GENERAL INTRODUCTION

1.1 Arsenic in the environment

Arsenic (Atomic number 33, Atomic weight 74.92) is the third element in the group VA of the periodic table. This metalloid is a member of the same family as Phosphorous. It is the 20th most abundant element in the earth's crust found at a concentration of 1.8 ppm. It occurs in phosphate rocks and in many industrial phosphates and mine tailings as an impurity, and also as a by-product in metallurgy of copper and other metals (Vircikova and Havlik1999, Bailey et al. 2002). Its concentration in soil ranges from 5.5 - 13 ppm, in streams ~2 ppb and in groundwater it is generally less than 100 ppb. Arsenic is a ubiquitous element present in various compounds in the earth's crust. The most common oxidation states are: -3, 0, +3 and +5. Arsenate and arsenite are also present in the soil solutions. It occurs naturally in sulphide minerals such as pyrite. In nature, arsenic (As) can be found in insoluble forms in combination with sulfur, such as AsS and As$_2$S$_3$, or as arsenopyrite (FeAsS), an iron-sulfur compound (Fig.1). The oxidation of these compounds gives rise to chemical forms that are more toxic to human life, such as arsenate (As(V)) and arsenite (As(III)). Arsenate is present in oxide environments, and it binds strongly to sediments. Arsenite can be obtained from arsenate under anaerobic conditions, and it is more toxic (Carepo et al. 2004). In most environments, arsenite is generally thought to be the more soluble and mobile form, which increases its potential toxicity. However, arsenate is the thermodynamically favorable form in most aerobic systems (Ferguson and Gavis, 1972; Tamaki, 1992). Estimated levels of arsenic in different sources are: sea water, 2-5 ppb; public water supplies, 5ppb (recommended limit 10ppb); uncontaminated soil, 5ppm; human food from plant sources, <0.5ppm. Fish and sea foods may contain much higher. With the exception of fish, most sea foods contain less than 0.25 µg/g arsenic. Many species of fish contain between 1 and
10 µg/g. Arsenic levels at or above 100 µg/g have been found in bottom feeders and shellfish. Marine organisms, such as shrimp, mussels contain naturally high concentrations of this element, typically ranging from 1 to 100 mg As/kg wet weight (Lau et al. 1987, DeGieter et al. 2002, NIFES Archive, 2004). An estimated average dietary intake in U.S. is about 0.9 mg/day and total body burden in adult is about 15-20 mg. An estimated 6 million people in West Bengal are presently drinking water contaminated with arsenic > 50 µg/L in an area of 38,865 km² (Chowdhury et al. 2001). According to WHO, intake of 1.0 mg of inorganic arsenic per day may give rise to skin lesions within a few years (RoyChowdhury et al. 2003). Contamination of the drinking water supplies with the inorganic forms (arsenite and ars enate) has often been reported and arsenic has been identified as major risk for human health in different parts of the world (Muller et al. 2003). The organic forms of arsenic are less toxic. As (III) is 100 times more toxic than As(V) (Neff,1997). In certain types of aquatic environments, such as the hypersaline Monolake of California, USA the dissolved arsenic concentration is extremely high (0.3mM) owing to the concentration effects of hydrologic and climatic factors and an abundance of hydrothermally based sources (Dowdle et al.1996). The predominant form of arsenic in water is usually arsenate (V) (Callahan et al. 1979, Wakao et al. 1988), but aquatic microorganisms may reduce the arsenate to arsenite and a variety of methylated arsenicals. Within anoxic soils, sediments and waters arsenic occurs primarily as As(III). Arsenic can be emitted into the environment from several natural sources, including volcanic eruptions. Weathering and sedimentation leads to wide natural distribution as weathering of sulphide rich rocks results in the formation of highly acidic (pH 3.0) and heavy metal laden effluents. At the abandoned Pb-Zn mining sites the pyrite rich tailings are subject to bioleaching which leads to the formation of acid waters heavily loaded with arsenic (Average conc. 250 ppm). Dissolved arsenic present in the seepage waters precipitates within a few meters from the bottom of the tailing dam in the presence of microorganisms eg. Acidithiobacillus ferrooxidans (Duquesne et al. 2003).
Thought to be pollution free and environment friendly, geothermal wells, used as a source of energy are also a source of arsenic into surface waters. Arsenite is often the predominant valence state of inorganic arsenic in geothermal source waters, although As(V) can be present with As(III)/As(V) ratios varying among different springs due to mixing with meteoric surface waters prior to discharge. These ratios are significantly influenced by redox transformations of different arsenic species by microorganisms (Nicholson, 1993; Mukhopadhyay et al. 2002). Hot spring waters typically contain 1-10 ppm arsenic and have been reported to bear up to 50 ppm, which implies that geothermal fluids are a significant source of Arsenic (Ghiring and Banfield, 2001). Forest fires can also disperse arsenicals to the wind. The multiplicity of industrial, agricultural and anthropogenic activities has enhanced the mobilization of heavy metals above the rate manageable by biogeochemical cycles (Fig. 1), thus resulting in an increased release of heavy metals in the environment. Among the anthropogenic sources of arsenic main are combustion of fossil fuels and smelting of non-ferrous metals. The above processes release arsenic as arsenic trioxide. Arsenic occurs in most coals in association with sulphur, when burned it accumulates on fly ash particles. The amount present on fly ash is significant. In the past 100 years commercially produced metal arsenites have been deliberately added to the biosphere as pesticides (Phillips and Tailor 1976). In arsenic rich environments a major concern is the potential for mobilization and transport of this toxic element to groundwater and drinking water supplies. In Bangladesh ∼57 million people have been exposed to arsenic through contaminated wells. This needs to understand the factors controlling the mobility and solubility of arsenic in aquatic system (Niggemeyer et al. 2001). 330 million people in the Indian subcontinent are at risk of As exposure and consequently disease through contaminated drinking water (Esquivel et al., 1998).
Fig. 1: Arsenic cycle in nature (Mukhopadhyay et al. 2002)
1.2 Chemistry of Arsenic Compounds

Arsenic, the 3rd member of group VA (Nitrogen family) of the periodic table after Nitrogen and Phosphorous was discovered by Albertus Magnus (Germany) in 1250. The origin of the name comes from the Greek word 'arsenikon' meaning yellow orpiment. The pure element is a steel gray crystalline solid that sublimes on heating and oxidizes readily in air. This element occurs as two modifications, yellow (sp. gr. 1.97) and grey (sp. gr. 5.73). Grey arsenic is the usual stable form with a melting point of 817°C and sublimation point of 613°C. Grey arsenic is a very brittle semi metallic solid. It is steel grey in colour, crystalline and tarnishes readily in air. When heated in air, arsenic readily forms arsenious oxide, As₂O₃ (also known as arsenic trioxide), which has a garlic like odour. Arsenic occurs in minerals combined with Sulphur, like As₄S₃ (orpiment) and realgar (As₄S₄). The lemon colour of orpiment and orange colour of realgar lead to their use in pigment and cosmetics in the past. Commercially arsenic is obtained as a byproduct of gold, silver and copper metallurgy. Also by heating the ore prsenopyrite (FeAsS) from which Arsenic sublimes on heating.

