Chapter I

Extreme Environments
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Microorganisms occupy almost every conceivable habitat on earth, most of these being moderate environments like neutral pH, temperatures between 20-40°C, pressure of 1 atm and an adequate concentration of nutrients, salt and moisture (Horikoshi 1991). The most concentrated and widespread occurrences of organisms are generally observed in these "moderate" environments.

It has also been known that there are extreme environments on earth which were thought to prevent the existence of life. In these habitats, environmental conditions such as pH, temperature and salinity concentrations are extremely high or low. Extreme environments are populated by groups of organisms that are specifically adapted to these particular conditions and are capable of surviving in these "extreme" environments. These organisms which thrive in extreme environments are called "Extremophiles" (Krahe et al 1996, Madigan et al 1997).

Extremophiles are capable of surviving in extreme of temperatures Thermophiles – surviving at high temperature and Psychrophiles – surviving at low temperatures, high pressure (Barophiles), high pH (Alkaliphiles), low pH (Acidophiles), high salt concentrations (Halophiles), low nutrient conditions (Oligotrophs), extremely dry conditions (Xerophiles), in presence of heavy metals (metal-tolerant organisms) and high levels of organic solvents (organic solvent tolerant organisms).

Extremophiles have developed special adaptations to survive in extreme conditions which include novel mechanisms of energy transductions, regulation of intracellular environment and metabolism, maintenance of structure and functioning of membranes and enzymes.
**Thermophiles**
- High temperature
  Thermal vents and hot springs may go hand in hand with chemical extremes

**Psychrophiles**
- Low temperature
  Arctic and Antarctic
  1/2 of Earth’s surface is oceans between 10°C & 40°C
  Deep sea -10°C to 40°C
  Most rely on photosynthesis

**Acidophiles**
- pH 0-1 of waters at Iron Mountain

**Alkaliphile**
- Alkaline
  Soda lakes in Africa and western U.S.
  e.g. Mono Lake
  Alkaline soda lake, pH 9
  salinity 8%

**Halophiles**
- Highly Salty
  Natural salt lakes and manmade pools
  - Sometimes occurs with extreme alkalinity
    - solar salterns, Owens Lake, Great Salt Lake
  coastal splash zones, Dead Sea
Most interesting practical applications so far:
- Many industrial processes involve high heat.
  - 45°C (113°F) is a problem for most enzymes.
- First Extremophile found 30 years ago.

**Thermophiles**
- Efficient enzymes to work in the cold.
  - Enzymes to work on foods that need to be refrigerated.
  - Perfumes - most don't tolerate high temperatures.
  - Cold-wash detergents.

**Psychrophiles**
- Enzymes to work in the cold.
  - Enzymes to work on foods that need to be refrigerated.
  - Perfumes - most don't tolerate high temperatures.
  - Cold-wash detergents.

**Acidophiles**
- Enzymes used to increase efficiency of animal feeds.
  - Enzymes help animals extract nutrients from feed.
  - More efficient and less expensive.

**Alkaliphiles**
- "Stonewashed" pants.
  - Alkaliphilic enzymes soften fabric and release some of the dyes, giving worn look and feel.

**Detergents**
- Enzymes to dissolve proteins or fats.
  - Alkaliphilic enzymes can work with detergents.

**Halophiles**
- Many possible applications using halophiles are being explored such as:
  - Increasing crude oil extraction.
  - Genetically engineering halophilic enzyme encoding DNA into crops to allow for salt tolerance.
  - Treatment of waste water.
The enzymes and various endogenous compounds produced by all extremophiles have various application in industry as seen in the table below.

### Table 1.1: Industrial applications of Extremophiles (Aguilar 1996; Cook et al 1996)

<table>
<thead>
<tr>
<th>EXTREMOPHILIC GROUP</th>
<th>ENZYMES, ENDOGENOUS COMPOUNDS</th>
<th>APPLICATIONS PRODUCTS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thermophiles 50-110°C</strong></td>
<td>Amylases, Proteases</td>
<td>Glucose, fructose for sweetness, paper bleaching, amino acid production from keratins, food processing, baking, brewing, detergents, biosensors, genetic engineering PCR, sequencing.</td>
</tr>
<tr>
<td><strong>Halophiles 3-20% salt</strong></td>
<td>Antibiotics, Carbohydrates, Compatible solutes, Membranes, Enzymes</td>
<td>Pharmaceuticals, Food coloring, Pharmaceuticals, Surfactants for Pharmaceuticals, Waste treatment peptide synthesis, enhanced oil recovery from wells.</td>
</tr>
<tr>
<td><strong>Psychrophiles 5-2°C</strong></td>
<td>Neutral proteases, Amylases, Lipases, Polyunsaturated fatty acids, Ice-protien, e-3 Fatty acids</td>
<td>Cheese maturation, dairy production, Detergent for cold water washes, Pharmaceutical, Artificial snow, Production of dietary supplements.</td>
</tr>
<tr>
<td><strong>Alkaliphiles pH &gt;9.0</strong></td>
<td>Transglutaminase, Amylases, Ureases, Cellulase 103, Cyclodextrins, Antibiotics</td>
<td>Detergents, Stabilization of volatile substances, Pharmaceuticals.</td>
</tr>
</tbody>
</table>
1.1 Alkaliphiles:

