expected length of amino acid sequence of our protein conducted through CLUSTALW (1.83) has revealed variability in the amino acid sequences of different strains of Bacillus cereus flagellin protein.

Peptide Mass spectrometry fingerprinting of ~36 kDa band isolated from CMG2K4 (Bacillus cereus) was used to search the Swiss-Prot and NCBInr databases using the Mascot search engine (Matrix Science) in order to identify the parent protein (Figure 3). Original, minimized and re-optimized sets of monoisotopic peptide masses were used to perform the searches but no exact match was found. 16 major peptide masses peaks (Figure 4) which were obtained as a result of digestion with trypsin were compared in SWISSPROT database http://c.expasy.org/sprot with peptide mass data of flagellin reported in other strains of Bacillus cereus. Maximum similarity found in exact match of 4 major peaks in Q45XJ8_BACCE ; Q4MU98_BACCE and Q4MU99_BACCE showed exact match of 3 major peaks; while Q4MU96_BACCE , Q4MU97_BACCE and Q45XK1_BACCE showed exact match with two major peaks of peptide masses of ~36 kDa protein of Bacillus cereus strain CMG2K4. There were several potentially modified peptides in all the six strains but minimum unmatched peptide masses were found to be 5 in Q4MU99_BACCE, Q45XJ8_BACCE, and Q45XK1_BACCE; while there were 6 in Q4MU97_BACCE, Q4MU98_BACCE; and 7 in Q4MU96_BACCE.

Figure 1

Figure 1a: Growth curve of CMG2K4 with and without NiCl to check induction through turbidity measurement.

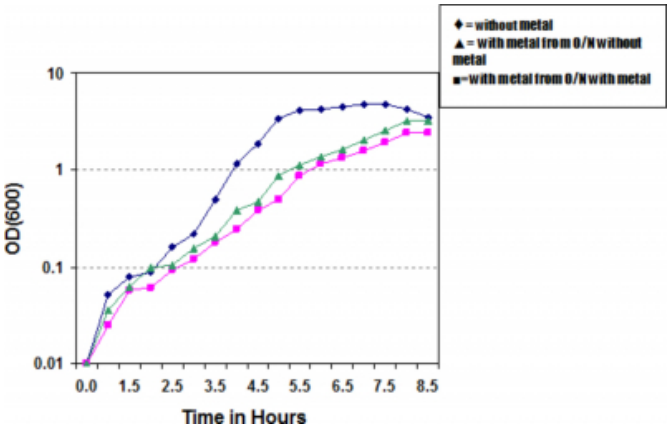


Figure 2

Figure 1b: Growth Curve of CMG2K4 with and without NiCl to check induction through viable count.

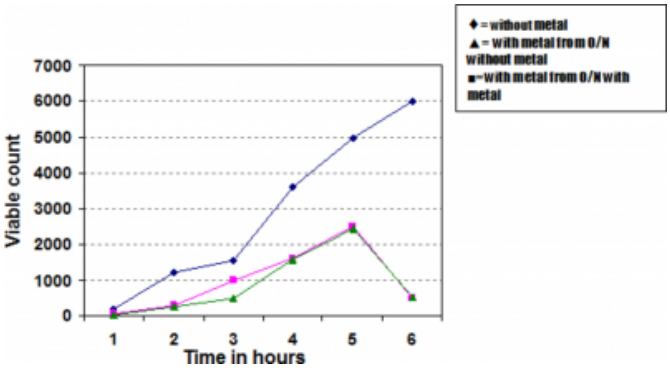
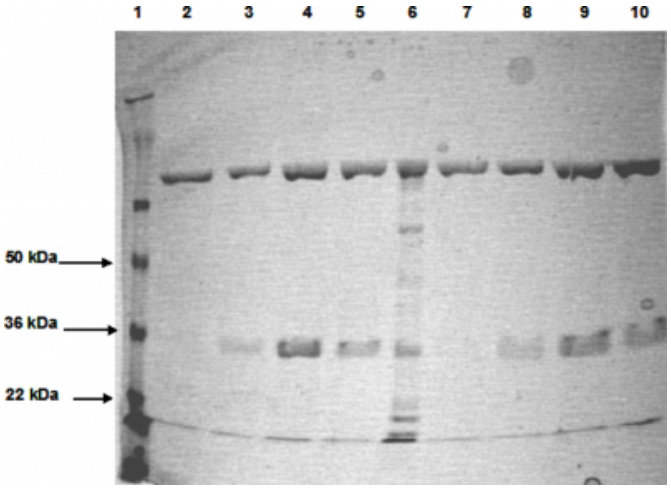