\[ \text{FeAsS} \rightarrow \text{FeS} + \text{As} \]

Arsenic and its compounds are poisonous. Arsenic compounds can be classified into three broad groups:

i) Inorganic arsenic eg. arsenate and arsenite

ii) Organic arsenic e.g. lewisite

iii) Arsine gas

Elemental arsenic resists water, acid and alkalis, tarnishes in air and burns in oxygen. Organic arsenicals such as lewisite, ethyl dichloroarsine (ED), methyldichloroarsine (MD) and phenyl dichloroarsine (PD) are well known chemical weapons or vesicants, quite potent in their action after mustards and
<table>
<thead>
<tr>
<th>CAS No.</th>
<th>Name</th>
<th>Synonyms</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>arsename</td>
<td></td>
<td>[1]</td>
</tr>
<tr>
<td></td>
<td>arsenite</td>
<td></td>
<td>[2]</td>
</tr>
<tr>
<td>124-58-3</td>
<td>methylarsonic acid</td>
<td>monomethyolarsonic acid, MMA</td>
<td>[3]</td>
</tr>
<tr>
<td>75-60-5</td>
<td>dimethylarsinic acid</td>
<td>cacodylic acid, DMA</td>
<td>[4]</td>
</tr>
<tr>
<td>4964-14-1</td>
<td>trimethylarsine oxide</td>
<td></td>
<td>[5]</td>
</tr>
<tr>
<td>27742-38-7</td>
<td>tetramethyolarsonium ion</td>
<td></td>
<td>[6]</td>
</tr>
<tr>
<td>64436-13-1</td>
<td>arsenobetaine</td>
<td></td>
<td>[7]</td>
</tr>
<tr>
<td>39895-81-3</td>
<td>arsenocholine</td>
<td></td>
<td>[8]</td>
</tr>
<tr>
<td></td>
<td>dimethylarsinoylribosides</td>
<td></td>
<td>[9]–[19]</td>
</tr>
<tr>
<td></td>
<td>trialkylarsonioribosides</td>
<td></td>
<td>[20], [21]</td>
</tr>
<tr>
<td></td>
<td>dimethylarsinoylribitol sulfate</td>
<td></td>
<td>[22]</td>
</tr>
</tbody>
</table>
Fig. 2: Structures of naturally occurring inorganic and organic arsenic species
Table 2: Other Arsenic compounds of environmental significance referred to in the text

<table>
<thead>
<tr>
<th>CAS No.</th>
<th>Name</th>
<th>Synonyms</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>1327-53-3</td>
<td>As(III) oxide</td>
<td>As trioxide, arsenous oxide, white As</td>
<td>As$_2$O$_3$ (or As$_4$O$_6$)</td>
</tr>
<tr>
<td>13768-07-5</td>
<td>arsenenous acid</td>
<td>arsenious acid</td>
<td>HAsO$_2$</td>
</tr>
<tr>
<td>7784-34-1</td>
<td>As(III) chloride</td>
<td>As trichloride, arsenous trichloride</td>
<td>AsCl$_3$</td>
</tr>
<tr>
<td>1303-33-9</td>
<td>As(III) sulfide</td>
<td>As trisulfide orpiment, auropigment</td>
<td>As$_2$S$_3$</td>
</tr>
<tr>
<td>1303-28-2</td>
<td>As(V) oxide</td>
<td>As pentoxide</td>
<td>As$_2$O$_5$</td>
</tr>
<tr>
<td>7778-39-4</td>
<td>arsenic acid</td>
<td>ortho-arsenic acid</td>
<td>H$_3$AsO$_4$</td>
</tr>
<tr>
<td>10102-53-1</td>
<td>arsenenic acid</td>
<td>meta-arsenic acid</td>
<td>HASO$_3$</td>
</tr>
<tr>
<td></td>
<td>arsenates, salts of ortho-</td>
<td></td>
<td>H$_2$AsO$_4$$^-$$^2$, AsO$_4$$^3$-</td>
</tr>
<tr>
<td></td>
<td>arsenic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>593-52-2</td>
<td>methylarsine</td>
<td></td>
<td>CH$_3$AsH$_2$</td>
</tr>
<tr>
<td>593-57-7</td>
<td>dimethylarsine</td>
<td></td>
<td>(CH$_3$)$_2$AsH</td>
</tr>
<tr>
<td>593-88-4</td>
<td>trimethylarsine</td>
<td></td>
<td>(CH$_3$)$_3$As</td>
</tr>
<tr>
<td>98-50-0</td>
<td>(4-aminophenyl)-arsonic acid</td>
<td>arsenilic acid, p-aminobenzene-arsenic acid</td>
<td>H$_2$N-AsO(OH)$_2$</td>
</tr>
<tr>
<td>139-93-5</td>
<td>4,4-arsenobis(2-aminophenol)</td>
<td>arsphenamine, salvarsan</td>
<td>HCHO$_2$N$_2$-As$_2$-As=As--O(0H)$_2$</td>
</tr>
<tr>
<td>121-59-5</td>
<td>[4-[aminocarbonyl-amino]phenyl] arsanic acid</td>
<td>carbarsone, N-carbamoylarsaniic acid</td>
<td>NH$_2$CO-NH-As$_2$-As=As--O(0H)$_2$</td>
</tr>
<tr>
<td>554-72-3</td>
<td>[4-[2-amino-2-oxoethyl]amino-phenyl] arsanic acid</td>
<td>tryparsamide</td>
<td>NH$_2$COCH$_2$NH-As$_2$-As=As--O(0H)$_2$</td>
</tr>
<tr>
<td></td>
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<tr>
<td>----</td>
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<td>----</td>
</tr>
</tbody>
</table>
| 121-19-7 | 3-nitro-4-hydroxyphenylarsonic acid | $\text{O}_2\text{N}$
| | | $\text{HO} - \text{Ar(OH)}_2$ |
| 98-72-6 | 4-nitrophenylarsonic acid | $\text{O}_2\text{N}$
| | | $\text{p-NO} - \text{Ar(OH)}_2$ |
| | dialkylchloroarsine | | $\text{R}_2\text{AsCl}$ |
| | alkyldichloroarsine | | $\text{RasCl}_2$ |
Introduction

phosgene oximes. DNA alkylation and/or inhibition of glutathione-scavenging pathways are two postulated mechanisms of its killer action. The onset of symptoms after exposure occurs in seconds (Table.1, Table2 and Fig.2).

