1.1 Introduction

Microbes modify many metals and, metalloids. At their ionic and/or molecular state/s that is. Their versatility in occupying almost all conceivable habitats and transforming infinite varieties of molecules are vital for Earth’s ecological functioning. Infact, the environmental stability and functions are governed by microbes. This happens through their metabolic pathways. Ecosystems are adversely affected due to imbalance caused either naturally or via human activities.

Pollution leads to health effects, causes diseases, affects societal harmony, brings about economic losses, resulting in global changes and shifts in species diversity in all ecosystems subjected to its deleterious facets. In the natural environment, microbes interact with metals and metalloids in all their states. It is thus not surprising that they should “modify” them, often advantageously. Metal ions are changed by both prokaryotes and eukaryotes for various cellular functions. Only Eubacteria and Archaea possess representatives which can oxidize metallic ions such as Fe(II), Mn(II), Co(II), Cu(I), AsO₂ or reduce Fe(III), Mn(IV), Co(III), AsO₄(Ehrlich, 1996). Further, these ions are reduced by some microbes respectively as Hg²⁺ or Ag⁺ to Hg⁰ and Ag⁰ without making use of the energy arising out of such processes (Summers and Sugarman, 1974). Interestingly, many microorganisms have a potential for resistance and/or tolerance.

Main focus here is on bacterial resistance/tolerance of bacteria to arsenic. Organic and metal pollution are of greater relevance, their detoxification is of practical significance. Among these, arsenic: As, a toxic metalloid, is a well known poison with atomic number 33 and molecular weight 74.9 that is ubiquitous in numerous environmental contexts throughout the globe. Albertus Magnus was the first to discover arsenic in 1250 (Rosen, 1999). It has the oxidation states of +5, +3, 0 and -3 resulting in a broad variety of arsenic compounds with diverse physical and chemical properties. Most common soluble forms being pentavalent [+5] As(V) and trivalent [+3] As(III) (Joshi et al., 2009). In the periodic table, As is placed under Group VA (metalloids). Metalloids are elements forming alloys with other metals. It also forms covalent bonds with C, H, O and S. Its mean abundance in the earth crust is 1.8 ppm, in soil it is 5.5 to 13 ppm, in streams it is less than 2 ppb (Mandal and Suzuki 2002) and in ground water it is generally less than 100 ppb.
In 2005, the Agency for Toxic Substances and Disease Registry (ATSDR) categorized As and its compounds as deadly poisons. Among the top 20 hazardous substances, As is ranked first by ATSDR and the USEPA. Nordstrom (2002) came up with 0.2 to 40 mg kg\(^{-1}\) As in soils as its typical range with a worldwide median concentration of 6 mg kg\(^{-1}\). Also, the more toxic, trivalent arsenite [+3] in its inorganic form (Hopenhayn, 2006) is amenable for greater cellular uptakes (Valenzuela et al., 2009) than the less toxic and less mobile pentavalent arsenate [+5].

Global cycles of most elements have undergone significant modifications due to anthropogenic processes. This is no exception with non-essential/toxic elements (Goering et al., 1999). Drinking water As permissible limit is recently revised from 50 ppb to 10 ppb by the World Health Organization.

Mandal and Suzuki (2002) reported As to be much higher in concentration in drinking water in several parts of the world including India. In India, some portions of Jharkhand, Uttar Pradesh, Bihar, West Bengal, Assam, Manipur and Chhattisgarh have so far been found to be contaminated with arsenic at concentrations much above the permissible limit. The microbial transformation of As and associated genetic regulatory pathways have been studied intensively by Oremland and Stolz (2005) during the past decade, in part due to water quality crises in Bangladesh, India and southeast Asia.

Microorganisms posses numerous regulatory genes responsible for different alterations of As, such as methylation, reduction and oxidation. All these processes contribute to the fate, transport and biogeochemical cycling of As. The microbial transformation of arsenate and extrusion of arsenite via the \textit{ars} operon (\textit{ars}C and \textit{ars}B respectively) has been well studied and these genes appear widely distributed throughout the Bacterial and Archaeal domains.

Oxidation of As(III) is commonly observed in soil, sediment and natural waters, and several arsenite oxidases have been characterized. The trivalent arsenite is converted to 100 times less toxic pentavalent arsenate by the process of oxidation. Both heterotrophs and chemoautotrophs mediate As oxidation. These arsenite oxidizing bacteria possess \textit{aox} gene that codes for arsenite oxidase enzyme responsible for arsenite oxidation (Branco et al., 2009; Cai et al., 2009). Thus oxidation of As[+3] to
arsenate is considered as a primary strategy of detoxification. This oxidation process can also be achieved by chemical methods but such methods are costly and result in secondary pollution. Hence, alternatively biological or bioremediation methods of arsenite remediation are preferred. Thus, realizing the importance of bioremediation of arsenite by biological oxidation I undertook the exploration and characterization of arsenite resistant bacteria. As Inskeep et al. (2005) suggest, distribution of \( aox \)-like genes in natural systems enables microbial oxidation of As(III) which is a critical component of the global As cycle.

My research has a focus on isolation of arsenite resistant bacteria from water and sediment samples followed by the identification of the bacteria. Further, the arsenite biotransformation potential in strains highly resistant (1000 ppm) to As is explored. The overarching aim here was to document ecological distribution of arsenic resistant bacteria (ARB) which is governed mostly by the degree of pollution due to arsenic. The main purpose of this study was to provide a current state of knowledge of metal-microbe interactions and to suggest scientific and technological approaches to address some gaps.

1.2 Objectives

- Ecology and quantitative analyses of arsenic resistant bacteria (ARB) in the coastal waters

  The rationale behind this objective was to study the prevalence and abundance of populations of bacteria tolerating arsenic which is of relevance in microbial ecology to understand the extent of its pollution and to realize the potential of such flora in detoxifying arsenic. Nature of the microbe-metal interactions is vital for metal toxicity alleviation. Investigation on the role of bacteria in the modification and sequestration of metals in natural environments is variously relevant. Study on the abundance of arsenic tolerant bacteria (ATB, capable of growth in media with less than 100 ppm As) and ARB (capable of growth in media with \( \geq 100 \) ppm As) will prove useful in detecting metal pollution in the coastal regions. Keeping in view of lack of studies on ATB and ARB from the coastal regions, this major objective was pursued.
• **Taxonomic and molecular characteristics of ARB**
  Many strains of native bacteria can sense arsenic, and detoxify it efficiently. Detailed study on the estuarine and marine bacterial strains was undertaken for this purpose. Screening for As tolerance, biochemical identification, physiological, biochemical and molecular biological characterization and phylogenetic analyses of the bacterial isolates highly resistant to arsenic isolated from different locations of River Mandovi (midstream, estuarine and marine zones) were carried out. Further, uptake and biotransformation of arsenite by a few strains were also investigated to understand certain physiological and biochemical aspects of arsenic resistant bacteria from the study region.

• **Profiling of proteins and characterization of genes responsible for As detoxification**
  Many bacteria have an inherent capability to detoxify arsenic and the genes responsible for detoxification are thought as inducible both by arsenate and arsenite. Since bioremediation steps benefit greatly by knowing the presence of inducible promoters of the *ars* operons, a detailed analyses on this is relevant.

• **Cloning and sequencing of genes involved in arsenic transformation/detoxification pathways**
  By cloning of, and/or screening for, genes responsible for arsenic resistance and, learning more about specific genes and proteins can help in understanding arsenite conversion to arsenate. Biochemical and molecular studies will help advance our information base. This will result in elucidation of tolerance mechanisms, structure, regulation and expressions of genes.