Alkaliphiles are defined as a diverse group of organisms that thrive in highly alkaline environments with optimum pH for growth being 9 or above. The term alkaliphiles denotes *alca* - Arabic soda ash, *phile* - loving. Based on the pH preference, alkaliphiles can be classified into two broad categories.

a) **Alkalitolerant** organisms that show optimal growth in the pH range of 7.0-9.0 but grow above pH 9.5.

b) **Alkaliphilic** organisms that show optimal growth between pH 10.0 and 12.0. These organisms can further be subdivided into

i) **Facultative alkaliphiles**: which show optimal growth at pH 10.0 or above but can grow well in neutral pH range and

ii) **Obligate alkaliphiles**: which show optimal growth above pH 10.0 but do not grow below pH 9.0 (Krulwich and Guffanti 1989).

The key difference between these two groups of alkaliphiles is in the membrane lipids. The fatty acid composition of the phospholipids in membranes of facultative alkaliphiles appears to have greater membrane integrity at near neutral pH values than the more unsaturated, highly branched fatty acids in membranes of obligate alkaliphiles (Clejan *et al* 1986). In order to more clearly distinguish between alkaliphiles and the more abundant alkaline – tolerant prokaryotes, the characteristic feature is the growth at high pH and an inability to grow well at near – neutral pH values such as 6.5 (Horikoshi, 1998). The lower pH limit and pattern of growth over a broad pH range also depends upon the particular growth substrate (Gilmour and Krulwich 1997, Krulwich *et al* 1997) and on strain differences that are not understood.
Alkaline Environments (Guffanti et al 1986). The optimal pH value of 9.5 is chosen for growth of alkaliphiles as it is approximately the upper limit of cytoplasmic pH range that is compatible with the growth of bacteria studied till date (Sturr et al 1994, Krulwich et al 1998). Among these bacteria that meet this criterion, the term facultative alkaliphile has been applied to species and strains that are able to grow between pH 6.5 – 7.5, whereas alkaliphiles that cannot grow in that range are termed obligate alkaliphiles.

In the present work "Alkaliphiles" were isolated from mangrove ecosystems of Goa (West Coast of India), namely Ribandar, Banastari, St.Cruz, Merces and Panjim. (Fig 1.1 & 1.2)

1.2 Mangrove Ecosystem:

Mangrove is one of the specialized ecosystems of the tropical areas. It is nutritionally rich due to the continuous shedding of foliage which gets decomposed to form detrital matter. The microbial flora of the mangrove ecosystems plays a significant role in the formation of detritus. These organisms though have continuous nutrients are affected by tidal variations, salinity and by anthropogenic substances added through run offs from the terrestrial ecosystems, such as excess of fertilizers, pesticides and by the activities of industries such as mining, shipbuilding etc. The interaction of microbial flora with such anthropogenic substances has resulted in the proliferation of physiologically diverse microflora in marine ecosystems. Among these are also the bacteria which have the ability to degrade hydrocarbons and
xenobiotics. This ecosystem is also found to develop specialized niches having specific conditions such as high pH, increase in salinity, high nutrients eg. Nitrogen and Phosphorus (Robertson 1992).

1.3 Aim and Scope of the work:

Study of microbial degradation of aromatic compounds at alkaline pH is important in natural ecosystems where the fate and toxicity of these contaminants is unknown, but the existence of such microorganisms would support functioning of the carbon cycle. Use of these microorganisms in removal of aromatic compounds from alkaline and/or industrial wastewater will support the environmental concern of industries and environmentalists.

Although much is known about the degradation of aromatic compounds at neutral pH, relatively very little information is available about such biotransformations occurring at an alkaline pH, more so with alkaliphiles.

The present work was therefore undertaken to study the alkaliphiles from mangrove ecosystems of Goa with respect to biodiversity, buffering capacities and their ability to biodegrade aromatic compounds under alkaline conditions.
Fig 1.1: Map of Goa showing the sampling sites
Fig 1.2  Mangrove ecosystems chosen as sampling sites