1.3 Uses of Arsenic

Present day uses of arsenic are mainly in electronics e.g. solar cells, optoelectronic devices, semiconductor applications, light emitting diodes and digital watches. Among the industrial uses are glassware, electrophotography, catalysts, pyrotechnics, antifouling agents, dyes and soaps. Arsenic is also used in alloys with lead, in storage batteries and in ammunitions, automatic body solders and radiators, battery plates (hardening agents). Other uses include pigments and dyes, preservatives of animal hides, glass manufacture and wood preservatives. Currently veterinarians employ an organic arsenical, sodium capasolate, for the treatment of heartworms in dogs. In 2005, the United states was again the world’s leading consumer of arsenic, mainly for CCA inspite of the voluntary ban on the consumption of CCA (Brooks, 2006). Arsenic is still not totally banned and is being used in some developing countries (Bentley and Chasteen, 2002; Mukhopadhyay et al., 2002; Lloyd, 2003; Rodriguez et al. 2003; Katz and Salem, 2005; Nachman et al. 2005; Jones, 2007). At present arsenic is being used increasingly to make Gallium Arsenide (GaAs) semiconductors for use in semiconductor diodes. As compounds had been widely used as pesticides and wood preservatives. The first antiseptic Salvorsan 606 and the African sleeping sickness drug Melarsen (Clesceri et al. 1998) also contain arsenic. During 18th, 19th and 20th centuries arsenicals were preferred for the control of agricultural pests before the widespread use of organochlorines. e.g. Paris green (CH₃)₂Cu.3Cu(AsO₂)₂ and Lead arsenate (PbHASO₄) were used in insecticides. White arsenic As₂O₃ was used as rodenticide, alkaline slution of As₂O₃ as an insecticide and herbicide and methyl arsenic sulphide (CH₃AsS) as a fungicide etc. The use in veterinary medicine as nutritional supplements and in the
treatment of various diseases dates back to 15th century. For the past centuries chronic feeding of small doses of various arsenic preparations has been reported to increase appetite, improve the level of activity, correct anaemia and improve the coats of animals. Arsenic was used as a feed additive which control enteric diseases of swine and poultry and to improve weight and feed efficiency of livestock. In the late 19th century, a preparation known as Fowler’s solution was in great demand which contains water, As_2O_3, KHCO_3 and alcohol as an accepted treatment for leukemia and dermatitis. Organic arsenicals such as Lewicite (L), ethyldichloroarsine(ED), methyldichloroarsine (MD) and phenyldichloroarsine (PD) are well known chemical weapons or vesicants quite potent in their action after mustards and phosgene oxime.

**1.4 Abiotic factors affecting Biogeochemical Cycling of arsenic**

Speciation determines how arsenic compounds interact with their environment. For example, the behaviour of arsenate and arsenite in soil differs considerably. Movement in environmental matrices is a strong function of speciation and soil type. In a non-absorbing sandy loam, arsenite is 5–8 times more mobile than arsenate (Gulens et al. 1979). Soil pH also influences arsenic mobility. At a pH of 5.8 arsenate is slightly more mobile than arsenite, but when pH changes from acidic to neutral to basic, arsenite increasingly tends to become the more mobile species, though mobility of both arsenite and arsenate increases with increasing pH (Gulens et al., 1979). In strongly adsorbing soils, transport rate and speciation are influenced by organic carbon content and microbial population. Both arsenite and arsenate are transported at a slower rate in strongly adsorbing soils than in sandy soils.
1.5 Biological activity of arsenicals

Till early 1990's it was believed that arsenic is not a mutagen (Rossman et al. 1980; Lee et al. 1985) by itself but it can act synergistically to enhance the mutagenic and clastogenic effect of known potent mutagens like MMS, EMS etc. (Jan et al. 1991). Arsenic is a weak inducer of chromosome aberrations and sister chromatid exchanges (Larramendy et al. 1981, Nakamuro et al. 1981, Wen et al. 1981, Wan et al. 1982, Lee et al. 1985). Now, Arsenic has been classified by USEPA as a human carcinogen. Arsenic can effect biochemical reactions. Trivalent arsenic can bind to the thiol groups of critically important proteins and pentavalent arsenic can replace phosphate in biochemical reactions and disrupt the formation of ATP in vitro. The toxicity of arsenate ion lies in its ability to mimic the PO₄ ion. Traversing into the molecular mechanism of arsenate toxicity reveals that arsenate resembles the ion phosphate both in size and valency, hence it gets preferably incorporated into ADP and gives ADP arsenate (ADP-As) instead of ATP. This molecule of ADP-As undergoes a futile cycle of hydrolysis where the cleavage of ADP-As bond is totally a wasteful process, yielding no energy for the cellular metabolic activities. Also abundance of arsenate in media leads to phosphate starvation. Arsenite on the other hand acts mainly by interacting with proteins and enzymes usually denaturing them or inhibiting their function. Arsenite (AsO²⁻ and AsO₃³⁻) has been shown to inhibit dehydrogenases such as pyruvate dehydrogenase, α-ketoglutarate dehydrogenase and dihydrolipoate dehydrogenases (Mahler and Cordes, 1966). As reported by Da Costa, 1972 As(III) uncouples the oxidative phosphorylation i.e. inhibition of oxidative phosphorylation by chemiosmosis.

What happens to arsenic when it enters the human body? The toxicity of arsenic to mammals is related to its absorption and retention in the body and varies with chemical form. The toxicity of arsenicals in decreasing order is; inorganic arsenites > organic trivalent compounds (arseoxides) > inorganic arsenates > arsonium compounds > elemental arsenic. Toxicity appears to be related to the solubility of arsenical in water. The low toxicity of elemental arsenic is attributed to
its near insolubility in water and body fluids. Inorganic arsenic is a potent mutagen as well as carcinogen. It is associated mainly with skin and lung cancers. It induces micronuclei, chromosomal aberrations (Oya-Ohta et al. 1996) and sister chromatid exchanges \textit{in vitro} in human lymphocytes as well as in cultured chinease hamster cell lines.

