Presence of Arsenite enhances biofilm formation of autotropic Arsenite-oxidising bacteria *Rhizobium naphthalenivorans* and promotes Arsenite As(III) oxidizing activity by protecting arsenite oxidase protein in the extracellular polymeric substance

This study will determine whether the presence of arsenite promotes biofilm formation of AOB. We will quantifythe oxidase protein AioA/AioB and the transcriptional regulator AioRof *Rhizobium naphthalenivorans*in the EPS of the biofilms and their suspended cells by western blotting.Compare the level of protein in both the cases, while also looking at the comparison of their oxidizing activities in presence and absence of As(III).Higher concentration of arsenite oxidase results in increased oxidizing activity of the biofilms. Quantitative and biochemical assay can be performed to measure the total protein and carbohydrate concentration in presence or absence of arsenite.

Experiments to address:

- **Selection of bacterial strain**-AOB and a biofilm forming *R.naphthalenivorans* can be grown in the tryptophan yeast extract in presence and absence of As(III).
- **Biofilm formation**-A mid-log phase culture can be diluted using Tryptic Soy Brothmedium. The diluted culture can be placed into a 48-well polystyrene microplate with different concentrations of As(III)[1]. A control well having zero concentration of As(III) can also be prepared. The plates canbe incubated at 30 °C for 6 days. Biofilm formation capacity was quantified by crystal violet assay[1].
- **Determination of As(III) oxidizing activity of biofilm and suspended cells**-Biofilms of *R.naphthalenivorans* can be grown in microplate in the presence and absence of As(III). Hemocytometer can be used to count the cell in the wells[1]. The biofilm can be sonicated to obtain suspended cells. These cells can be adjusted to the cell numbers as of biofilms. Bacterial As(III)-oxidizing activities of the biofilms and suspended cells were evaluated in PBS with As(III) by incubating at 30 °C[1]. At an appropriate interval, cultures was removed for measuring the concentrations of As(V) and As(III)[1].
- **Extraction of EPS from bacterial strain**-Overnight bacterial cultures can be inoculated in LB broths with and without As(III)[2]. Followed by incubation at 37º C for 3 days. Bacterial cells can be removed by the centrifugation. Supernatants can be separated and incubated with absolute ethanol overnight at 4º C[2]. After incubation, precipitated EPS can be collected by centrifugation.

- **Western Blot**-SDS-PAGE and Western immunoblotting can be carried out to separate and detect As(III) oxidase protein AioA/AioB and the regulator AioR in the EPS pellet of bacterial strain with and without As(III). Antibiotic specific to AioA, AioB and AioR can be used to specifically bind in the western blot. Western blotting of suspended cell grown in the same conditions can also be performed. The level of proteins in EPS of biofilms

and suspended cell in presence and absence of As(III) can be measured as well as compared by using bovine serum albumin (BSA) standard.

- **Biochemical characterization of EPS**-The weight of EPS extracted from biofilm in presence and absence of As(III) can be measured. Total protein content of EPS can be estimated by Bradford assay. Total carbohydrate contents of EPS can be measured by using colorimetric phenol sulfuric acid[2].

Expected outcomes:

- Quantitative analysis of biofilm at different concentration can be performed by standardized crystal violet assay. As expected, if arsenite enhances biofilm, the biofilm at highest concentration of As(III) have the maximum absorbance.
- If As(III)-oxidizing activity of the biofilm in presence of As(III) is higher than their suspended cells then it will be proved that biofilm formation promotes oxidizing activity. If biofilm formation doesnot promote oxidizing activity then the suspended cells will have higher or equal activity in comparison of the biofilm.
- Quantitative analysis of AioA, AioB and AioRproteinsof EPS and suspened cellwill be performed by western blot. If EPS protects arsenite oxidase, the level of proteins will be higher in the EPS in comparison of the suspended cells.
- The weight, total protein and carbohydrate of EPS of biofilm in presence of As(III) will be expected to be higher. This will prove that biofilm formation is enhanced by the presence of arsenate.

References:

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