Polish J. of Environ. Stud. Vol. 15, No. 3 (2006), 517-520

Letter to Editor

A Preliminary Study on Tellurite Resistance in *Pseudomonas* spp. Isolated from Hospital Sewage

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Received: May 25, 2005
Accepted: December 27, 2005

Abstract

A total of 48 *Pseudomonas* spp., isolated from the Çukurova University Balcalı Hospital sewage in Adana, were characterized on the basis of morphological, cultural and biochemical characteristics. To improve our understanding of the ecology of tellurite resistance in bacteria, the minimum inhibition concentrations (MIC) of potassium tellurite (K_2TeO_3) for growth were used to determine metal tolerance of the isolated strains. Most of the strains tolerated to 55 μgr/ml potassium tellurite. Only strain *Ps 37* tolerated relatively high concentrations of tellurite (80 μgr/ml). 27% of the strains possessed plasmid mediated tellurite resistance (Tel^r).

Keywords: heavy metals, tellurite, *Pseudomonas*

Introduction

Resistance to heavy metals has been of interest to microbiologists for many years. Toxic ions are mobilized from industrial activities and eventually are accumulated through the food chain, leading to serious ecological and health problems [1]. Despite these toxic stresses, microorganisms have evolved resistance mechanisms to deal with metal toxicity, which includes volatilization, extracellular precipitation and exclusion, binding to the cell surface and intracellular sequestration [2].

Many species of bacteria have genes that control resistance to specific toxic heavy metals. This resistance often is determined by extrachromosomal DNA molecules (plasmids). The same mechanisms of resistance occur in bacteria from soil, water, industrial waste and clinical sources [3]. Bacterial plasmids encode resistance systems for toxic metal ions, including Ag, Cd, Co, Hg, Ni, Pb, Te, Zn and other toxic heavy metals [4]. Plasmid-determined resistance to toxic metal ions has been demonstrated for many bacterial species and is a useful selectable marker for these DNA molecules [5].

Tellurium is occasionally found native, but is more often found as the tellurite of gold, and combined with other metals. Tellurium compounds are used in the film and rubber industries and in the manufacture of batteries, and are found in fairly large amounts in the human body [6, 7]. Tellurium improves the machinability of copper and stainless steel and its addition to lead decreases the corrosive action of sulfiric acid on lead and improves its hardness. New applications include a germanium-antimony-tellurium compound used for optical storage on digital video discs [8]. Although tellurium is not an essential nutrient and is relatively rare in the environment, it is also considered to be extremely toxic, and clinical manifestations of toxicity are observed at very low concentrations [9]. It is shown that tellurite resistance is found in both Gram-positive and Gram-negative bacteria, but Gram-negative bacteria is frequently encoded by

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cryptic or conjugative plasmids [10, 11]. Several chromosomal Te^r systems have also been characterized [12]. The natural resistance to TeO_3^{2-} has also been used in the identification of *Corynebacterium* species. Tellurite resistance is also characteristic of *Streptococcus faecalis* and most *Staphylococcus aureus* [13]. The mechanism of resistance is not fully known yet, but is systematically results in the reduction of tellurite to elemental tellurium [10, 14]. The two conclusions from sequencing studies of plasmid-determined tellurite resistance operons are: 1) that several systems seem different and 2) that the predicted amino acid sequences have not led to a mechanistic model for resistance [4]. Potassium tellurite (K_2TeO_3) is toxic to most microorganisms, particularly Gram-negative bacteria [6]. It has been suggested that tellurite toxicity stems from its strong oxidizing ability, which might interfere with many cellular enzyme processes [12].

Several chromosome and plasmid-encoded metal resistance genetic systems have been studied in *Pseudomonas* as well as in related bacteria [15]. At least five genetically distinct chromosomal and plasmid-borne bacterial tellurite resistance systems have been described [12, 16, 17]. The existence of different types of plasmid encoded Te^r determinants suggests that this resistance is important to Gram-negative bacteria in the environment in which tellurium compounds appear to be relatively abundant [6]. The emergence of several unrelated Te^r determinants among a wide range of bacterial species, including human pathogens, suggests that these determinants provide some selective advantage in natural environments, which may be unrelated to the Te^r phenotype [18].