Figure 3

Figure 2: SDS-PAGE for CMG2K4 Protein in Presence Of Nickel (Stationary Phase Culture)



Lane 1= Standard (See Blue 2 Pre-Stained Invitrogen)

2-5 & 7-10= CMG2K4 Protein in Presence of Nickel (2, 5,10, & 8µl)

Lane 6= CMG2K4 Protein without Nickel

Figure 4

Figure 3: Mass Spectrum Of ~36 kDa Protein Band of CMG2K4

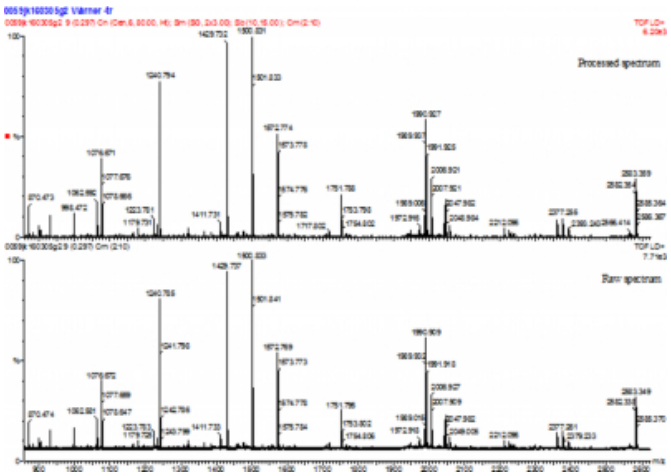


Figure 5

Figure 4:Partial data set (major peaks only).

900.562	930.488	998.475	1062.593	1064.605	1076.671
1240.794	1429.733	1500.831	1572.774	1751.789	1989.938
2005.926	2045.982	2360.246	2582.356		

DISCUSSION

The present studies were initiated with the aim to identify indigenous bacteria having potential for heavy metal tolerance and to ruminate about the genetic mechanisms involved. Metal contaminated sites are the potential source of metal tolerant bacteria (Badar et al., 2001). For this study bacterial strains were collected from the soil contaminated with heavy metals such as automobile workshops, where battery servicing, welding and electroplating is performed commonly.

Among several heavy metal tolerating strains, CMG2K4 was found to tolerate $NiCl_2$ and accumulate it. As CMG2K4 was accumulating $NiCl_2$ as well so it was suggested that in CMG2K4 the mechanism of tolerance is not the active efflux of heavy metal ions from the cytoplasm to the extra cellular medium as generally reported for various heavy metals (Rensing et al., 1997).

Partial 16S-rDNA sequencing technique was used to identify CMG2K4 which was identified as Bacillus cereus. CMG2K4 showed couple of contrasting characters with respect to already known mechanisms of tolerance; firstly CMG2K4 does not contain any plasmid, contrary to the reported mechanisms of nickel tolerance (Liesegang et al., 1993, Grass et al., 2000); secondly, constitutive nickel tolerance mechanism was observed which was evident through optimal density and viable count of growth curves with and without nickel in contrast to the reported mechanisms of tolerance in nickel (Hong et al., 1996).

