The extremophilic microorganisms have provided an interesting and challenging platform to the researchers, since the time of their discovery. Besides growth under the extreme conditions, the production of the valuable molecules, such as enzymes, antibiotics and hormones has fascinated the scientific community. The extremophiles primarily include Halophiles, Thermophiles, Barophiles, and Ps eryophiles and Acidophiles under one roof (Austin, 1988; Herbert and Sharp, 1992; Singh, 2006).

Among the extremophiles, the thermophilic bacteria, actinomycetes and fungi are the organisms which can grow and produce such compounds optimally at high temperatures, mainly between 45 and 80°C. Many thermophiles are archaea, which can grow at even high temperatures. The thermophiles are found in various geothermally heated regions of the Earth, such as the hot springs and the deep sea hydrothermal vents as well as in the decaying plant matters, such as peat bogs and compost. The thermophilic microorganisms are further subcategorized on the basis of their temperature tolerance. For instance, the facultative thermophiles, can grow within the temperature range from 50 to 65°C, but also grow at 37°C; the obligate thermophiles have maximum growth temperature from 65 to 70°C, and will not grow below 40°C; the extreme thermophiles can grow between 40 and 70°C with an optimal growth temperature of about 65°C. The hyperthermophiles, mainly comprising of archae, are capable of growing at over 90°C, with a range of the optimal temperatures from 80 to 115°C (Brock, 1986; Singh et al., 2012).

The thermal springs are high temperature aquatic ecosystems and are widely distributed throughout the world. They are largely distributed in the volcanically active areas. These habitats are of special interest from an ecological and evolutionary point of view, as the inhabiting microorganisms have developed mechanisms to thrive at different temperatures.
It is very interesting to explore the diversity and phylogeny of the microbial communities, as it may furnish some valuable information of various adaptive mechanisms employed during the evolution. The concept of the microbial diversity and phylogeny further bifurcates into the culture-dependent and the culture-independent (metagenomics) approaches. The conventional culture-dependent methods have been used for the measurement of the microbial diversity and phylogeny for many years. These methods are fast, inexpensive and can furnish information of the active, heterotrophic components of the community. However, now it is established that only 0.1-1% of the total bacterial community can be cultivated in the laboratory (Hill et al., 2000). Thus, the majority of the bacterial community remains non-cultivable and thus inaccessible. In addition, sometimes, it is very difficult to culture and maintain the pure microbial strains in the laboratory conditions either due to the need for some special physical conditions, fastidious nutritional requirements or delayed growth. During the process of the enrichment and culturing, it may happen that the dominant microbial species get a selective advantage, while some of the recessive microbial communities may be eliminated. This may project a false conclusion regarding the microbial diversity. Therefore, the need of metagenomic approach is now gradually realized. Metagenomics has in a way bypassed the bias of cultivation of the microbial strains by the enrichment techniques.

During the recent years, a number of culture-independent molecular techniques have been employed using polymerase chain reaction (PCR) – mediated amplification of the 16S rRNA gene sequence from the environmental DNA (Hayashidani et al., 1995). These studies reported on the existence of a large number of phyla that were poorly or not represented by the cultured bacterial strains (Dunbar et al., 1999; Kaiser et al., 2001). Moreover, the culture-independent methods require a high quality intact DNA in terms of purity and quantity. The advancements in molecular biology and bioinformatic tools have contributed significantly to the accuracy, adequacy, reliability and reproducibility of the unexplored diversity. However, each method has its own benefit and limitation. Therefore, the best way to study the microbial diversity is to use the concept of the polyphasic taxonomy, a combination of the conventional and molecular methods for a clear picture.
In another dimension, the enzymes of the thermophilic microorganisms, referred as ‘thermozymes’ have unique characteristics of the catalysis and stability at the elevated temperatures, broad pH and various chemical denaturants. Therefore, the thermozymes can be used in several biotechnological processes efficiently, replacing the enzymes of the mesophilic microorganisms (Demirijan et al., 2001; Singh et al., 2012). The isolation of large number of the thermophilic microorganisms from different habitats would lead to the discovery of new thermostable enzymes with improved properties (Antranikian et al., 1999; Singh et al., 2012). In addition, as a consequence of the growth at high temperatures and unique macromolecular properties, the thermophilic bacteria possess high metabolic rates, physically and chemically stable enzymes and lower growth but higher end product yields than their mesophilic counterparts. Reactions at higher temperatures have added benefits of the decreased viscosity and enhanced diffusion coefficient of the substrates leading to the equilibrium displacement in endothermic reactions (Kumar and Swati, 2001). Thus, many possibilities for the industrial processes have emerged with thermostable enzymes (Leuschner and Antranikian, 1995; Haki and Rashit, 2003; Arikan, 2008, Singh et al., 2012).