1.6 Arsenic Resistant Bacteria
Arsenic resistance in bacteria is a widespread phenomenon. The resistant bacteria fall into diverse taxonomic groups. \textit{E.coli}, \textit{Pseudomonas} spp., \textit{Acidiphilium multivorum}, \textit{Alcaligenes} sp., \textit{Desulphotomaculum} sp., \textit{Aeromonas} sp., \textit{Exiguobacterium} sp. etc. represent the gram negative community, while \textit{Bacillus} spp., \textit{Staphylococcus aureus}, \textit{Thiobacillus} sp., \textit{Acinetobacter} sp., \textit{Clostridium} sp., \textit{Sulfurosprillum barnesii} (Stolz et al. 2002) are among the important members of gram negative group which show high levels of resistance towards arsenic. Some archebacteria have also been found to possess arsenic resistance, e.g. \textit{Ferroplasma acidarmanus}’ Fer1 is an arsenic-hypertolerant acidophilic archaeon isolated from the Iron Mountain mine, California; a site characterized by heavy metals contamination (Austin et al.2007). \textit{Halobacterium} sp. NRC-1, which is an extremely halophilic archaeon possess arsenic resistance genes on its plasmid pNRC-100 (DasSarma et al. 2006).

1.7 Antibiotic resistance in arsenic resistant bacteria
Heavy metal resistance and drug resistance are often linked and are present on the same plasmid, e.g. mercury is frequently specified by drug resistance plasmids and is also common in soil \textit{Pseudomonas} and Bacilli. Plasmid determined copper resistance has been reported on an antibiotic resistance plasmid, in \textit{E. coli} isolated from pig fed copper supplements as growth stimulants. Arsenic resistances are governed by plasmids that also code for antibiotic and other heavy metal resistances. For example, in Tokyo in the late 1970s both heavy metal resistances
and antibiotic resistances were found with high frequencies in *Escherichia coli* isolated from hospital patients, where as heavy metal resistance plasmids without antibiotic resistance determinants were found in *E. coli* from an industrially polluted river. Selection occurs for resistances to both types of agents in the hospital, but only for resistance to toxic heavy metals in the river environment (Shukla et al. 2006). Virdi et al., 2001 have reported an arsenic resistant strain of *Yersenia enterocolitica* which was resistant to five antibiotics. MIC of five antibiotics namely amikacin, gentamicin, tetracycline, ciprofloxacin, and nitrofurantoin for pork isolates of *Yersinia enterocolitica* increased two- to eightfold after bacteria were grown in the presence of 5 mm arsenite. For *Y. enterocolitica* isolates obtained from wastewater (sewage effluents), an unequivocal increase in MICs was seen with amikacin and gentamicin. Rajini Rani et al. (1992) studied a *Pseudomonas* sp. isolated from the Bay of Bengal (Madras coast) contained a single large plasmid (pMR1) of 146 kb. Plasmid curing was not successful with mitomycin C, sodium dodecyl sulfate, acridine orange, nalidixic acid or heat. Transfer of mercury resistance from marine *Pseudomonas* to *Escherichia coli* occurred during mixed culture incubation in liquid broth at 10^{-4} to 10^{-5} ml^{-1}. However, transconjugants lacked the plasmid pMR1 and lost their ability to resist mercury. Transformation of pMR1 into *E. coli* competent cells was successful; however, the efficiency of transformation (1.49Ã—10^2 Hg^+ transformants µg^{-1} pMR1 DNA) was low. *E. coli* transformants containing the plasmid pMR1 conferred inducible resistance to mercury, arsenic and cadmium compounds similar to the parental strain, but with increased expression. The mercury resistant transformants exhibited mercury volatilization activity. A correlation existed between metal and antibiotic resistance in the plasmid pMR1.

1.8 Biotransformation

In aqueous environment bacteria and other microorganisms interact with arsenic compounds in different ways. Some bacteria, fungi and algae are able to reduce...
arsenate into arsenite and finally into trimethylarsine (Woodfolk and Whitelay 1962, Sehlin and Lindstrom 1992). Studies with *Methanobacterium* M.O.H. (McBride and Wolfe 1971) have shown that the reaction is as follows:

\[
\begin{align*}
2e^- & \quad RCH_3 & \quad O & \quad RCH_3 & \quad O & \quad 2e^- \\
AsO_4^{3-} & \rightarrow AsO_2^- & \rightarrow CH_3-AS-OH & \rightarrow CH_3-AS-OH & \rightarrow CH_3-AS-H \\
OH & \quad CH_3 & \quad OH & \quad CH_3 & \quad OH
\end{align*}
\]

arsenate arsenite methylarsonic acid Dimethyl arsinic acid (cacodylic acid) Dimethyl arsine

Dissimilatory reduction of arsenic (V) has been shown to occur in at least nine different genera scattered throughout the bacterial domain (Newman et al. 1998, Stolz and Oremland 1999, Ghihring and Banfield 2001 and Niggemyer et al. 2001) and has also been observed in hyperthermophilic archea (Huber et al. 2000). These microorganisms are either strict anaerobes, facultative anaerobes or microaerophiles, capable of utilizing arsenate as the terminal electron acceptors. However sufficient evidence is present in favour of aerobic bacteria being involved in the reduction of arsenate (Jones et al. 2000, Macy et al. 2000, Macur et al. 2001, 2004). *Pseudomonas* spp. and *Alcaligenes* spp. are able to reduce arsenate to arsenite and both to arsine (AsH₃) anaerobically (Cheng and Focht 1979). On the other hand some microbes can oxidize reduced arsenic, i.e. arsenite into arsenate (Sehlin and Lindstrom 1992, Macur et al. 2004). In *Alcaligenes faecalis* the arsenite oxidising activity was found to be inducible by arsenite or arsenate (Osborne and Ehrlich 1976). Normally the bacteria which are involved in biotransformation are themselves resistant to arsenic to certain levels. The occurrence of arsenate resistant bacteria has been reported across oxic-anoxic boundaries (Saltikov et al. 2003). In that context it is necessary to mention that arsenate resistant bacteria are not necessarily arsenate respiring, which are till
now reported to be isolated from anoxic waters and sediments and show a very high tolerance towards arsenite (\(\sim 10\text{mM} - 60\text{mM}\)), which is only upto 1 mM for non-arsenate respiring arsenate resistant bacteria. Microorganisms can mediate a variety of reactions including reduction, oxidation and methylation. A number of bacteria reduce As(V) to As(III) as a detoxification mechanism based on the enhanced outward mobility from the cell of As(III) (Dowdle et al. 1996). In 1994-95 first reports of some novel strains of bacteria (Ahmann et al. 1994 and Laverman et al. 1995) capable of respiratory growth by coupling the reduction of As(V) to As(III) with the oxidation of lactate were published. Thermodynamic calculations showed that this reduction is sufficiently exergonic to sustain growth. Reduction of As(V) to As(III) in anoxic sediments is carried out by bacterial dissimilatory arsenic reduction (DAsR). The biogeochemical cycle of this element depends on microbial transformation which affects the mobility and distribution of arsenic species in the environment. Several bacteria involved in the transformation process consisting reduction, oxidation and methylation of arsenic species have been described (Muller et al. 2003). Knowledge of bacterial biotransformation has led to the exploration of alternative methods for arsenic remediation based on its biological oxidation. Several arsenite oxidising bacteria have also been isolated, starting with an *Achromobacter* strain. Since then different arsenite oxidising bacteria including several *Pseudomonas* strains, *Alcaligenes faecalis*, *Thiobacillus ferrooxidans* and *Thiobacillus acidophilus*; bacteria from the *Agrobacterium /Rhizobium* branch of \(\beta\)-Proteobacteria and bacteria of the *Thermus* genus have been described. Recently a bacterium belonging to the Zoogloae branch of \(\beta\)-Proteobacteria was isolated from an Arsenic contaminated environment. This strain ULPAs1 is able to efficiently oxidize arsenite to arsenate. Due to its increased resistance to As(III) as well as other heavy metals this strain is a good candidate for bioremediation of environments heavily contaminated with arsenic (Weeger et al. 1999).
1.9 Bioaccumulation of arsenic by microorganisms and bacteria