CMG2K4 did not indicate presence of any reported genes with respect to nickel tolerance in presence of respective standards through PCR. Knowing the fact that bacterial transcriptional activator proteins respond to different toxic environmental compounds such as heavy metals, antibiotics or other drugs (Summers, 1992), a different strategy was adopted to locate genetic basis of tolerance against nickel in CMG2K4. Protein contents were extracted from whole cell extract of CMG2K4 grown with and without $NiCl_2$. Crude protein extract of CMG2K4 showed an intense band of ~36 kDa molecular mass which was found to be the band of only over expressed protein in presence of nickel. All the other bands diminished as compared to control both in log phase culture and stationary phase culture. These results indicate that the ~36 kDa band protein is a constitutive protein of CMG2K4 which might play active role for survival even in the presence of high concentration of nickel.

10 amino acids N-terminal sequence of ~36 kDa protein showed ~100% identity with Flagellin protein from B. cereus using MPsrch program identified in ~36 Kda protein as Flagellin (www.ebi.ac.uk) (Waterman and Smith, 1981). Most of the sequences of flagellin protein which showed homology with our ~36 kDa protein of CMG2K4 were of Bacillus cereus and CMG2K4 was also identified as Bacillus cereus, which supports that the homology is not coincidental. Furthermore, length of amino acid sequence of the protein is expected to be 327 and the reported length of some of the Flagellin protein sequence came out to be very close to this number i.e., 284-397.

Several strains of Bacillus cereus have been completely sequenced (Ivanova et al., 2003, Rasko et al., 2004), but the flagellin protein sequences which matched with the 10 amino acid long sequence of CMG2K4 protein were mostly Bacillus cereus G9241 (Hoffmaster et al., 2004). Multiple sequence alignment through CLUSTALW. (1.83) in EBI, Uni-Prot database (Lopez 1997, Higgins et al. 1994) at http://www.ebi.ac.uk/clustalw, of amino acid sequence of flagellin protein of six selected strains of Bacillus cereus on the basis of having amino acid sequence length close to the expected length of the studied protein i.e., 327 amino acids, showed N-terminal and C-terminal homology of the sequences but variability was found in the middle C-terminal half of the sequence.

In order to confirm the identity of ~36kDa protein, protein mass fingerprinting was performed by MALDI-Mass Spectrometry which resulted in several peptide peaks, out of which major peaks were analyzed in Swiss-Prot database at http://www.expasy.ch through Proteomics server using FindMod tool. Major peptide peaks of ~36 kDa protein with that of other reported peptides peaks of flagellin protein of Bacillus cereus strains were compared. All the exactly matched peptides were located at the conserved terminal sequences.

N-terminal sequencing, MPsrch sequence searches, multiple alignment and Peptide mass fingerprinting by MALDI mass spectrometry confirmed that the over expressed ~36 kDa protein from nickel tolerant strain of B. cereus CMG2K4 is in fact flagellar protein, Flagellin.

Flagellin is a component of flagellar filament which provides motility to the bacteria. Potential benefits of motility include increased efficiency of nutrient acquisition, avoidance of toxic substances and to access optimal colonization sites within them (Ottemann and Miller, 1997). It has been reported that synthesis of flagellin gets repressed by various antibiotics belonging to different groups having different mode of actions (Kaldalu et al., 2004). The flagellin protein inspite of being recognized as transcriptionally active against different antibiotics, it has not been reported with respect to its role in heavy metal tolerance. This has been revealed as a result of present finding.

CONCLUSIONS

N-terminal sequencing, MPsrch sequence searches and multiple alignment has confirmed that the over expressed ~36 kDa protein from nickel tolerant strain of B. cereus CMG2K4 is in fact flagellar protein, Flagellin. The flagellin protein inspite of being recognized as transcriptionally active against different antibiotics, it has not been reported with respect to its role in heavy metal tolerance. This has been revealed as a result of present finding.

