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PRAMOD K. SHARMA, MURTHY A. VAIRAVAMURTHY, and JAN KIELECZAWA

Department of Applied Science, Department of Biology
Brookhaven National Laboratory, Upton, NY 11973

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ANAEROBIC RESISTANCE TO HIGH LEVELS OF CADMIUM AND OTHER TOXIC METALS IN A FACULTATIVE ANAEROBE ISOLATED FROM PRISTINE SALT MARSH SEDIMENTS

PRAMOD K. SHARMA¹, MURTHY A. VAIRAVAMURTHY, and JAN KIELECZAWA²

Department of Applied Science, Department of Biology²
Brookhaven National Laboratory, Upton, NY 11973
¹E-mail: psharma@bnl.gov

We have isolated many Cd (II) resistant bacterial strains from relatively pristine sediments collected from salt marshes in Shelter Island, New York. Detailed studies are being performed on one isolate, strain Cd-1. Strain Cd-1 is metabolically diverse, halotolerant, Gram-negative, facultative anaerobe. It can resist high amounts of Cd (II), Cr (VI), As (V), Se (IV), Co (II), Pb (II), or Zn (II) under defined anaerobic conditions. With pyruvate as the energy source, Cd-1 can grow well at examined Cd (II) concentrations ranging up to 15 mM. It can resist Cd (II) with or without marine level NaCl concentration, under acidic or neutral conditions. It can resist Cd (II) under aerobic conditions as well. These features are novel for a heavy metal resistant bacterium.

FT-IR spectroscopy of intact cells grown without and with Cd (II) showed differences in spectral patterns in regions between 1250 to 1200, 1200 to 900, and 900 to 600 cm⁻¹ suggesting biochemical changes due to Cd (II) stress. More direct biochemical approaches including protein electrophoresis, protein sequencing, and phospholipid fatty acid (PLFA) analysis showed that a 37 kD outer membrane protein, OmpC, and an unsaturated PLFA, palmitoleate, are overproduced in cells grown with Cd (II). OmpC is a porin [1]; it however can act as a selective channel for cations [2]. Porins can aid in chemiosmotic heavy metals efflux in Gram-negative bacteria [3]. Increase in unsaturated fatty acid content can increase membrane fluidity [1, 4, 5], which in turn may aid in better toxic metal efflux by ATPases [4, 5].

With added Cd (II), rapid growth occurred at low pH (4 to 6) and high NaCl concentrations (40 mM). CCCP, a proton uncoupler, inhibited growth on Cd (II) containing nutrient agar plates showing that chemiosmotic efflux system was involved in Cd (II) resistance. It however is not clear whether Na⁺/H⁺ antiporter in effect was Cd²⁺/H⁺ antiporter. NapA (Na⁺/H⁺ antiporter in Enterococcus hirae) expression responds to Na⁺ concentration in the growth medium [6]. Interestingly, OmpC expression also responds to increasing external NaCl concentration [1].
Cd-1 contains a megaplasmid, pCD1-150, with a size range between 150 to 200,000 base pairs. Around 60,000 bases have been sequenced using shotgun approach. DNA fragments with a high sequence homology to a plasmid-coded cation-proton antiporter and a putative cation transporting P-type ATPase that confer Ag\(^+\) resistance in *Salmonella typhimurium* [7] have been localized. A DNA fragment with a high sequence homology to a plasmid-coded membrane fusion protein in Ag\(^+\) resistant *S. typhimurium* [7] has also been localized. Therefore, deduced components of chemiosmotic Cd\(^{2+}\) efflux system in strain Cd-1 include an inner membrane cation/proton antiporter, a membrane fusion protein that brings together the inner and outer membrane, and an outer membrane protein, OmpC. This system is homologous to chemiosmotic Czc [Co (II), Zn (II), and Cd (II)]-resistance system in *Alcaligenes* [3, 8, 9].

Our physiological data shows that a Cd\(^{2+}/2H^+\) antiporter was functional in strain Cd-1, it however is not clear whether Cd (II)-translocating ATPase was also functional under the conditions used here.

Although Cd-1 resists Cd (II) via efflux system(s), it also transforms Cd to CdS when sulfate is the sole S source. CdS production increases when thiosulfate is added to the growth medium. Transformation of soluble Cd (II) ions to insoluble CdS particles is of interest from bioremediation standpoint. Recent studies by other workers [10, 11] show that aerobic cultures of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* can also transform Cd (II) to CdS with sulfate or thiosulfate addition to the growth medium.

CdS production was monitored by X-ray absorption (XANES and EXAFS) spectroscopy of washed cells. CdS was thus associated with the cells. EXAFS data however shows that complexes other than CdS were also formed. Additional studies are needed to determine the nature of these complexes, accomplished work nonetheless clearly shows that multiple Cd (II) resistance mechanisms were operational in strain Cd-1.

Biolog and also nearly complete 16S rRNA sequence analysis shows that strain Cd-1 belongs to genus *Klebsiella*. Related strains, including *K. planticola*, are ubiquitous in nature, and are associative nitrogen-fixers [12]. Cd-1 thus may not only play a role in heavy metal speciation in a variety of environments, but it may also influence primary macrophyte production in coastal marshes and other suitable environments. Biogeochemical implications of this work are thus of significance.

From practical applications viewpoint, our data shows that Cd-1 is an excellent candidate for subsurface bioaugmentation processes. Large amounts of this bacterium can be grown aerobically in a short period and subsequently introduced into anaerobic subsurface environment for metal precipitation. Appropriate growth conditions or genetic manipulations may be used to enhance its ability to immobilize even higher amounts of Cd (II). As mentioned earlier, thiosulfate addition to growth medium helps in greater CdS production.

White et. al. [13] recently proposed that a sequential aerobic-anaerobic process may be used for accelerated bioremediation of soil contaminated with toxic metals including Cd (II). Here sulfuric acid producing sulfur-oxidizing
bacteria bioleach the metals, leachate metals then are precipitated as insoluble sulfides by sulfate-reducing bacteria. However, another study [14] by the same group indicates that sulfate-reducing bacteria can not tolerate high amounts of Cd (II). We therefore propose that high level Cd (II) resistant strains such as strain Cd-l or P. aeroginosn [11] may be more suitable for the metal precipitation step. Both strains can use thiosulfate, consequently they can transform high amounts of Cd (II) to CdS. It should however be noted that although both strains are of non-clinical (marine) origin, it is not clear whether they pose a serious health risk. Environmental Klebsiella lack capsular types K1 to K6, while the pathogenic strains possess them [12]. Cd-1 thus may be a safe bacterium for accelerated heavy metal bioremediation processes.

CONCLUSIONS

- This is first report on the ability of a marine bacterium to resist high levels of Cd (II) and many other toxic heavy metals under anaerobic conditions.
- This is the first report that shows that multiple Cd (II) resistance mechanisms, i.e., efflux, biotransformation, and bioaccumulation were operational in a pure culture under anaerobic conditions.
- This is the first report that indicates that both chemiosmotic antiporter and P-type ATPase may be involved in Cd (II) resistance in a Gram-negative bacterium.
- This is first report that shows that OmpC and palmitoleate levels increase in bacterial cells in response to Cd (II) stress.
- We show that NaCl plays an important role in Cd (II) resistance in Cd-1.

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