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Review Article

A Review on Detoxification and Bioremediation of Antimony by Bacterial Species

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Abstract

Rising pollution and heavy metal contamination is one of the most vital concerns across the globe. Presently, research on antimony pollution grabs the attention due to its application in industry. Heavy metal pollution and contamination needs proper regulation and monitoring. Hence, in this article we have encompassed the significant molecular mechanisms such as the operons and efflux transporters involved as well as biochemical processes like oxidation, reduction and biomethylation mediated by As and Sb resistant bacteria that considerably help in bioremediation. This article summarizes most of the common techniques employed by bacteria to combat Sb pollution.

Keywords: Bioremediation; Heavy Metal Resistance; Detoxification; Operon

Introduction

The term heavy metal briefly explains any metallic element that has high density and is extremely toxic or poisonous even in low concentration when incorporated in the cell system. They usually have high atomic weight and high atomic number. They are considered as the natural components of earth which holds larger significance in nature when scrutinised independently.

Antimony (Sb) is positioned in Group 15 of the Periodic Table, and it lies directly below arsenic (As) and shares some similar chemical and toxicological properties [1,2]. Antimony (Sb) being the less common natural element is a strong chalcophile which frequently coexists in sulphidic mineral phases such as stibnite Sb_2S_3 and $Sb(OH)_6^-$ [3,4]. In the aqueous environments at neutral pH, Sb(III) is more prevalent in the form of $Sb(OH)_3$ under anoxic conditions while Sb(V) dominates in oxic conditions [5]. Contrary to neighbouring element arsenic (As), the biogeochemistry of Sb has received less attention as a result there has been a very limited understanding about its behaviour as an environmental contaminant [6]. Antimony is commonly associated with arsenic (As) and both elements exhibit similar geochemical properties and toxicological effects that depend on their chemical form and oxidation state. Antimony and arsenic can exist in four oxidation states (-III, 0, III and V), while they are mainly found in two oxidation states, trivalent (III) and pentavalent (V), in natural systems [3].

Antimony (Sb) is a naturally occurring metalloid capable of forming toxic products and is a suspected carcinogen and has been classified as a priority substance [7,108]. It is the ninth most mined element in the world [8], and it also occurs in nature as Sb_2O_3. As a very toxic metal, Sb can be widely found in soil and aquatic systems (mainly fresh and marine water) in the form of stibnite (Sb_2S_3). Soil enriched with antimony is a direct manifestation caused by the mobilization of antimony from mineral ores, discarded mines and other activities such as mining, mineral smelting along with drugs and pesticides production [9,10]. Diantimony trioxide Sb_2O_3 is used

as a catalyst in the production of polyethylene terephthalate (PET) and as a flame retardant in the production of plastics, textiles and rubber [11]. The toxicity level of antimony varies in environmental species along with their different oxidation states. Sb(III) has been found to be highest potential toxicity as compared to Sb(V); followed by inorganic forms [12].

Anthropogenic activities, such as metal (metalloid) mining, smelting and the burning of fossil fuels, have led to the release of a large amount of antimony (Sb)-containing compounds into the environment, causing serious Sb pollution in some regions of the world [13]. Natural sources of Sb in the environment can include volcanism, weathering of Sb-bearing crust rocks, minerals, etc. [14]. Antimony is also widely used in the manufacture of small arms and ammunition semiconductors, batteries, alloys, pigments, catalysts, etc. [5,15]. As toxicity and mobility of Sb strongly depend on its chemical speciation [16], it is of utmost importance that proper cultivation of its chemistry and impact in ecosystems is ensured. Human exposure to antimony can directly result in liver, lung, and cardiovascular diseases which can prove to be extremely fatal. This mainly happens due to its affinity to the thiol groups of glutathione and proteins [7,13]. In recent years, antimony has been regarded as a genotoxic element and has also been viewed as an "emerging" contaminant.

With extensive research peaking, it was observed that Sb(V) acts as a predominant species in oxygenated systems while Sb(III) happens to be the main constituent in species inhabiting Sb-dominated anoxic and pore waters [17]. Although its presence has been detected in surface water where the availability of this metal is mainly due to the activity of phytoplanktons. Presence of Sb was also documented in the aquatic environment because of rock weathering, soil runoff and anthropogenic activities. Concentration of Sb in freshwaters was reported ranging from a few ng/l to a few µg/l depending on location. and in sediments the range order was of a few µg/g. Studies and surveys also indicate that higher concentrations of Sb in the environment and ecosystem is mainly due to anthropogenic sources mostly in places which are in proximity to smelting plants [10]. Despite the toxicity of associated elements, many indigenous microorganisms can survive and thrive in soils and contaminated waters [18].

The US environmental agency considers Sb as one of the most hazardous contaminants, furthermore the maximum contaminant level of Sb in drinking water is 6 µg/l according to US EPA reflecting the overall toxicity of the element (US Environmental Protection Agency, 1999). Sb speciation greatly influences the toxic effects in general which naturally sheds light upon various species surviving in Sb rich area. One of the most decisive observations regarding antimony was the toxicity of its oxidative states. As mentioned before, Sb(III) showed highest toxicity followed by Sb(V) and then organo-antimonial [19].

Since the inception of various industries, heavy metal pollution has always been a paramount cause of concern. One of the major reasons for this concern is due to the contaminated waters crossing their legal limits. To counteract such adulteration, different chemical processes have been designed and proposed for successful removal of antimony from any aqueous medium. A brief review of the paper [20], reports an analysis on the chemically motivated removal techniques such as coagulation/flocculation, membrane separation and other electrochemical methods. These chemical processes have been successful in eradicating heavy metals from drinking water yet various factors affected its functioning and limited its impact. It was during such hindrances that the scientific world started taking keen interest in employing microbial communities and other natural components to combat the effects of pollution. During 2009, in addition to all the existing papers, it was reported that in comparison to Sb(V), Sb(III) had a tendency to reach its critical biological targets faster along with the potential to retain itself for a longer period of time in the body [21]. This observation further solidified the importance of Sb detoxification as Sb(III) is a more abundant element in nature than Sb(V).

Considering the above listed human activities and natural incidents have increased the exposure to Sb resulting in accumulation of antimony in the environment as well as in the human community. This shift in dimension of combating effects of pollution ultimately led to a massive increment in research and scientific exploration about the general condition of antimony contamination in the world.