The thermophilic microorganisms are difficult to isolate and maintain in pure culture. The major challenge for the thermophilic microorganisms is their survival and production of active and stable enzymes and other bioactive molecules at high temperatures. In addition, it is highly relevant to explore the diversity, molecular phylogeny and production of enzymes and other valuable compounds. Therefore, the present research focused on two aspects: the diversity and the biocatalytic potentials of the thermophilic bacteria isolated from the Tulasi Shyam hot spring reservoirs in the Gir Forest, located in the Gujarat State, India.
Aims and Objectives of the Research

The present study focuses mainly on two aspects, the diversity and the biocatalytic potentials of thermophilic bacteria isolated from the natural hot spring reservoir, Tulasi Shyam in the Gujarat State, India.

- Physicochemical characterization of the water and soil samples, collected from the natural hot spring reservoir
- Isolation of the thermophilic, thermotolerant and thermoalkalitolerant bacteria
- Assessment of the seasonal variation on the bacterial population dynamics in the particular ecosystem by the culture-dependent and the culture-independent (the sequence based metagenomic) approaches, using various statistical diversity indices
- Assessment of the bacterial diversity by a combination of various conventional microbiological techniques and the advanced molecular techniques, such as 16S rRNA sequencing and amplified 16S rDNA restriction analysis (ARDRA)
- Construction of the phylogenetic tree, using suitable bioinformatics software based on the sequence homology to judge the population heterogeneity
- Sequence-based metagenomic approach, i.e. optimizing the isolation of the total environmental DNA, followed by the 16S rRNA gene amplification using a set of universal eubacterial primers. Analysis of the amplified products by the denaturing gradient gel electrophoresis (DGGE) to investigate the bacterial diversity
• The biocatalytic potentials of the thermophilic bacteria: the screening for the extracellular enzymes on selective media plates, mainly amylase, protease, cellulase and lipase

• Optimization of various physicochemical parameters, affecting the production of the α-amylases

• The purification and characterization of the thermostable α-amylases with respect to the enzyme stability, enzyme kinetics, solvent tolerance and protein denaturation

• Immobilization of the purified enzyme to enhance the stability and reusability

• Investigation of the various thermodynamic parameters of the purified enzymes: deactivation rate constant, half-life, changes in enthalpy, entropy, activation energy and Gibb’s free energy

• Investigation of the structural attributes of the purified enzyme by the Circular Dichroism (CD) spectroscopy, followed by K2D3

• Application of the purified and immobilized enzyme in the starch hydrolysis process by the calculation of the dextrose equivalent (DE) values
A GLIMPSE ON THE RESEARCH

Molecular Diversity and Phylogeny

Culture dependent Diversity

Conventional Techniques
- Isolation, Gram reaction & morphology
- Growth optimization
- Biochemical properties & Antibiogram

Molecular Techniques
- Genomic DNA isolation
- ARDRA
- 16S rRNA sequencing

Culture independent Diversity

Optimization of the metagenomic DNA isolation
- PCR amplification of 16S rRNA gene
- DGGE

Production optimization, single step purification and characterization of the α-amylases

Natural Hot spring reservoirs in Tulasi Shyam, India

Screening for the Biocatalytic Potentials

Bacillus amylobiophosphatans TSWK1-1
- Immobilization & characterization
- A comparative profile on native and immobilized enzymes

Bacillus sp. TSSC-1
- Evaluation of thermodynamic parameters, related to the enzyme stability
- Application in the starch hydrolysis

A. beppuensis TSSC-3
- Investigation of the changes in the secondary structure of the pure amylase by CD spectra