This preliminary study describes the isolation and characterization of the tellurite resistance of *Pseudomonas* spp. from Çukurova University Balcalı Hospital sewage. It is also aimed at determining whether the resistance gene is encoded by a plasmid or by chromosome. We thought that it was important to understand the molecular origins of this resistance in terms of their distribution in different bacteria. The understanding of bacterial metal resistance systems has been useful for both environmental sciences and medicine.

Experimental Procedures

Sampling Site and Isolation of Microorganisms

Samples were taken from sewage in Çukurova University, Balcalı Hospital in Adana, and kept in sterilized flasks under refrigeration until processing in a laboratory. Samples were plated onto LB Agar by serial dilution technique. The colonies formed after 24 h of growth at 30°C were selected according to their colony morphology. All of the strains' physiological and morphological properties were determined. Colonies were identified in our laboratory following Bergey's Manual of Determinative Bacteriology [19].

Determination of MICs

For testing tellurite resistance, potassium tellurite (K_2TeO_3) in varing concentrations were added to sterilized nutrient agar plates which were then spot inoculated. Plates were incubated at 30°C for 2 days [20]. 20 to 100 μgr/ml concentrations of tellurite were chosen for the differentiation between metal-resistant and sensitive strains. When grown in the presence of potassium tellurite, bacteria form black colonies due to the deposition of intracellular crystals of what has been shown to be metallic tellurium [21, 22]. The lowest concentration of tellurite without growth was defined as the MIC. The sensitive and resistant ranges of MIC has been described on the bases of the standard strain of *E. coli* 0157 (MIC 1 μg/ml of K_2TeO_3). All the experiments were replicated three times.

Plasmid Curing

To determine if the resistance gene is encoded by a plasmid or not, ethidium bromide was used to eliminate the plasmids from the strains, and also heat treatment was applied as a second control [20, 23]. Nutrient Agar containing ethidium bromide curing agent was inoculated with strains and incubated overnight at 37°C. Colonies were tested for resistance to potassium tellurite. Elevated temperature treatment was applied as a second control [20].

Plasmid Isolation and Electrophoresis

The plasmid DNAs were isolated from the *Pseudomonas* strains carrying plasmid-encoded resistance by a minipreparation method [24]. The isolated plasmids were characterized by agarose gel electrophoresis according to the standard procedure of Sambrook *et al*. [25]. Agarose gel electrophoresis through a horizontal slab gel of 0.8% agarose submersed in TBE (Tris-HCI, Boric Acid, EDTA) runnig buffer at 70 V for 2 hr were performed. DNA bands were stained with Ethidium Bromide for 15 min. and visualized on a UV transilluminator. Molecular weight of the plasmids were determined by using a computer program (DNA Size Version I) [26].

Results and Discussion

Firstly, it was aimed at investigating that MIC of the potassium tellurite on *Pseudomonas* spp. isolated from hospital sewage. 48 *Pseudomonas* sp. strains were selected and identified on the basis of morphological, cultural and biochemical characteristics.

In experiments which determinated MIC values of the strains, the medium supplemented with appropriate K_2TeO_3 concentrations. The MIC was then recorded as

the concentration of metal just prior to the concentration that inhibited growth. The sensitive and resistant ranges of MIC have been described on the bases of the standard strain of *E. coli* 0157 (MIC 1 μgr/ml of K_2TeO_3).

One of these strains, namely *Ps 37*, exhibited a high resistance to the K_2TeO_3 as compared to the other strains. The MIC value of *Ps 37* were found to be 85 μg/ml of K_2TeO_3. The rest of the strains' MIC values were found to be 60 μg/ml, except *Ps 13* (MIC 65 μg/ml). But it is well known that there are no currently acceptable concentrations of metal ions which can be used to distinguish metal-resistant and metal-sensitive bacteria [27].

Potassium tellurite is toxic to many microorganisms at concentrations as low as 1 μg/ml [11, 13, 28]. But Yurkov *et al*. [29] reported that the highest level of resistance to tellurite (MICs between 2,300 and 2,700 μg of K_2TeO_3 per ml) was observed for cells of *Erythromicrobium hydrolyticum*, *Erythromicrobium ursincola*, or *Erythromicrobium ramosum* grown in the presence of acetate. Tomas and Kay [30] stated that tellurite is highy toxic (1 μg/ml) toward *Escherichia coli* and mutants were resistant to low levels of tellurite (10 μg/ml). High tellurite resistance has also been shown in the case of some photosynthetic bacteria [29, 31].