Mechanisms adopted by bacteria to thrive in Antimony rich environment

Many microorganisms adopt several intrinsic mechanisms to counter the effects of toxic metalloid intrusion without disrupting

its cell machineries. They indigenously developed methods which renders such organisms an additive advantage to survive in areas contaminated by toxic metalloids. As antimony is not an essential trace nutrient, it becomes difficult to cultivate the plausible mechanisms adopted by resistant organisms. Our knowledge on antimony stands very limited and hence in this paper we have tried to summarise the most common mechanisms adopted by bacterial cells to counter the ramifications of Sb toxicity. In this article, we have broadly classified the methods adopted by bacterial cells for detoxification of antimony into four parts and each of the topics discussed brings forth the significance it has in bioremediation-

- Sb uptake and efflux- Resistant genes, efflux transporters, operons involved and remediation through biosorption.
- Oxidation and Reduction
- Antimony resistant bacteria in various other ecosystems and its relevance in element cycle in nature.
- Biomethylation.

Antimony uptake and Efflux: (Resistant genes, Efflux Transporters, operons involved and Biosorption)

Sb(III) uptake facilitated by GlpF efflux protein-

GlpF or glycerol uptake facilitator protein is an aquaglyceroporin, belonging to the ABC transporter protein family which helps in uptake of glycerol with limited permeability to water and uncharged ions [22]. GlpF was the first aquaglyceroporin in *E. coli* that facilitated Sb(III) influx from outer environment to the cytoplasm of the cell [23]. GlpF plays a very a crucial role in the uptake of glycerol in *E. coli* cells that naturally enables Sb influx in cells too as Sb mimics the structure of glycerol thereby forming a pseudo compound which is analogous to the structure of glycerol [22]. Furthermore, it was noticed that disruption of GlpF protein led to inhibition in Sb uptake. This modification paved the path for a possible bioremediation method including the significance of ars operon, which rose to prominence in the Sb detoxification process.

Significance of ars operon in antimony detoxification

Arsenic also has been a major cause of concern when it comes to environmental pollution. It is regarded as the most prevalent toxic metal, derived as a by-product due to numerous activities both natural and man-influenced. Therefore, few microorganisms have adopted or developed some intrinsic mechanisms to sustain life in various arsenic enriched environments. One such dominant mechanism is the proteins and genes involved in the ars operon. This ars operon was said to be effective in providing resistance against antimony.

The *ars*RDABC operon of the conjugative R-factor R773 was said to be the most dominant set of genes that conferred resistance to inorganic As(III) and Sb(III) in *E. coli* [24,25]. The ars operon of the *E. coli* conjugal plasmid R773 has five genes namely, arsR, arsD, arsA, arsB and arsC. The arsR and arsD genes encode for metalloregulatory proteins. The arsR and arsD genes are also responsible for encoding repressors that control the basal and upper levels of ars operon expression, while the arsABC genes encode the structural components of the arsenic resistance mechanism. ArsA is an ATPase which forms a complex with ArsB, the transmembrane arsenite efflux pump. ArsC on the other hand was responsible for encoding a small, cytoplasmically located reducing enzyme called arsenate reductase which reduces arsenate to arsenite, which can then be pumped out of the cell. The arsB protein is capable of exporting arsenite even in the absence of arsA. The arsA and arsB genes encoded the subunits of an ATP-driven arsenite pump. Whereas arsA protein functioned as the catalytic subunit of the pump. In the absence of the arsA gene, the arsB gene product alone provides partial arsenite resistance, most likely by functioning as a secondary uniporter. Furthermore, it was revealed that arsenate resistance was also conferred by reduction to arsenite by the arsC gene product; the resulting arsenite then got extruded by the transporter system. Hence, this ultimately led to the conclusion that arsenite resistance was a result of the catalytic function of the arsB gene product [24,25]. Further research established that a new class of transposon was discovered named Tn2502 located on plasmid pYV of *Yersinia enterocolitica*, which divergently transcribed the gene arsH a homolog to arsRBC. It provided resistance against arsenite and arsenate [26,27]. The presence of this gene either in cis or in trans was said to be essential for arsenic resistance in *Yersinia enterocolitica*. In a study conducted by Chen., *et al*. demonstrated that ArsH was an organoarsenic oxidase that took part in oxidation of trivalent herbicides such as monosodium methyl arsenate and aromatic arsenicals to its pentavalent species [28].

After extensive research on ars operon, it was found that ars operon in *E. coli.* equally conferred resistance against antimony. This discovery paved the path for further exploration of antimony

and arsenic oriented bioremediation processes with the help of ars operon.

As study and research further advanced, few more facts were taken into consideration on ars operon. It was noted that plasmid-encoded arsenical resistance (ars) operons in both gram-positive and gram-negative bacteria showed high level metalloid resistance. It was also documented that the chromosomally encoded ars operon when subcloned into a multicopy plasmid conferred a moderate level resistance to both arsenite and antimonite in *Escherichia coli* [29]. Besides, when this operon was removed from the chromosome, the cells exhibited hypersensitivity to arsenite, antimonite, and arsenate. Hence it was concluded that *Escherichia coli* R factor R773 encodes for a transport system that extrudes arsenate, arsenite, and antimonite; thereby lowering the intracellular concentration of toxic oxyanion that basically conferred the resistance. This efflux pump was arsAB which hydrolysed ATP and derived energy that helped in the expulsion of antimony from the cell. These plasmid-encoded metalloid resistance were widespread in bacterial species even before the emergence of resistance to most antibiotics.

As understanding of ars operon further increased it was noted that in the absence of the arsA gene, the arsB protein functions similarly to the staphylococcal protein; presumably both having similar functions that is, secondary arsenite porters. The levels of similarity of the chromosomal ars gene products with their R773 counterparts was 75, 90, and 94%, respectively. The lack of arsD and arsA genes gave the chromosomal arsRBC operon a physical structure more like structures of the ars operons from plasmids of gram-positive bacteria, even though the gene products exhibited only moderate (57% for arsB) to poor (19% for arsC) similarity [29]. Furthermore, as chromosomal ars operon conferred low-level resistance to the oxyanions, the cloned genes increased resistance when expressed on a multiple copy of plasmids. The deletion of the ars genes from the chromosome resulted in hypersensitivity to arsenite. It was considered that hypersensitivity to arsenite gets also reversed by expression of the operon from a plasmid [29]. The whole mechanism and noteworthiness of ars operon in arsenite resistance invoked the curiosity of scientists working on antimony resistance and detoxification. Vigorous research led to another precise and cohesive denouement about the greater significance of arsAB complex in Sb efflux from the cell. As arsB was the most widespread determinant of arsenic resistance in bacteria and archaea, it was further noticed that primary level transportation of Sb(III) was catalysed by the arsB protein along with everted carrier protein vesicles that accumulated Sb(III) with the help of energy supplied by NADH oxidation. This led to the discussion about dissipation of either of the membrane potential or the pH gradient. On the contrary, neither of their dissipation did not prevent Sb(III) uptake. While dissipation of both completely uncoupled the carrier protein that is the GlpF protein. This indicated that transport of the metalloid was dependent on electrical or the chemical component of the electrochemical gradient. Reciprocally, Sb(III) transport via arsB dissipated both pH gradient and membrane potential. These results strongly suggested that arsB was considered an antiporter that catalyses metalloid exchange [30].

