Introduction
Bio-active metabolites produced by microbes and other living organisms exert biological activity by virtue of their structure on other living cells such as micro-organisms, plants, higher animals and humans. Traditionally, microbes are known for production of antibiotics, which is the largest group of bio-active compounds. However, bacteria, fungi and even micro-algae are reported to produce a diverse range of pharmacologically and immunologically active molecules such as enzymes, enzyme inhibitors, anti-parasitic agents, insecticides, phyto-hormones etc. In the recent years, various advances have been made in virtually every field of drug discovery, and the total number of compounds with clearly defined biological activity isolated from natural sources is close to 100,000 out of which about 10,000 compounds are of microbial origin [37]. Besides this, the potential of micro-organisms to convert and transform natural products has been widely exploited for the production of bio-active compounds, particularly steroid molecules.

The present thesis is therefore focussed to screen the ability of micro-organisms from marine and estuarine sources for steroid transformation and L-asparaginase production. The work was diverted towards studies on:

1) L-asparaginase producing bacteria

And

2) Bacteria capable of steroid transformation in an organic-aqueous biphasic system (Cholesterol was selected as a model steroid compound). Since Goa has a tropical climate and extensive coastline known to harbour rich bio-diversity, all the bacterial cultures used in this study were isolated
from sediment samples collected from the Arabian Sea and Mandovi estuary.

1) **L-asparaginase (L-asparagine aminohydrolase E.C. 3.5.1)**

This enzyme cleaves the amino acid L-asparagine to L-aspartic acid and ammonia is of extreme importance in the treatment of certain cancers and tumors particularly acute lymphoblastic leukemia (A.L.L.) [320]. Leukemic cells have a lower asparaginase synthetase activity and a higher requirement for asparagine as compared to normal cells. The amino acid starvation leads to apoptosis or cell suicide. L-asparaginase selectively suppresses the synthesis of ribosomal proteins at the level of mRNA translation. The medical use of the enzyme was initiated in the early 1960s when its significance in destroying cancer cells was reported by Broome [58]. Currently, the industrial production of L-asparaginase is by *E.coli* and *Erwinia chrysantheni*. These enzymes are highly effective in cancer treatment, however there are drawbacks such as toxicity effects induced by immunological reactions and the need for extensive purification protocols to ensure complete removal of endotoxins and related impurities. Hence the search for a source of asparaginase continues. For clinical activity, the enzyme besides having a high affinity for asparagine should be stable and active at 37 degrees C and pH 7.4 [352]. Studies were therefore diverted towards:

a. Isolation of L-asparaginase producing bacteria from marine and estuarine habitats.
b. Determination of L-asparaginase activity of the bacterial isolates at 37 degrees C and pH 7.

c. Optimisation of enzyme activity.

d. Identification of selected isolates.

2) **Bio-transformation of steroids** is a multi-million dollar industry having numerous pharmaceutical uses. The major limiting factor in this process is the extremely poor solubility of steroids in the aqueous medium (< $10^{-2}$ to $10^{-3}$ g/l), which lowers the transformation rates and increases monetary implications. It is therefore desirable that solubility conditions be improved during bio-transformation. It has been established that cholesterol dissolved in organic solvents at a high concentration is converted at a much higher rate with cells or enzymes suspended in the water-phase [269]. However, organic solvents are toxic to all ordinary bacteria even in low concentrations. Therefore, to have adequate production of chemicals by micro-organisms in such a two-phase system, it is necessary to develop micro-organisms which contain the relevant enzymes in sufficient amounts and with high specific activities, even in the presence of the generally destructive organic phase. This problem can be overcome by using organic solvent tolerant bacteria (OSTB) which can carry out the desired bio-transformations in an organic solvent saturated system. OSTB are a relatively novel group of extremophilic microbes which have developed various adaptations to withstand solvent toxicity. They have tremendous potential in industrial processes involving non-aqueous bio-catalysis and transformation in presence of an organic phase [91], as the cells and enzymes of OSTB are found to be active in the
presence of organic solvents [297, 298]. It has been reported that the number of OSTB in marine habitats is much higher than in soil [201]. Hence, the present work was undertaken to isolate organic solvent tolerant cholesterol transforming bacteria from this ecosystem. An attempt was therefore made to study the following aspects:

a. Isolation of bacteria capable of cholesterol transformation in an organic–aqueous biphasic system from marine/estuarine habitats.

b. Identification of selected bacterial isolates.

c. Development of a suitable organic-aqueous biphasic fermentation system for cholesterol transformation

d. Identification of the intermediate obtained from cholesterol.

e. Study of the solvent tolerance of these isolates.