1.1 Overview of the Introduction

Arsenic is a notoriously toxic and omnipresent metalloid in the environment. Constant exposure to an even small amount of inorganic arsenic can lead serious health problems, such as ‘arsenicosis’ and cancer (Tchounwou et al., 2004). It has recently become one of the major environmental toxicants due to its increased application in fertilizers, wood preservatives, insecticides, fungicides, and semiconductors. The burning of fossil fuels and mining have broadened the global arsenic, which leads to the accumulation of the huge quantity of arsenic in the nature. Atmospheric depositions and Riverine inputs dump the pollutants ultimately into the marine environment. In the unpolluted marine environment, the concentration of arsenic is comparatively invariable; the possibility of anthropogenic inputs of arsenic can lead to its high concentration in the coastal areas and estuaries (Kalia and Kambholja, 2015). Also, natural sources like volcanoes, microbial mobilization, marine hydrothermal fluids, etc. are the major contributors to its pollution. Ultimately, the toxic metalloid is transported from these sources (natural and anthropogenic) into the marine environment. Coastal regions and estuaries of many countries including Bangladesh, India, China, Japan, Republic of Korea, Australia and the United States of America have elevated arsenic pollution (Kalia and Kambholja, 2015).

Worldwide millions of people are at risk due to long-term arsenic contaminated groundwater consumption which has become a global public health problem (Milton and Rahman, 2002; Nordstrom DK, 2002). Arsenic pollution of groundwater has led to a considerable epidemic of arsenic toxicity in many parts of India and Bangladesh. It is anticipated that around 85 million people are drinking elevated (above 10 ppb of World Health Organization’s (WHO) standard) levels of arsenic-containing groundwater. Unintentional use of arsenic contaminated groundwater for irrigation purposes and consumption of arsenic contaminated seafood are the major area of concern.

Notorious for its toxicity, arsenic the 33rd element of the periodic table discovered in 1250 by Albert Magnus. Both metallic and nonmetallic properties, classifies it as a metalloid. It sublimes at 613°C, melts at 817°C, has a density of 5.27 g/cm³. It is present in wide variety of chemical forms in
the environment, and its toxicity highly depends on its chemical form. It occurs in four oxidation states: $\text{As}^{+5}$, $\text{As}^{+3}$, $\text{As}^0$, and $\text{As}^{-3}$. Inorganic forms of arsenic are predominant (~ 80%) in nature than organic forms, and Pentavalent arsenic [arsenate, $\text{As}^{+5}$] is the ubiquitous and dominant form of inorganic arsenic found in the marine environment.

Removal of this toxic metalloid has become an essentiality to keep drinking and irrigation water safe. Physicochemical methods for instance adsorption or ion exchange and chemical precipitation are used to eliminate toxic metals from contaminated water. Still, physicochemical methods are relatively costlier and risk of secondary pollutions always associated with it. Therefore, current recognition requires making of economic, eco-friendly methods. This stimulated interest in the studies on biological remediation of metal (Patel et al., 2007; Joshi et al., 2009). Among biological remediation, there are many candidates like plants, animals, and bacteria. Bioremediation points of view the plants and animals are less eligible candidates and have some disadvantages, such as slow growth rate, complex growth requirements, and tedious downstream applications. The bacterial cells are competent of eliminating toxic metal from their environs could be utilized as a substitute or to enhance known techniques of arsenic removal and the better candidates for bioremediation.

New remediation strategies could be used to develop more precise techniques through the combination of biological, chemical, and physical methods. Developments in genetic manipulation techniques build it possible to produce excellent arsenic accumulating bacteria that can able to cleanse polluted water and soil. Although a lot remains to be learned regarding metal resistance mechanisms, the future application of these processes has stimulated environmental remediation potential.

In retort to toxicity, bacteria have developed several pathways for arsenic detoxification such as reduced arsenic uptake, arsenic reduction, arsenic oxidation and methylation. Arsenic resistance genes (denoted as $\text{ars}$ genes) are widespread in microbes and are localized to $\text{ars}$ operon present either in the chromosome or plasmid (Kotze et al., 2006; Cervantes et al., 1994; Silver and Walderhaug, 1992). Many microbes, $\text{ars}$ operon possess
three genes *arsR* (Regulatory gene), *arsB* (gene codes for Arsenite efflux protein), and *arsC* (Gene codes for arsenate reductase protein) (*Cervantes et al.*, 1994). While certain genetically modified bacteria have been developed in their potential use for arsenic bioremediation is not yet established (*Sauge-Merle et al.*, 2003). Currently, the primary area of interest is to find natural inhabitant bacteria having the high capacity of arsenic tolerance and accumulation naturally. Presently the center of attention on marine bacteria increased for bioremediation of arsenic. Since, the marine environments are most unstable and difficult environment, and bacteria capable to survive in such conditions are more competent for the adaptations and may provide potentiality for arsenic bioremediation.

1.2 The aim of the study:

High level of interest is designed to develop methods for cleaning up arsenic contaminated sites at minimal costs with least environmental side effects. Microorganisms which have the ability to accumulate arsenic and transform it into the less toxic form might be the valuable candidate for the elimination of extremely toxic arsenic. Marine environments possess the most adverse conditions, and the bacteria capable of surviving in such harsh environments are more capable for adaptations. Hence, bacteria inhabitant of the marine environment may be the better candidates for arsenic bioremediation.

Thus, to get some insight on how marine bacteria tackle arsenic and how they detoxify it for their survival, the present study was divided into following main areas:

- Sample (Surface Seawater and Sediment) collection from the coastal environment:
  Coastal area near to Vapi and Kavi, and Alang–Sosiya Ship Breaking Yard, Gujarat, India were selected as the marine environment for sample collection. Nearby industries possess the high level of influence on these coastal regions and responsible for pollution of the variety of contaminants. However, till date, the arsenic contamination was not reported at these locations. Initially, it was necessary to evaluate total arsenic contents before planning to search for arsenic tolerant marine
bacteria. Coastal surface sediment and seawater samples from sampling sites were collected, processed and assessed by ICP-OES for arsenic concentrations.

- Marine bacteria have been isolated on arsenic containing medium and screened for maximum levels of arsenic tolerance.
- Arsenic hyper-tolerant marine bacteria were identified at species level.
- Growth kinetics was studied and optimization of growth conditions in selected strains.
- Time-dependent accumulations of arsenate in selected strains were carried out. TEM-EDAX has been used to locate and assess the accumulation of arsenic in selected isolates.
- Arsenate reduction was investigated during the mid-log growth phase (which showed the maximum accumulation of arsenic) using pH measurement of extracellular medium and Silver Nitrate Test.
- Amplification of genes responsible for arsenic resistance (ars) in selected isolates was performed to get insight the probable mechanism of arsenic detoxification in selected marine bacterial isolates.