Ant operon in Antimony-resistant bacteria

An experimental study in 2021, talks about the significance of ant operon in antimony resistance posed by bacterial species. The study documents that ant operon significantly confers resistance to Sb(III). This operon was present in the bacterium *Comamonas testosterone* JL40 isolated from the antimony mining site. Furthermore, it was seen that operon was transcriptionally regulated by the product of the first gene in the operon, antR. AntR belongs to the member of the ArsR/SmtB family of metal/metalloid responsive repressor resistance. In addition to that antR was also isolated and purified from *C. testosterone* and its response to metalloids were documented. It responded in the order of Sb(III) methylarsenite(MAs(III)>>As(III)). The structure of antR was observed to be homodimeric in nature and it adopted the classical ArsR/SmtB topology and characteristics. This was the first report on the structure of AntR and its behaviour post the binding of a transcriptional repressor in an antimony rich environment [31].

Further research by [32], on bacterium *Comamonas testosterone* JL40 led to the identification of a novel bacterial P1B- type antimonite Sb(III)-translocating ATPase from *C testosterone* that directly conferred Sb(III) resistance. Comparative proteomics analysis of strain JL40 showed results that indicate an upregulation of the ant operon in the presence of Sb(III) [32]. The ant operon is said to have three main genes, AntC, AntR and AntA. AntR as stated earlier belongs to the ArsR/SmtB family of metalloregulatory proteins while AntA belongs to the P1B family of P-type translocating ATPases. AntA considerably had both similarities and dissimilarities from the other members belonging to the P1B- subfamily hence it was considered to have unique properties and was said to be the first member of subfamily P1B-8.

It was also noticed that the expression of antA in *E. coli* AW3110 (ars) conferred resistance to Sb(III) and reduced its intracellular concentration while it did not provide any resistance to As (III) or any other metal. Later, it was seen that everted membrane vesicles from cells expressing antA accumulated Sb(III) but not As (III). Besides, it was also documented that the antC, a small protein possessed a potential Sb(III) binding site and its co-expression with antA considerably increased resistance to Sb(III). This study helps us understand the biogeochemical movement of antimony by bacteria and its importance in bioremediation studies.

A novel efflux transporter-arsK

In a comprehensive study performed an experiment to study the function of a major facilitator superfamily gene-arsK, its expression in *Agrobacterium tumefaciens* GW4 and its role in biogeochemical cycle [33]. The study showed that arsK was a novel efflux transporter and its expression was induced by arsenic and arsenate. It was also taken into consideration that arsK was phylogenetically divergent from other arsenic efflux proteins. To test the function of the arsK gene in the ars operon; the arsK gene from strain GW4 was cloned under the control of the lac promoter, into a plasmid. Then it was expressed under arsenic-hypersensitive strain AW31110, and the resistant arsenicals genes were assayed. The results elucidated the importance of arsK and the resistance it conferred to the bacterial cell. It was seen that arsK was clearly responsible for the resistance conferred to *Agrobacterium* against As (III), Sb(III), R Oxarsone (III), and Methyl As (III); as it reduced cellular accumulation of such elements. Although the complexities and the intricate mechanism involved remains unexplored, the study opened new fronts for further research on *ars*K gene and its independent significance in Sb resistance.

Na+:H+ Antiporter and the genes involved in *Agrobacterium tumefaciens* for antimonite bioremediation

In recent studies regarding the genetics underlying As (III) oxidation in *Agrobacterium tumefaciens*, it was discovered that regulatory (aoxR) and Na+:H+ antiporter (mrpB) mutants are defective in As (III) oxidation [34,35]. Under further investigations, it was found that the wild-type *A. tumefaciens* strain was also capable of oxidizing Sb(III).

The wild-type *A. tumefaciens* strain 5A and the aoxR and mrpB mutants were all found to oxidize Sb(III) at the same rate. As expected for these culturing conditions and genotypes expression of the As (III) oxidase structural genes, aoxAB, was observed in the wild-type strain but not in the aoxR regulatory mutant [34,35]. This provided evidence that Sb(III) oxidation occurred in the absence of aoxAB expression and that Sb(III) does not induce aoxAB expression from an Sb(III)-sensitive promoter within the aox operon. This means regulatory mutant and wild-type *A. tumefaciens* are capable of Sb(III) oxidation, but regulatory mutant is defective in case of As (III) oxidation. Therefore, the complete lack of aoxAB expression in experiments with this mutant provides strong evidence that the As (III) oxidase enzyme is not involved in Sb(III) oxidation in this organism. Further evidence suggesting that Sb(III) oxidation occurs by a separate biochemical pathway comes from the results obtained with the mrpB mutant. As because mrpB is part of a multi-subunit Na+:H+ antiporter (reviewed in [36]) that was found to be essential for As (III) oxidation [35], it nevertheless is not required for Sb(III) oxidation. The report from genetic study shows that Sb(III) oxidation occurs in the absence of enzymes and cellular functions that are essential for As (III) oxidation, As (III) oxidase enzyme. From the study [37], we come to a conclusion that cellular mechanisms and enzymes for Sb(III) oxidation are different from those used for As (III) and the growth responses exhibited by the organism suggested Sb(III) is considerably more toxic than As (III).

Trivalent metalloid/ H+ Antiporter - Acr3p and its homolog Yqcl

Arc3p and its homolog Yqcl which are mainly found in *Actinobacteria* and *Alphaproteobacteria* was said to have the potential to substitute for arsB which functioned as an Sb(III) efflux pump [38,39]. Acr3p was also identified in *Saccharomyces cerevisiae* [40]. Acr3p which was found in archaea and eukaryotes consisted of three-gene cluster (acr1, acr2, and acr3) and was directly responsible for Sb(III) resistance. Studies from [41], showed that deletion of acr3 in *Agrobacterium tumefaciens* 5A resulted in more sensitivity to Sb(III) but the reason for this occurrence remains unknown. In addition, it was discovered that Acr3 also conferred resistance yeast and *Leishmania* sp. Although in *Leishmania* sp. Sb(III) entered through an aquaglyceroporin named AQP1 correlated well with the Sb(III) accumulation in *Leishmania* cells [42].