Arsenic is toxic to life forms at all concentrations and does not have any physiological role except in some bacteria which use it as an electron acceptor for anaerobic respiration (Laverman, et al. 1995). Arsenic resistance in bacteria is a well studied phenomenon, although until recently (Anderson and Cook, 2004), the presence of arsenic resistance in the genus *Aeromonas* was not reported. These scientists from New Zealand, have reported it for the first time and the strain was PCR negative for the *ars* operon genes. Although the presence of Fe, Mn, Cu, Ni and Zinc accumulating bacteria have been reported in soil and rhizosphere, arsenic accumulating bacteria are lesser known and explored. Some engineered bacteria having plant arsenic hyperaccumulating genes have also been developed for this purpose (Sauge- Merle et al. 2003; Kostal et al. 2004,). However there have been no reports of arsenic bioaccumulation in mesophilic bacteria and only some thermophilic bacteria forming reddish brown biofilms from hot springs (Tazaki et al. 2003a,b) have been known to have this property, where in it is found to accumulate as Fe-As in the cell walls.

1.10 Biochemical Basis of Resistance

The bacteria are able to tolerate arsenic as well as resist its presence in the medium by virtue of specialized enzymes such as arsenate reductase, arsenite oxidase and methyltransferase (Mukhopadhyay et al. 2002). All these enzymes are coded by the genes of bacterial *ars* operon(Fig.3.1) The enzyme arsenite oxidase is responsible for oxidation of As(III) to As(V) which is a much less toxic form. Arsenate reductase is another enzyme responsible for detoxification. This enzyme converts As(V) to As(III) and makes it ready to be effluxed out of the cell by means of a membrane bound ArsB pump. Methyltransferases function by removing the methyl group of organic arsenicals and converts them to As(III), which can be easily effluxed out of the cell(Fig.3.3).
1.11 Arsenate reductases

An initial step in arsenate metabolism is the enzymatic reduction of arsenate As(V) to As(III). At least three families of arsenate reductase enzyme have arisen apparently by convergent evolution. These are:

i) *E.coli* plasmid (R773) and chromosomally encoded arsenate reductase. Apart from *E.coli* this family includes *H. influenzae*, *N. gonorrhoeae*, *Azotobacter* sp. etc.

ii) *S. aureus* pl258 family which includes pSX267, *B.subtilis* and human arsenic resistance operon.

iii) Yeast (Acr2p reductase family) found in *Saccharomyces cerevisiae*.

Pathways for arsenic detoxification exist in all organisms including bacteria and yeast.( Bhattacharjee et al. 1999). These thiol linked reductases are required to confer resistance to As(V) in both prokaryotes (Gladysheva et al.1994 and Ji et al. 1994) and eukaryotes (Bobrowicz et al.1997, Mukhopadhyay and Rosen1998). These enzymes arose independently at least three times by convergent evolution. The *S. aureus* pl258 *ArsC* is homologous to low molecular weight protein phosphotyrosine phosphatases (lmwPTPases) (Bennett et al. 2001). Acr2p is homologous to Cdc25a cell cycle protein tyrosine phosphatase (Fauman et al.1998). The R773 *ArsC* purified from *E.coli* requires reduced glutathione(GSH) and small thiol transfer protein glutaredoxin (Grx) for its arsenate reductase activity (Gladysheva et al.1994). *ArsC* of *E.coli* has a single cysteine residue Cys12 (Liu et al., 1995) that forms a covalent thiolate-As(V) intermediate (Martin et al. 2001). Glutaredoxin (Grx) is required to reduce the enzyme bound ES-As(V) to an ES-As(III) intermediate (Gladysheva et al. 1994, Shi et al 1999). The *E.coli* glutaredoxins (3 types) have a consensus sequence Cys-Pro-Tyr-Cys. Mutation in N terminal Cys causes loss of activity (Shi et al 1999).

