Relation between As(III) oxidation potential and siderophore production: a study of tannery As(III) oxidizers

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ABSTRACT: Herein we report the relationship between bacterial ability in arsenic (As) transformation, As resistance and siderophore production. It was investigated using different 40 As-resistant bacteria isolated from the tannery environment. Out of the bacterial genera isolated the 7 As(III) oxidizing genera and 4 As resistant genera produced siderophore. The bacterial genera producing more siderophores showed greater As(III) oxidation and As(III) resistance than those producing less siderophore.

1 INTRODUCTION

Arsenic (As) is a potent poison present in the environment in different oxidation states depending on the existing environment. The As(III) form is more toxic which is present in reducing environment than As(V) present in oxidizing environment (Oremland and Stolz, 2003). The microbes evolved number of strategies to survive against the toxic environment. One of these is the production of iron chelator i.e., siderophore in the environment. The siderophores are low molecular weight organic compound secreted by microorganisms in the environment (Miethke and Marahiel, 2007). Nearly, all living organisms require iron for metabolic activities and growth. It is not readily available to microorganisms and plants under normal conditions. The basic important role of siderophores is involved in the high affinity acquisition and receptor dependent transport of ferric ion. Regulation of the siderophore production is based on the concentration of iron in the environment (Miethke, 2013). The function of these compounds is to scavenge iron from the environment and to make it available to the microbial cell. Iron is soluble in ferrous form and can be easily acquired by microbial cell (Andrews *et al.*, 2003). The production of iron binding compounds, siderophores is the most studied mechanism amongst the various means used by microorganisms (Grove 1980; Jappelli & Marconi, 1997). Herein we studied the relation between different genera for As(III) oxidation and siderophore production isolated from tannery environment.

2 METHODS

2.1 Isolation and identification of arsenite oxidizing bacteria

Tannery soil and effluent samples were taken from waste disposal site Miraj, India. In enrichment, 3 g

soil was inoculated in Tris-mineral medium, supplemented with 0.04% yeast extract (TMM) and 5 mM of As(III). Flasks were incubated on shaker for As(III) at 30°C for 5 days and 3 mL of this enrichment culture was inoculated in fresh medium. This procedure was repeated two times. The isolates yielded were initially screened by using silver nitrate plate assay. Sequencing of 16S rRNA gene was performed using genomic DNA isolated by alkaline lysis method (Fig. 1).

2.2 Screening of arsenite oxidizers and siderophore producers

Arsenic oxidation ability isolated strains were estimated by using molybdenum blue method (Bagade *et al.*, 2016). Siderophore production ability was tested on the universal CAS plate assay (Schwyn *et al.*, 1987).

3 RESULTS AND DISCUSSION

3.1 Isolation and identification of arsenite oxidizing bacterial strains

Out of the total 40 heterotrophic cultures isolated from enrichment of tannery effluent and soil, 18 different genera were identified. The isolates were identified by partial (800 to 1300 bp) sequencing of 16S rDNA and checked for its As transformation ability. The isolates after sequencing showed bacteria belonging to different total 11 genera namely *Achromobacter*, *Ochrobactrum*, *Sinorhizobium*, *Alcaligenes*, *Agrobacterium*, *Bordetella*, *Rhizobium*, *Stenotrophomonas*, *Bacillus*, *Microbacterium* and *Pseudomonas*.

3.2 Screening of arsenic redox isolates and siderophore producers

Out of the total cultures screened, seven cultures showed As(III) oxidizing ability which were grouped according to the genera they belonged. The seven genera showed As(III) oxidizing ability

Table 1. Different bacterial genera showing arsenic oxidizing genera and/or siderophore producing ability.

Isolate	As(III) oxidation	CAS positive
<i>Achromobacter</i> sp. T-14	+	+
<i>Ochrobactrum</i> sp. T-5	+	+
<i>Sinorhizobium</i> sp. T-20	+	+
<i>Alcaligenes</i> sp. R-3-2	+	+
<i>Agrobacterium</i> sp. R-7	+	+
<i>Bordetella</i> sp. R-8	+	+
<i>Rhizobium</i> sp. R-6-2	+	+
<i>Stenotrophomonas</i> sp. R-4-1	—	+
<i>Bacillus cereus</i> sp. R-4-2	—	+
<i>Pseudomonas</i> sp. T-1-1	—	+
<i>Microbaterium</i> sp. T-2	—	+

Figure 1. Representative figure of few bacterial genera showing yellow zone around the cultures indicating CAS test positive.

are namely *Achromobacter*, *Ochrobactrum*, *Sinorhizobium*, *Alcaligenes*, *Agrobacterium*, *Bordetella*, *Rhizobium*. While the four genera *Stenotrophomonas*, *Bacillus*, *Microbacterium* and *Pseudomonas* only showed siderophore producing ability (Table 1).

There was a relation between biotransformation of arsenicals viz., As(III), As(V) and siderophore production. Out of the bacterial genera isolated the As(III) oxidizer and As(V) reducers the bacterial strains producing more siderophores showed greater As(III) oxidation and As(III) resistance than those producing less siderophore.

4 CONCLUSIONS

We found in our study that arsenite oxidizer showed siderophore production ability that could be an

exception for previously reported results. There are total 29 isolates obtained from this study distributed in 11 different genera having the ability of siderophore production.

There is a strong correlation we found from our study that pathogenic bacteria specifically synthesize the siderophore. Due to huge diversity and growth condition we have not performed screening of chemical type of siderophore by different isolates. However, it is quite interesting to observe that those isolates responsible for As speciation can also synthesize the siderophore.

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