Remediation through biosorption

Biosorption is defined as the ability of biomaterials to accumulate heavy metals from wastewater through metabolic

mediation such as using ATP or spontaneous physiochemical pathways of uptake (devoid of ATP consumption) or the general property of certain inactive non-living microbial biomass which bind and concentrate heavy metals from a highly diluted aqueous solution [43-45]. Biosorption has mainly been attributed to the cell wall structure containing functional groups such as amino, hydroxyl, carboxyl and sulphate, acting as binding sites for metals via electrostatic attraction, ion exchange and complexation. The process of biosorption by dead biomass proved to be a promising technology for the removal of heavy metals from aqueous solutions [20]. Biosorption capacities for Sb(III) were found to be in the range of 1.81-4.88 mg/g (pH 4.0, 25°C) depending on the ionic strength [46]. The biosorption process usually follows the pseudo-second-order rate kinetics. Carboxyl, hydroxyl and amino groups are supposedly involved in Sb(III) biosorption by surface complexation and hydrogen bonding with protein structures. The biosorption process is further accompanied by oxidation of Sb(III) to Sb(V), in a percentage below 7%, and this reaction was dependent on pH and time [20].

Based on the importance of biosorption, studies indicated the adsorption of Sb(III) and Sb(V) by environmental microbes (e.g., bacteria, fungi, algae, moss). Studies from [46], probed into the biosorption processes of Sb(III) and Sb(V) including the mechanism involved in the bacterial species like that of cyanobacteria and *Microcystis,* which was isolated from Taihu Lake in China. *Microcystis* was said to have a large adsorption potential for Sb(III) and Sb(V).

Oxidation and reduction

In biogeochemical cycling of elements, the most significant methods of detoxifications arise from redox reactions. It can be an intrinsically employed method or a method that's human manipulated. Likewise, bacteria when involved in detoxification of antimony engages in redox reactions for nullifying the consequences that take place due to heavy metal toxicity. In this paper we also discuss the redox induced biotransformation and the plausible pathways of oxidation and reduction by bacterial cells. Till date biotransformation remains as the most effective method in bioremediation [47].

Oxidation

Microorganisms oxidize the highly toxic Sb(III) to the less toxic Sb(V) in natural environments, playing a vital role in microbial detoxification. As Sb(V) is a thermodynamically stable species in aerobic environments, it has been considered ten times less toxic than Sb(III) [48].

An increasing number of researchers are gradually focusing on Sb microbial oxidation processes and oxidation mechanisms in order to improve detoxification methods. At present many Sb(III)-oxidizing bacteria have been isolated from Sb-contaminated soils and sediments [2,3,15,49,50].

Antimony oxidising bacteria and its variations

Stibiobacter senarmontii, an Sb(III)-oxidizing bacterium, is one of the earliest bacteria recovered which was apparently capable of oxidising antimony in the presence of oxygen with the support of chemoautotrophic growth [51]. Most of the Sb(III)-oxidizing strains that have been described since early studies and the studies discussing about *Stiobacter senarmontii* states that the strains can oxidise Sb(III) during heterotrophic growth. This indicates that the process may serve as a cellular detoxification mechanism instead of conservation of energy that takes place during oxidation to support the biochemical incorporation of carbon dioxide into the organic matrix of the cell.

Further studies and research led to the discovery and isolation of more than 60 Sb(III)-oxidizing strains from mining soils and contaminated sediments with the increased focus on bacterial Sb(III) oxidation. The Sb(III)-oxidizing strains identified thus far belong to 17 genera, including *Pseudomonas* (22 strains), *Comamonas* (10 strains), *Agrobacterium* (8 strains), *Stenotrophomonas* (3 strains), *Acinetobacter* (7 strains), *Variovorax* (3 strains), *Paracoccus* (2 strains), *Aminobacter* (1 strain), *Sphingopyxis* (2 strains) Arthrobacter (1 strain), *Bacillus* (1 strain), *Janibacter* (1 strain), *Stibiobacter* (1 strain), *Thiobacillus* (1 strain), *Hydrogenophaga* (1 strain), *Cupriavidus* (1 strain), and *Sinorhizobium* (1 strain) [2,3,37,50-54]. Amongst all these Sb(III)-oxidizing strains, *Pseudomonas, Comamonas, Agrobacterium,* and *Acinetobacter* are four major genera that make up 34%, 15%, 12%, and 11% of known Sb(III)-oxidizing strains, respectively. Amongst the 65 strains mentioned above, only two thus far appear to be *lithoautotrophs*. Unlike the case for As(III) to date, there are no examples of anaerobes that can oxidize Sb(III) by using it as an electron donor to support anoxygenic photosynthesis [55].

All these Sb(III)-oxidizing strains were classified into *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*,

and *Actinobacteria*. Among all of these Sb(III)-oxidizing strains, 49% belong to *Gammaproteobacteria*. Amongst which *Pseudomonas* and *Acinetobacter* were the two most common species. *Comamonas* strains belong to *Betaproteobacteria*, while *Agrobacterium* strains are members of the *Alphaproteobacteria*. The strains belonging to *Betaproteobacteria* showed the highest Sb(III) oxidation rate; for example, *Comamonas testosteroni* S44 was responsible for complete oxidation of 50 µM Sb(III) to Sb(V) within 3 days [2,56]. Interestingly it was noted that *C. testosteroni* S44 could not oxidize As(III) [57], indicating that the molecular mechanism of Sb(III) oxidation may sometimes differ as that of As(III) oxidation (Lehr., *et al*. 2007). In addition to that it was noticed that Sb-dependent chemoautotrophic growth of *V. paradoxus* strain IDSBO-4 was able to oxidize ~500 µM Sb(III) to Sb(V) over an incubation period of 10 days [50]. Hence, it can be very well seen that oxidation by bacterial species in general is an important aspect in bioremediation of Sb.

Biotic antimonite oxidation mediated by aioA

Bacterial As(III) oxidation involves the As(III) oxidase aioAB or arxAB [58,59]. AioAB functions as an aerobic As (III) oxidase [60] while arxAB catalyzes the anaerobic oxidation of As(III) [59]. It was suggested through *in vitro* processes with purified As(III) oxidase from *Rhizobium* sp [61]. Strain NT-26 provides direct evidence of aioAB encoding for As(III) oxidase which was successful in expressing itself only in the presence of As(III) and not Sb(III) [61]. However, with *in vivo* experiments it was seen that a mutation in aioA (the gene encoding the larger subunit of As(III) oxidase) reduces the ability to oxidise Sb(III) by nearly one-third of the wild type [61].