1.12 Protein profile of arsenate resistant bacteria

In *E. coli* arsA and B together form a complex which functions as a primary arsenite pump. In *S. aureus* arsB alone is sufficient to act as a chemiosmotic
secondary transport system for arsenite resistance without the presence of an ArsA ATPase (Cervantes et al. 1994). ArsC is an arsenate reductase that mediates reduction of arsenate prior to arsenite efflux. The size of ArsC is 12-15 KDa is a soluble enzyme which couples the oxidation of thiols from glutathione/glutaredoxin or thioredoxin to the reduction of As(V) to As(III) (Ji and Silver 1992, PNAS). The ars operon as such is induced by As(III) and Sb(III) (Wu and Rosen, 1993). Two regulators ArsR and ArsD are repressors which control basal and maximal levels. Both ArsR and ArsD bind to the same operator site, immediately upstream of the ars operon, but with different affinities (Wu and Rosen 1993, Xu et al. 1996, Afkar et al., 2003). ArsR exhibits a 100 times greater affinity for the ars operator and requires 10 times less As(III) to relieve in vivo repression of the ars operon, than does ArsD. The proposed model for regulation is that ArsR controls ars transcription when As(III) concentrations are low, where as ArsD controls transcription in environments where As(III) is high. The ArsR protein of R773 has two arsenite binding cysteines, viz. cys32 and cys 34 necessary for induction. The haloalkaliphilic low GC gram positive bacterium Bacillus selenitireducens strain MLS 10 has ArrB which is 26.3 KDa(230 aa, 693 bp) protein with 4 FeS binding domains. It shows 66% similarity and 50% identity with ArrB from Shewanella sp. strain ANA-3. The Shewanella sp. ArrA is a large,95 KDa molybdenum containing enzyme and ArrB is a 26KDa enzyme with several FeS clusters and both belong to DMSO reductase family. (Afkar et al. 2003). Both these enzymes are required for As(V) reduction. ArrA is thought to be the subunit which binds As(V) and reduces it to (III), while arrB helps in conducting the electrons. P. putida has another extra gene arsH, in two copies in its chromosomal ars operon. Two copies of this gene are present viz. arsH1 (241a.a.) and arsH2(223 a.a.). P. aeruginosa has ars H homologue with 87% a.a. similarity, which is reduced to 74% only in case of Y. enterocolitica and T. ferrooxidans ars H1 and H2 are 44% homologous to plant NADH oxidoreductases (190 aa similar segment) and 47% similar to B. subtilis. Neyt et al. 1997 suggested that arsH might be a transcriptional activator because a
plasmid containing the ars RBC genes alone of *Y. enterocolitica* pYV did not cause any increase in arsenic resistance upon transformation. Another relatively uncommon gene ArsM (arsenite S-adenosylmethionine methyltransferase) catalyses the formation of a number of methylated intermediates from As(III) with trimethylarsine as the end product. This gene was found to be present in *Rhodopseudomonas palustris*. Its molecular weight is 29.6 KDa (283aa). The ars operon in the skin element of *B. subtilis* codes for four proteins –ArsR, ORF2, ArsB and ArsC showing 33%, 18% and 62% homology with the ars operon of *E. coli* R773 (Sato and Kobayashi, 1998). Two novel proteins have been found in case of *Streptomyces* sp. strain FR-008 (Wang et al. 2006) designated as ArsO and ArsT. ArsO is a flavin binding monooxygenase and ArsT is a thioredoxin reductase. ArsO protein is 57% identical to a putative monooxygenase (ZP_00420634) from *Burkholderia vietnamiensis* G4. ArsT showed extensive homology to numerous thioredoxin reductases, such as thioredoxin reductases from *Streptomyces coelicolor* A3(2) show 71% amino acid identity.

1.13 Molecular basis of Arsenic Resistance

**Human arsenic resistance determinants**

Genetic determinants for arsenic resistance have been identified in yeast, fungi, plants mammals as well as bacteria. In mammals biomethylation takes place and the methylated metabolites are excreted by the kidney in urine. DMAA being the major compound. In humans three genes have been identified as being involved in arsenic biotransformation:

i) Purine nucleoside phosphorylase involved in the reduction of arsenate(V) into arsenite(AsIII).

ii) Glutathione S transferase omega (GSTO) involved in the reduction of MMA(V) i.e. monomethyl arsenic(V) to MMA(III).
iii) As(III) methyltransferase (CVT19, recently designated as As3MT). This enzyme is proposed to be capable of entire gamut of arsenic biotransformations that begin with arsenite and end with Dimethyl Arsenic(V) or DMA(V).

There is a strong correlation between genotype and arsenic metabolism. Till now 23 polymorphic sites have been selected by the researchers to study this effect. Two phenotypes were selected for this study by Prof. Klimecki and their research group at the University of Arizona (Yu et al. 2003). These are: i) the ratio of As(III):As(V) in urine and ii) DMA(V):MMA(V). These scientists discovered that there are 3 polymorphic sites in As(3)MT gene which are significantly associated with D:M ratios in the total population. This effect is more pronounced in children below 18 years. Children generally have a higher D:M ratio than adults. In brief we can say that there is a genetic association in humans that is influenced by developmental stage.

The bacterial ars operon

The ars operon was first discovered in the plasmids of Staphylococcus aureus (p1258) by Ji and Silver in 1992. Later on this operon was found to be present in Staphylococcus xylosus also by Rosenstein et al. (1992) on plasmid pSX267. Bacterial detoxification of arsenic is generally a two step process, i.e. first reduction of arsenate to arsenite (Ji and Silver 1992, Ji et al. 1994) followed by arsenite efflux involving one or both of the following efflux systems:

(i) Simple arsenite efflux system mediated by one ArsB efflux protein, which is encoded by the arsB gene located on a plasmid in Staphylococcus xylosus (Rosenstein et al 1992) and also located on chromosomal genome in E.coli (Diorio et al. 1995), Pseudomonas fluorescens and Pseudomonas aeruginosa (Cai et al. 1998).

(ii) de lux arsenite transporters composed of an ArsB pore + an ArsA ATPase, e.g. plasmid encoded arsenical resistance in E.coli and Acidiphilium multivorum (Suzuki et al. 1998). The E.coli plasmid R773 ars operon contains five genes viz. arsR, D, A, B, C encoding an arsenate reductase (ArsC) that reduces arsenate to arsenite, a
Fig 3.1 Different families of ars operons
Figure 3.2: Genes and products for arsenic resistance in gram positive and gram negative bacteria. Alignments and functions of arsenic resistance genes with amino acid sizes of predicted products and percent identities between amino acid products (Silver and Phung, 1996).
**Fig 3.3: Pathways of arsenic detoxification in prokaryotes and eukaryotes.**

*E. coli*, a typical prokaryote, accumulates As(V) by phosphate transport systems such as the Pst ABC ATPase. As(V) is reduced to As(III) by ArsC, a Grx-GSH-linked enzyme. The resulting As(III) is pumped out of the cell by the ArsB (oxyanion pump)/ArsA (ATPase) system. The eukaryote *S. cerevisiae* uses a similar cycle of AS(V) uptake, reduction and extrusion, but the proteins that catalyze these reactions are the products of independent evolution from the bacteria. Phosphate transporters such as Pho84p accumulate As(V). Acr2p, a Grx-GSH-linked enzyme, reduces As(V) to As(III). As(III) can be extruded from the cells by the Acr3p carrier. Alternatively, As(III) can be conjugated with GSH to form As(GS)_3. The Ycf1p ABC ATPase removes As(III) from the cytosol by pumping As(GS)_3 into the vacuole.
membrane bound anion translocating ATPase (ArsA) and ArsB an inner membrane protein that forms the arsenite conducting channel. The ars operon serves as a detoxification mechanism by lowering the intracellular arsenic concentration, thus conferring resistance to As(III) and As(V) (Saltikov and Olson, 2002). While arsR is a trans acting inducer responsive repressor, ArsD is an inducer independent protein controlling basal and upper level of expression (Wu and Rosen 1993, Rosen et al.1995, Shi et al.1996, Xu et al.1996). Gram negative plasmid borne ars operons share highly homologous sequences yet are highly divergent from their gram positive counterparts. Sequences homologous to the E.coli chromosomal ars operon are also highly conserved among enterobacterial genera. The P. aeruginosa RBC- type ars operon appears to be conserved in P. fluorescens but not in other arsenic resistant Pseudomonas spp.. Chromobacterium violaceum also possesses a chromosomal arsenic resistance operon of the arsRBC type (ORFs CV2438, CV2439 and CV2440) that comprises a regulatory protein arsR, a membrane-bound protein responsible for arsenite efflux arsB, and an arsenic reductase, arsC (Vasconcelos et al., 2003).