In our study, when the strains were grown in potassium tellurite-containing media, dark coloration of the bacterial colony was observed. It was reported that resistant bacteria produce jet-black colonies on solid medium supplemented with K_2TeO_3 as the result of internal deposition of elemental tellurium [32, 33]. All strains were tested, whether the resistance gene was encoded by plasmid or not. Ethidium bromide and heat were used for plasmid-curing experiments as curing agents. Among the resistant strains, 27% of strains were found to have plasmid-encoded tellurite resistance but, interestingly, not *Ps 37*. The plasmids were isolated from the strains carrying plasmid-encoded resistance. According to electrophoresis experiments, only one plasmid was detected in these strains. The molecular sizes of these plasmids were found to be approximately 22 kb (Fig. 1). To confirm if the resistance gene is encoded by this plasmid DNA, the electrophoretic profile of the plasmids isolated from the original (non-cured) strains were compared with the cured strains. According to the gel electrophoresis, plasmids 22 kb in size were not detected in cured strains, This results indicated that the tellurite resistance gene carried on this plasmid. It was reported that different tellurite resistance system in the class of plasmids that includes the well studied board hosted the range plasmid RP4. This system is initially "cryptic" in that the cells with the plasmid are tellurite-sensitive. However, there is a low frequency mutation on the plasmid that results in a tellurite resistance determinant on RP4. There is no physical rearrangement associated with the mutation, but tellurite resistance was found on a 4.5 kb transposon. Tn521, which can hop to other plasmids [34]. The other well studied determinants of plasmid governed tellurite resistance, including those from IncH plasmids pMER610, pHH1508a, and R478 and a "cryptic" determinant on IncPα plasmid RK2 that was activated by mutation [10, 11, 22, 35]. There is even a chromosomal determinant of tellurite resistance in *E. coli* [35]. It differs in sequence and gene number from the plasmid systems. Therefore, the two unexpected conclusions from sequencing studies of plasmid-determined tellurite resistance operons are: first, that several systems seem different and second, that the predicted amino acid sequences have not led to a mechanistic model for resistance. Several mechanisms of tellurite resistance have been proposed, including enzymatic reduction, reduced uptake, and enhanced efflux [10, 11, 22, 35]. However, convincing data for any mechanistic hypothesis have been lacking, and that there is any clear mechanism of tellurite resistance was underlined by some authors [10, 36].

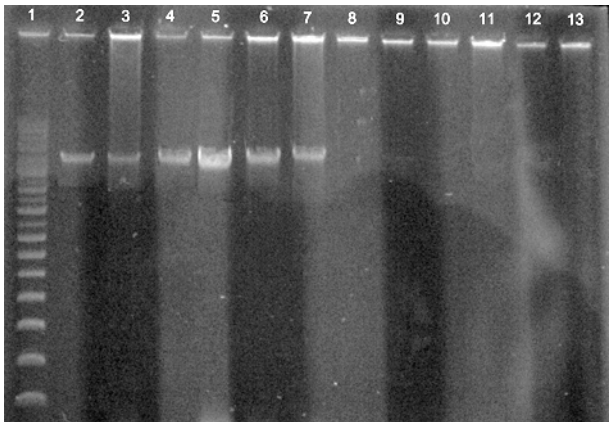

Fig. 1. Separation profile of original (non-cured) and cured strains on agarose gel. Lane 1: DNA ladder (11 supercoiled fragments, Sigma D5292); Lane 2 to 7: Original (non-cured) strains of *Ps 1, Ps 4, Ps 5, Ps 7, Ps 10* and *Ps 13* respectively; Lane 8 to 13: Cured strains of *Ps 1, Ps 4, Ps 5, Ps 7, Ps 10* and *Ps 13*.

Te-containing compounds are relatively rare in the environment. There is limited scope for most bacteria to come into contact with tellurite, except in highly polluted environments, and most human pathogens might never be exposed to such compounds. Te^r might not constitute a distinct resistance determinant in all bacteria [12]. It is likely that tellurite resistance and the ability to reduce tellurite are related, although such a relationship has not yet been proven [37].

The occurrence of bacteria which possess plasmid-encoded resistance to tellurium compounds in nature it is might be developed under intensive heavy metal stress. Like other heavy metals, tellurium compunds are used in some medical devices. These compounds are also used for tooth-filling material in dentistry. Finding new plasmids which encode tellurite resistance in these bacterial isolates indicates that hospital sewage is an ideal environment for developing an bacterial heavy metal resistance.

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