Role of homologous aioA genes in *Hydrogenophaga taeniospiralis* and *Variovorax paradoxus*

Terry., *et al*. conducted an experiment, two bacterial strains from contaminated mine sediments *Hydrogenophaga taeniospiralis* strain IDSBO-1 and *Variovorax paradoxus* strain IDSBO-4 were isolated and were allowed to grow on tartrate compounds [50]. They were both equally responsible for the oxidation of Sb(III) using either nitrate or oxygen respectively as a terminal acceptor of electrons. Both the isolates were from the Comamonadaceae family and showed 99% similarity to species of this family. These novel strains possessed a gene with homology to the aioA gene which encodes for As(III) oxidase. Furthermore it was seen that both the strains could achieve As(III) oxidation aerobically, but only strain IDSBO-4 oxidised Sb(III) in the presence of air while IDSBO-1 could achieve this only via nitrate respiration [50]. The results further suggested that both the isolates were capable of chemolithoautotrophic growth using As(III) as primary electron donor and indicated a 90% similarity to other As(III) oxidizing bacteria. IDSBO-4 also demonstrated oxidation of Sb(III) from Sb(III) tartrate including the incorporation of radiolabelled bicarbonate indicating the possible Sb(III) dependent autotrophy [50]. Lastly, it was noticed that Enrichment cultures produced the Sb(V) oxide mineral mopungite and extremely Lee's amounts of Sb(III) bearing seanaramonite as precipitate [50].

Antimonite oxidation mediated by hydrogen peroxide

Hydrogen peroxide is considered to play a key role in the redox chemistry of several trace elements in aquatic environments [62-65]. It was noted that katA, a gene that is present in the Sb(III) oxidizing strain of *A. tumefaciens* played an important role in Sb oxidation [28]. It was seen that Sb(III) oxidation rate was increased by the disruption of katA. This led to the conclusion that cellular hydrogen peroxide concentration may have been the cause of consistent growth [66]. Khakimova., *et al*. in later studies stated that hydrogen peroxide was able to induce the expression of katA in strain GW4 [67]. Moreover, hypothetical model of IscRs regulation of bacterial Sb(III) oxidation summarized the significance of hydrogen peroxide, which gets produced under bacterial oxidative stress response in the presence of Sb(III) and in turn hastens oxidation of Sb(III) to Sb(V) under alkaline conditions [56]. Hence, this report clearly highlights the importance of hydrogen peroxide in antimonite oxidation.

Bacteria responsible for antimonite oxidation in antimony-contaminated soil revealed by DNA-SIP coupled to metagenomics:

A research in 2021 revealed that Sb(III) oxidation can minimize antimony toxicity through DNA-SIP protocol coupled to metagenomics [68]. DNA-SIP protocol is basically stable isotope probing (DNA-SIP) is a powerful method that links identity to function within microbial communities. The combination of DNA-SIP with multiplexed high throughput DNA sequencing enables simultaneous mapping of *in situ* assimilation dynamics for thousands of microbial taxonomic units [69]. Sb(III) oxidizing microcosms were established using Sb-contaminated rice paddies as inocula. This led to the observation that showed an increased amount of copies and transcription of the aioA gene (arsenate

oxidizing gene) in the microcosms during the biotic Sb(III) oxidation. Thus, proving that Sb contamination influences the genes involved in bacterial cells. Several other Sb(III) oxidizing bacteria were also identified using a combination of DNA-SIP and shotgun metagenomic. These were *Paracoccus, Rhizobium, Achromabacter* and *Hydrogenophaga.* Facultatively auxotrophic *Paracoccus* sp. have been reported to catalyze Sb(III) oxidation under both oxic and anoxic conditions [54,70]. *Rhizobium and Achromobacter members* were previously described as autotrophic As(III)/Sb(III) resistant bacteria [2,61,71]. *Hydrogenophaga* was identified as an arsenite oxidizer containing the aioA genes [72-74]. Metagenomic analysis further indicated the presence of the aioA genes in these putative Sb oxidizing bacteria. The study further focused on autotrophic Sb oxidizing bacteria and the microbes involved. However, the presence and involvement of heterotrophic bacteria might also play a critical part in the bioremediation, which still needs more investigation.

Antimony oxidation in *Agrobacterium tumefaciens* and the significance of anoA

Microbial redox transformation is presumed to be an important antimony biogeochemical cycle in nature.

Proteomics analysis as well as reverse transcriptase along with PCR- analysis of Sb(III) gave some crucial leverage points about the importance of oxidation and reduction in the antimony cycle. It was revealed that an oxi-bacterium commonly known as *Agrobacterium tumefaciens* GW4 possessed an oxido-reductase anoA which is also widely distributed in other microorganisms, it was seen to have the capability to oxidise Sb(III). Sb(III) which has an oxidation state of +3 happens to be more toxic in nature hence when oxidised to state +5 that is Sb(V); the associated antimony-based compound become less toxic in nature, thereby this oxidation state change also adds to the list of possible bioremediation techniques. Later, it was seen that deletion of anoA reduced Sb(III) resistance and decreased antimonite oxidation by 27%.

While it was also noted that anoA complemental strain was like the wild type strain of GW4 and GW4 anoA overexpression increased Sb(III) oxidation by 34% [75].

Furthermore, an addition of Sb(III) upregulated anoA expression and cloning of anoA to *E. coli* demonstrated direct transferability of this activity. With further advancement of this study, it was seen that a His-tag purified anoA in certain cases required NADP+ as cofactor exhibited a K_m for Sb(III) of 64 +/- 10 µM and a V_{max} of 150 +/- 7 nmol/min/mg.

This study therefore contributed to important initial steps that ensured a better understanding of microbe-antimony interactions and the knowledge required to gain a perception about the microbial participation in antimony biogeochemical cycling in nature although the exact function and the regulation role of anoA particularly requires more cultivation and clarity [76].

The phoB gene in *Agrobacterium tumefaciens*

During the study of microbial oxidation of antimony [76], Sb(III) to Sb(V) and Sb resistance conferred by the presence of anoA also found that the expression of phosphate transporters was induced by Sb(III) in *Agrobacterium tumefaciens* GW4. This was revealed in the proteonomic analysis. Thus, predicting that the phosphate regulator phoB may regulate bacterial Sb(III) oxidation and resistance. The study gave a comprehensive detail about the significance of phosphate transporters in detoxification methods employed by *Agrobacterium.* The results obtained after emphasised genomic analysis showed the presence of three phoB (named $phoB_1$, $phoB_2$ and phoR gene) in GW4. The reported assay also showed that both $phoB_1$ and $phoB_2$ were induced in low phosphate condition (50 µM) and it was seen that only was induced by Sb(III).