Plasmid linked arsenic resistance was known since a long time (Novick and Roth 1968). It was first reported in Staphylococcus aureus (1968) and later in E.coli plasmid R773 (Chen et al. 1986). The presence of ars operon genes in the chromosome of E.coli was only recently discovered (Carlin et al.1995 and Diorio et al.1995). Carlin et al. have reported the presence of ars operon in all the strains of E.coli including JM109, through southern hybridization. Diorio et al. (1995) showed that Pseudomonas aeruginosa also contains an ars operon homolog. Acidiphilium multivorum plasmid pKW 301 contains RDABC type ars operon (Suzuki et al. 1998). Sulfolobus acidocaldarius is a mineral leaching archaebacterium known to harbour these genes (Sehlin and Lindstrom, 1992). The presence of ars operon was detected in Aciditiobacillus caldus, Deinococcus indicus, Pseudomonas fluorescens Desulfitobacterium sp., Thiobacillus ferrooxidans as well. The arsenate reductase gene is almost universally present in all known arsenate resistant bacteria and its presence is an indication of ars operon being present in that
particular bacteria. The length of arsC gene in terms of nucleotides varies from species to species (Fig.3.2, Table 3).

Atypical ars operons

*Lactobacillus plantarum* plasmid pWCFS 103 has only arsB gene in its ars operon, along with arsR and two copies of arsD gene (RDDB type). arsC and arsA are not present (Kleerebezem et al.2003; Kranenburg et al. 2005). *Streptomyces* sp.strain FR008 has a somewhat different arrangement of ars operon genes on its linear plasmid pHZ227. It has RBOCT type system, where 'O' stands for putative flavin binding monooxygenase and 'T' stands for thioredoxin reductase. (Wang et al. 2006). Another example of unique ars operon arrangement is *Bacillus* sp. which has 4 genes arsR, orf2, arsB and arsC. *Halobacterium* sp. NRC1 in its plasmid pNRC100 harbours an arsADRC-R2M type operon, where M = arsenic (III) methyl transferase (DasSarma et al.2006).

The arr Genes : The arr operon has only recently been identified and relatively little is known about its regulation and biochemistry (Saltikov and Newman 2003). It consists of two genes arrA and arrB. It was first discovered in *Shewanella* sp.ANA3. Sequences of both ArrA and ArrB are highly conserved and their presence in the environment can be detected by RT-PCR.

1.14. Primers designed so far to study the ars operon genes

The first systematic study in the field of design and application of ars operon specific primers was carried out by Saltikov et al.(2002). These scientists successfully used primers for each of the three ars operon genes, viz. ars A, arsB and arsC present in most enteric and many gram negative bacteria. These primers are as follows:

arsA-1-F 5' TCCTGGATTGTCGGCTCTTG 3'
arsA-1-R 5' ATCTGTCAGTAATCCGGTAA 3'
arsB-1-F 5' CGGTGGTGTGGAATATTGTC 3'
arsB-1-R 5' GTCAGAATAAGAGCCGCACC 3'
Introduction

Macur et al. (2004) used four sets of primers for amplification and phylogenetic studies of \( \text{arsC} \) among diverse groups of bacteria. These sets of primers for \( \text{arsC} \) genes were designed based on different groupings of \( \text{arsC} \) sequences of 17 characterized and putative \( \text{arsC} \) genes which were obtained from GenBank and aligned using different softwares.

Primer set 1 was derived from the \( \text{arsC} \) genes of enteric bacteria (F 5'-TGAGCAACATYACCAT-3' and R 5'-TTATTTCAGYCGTTTACC-3'; corresponding to positions 1-426 of \( \text{E. coli} \) \( \text{arsC} \)).

Primer set 2 was derived from the \( \text{arsC} \) genes of Gram-positive bacteria (F 5'-ATTTAYTTTATATGYACAG-3' and R 5'-GATCATCAAAAACCCCAAT3'; corresponding to positions 16-317 of the \( \text{Bacillus subtilis} \) \( \text{arsC} \)).

Primer set 3 was derived from the \( \text{arsC} \) genes of \( \text{Pseudomonas aeruginosa} \) and \( \text{P. putida} \) (F 5'-AGTCCTGTTCATGTGYAC-3' and R 5'-TGGCGTSGAAYGCCG-3'; corresponding to positions 6-365 of the \( \text{P. aeruginosa} \) \( \text{arsC} \)).

The fourth primer set was designed from the annotated \( \text{arsC} \) in the \( \text{Agrobacterium tumefaciens} \) genome sequence (GenBank Accession No. AE008073) (F 5'-ATGTCCGATTATTTCAATCCG-3' and R 5'-TTTCCTTCAATTGCGAGACCTGC-3'; corresponding to positions 11-416 of the \( \text{A. tumefaciens} \) \( \text{arsC} \)).

Another significant contribution in this field was of Sun et al. (2004) who chose a group of 13 bacteria with the best aligned homologous regions of the \( \text{arsC} \) gene from 120 \( \text{arsC} \) sequences to design 2 primer pairs.

\[ \text{amlt 42f} \ 5'TCGCGTAATACGCTGGAGAT \ 3' \]

\[ \text{amlt 376r} \ 5'ACTTTCTCAGGAGATGATCCG \ 3' \]
Table 3: Length of ars C gene in various bacterial species.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Length of arsC (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>425</td>
</tr>
<tr>
<td><em>Klebsiella sp.</em></td>
<td>414</td>
</tr>
<tr>
<td><em>Yersinia enterica</em></td>
<td>492</td>
</tr>
<tr>
<td><em>Acidiphilium multivorum</em></td>
<td>425</td>
</tr>
<tr>
<td><em>Desulfovibrio Ben RA</em></td>
<td>425</td>
</tr>
</tbody>
</table>
**1.15 Genome walking approach for cloning of ars operon**

Use of genome walking technique in cloning bacterial genes and operons is very common. e.g. in 1998 Rudd et al. have reported the cloning of Suiyisin (involved in hemolysis) gene from *Streptococcus suis* by using this technique. They successfully cloned and over expressed it in *E. coli*. Carlin et al.(1995) showed that EcoRV fragment contains complete arsC gene and half of the arsB gene of the chromosomal ars operon of *E.coli*. They further showed that there are no HincII sites in the operon and the 3.2 Kb fragment thus generated contains the complete ars operon in *E.coli*. Another example is the discovery of the DABC type ars operon in the gram negative strain Shewanella sp.ANA-3( Saltikov et al.,2003) where they have used the technique of primer walking to elucidate the complete nucleotide sequence of the ars operon ofANA-3. Therefore, this technique is quite applicable for studying ars operons if we know the complete or partial sequence of one of the downstream (near the 3' end) genes from the complete operon.