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References

1. Ahmed N, Nawaz A, Badar U: Screening of copper tolerant bacterial strains and their potential to remove copper from the environment. Bull Environ Contam Toxicol 2005, 74: 219-226
2. Altschul S F, Gish W, Miller W, Myers E W, Lipman D J: Basic local alignment search tool. J Mol Biol 1990, 215: 403-410
3. Ausbel F M: Current protocols in molecular biology. Edited by Ausubel F M, Brent R, Kingston R E, Moore D D, Seidman J G, Smith J A, Struhl K 1994
4. Badar U, Abbas R, Ahmed N: Characterization of copper and chromate resistant bacteria isolated from Karachi tanneries effluents. In: Industrial and Environmental Biotechnology. Edited by Ahmed N, Qureshi F M, Khan O Y. Horizontal Scientific Press UK 2001, 43-54
5. Baines D: Analysis of purity: Detection of Impurities: Sodium dodecyl sulphate-Polyacrylamide gel electrophoresis. Protein Purification techniques. In: A practical approach. Edited by Roe S, 2000, Chap. 3 (2): 27-49
6. Bopp L H, Ehrlich H L: Chromate resistance and reduction in Pseudomonas fluorescens strain LB300. Arch Microbiol 1988, 150: 426-431
7. Bradford M M: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal.Biochem 1976, 72: 248-254
8. Carson B C, Ellis H. V, McCann J L: Toxicology and Biological Monitoring of Metals in Humans: Including Feasibility and Need. Lewis publishers, Chelsea, Michigan 1986