Furthermore, gene knockout/complementation of Sb(III) oxidation and Sb(III) tests showed that deletion of $phoB_2$significantly inhibited the expression of anoA and decreased bacterial Sb(III) oxidation efficiency thereby reducing Sb(III) resistance. On the other hand, deletion of $phoB_2$ did not affect anoA expression level nor suppressed Sb(III) resistant/oxidation activity. Later, an electrophoretic mobility shift assay (EMSA) was done which indicated about binding with the promoter sequence of anoA.

Site directed mutagenesis and short fragment EMSA revealed the exact DNA binding sequence for protein-DNA interaction. The results indicated that was also associated with Sb(III) resistance.

The regulation method provides a great insight into the contribution of bacterial species in probable detoxification strategies and their survival capabilities in Sb rich environments. The methods above mainly constitute the basics of Sb oriented bioremediation.

Reduction

Some microorganisms have the potential to reduce Sb(V) to Sb(III) in the environment, especially under anaerobic conditions. In the paper [77] Hockmann., *et al*. it was noted that the endogenous microbial community reduced Sb(V) to Sb(III) under anaerobic conditions from a military shooting range soil, utilizing the lactate as an electron donor. According to a study [79], it was reported that Sb(V) was reduced to Sb(III) by a natural microbiological population in Sb-contaminated stibnite mine sediment and that the rates of Sb(V) reduction were enhanced by amendment with acetate. The reduction of Sb(V) is coupled to anaerobic heterotrophic respiration, according to radioisotope experiments, where Sb(V) is the terminal electron acceptor and acetate is the electron donor. Another discussion [80], demonstrated that some autotrophic bacteria from sediment samples of a flooded mine pit can leverage hydrogen gas () as an electron donor for Sb(V) reduction and generate the mineral precipitate Sb_2S_3.

Bio-reduction using Hydrogen as Electron Donor:

In the experiment conducted by Lai., *et al*. [79], on autotrophic microbial Sb(V) reduction using hydrogen gas as an electron donor was another crucial demonstration showing the significance of antimonate reduction and its positive after-effects in biogeochemical cycling and detoxification of antimony. As it was noted that trivalent antimony compounds are more toxic than the pentavalent state; as because the trivalent form of Sb can readily precipitate with sulphide when its strongly adsorbed by the Fe_3OH at neutral pH and the precipitate was removed by the process of centrifugation or filtration [80]. In this experiment the stoichiometry, Sb(V) reduction was intricately studied using hydrogen or lactate as the electron donor through EDS and SEM. Later, it was seen that *Rhizobium* was dominant in the hydrogen fed cultures implying its importance for Sb(V) reduction; while on the other hand lactate fed cultures contained more fermenters or heterotrophic microorganisms. Thus, supporting the claim of Sb(V) remediation using hydrogen as an inorganic electron donor.

Bio-reduction of antimonate

The knowledge about Sb(V) reduction, unlike Sb(III) oxidation remains considerably elusive. Antimonate reduction in general occurs in anaerobic conditions [5]. Kulp., *et al*. [78] reported about anaerobic bacterial reduction of Sb(V) in anoxic sediments. In an experiment [81], Abin and Hollibaugh isolated a bacterium that was capable of using Sb(V) as a terminal electron acceptor for anaerobic respiration and the results showed the presence of Sb(III) precipitate in the form of microcrystals of antimony trioxide. The bacterium designated strain MLFW-2 was a sporulating member of highly branched lineage within the order of *Bacillales* (Phylum *Firmicutes*). The report provided by Abin and Hollibaugh was a breakthrough in determining the importance of antimonate reduction which unequivocally suggested that a bacterium was fully capable of growth and reproduction by conserving energy from the reduction of antimonate. when isolated could generate energy during anaerobic Sb(V) reduction. However the molecular mechanisms and enzymes involved still require more clarity [81].

Furthermore, bacterial Sb(V) reduction can be a promising bioremediation strategy as because Sb(III) readily precipitates with sulphide and is strongly absorbed by Fe phases in a reducing environment [77,82]. A study by Hockmann., *et al*. indicated that Sb(V) reduction to Sb(III) was more rapid in anaerobic calcareous soil when indigenous microorganisms were present [77]. Subsequently, the Sb(III) generated bound to the surface of iron hydroxides, which led to the immobilization of Sb. In addition, A sulfate-reducing bacteria (SRB) was employed to remove Sb(V) from Sb mine drainage too [83]. The SRB converted sulfate ions into sulphide that was responsible in the reduction of Sb(V) to Sb(III) following which the results showed the precipitation of stibnite (Sb_2S_3) [79]. Furthermore, a chemoautotrophic microorganism belonging to the Rhizobium genus was identified and the isolate was able to use hydrogen as the sole electron donor for the reduction of Sb(V). This resulted in the production of Sb(III) precipitate in the form of Sb_2O_3 [79].

Warshina., *et al*. [84], concluded the complete genome sequencing of *Geobacter* sp. SVR strain which was said to be an antimonate-reducing bacterium. This strain was isolated from antimony rich soil in Nakase, Japan. It was noted that SVR strains proliferated using antimonate as an electron acceptor providing further insights into the antimonate reduction mechanism.

Antimonate reduction mechanisms demonstrated or initiated by bacterial cells further opens pathways for employing more advanced techniques in bioremediation of Sb, this equally holds promise for anaerobic biotreatment of wastewater containing toxic Sb(V). With further research in future the possibility of discovering the 'now' unknown Sb(V) efflux transporter also seems very likely.

Figure 1: Schematic representation of various Sb efflux and oxidizing mechanisms.