**1.16 Biosensors for arsenic**

Biosensors offer great sensitivity and selectivity for the detection and quantification of substances that might otherwise be difficult to monitor. Limited understanding of the biochemistry involved in the response of higher organisms to arsenic restricts the applicability of biosensor using higher organisms. However the information gathered from biosensors can at least be quantitatively incorporated into risk assessments. Bacteria can be used as biosensors to demonstrate the toxicity of a variety of environmental media including soil, sediment and water by coupling bacteria to transducers that convert a cellular response into detectable signals. Biosensors are made by pairing reporter gene
that generates a signal with a contaminant sensing component that responds to chemical or physical change.

Various biosensors have been developed and tested at a research level for detecting bioavailable arsenic (Ji and Silver 1992, Corbisier et al. 1993, Tauriainen et al. 1997, 1999, 2000 Roberto et al. 2002, Petanen and Romantschuk 2003). Both gram negative and gram positive arsenic resistance systems have been utilized in biosensors as the arsenic sensing component (Roberto et al. 2002). Constructed biosensors till date have used β-galactosidase, GFP and luciferase (from firefly and bacteria) as the reporter gene coupled with various combinations of arsenic resistance mechanism components. Various strains of bacteria have served as the host bacteria like E. coli, Bacillus spp., S. aureus etc. The results obtained by biosensors are compatible with and comparable with chemical analysis, while being free from chemical extractions and analytical procedures. (Turpeinen et al. 2003, Flynn et al. 2002, Petanen and Romantschuk, 2002). The detection limits of biosensor depends on its design. It has been demonstrated as low as 7ppb for As(III) and and 35ppb for As(V) (Turpeinen et al. 2003). Once the bacteria are engineered to respond to a particular compound, they can be easily and inexpensively grown producing an endless supply of metal sensing cells. Ji and Silver (1992) studied the regulation of ars operon in S. aureus plasmid pl258 using different strains of bacteria as host. Through gene fusion Corbisiera et al. (1993) combined the lux AB gene encoding luciferase gene from V. harveyi and the ars operon of the S.aureus plasmid pl 258 and achieved similar results. He studied the expression in E. coli and S. aureus and found different patterns of induction wherein the S.aureus sensor was inducible with antimonite also in addition to arsenate, arsenite and bismuth which can act as inducers in both the strains. In the year 1997 Scott et al. constructed a biosensor by linking the ars P/O region of E. coli plasmid R773 with lacZ(β-galactosidase) + arsR whose activity was measured electrochemically. This plasmid construct was designated as pBGD23 and was
introduced into *E. coli* JM109. These authors suggested that the sensitivity of this biosensor can be improved by using a luciferase gene in place of *lac Z* gene. Later, Tauriainen et al. (1997) have constructed a luminescent biosensor for the measurement of bioavailable arsenite and antimony. The arsenate responsive promoter and regulatory element *arsR*(380 bp) of *S. aureus* pl258 were fused with the firefly luciferase (*lucFF*) gene in a reported plasmid construct pTOO21(7.6Kb). Expression was studied in *S. aureus* RN4220, *Bacillus subtilis* BR 151 and *E. coli* MC1061 based expression systems. The detection limit was 3.3µM for arsenite, 330µM for arsenate. Later this sensor was improved in 1999 using *E.coli* R773 promoter and regulatory elements. It demonstrated greater sensitivity than its predecessor pTOO21. This plasmid pTQO31 is able to detect both As(III) and As(V) with equal strength and strain MC1061 proved to be a better host in this case. It had a detection range of 33nm-1mM for As(III) and 33nm-33mM for As(V). *E. coli* tends to be the most sensitive host (Tauriainen et al. 2000). But *P.fluorescens* has been recommended as a host for soil samples because it is native to soil and it requires less amount of substrate to reach maximum luminescence (Petänen et al. 2002). A disadvantage of biosensors is that it can only be assumed that the fraction of arsenic bioavailable to bacteria is equal to the fraction bioavailable to the higher organisms. Petanen et al. 2002 developed two arsenic sensing strains using *lucGR* gene encoded for luciferin from the click beetle. The promoter and *arsR* were used from pl258 and pR773 as the arsenic sensing components for pTPT21 and pTPT31, respectively. The construct pTPT31 performed much better with a minimum detection limit of 10nm of arsenite. Soon after that Roberto et al. (2002) have developed in their laboratory an arsenic biosensor by coupling *arsR*, *arsD* and their promoter with the gene encoding for GFP from the marine jellyfish *A. victoria*. *E.coli* was used as a host and the sensor was able to detect As(III) and As(V) in a range of 1-10,000 ppb.
In the present study I have isolated several interesting arsenate resistant bacteria from marine environment of Goa and characterized them w.r.t. identification by biochemical tests, FAME analysis as well as 16s rDNA approach; tolerance limits, mechanisms of resistance, antibiotic sensitivity, bioaccumulation, biotransformation and also tried to amplify the \textit{arsC} gene from highly resistant isolates. Cloning of the putative \textit{ars} operon (partial) fragment (2.5Kb) from the 30 Kb plasmid of one of our isolates \textit{Vibrio} sp. SI9 by applying the Genome Walking technique is our achievement worth mentioning.

The limited availability of scientific reports about biodiversity, biotransformation of arsenate/arsenite, structure and function of genetic determinants of arsenate resistant bacteria from our country and no such reports from Goa in particular motivated me to carry out a detailed study including screening and identification; uptake and biotransformation of arsenate; physiological, biochemical and molecular biological characterization and phylogenetic analysis of isolates highly resistant to arsenic, from different econiches of Goa viz. marine, estuarine and sewage water habitats.

These studies will make the foundation of developing arsenate hyperaccumulating strains for bioremediation and can also help us in the development of microbial biosensors utilizing the inducible promoter of the \textit{ars} operon from the highly arsenate resistant bacteria.