9. Cumming R H, IcetonG: Cell disintegration and extraction techniques. Protein Purification techniques. In:A Practical approach. Edited by Roe S, 2001, Chap. 5 (2): 83-108.
10. Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins M R, Appel R D, Bairoch A: Protein Identification and Analysis Tools on the ExPASy Server. The Proteomics Protocols Handbook. Walker,J.M. Humana Press 2005
11. Grass G, Grosse C, Nies D H: Regulation of the cnr Cobalt and Nickel Resistance Determinant from Ralstonia sp. Strain CH34. J Bacteriol 2000, 182: 1390-1398
12. Grosse C, Grass G, Anton A, Franke S, Navarrete A, Lawley B, Brown N L, Nies D H: Transcriptional organization of the czc heavy-metal homeostasis determinant from Alcaligenes eutrophus. J Bateriol 1999, 181: 2385-2393
13. Higgins D, Thompson J, Gibson T, Thompson J D, Higgins D G, Gibson T J: CLUSTAL W: improving the sensitivity of progressivemultiple sequence alignment through sequence weighting,position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994, 22: 4673-4680
14. Hoffmaster A R, Ravel J, Rasko D A, Chapman G D, Chute M D, Marston C K, De B K, Sacchi C T, Fitzgerald C, Mayer L W, Maiden M C J, Priest F G, Barker M, Jiang L, Cer R Z, Rilstone J, Peterson S N, Weyant R S, Galloway D R, Read T D, Popovic T, Fraser C. M: Identification of anthrax toxin genes in a Bacillus cereus associated with an illness resembling inhalation anthrax. PNAS 2004, 101: 8449-8454
15. Hong S, Candelone J P, Turetta C, Boutron C F: Changes in natural lead, copper, zinc and cadmium concentrations in central Greenland ice from 8250 to 149,100 years ago: their association with climatic changes and resultant variations of dominant source contributions. Earth and Planetary Science Letters 1996, 143: 233-244
16. IARC (International Agency for Research on Cancer): Chromium, Nickel and Welding, IARC Monographs on the Evaluation of Carcinogenic Risks of Chemicals to Humans. World Health Organization, Lyon 1990: 249-256
17. Ivanova N, Sorokin A, Anderson I, Galleron N, Candelon B, Kapatral V, Bhattacharyya A, Reznik G, Mikhailova N, Lapidus A, Chu L, Mazur M, Goltsman E, Larsen N, D'Souza M, Walunas T, Grechkin Y, Pusch G, Haselkorn R, Fonstein M, Ehrlich S D, Overbeek R, Kyrpides N: Genome sequence of Bacillus cereus and comparative analysis with Bacillus anthracis. Nature 2003, 423 (6935): 87- 91
18. Kaldalu N, Mei R, Lewis K: Killing by Ampicillin and Ofloxacin Induces Overlapping Changes in Escherichia coli Transcription Profile. Antimicrobial Agents and Chemotherapy 2004, 48 (8): 890-896
19. Liesegang H, Lemke K, Siddiqui R A, Schlegel H G: Characterization of the inducible nickel and cobalt resistance determinant cnr from pMOL28 of Alcaligenes eutrophus CH34. J Bacteriol 1993, 175: 767-778
20. Lopez R: ClustalW WWW Service at the European Bioinformatics Institute. http://www2.ebi.ac.uk/embnet.news/vol4_3/clustalw1.html. The ClustalWWW server at the EBIembnet.news 1997
21. Malakul P, Srinivasan K R, Wang H Y: Metal toxicity reduction in naphthalene biodegradation by use of metal-chelating adsorbents. Appl Environ Microbiol 1998, 64: 4610-4613
22. Mergeay M, Nies D H, Schlegel H G, Gerits J, Charles P, Van Gijsegem F: Alcaligenes eutrophus CH34 is a facultative chemolithotroph with plasmid-bound resistance to heavy metals. J Bacteriol 1985, 162: 328-334
23. Morris C: Academic Press Dictionary of Science and Technology. Academic Press, San Diego 1992
24. Nies D H, Silver S: Plasmid-determined inducible efflux is responsible for resistance to cadmium, zinc and cobalt in Alcaligenes eutrophus. J Bacteriol 1989, 171: 896-900
25. Ottemann K M, Miller J F: Roles for motility in bacterial-host interactions. Mol. Microbiol 1997, 24: 1109-1117
26. Rasko D A, Ravel J, Okstad O A, Helgason E, Cer R Z, Jiang L, Shores K A, Fouts D E, Tourasse N J, Angiuoli S V, Kolonay J, Nelson W C, Kolsto A B, Fraser C M & Read T D: The genome sequence of Bacillus cereus ATCC 10987 reveals metabolic adaptations and a large plasmid related to Bacillus anthracis pXO1. Nucleic Acids Res 2004, 32 (3): 977- 988
27. Rensing C, Mitra B, Rosen B P: Insertional inactivation of dsbA produces sensitivity to cadmium and zinc in Escherichia coli. J. Bacteriol 1997, 179: 2769-2771
28. Schmidt T, Schlegel H G: Combined nickel-cobalt-cadmium resistance encoded by the ncc locus of Alcaligenes xylosoxidans 31A. J Bacteriol 1994, 176: 7045-7054
29. Silver S, Ji G: Newer systems for bacterial resistance: New surprises. Annu Rev Microbiol 1994, 50: 753-789
30. Silver S, Walderhaug M: Gene regulation of plasmid- and chromosome- determined inorganic ion transport in bacteria. Microbiol Rev 1992, 56: 195-228.
31. Summers A O: Untwist and shout: a heavy metal-responsive transcriptional regulator. J Bacteriol 1992, 174: 3097-3101
32. Summers A O: Current Perspectives in Microbial Ecology. American Society for Microbiology, Washington D C 1984
33. van Valiet A H M, Kuipess E J, Waidner B, Davies B J, Vries N, Penn C W, van der C M J E, Kist M, Bereswill S, Kusters J G: Nickel-Responsive Induction of Urease Expression in Helicobacter pylori is Mediated at the Transcriptional Level. Infect Immun. 2001, 69: 4891-4897
34. Waterman M, Smith T: Identification of common molecular subsequences. J Mol Biol 1981, 147: 195-197
35. Wilkins M R, Gasteiger E, Gooley A A, Herbert B R, Molloy M P, Binz P A, Ou K, Sanchez J C, Bairoch A, Williams K L, Hochstrasser D F: High-throughput mass spectrometric discovery of protein post-translational modifications. J Mol Biol. 1999, 289 (3): 645-657

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