Antimony resistant bacteria in other ecosystems and its relevance in element cycles

Bacterial endophytes resistant to antimony

An Endophytic bacterial strain showing resistance to high antimony concentration was first isolated from the roots of *Hedysarum pallidum* Desf., a Sb accumulator *Fabacea* growing on mining soils. This was a significant step in the intensive research regarding bacterial Endophytes resistant to antimony. The isolated strain was identified as *Serratia marcescens* species. Further analysis of the strain showed miraculous growth compared to the control when it exhibited a minimum inhibitory concentration (MIC) to its growth at 450 mM of Sb. In the presence of excessive concentrations of Sb, corresponding to 30 mM of Sb that is 3652 mg/L of Sb, despite the prevalent factors the strain maintained a stable growth. The Sb toxicity was responsible for a considerable amount of increase ($p < 0.05$) in hydrogen peroxide and in the strain antioxidant biomarkers such as proline, catalase, ascorbate peroxidase, peroxidase and SOD (superoxide dismutase). Positive and effective correlations ($p < 0.05$) were found between oxidative and antioxidant biomarkers thereby emphasising on the interrelationships between them in fighting against oxidative stress. Although this bacterial cell is considered pathogenic in higher mammals, it provides a beneficial solution to its host's growth in Sb contaminated soil. Further cultivation yielded greater results that showed *S. marcescens* culture medium caused high accumulation of hydrogen peroxide in the bacterial cells leading to lipid peroxidation and therefore a decrease in its growth. This indicates that the bacterial cell directed its focus on survival more than that of growth. This adaptation gave an insight on how during Sb stress the strains implement its antioxidant molecules to fight the stress induced by excessive Sb presence. Although after a certain threshold of 1216.6 mg/L Sb, only the sensitive molecules such as proline CAT and APX remain as the only ones involved in fighting Sb stress. Ability of the strains to survive and produce enough biomass and biomarkers attests to its high resistance to that of its host *Hedysarum pallidum*. This clearly suggested that the bacterial cell is involved in its host's aptitude to grow in Sb contaminated soil. Thereby making this bacterium a potential candidate for bioremediation of antimony contaminated soil [85].

Microbiome interaction in antimony contaminated rice paddy

Cultivation of the microbiome of rice paddies where antimony and arsenic contaminants dominated the region gave a leveraged interest in remediation study. In the study conducted by Li., *et al*. [86], six rice paddy fields near an active Sb mining area were investigated. The As and Sb concentration of all samples were elevated compared to the background level in China. Nitrate, total As, total Sb and Fe(III) were the major determinants of the microbial community in that region. The understanding of the microorganisms in paddy fields led to the identification of seven bacterial taxa i.e., *Bradyrhizobium, Bryobacter, Candidatus solibacter, Geobacter, Gemmatimonas, Halingum* and *Sphingomonas.* These taxa were strongly correlated with As and Sb contaminant fractions and were said to have intrinsic mechanisms to metabolize As and Sb. This study concluded that many soil microorganisms can survive in arsenic and antimony enriched sites. Because these contaminants have the potential to accumulate in rice and jeopardize the life of residents hence research and proper study regarding the microorganisms thriving in such areas is of utmost tin transportation and transformation of As and Sb in paddy soil.

As of now the identification of the bacterial cells have been the primary step further research into their interactions can prove to be a vital step in promoting sustainable and safe production of agro-based products.

Effects of different antimony contamination levels on paddy soil bacterial diversity and community structure

In the study [86] conducted in 2021, to cultivate the response mechanism of paddy soil microorganisms to contamination by antimony (Sb) alone. The experiment involved the addition of

$K(SbO)C_4H_4O_6.1/2H_2O$ with different contents to un-contaminated paddy soil. After which related studies were carried out. 16sRNA was then sequenced in V3-V4 regions of the paddy soil bacteria with different Sb contamination levels. Followingly the α diversity, species enrichment and separation of paddy soil microorganisms were analysed. The biochemical behaviour and influence of Sb fractions on bacterial communities and the ecological functions were simultaneously studied. The results showed that the contents of the Sb_{tot} and Sb(V) increased with increase of contamination level and it was also documented that the difference was significant among the groups. For Sb_{exe} and Sb_{srp} a slight amount of difference between the S100 and S200 groups but significant difference was documented among other groups. Maximum increase in diversity was documented in the S200 group while minimum was documented in the control group. Analysis of relative importance demonstrated that Sb(III) and Sb_{srp} were the main Sb fractions affecting and determining the diversity index of the bacterial community. In addition, the results of principal coordinate analysis (PCoA) showed that there was significant difference in the bacterial community in the control group and in the soil with different contamination levels of Sb. On the basis of diversity analysis, it was noted that Proteobacteria, Actinobacteria and Bacteroidetes were the main dominant phyla in paddy soil with different Sb concentrations and their enrichment including their separation was greater than those of other dominant phyla. Furthermore, through the Bayesian network interference, it was shown that Sb_{tot} affected the *Sphingomonadaceae* and Sb_{srp} affected *Burkholderiaceae*, *Xanthomonadaceae* and *Acidobacteriaceae*. Sb(V) on the other hand mainly impacted *Flavobacteriaceae*, *Rhodopirillaceae* and *Acidobacteriaceae*.

The above results and analysis basically shed light upon the scientific basis for the biochemical restoration potential of paddy soil with different Sb contamination thereby proving that Sb significantly plays a role in diversification of bacterial cells and its moulding of its survival mechanisms in such contaminated areas. The thriving of such a bacterial community can benefit researchers in employing these microbes to develop natural techniques in order to curb the effect of heavy metal pollution.

Bacterial influence in antimony enriched plant ecosystem

It was seen in various studies and research that bacteria-assisted phytoremediation too proved to be a valuable remediation strategy for metal contaminated soils [87,88]. It was assumed that Sb(III)-oxidizing bacteria with PGP characteristics might give an additive advantage of reducing the toxicity of Sb(III) and thereby helping in promoting plant growth in antimony contaminated soil but their actual role in soil plant system remains considerably elusive [89,90]. In a study by Gu., *et al*. [92], it was seen that some Sb(III) oxidizing bacteria with plant growth promoting (PGP) characteristcs contributed largely in the alleviation of Sb toxicity in plants. Furthermore, it was seen that Sb(III) oxidizing bacterium *Bacillus* sp. S3 possessed Indole acetic acid (IAA) production including the activity of 1-aminocyclopropane-1-carboxylate deaminase (ACC deaminase) and they were the only two PGP strategies documented in *Bacillus* sp S3. Later on it was noted that despite the production of IAA and the ACC deaminase activity sharply decreased under Sb stress. After the innoculation of *Bacillus.* sp S3 in Arabidopsis plants it was observed that the plant biomass and chlorophyll content significantly increased, elevation in peroxidation of membrane lipids was seen, it also decreased antioxidant enzyme activity and most importantly it reduced the transcription of Sb transporters including ROS related enzymes in Arabidopsis. The noteworthy aspect of this study was that the inoculation of *Bacillus* sp S3 not only decreased Sb accumulation but was also successful in reducing the percentage of Sb(III) out of the total concentration of Sb in arabidopsis [91]. Such an experiment led to a more distinct understanding of the importance of bacteria in Sb bioremediation. As phytohormone IAA is known to be involved in various activities that have a positive effect on plant growth [92,93]. Any manipulation that can increase the IAA effect also can enhance the survival chances of plants in an antimony contaminated environment.

Arsenic and antimony co-contamination- influence in soil microbial community and its relevance in element cycles in nature

In a study by Li., *et al*. [86], it was documented that microorganisms have the capability to mediate Sb and As transformation thereby changing their toxicity and mobility. The innate influence of As and Sb has been extensively characterized however how their co-contamination influences microbiome metabolic potential remains ambiguous. In this study, two contrasting sites were selected located in Shimen Realgar mine, the largest mine in Asia to explore the adaptability and response of the soil microbiome to As and Sb co-contamination and the impact on

microbial metabolites. It was noted that As and Sb were the driving force that reshaped and redesigned the community composition. It was also noted geochemical parameters pertaining to the co-contamination played a crucial role in the bacterial community. *Bradyrhizobium, Nocardioides, Sphingomonas, Burkholderia and Streptomyces* were predicted to be tolerant to high concentration of As and Sb. Co-occurrence network analysis revealed that the genes related to C-fixation, nitrate/nitrite reduction N-fixation and sulfate reduction showed insignificant amount of correlation with the As and Sb fractions, suggesting that As and Sb biogeochemical cycling might interact with and benefit from C, N and S cycling. The results ultimately indicated that Sb and As concentration not only influences Arsenic related genes but also influences genes correlated with microbial C, N and S cycling.

Biomethylation of antimonite

Biomethylation has been considered a major detoxification process for arsenicals and antimonial elements especially when the processes are manipulated and strategized separately under human surveillance and research. It was seen that inorganic Sb when methylated had the potential to influence environmental toxicity and its bioaccumulation [94].

Andreae., *et al*. 1981 first reported about stibine (SbH_3), monomethyl stibine and dimethyl stibine in natural aquatic systems. Followingly it was observed that volatile Sb and methylated species too in addition coexisted in freshwater, sea water, geothermal hotspots, sewage soils sedimentary deposits and other landfill gas areas [95-98] elucidated on the presence of pondweed (*Potamogeton pectinatus*), moss (*Drepanocladus* sp.), and liverwort in plants that opened the debate of utilising their general interaction and biomethylation reactions as a plausible detoxification method in addition this also encouraged advanced level study of methylated species thriving in antimony rich ambience. As of now Sb methylation has been identified in strains of fungi, methanogenic archaea and bacteria. There has been a considerable amount of research in antimony based fungal species and the toxic gases generated by Sb methylation by *S. brevicaulis* which is said to have been responsible for the spread and outbreak of SIDS (sudden infant death syndrome) [99]. Further studies concluded that along with *S. brevicaulis*, a mix of common environmental bacillus strain in crib mattress contributed to the formation of TMSb but whether it was accountable for the spread of SIDS remains unclear [100,101].

Anaerobic digestion of sewage sludge gave 3 methanobacteria (*Methanobacterium formicicum*, *Methanosarcina barkeri*, and *Methanobacterium thermoautotrophicum*), a sulfate-reducing bacterium (SRB) (*Desulfovibrio vulgaris*), and a peptolytic bacterium (*Clostridium collagenovorans*) these bacterial cells were said to have been responsible in producing TMSb in their culture headspace following which they too engaged in step wise reduction of methylated Sb by producing stibine, monomethyl stibine and dimethyl stibine [102]. Further studies also stated that stimulation of Sb biomethylation by strain of methanogenic archaea and SRB [103]. It was also seen that gram+ve bacteria *Clostridium glycolicum* AS-I had a considerable impact in conversion of inorganic Sb into volatile derivatives of STB DMSb TMSb [104]. Although, one of the major causes of concern while pursuing this experiment was despite the use of organic Sb as a substrate, the production of TMSb was only accomplished the transformation of trimethyl bromo antimony in pure culture strains of *Pseudomonas fluorescens* K27 [105]. In addition, low yields of MMSb, DMSb and TMSb by an aerobic *Flavobacterium* sp. also indicated about a fluke reaction instead of a well-defined molecular resistance reaction. Despite having a vagueness pertaining to the molecular mechanics of Sb biomethylation it was concluded that Sb methylation was a significantly slower process than that of arsenic methylation. Furthermore, it was seen that Sb methylation was enhanced with the presence of As while As biomethylation was subsequently inhibited due to the presence of Sb this mostly was caused due to their similarities in physicochemical properties and their co-occurrence [106]. Therefore, it is of utmost importance to understand the importance of arsenic in antimony biomethylation. Furthermore, it was found that Sb biomethylation by *S. brevicaulis*, *Flavobacterium* sp. and *Cryptococcus humicolus* was enhances by the presence of arsenic [94,106]. Challenger's experiment [107] was pioneer to establish the pathway of step wise reduction of radioactive Sb remains as the most defined mechanism of Sb biomethylation. Biomethylation has evolved from time to time as a plausible remediation process. Sb biomethylation and its step wise reduction following which a proper efflux of the element can be the most vastly effective method for detoxification. Although the mechanisms remain unclear, this field of research opens a plethora of opportunities for further research and developmental studies in bacterial detoxification methods.

Conclusion

The whole report discusses the various kinds of bioremediated mechanisms adopted by bacterial cells to counter the ramification of toxicity induced by Sb as an element. The report not only sheds light upon the plausible mechanisms but also about the human manipulations done on genetic components of bacteria which serves as probable detoxification strategies. In recent years, heavy metal pollution has been an ever-growing cause of concern that has posed threat to our environment and in turn has had the potential to not only disrupt microbial ecosystems but also wreak havoc in higher eukaryotes such as plants and humans.

Human beings without any debate remain as the most vulnerable species when it comes to harmful effects of heavy metal contamination and toxicity. Therefore, strategically designed bioremediation processes not only ensure safety against contaminants but also improves genetic modification in various biological fields which naturally generates more agricultural output and a general upgradation in normal lifestyle. As seen above how advanced study in heavy metal toxicology and microbiology enables us to utilize microbial interactions in our environment, it also subsequently increases the chance to put forward a stronger rebuttal against the vicious aftermath of any kind of hazardous contamination. Hence, with this article we precisely discuss the importance of operons, efflux transporters, oxidation and reduction processes, biogeochemical cycle of Sb and the significant self- developed intracellular Sb resistant processes by bacteria. As scientific interests in Sb metabolism and its impact on the environment continues to grow steadily, it is inevitable that in upcoming years more research will yield much better results that will lead to the development of revolutionary methods which when implemented, can free the world from unhealthy heavy metal pollution.

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