ABSTRACT

The microbial population of soils is made up of five major groups including bacteria, actinomycetes, fungi, algae, and protozoa, and among these groups, bacteria are the most abundant group (Alexander, 1961) and the most important microbe for decomposing waste. Bacteria use wastes for their own metabolism and finally, they produce some simple and useful compounds which are important for soil health, plant growing and overall to keep well balance of the natural ecosystem. Microbes live in an environment where the nutrients are mainly macromolecular in nature. These nutrients are not utilizable by the microbes unless cleaved into smaller molecules that they can absorb. The cleavage of macromolecular nutrients into smaller molecules is accomplished by the enzymes secreted by the microbes themselves. Enzymes are well-known biocatalysts that perform a multitude of chemical reactions and are commercially exploited in the detergent, food, pharmaceutical, diagnostics, and fine chemical industries. More than 3000 different enzymes described to date the majority have been isolated from mesophilic organisms. These enzymes mainly function in a narrow range of pH, temperature, and ionic strength. Moreover, the technological application of enzymes under demanding industrial conditions makes the currently known enzymes unrecommendable. Thus, the search for new microbial sources is a continual exercise, where one must respect biodiversity.

Keratinases are proteolytic enzymes in nature. It was classified as proteinase of unknown mechanism as recommended by the Nomenclature Committee on the International Union of Biochemistry (1978) with EC number 3.4.99 (Owen et al., 1983). Recently, some of the workers defined keratinase as serine protease due to its 97% sequence homology with alkaline protease and it is also inhibited by the same inhibitor that inhibits serine protease (Wang et al., 1995; Taha et al., 1998 and Bressollier et al., 1999). Keratinases are produced only in the presence of keratin-containing substrate. It mainly attacks the disulfide (-S-S-) bond of the keratin substrate (Bockel et al., 1995). The keratinase production by various microorganisms were reported by a number of workers. It was found that keratinase produced by fungi, Streptomyces and bacteria were produced in nearly at alkaline pH and almost thermophilic temperatures. These enzymes have wide range of substrate specificity such as it can degrade other fibrous protein fibrin, elastin, collagen and other non
Abstract

fibrous protein like casein, bovine serum albumin gelatin etc. (Noval et al., 1959; Mukhapadhayay et al., 1989; Dozie et al., 1994; Lin et al., 1995; Letourneau et al., 1998; and Bressollier et al., 1999). Keratinophilic bacteria are able to synthesize a variety of important biotechnological and industrial enzymes, such as amylase, protease, cellulase, lipase, and keratinase. Amylase catalyzes the hydrolysis of starch, protease catalyzes the hydrolysis of proteins, cellulose catalyzes the hydrolysis of cellulose, lipase catalyzes the hydrolysis of lipids and keratinase catalyzes the hydrolysis of keratins. These extracellular enzymes are widely used in the food, beverage, leather processing, textile and detergent industries as well as wastewater treatment. In addition, microbial enzymes are more stable than plant or animal enzymes.

The present work was carried out on keratinase producing bacteria. The study was done on different feather dumping sites soil around Aurangabad, Maharashtra. Soil samples from different feather dumping sites were collected aseptically and brought to the laboratory and analyzed. The bacteria were isolated by serial dilution method. Screening of keratinase producing bacteria was done. Characterization of efficient keratinase producing bacteria and enzyme partial purification and characterization was done. The data obtained during the study, compiled into the following chapterwise.

Chapter I - Introduction and review of literature

This chapter contains details of keratin substrates, literature regarding the keratinase producing microbes. It also gives information about the role of the keratinase enzyme in feather waste management.

Chapter II – Sample collection, isolation, and screening of bacteria

This chapter deals with the collection of soil samples from different feather dumping sites, enrichment of soil samples in minimal salts medium and isolation of bacteria by serial dilution method on nutrient agar plates. It also deals with the screening of keratinase producing bacteria with the help of skim milk agar plates and feather meal agar plates method. A zone of clearance was observed on keratinase positive isolates. The bacterial strain which showed the largest zone of clearance was selected for further study.
Chapter III – Identification and characterization of bacteria

This chapter deals with the identification of selected bacteria by Gram’s stain method, by performing various biochemical tests such as IMViC test, Carbohydrate utilization tests and finally, molecular identification by 16 S rRNA sequencing.

Chapter IV – Isolation, purification and biochemical characterization of keratinase

This chapter deals with the production of the keratinase in feather meal broth by submerged fermentation, media optimization for the keratinase producing bacteria by a single factor at a time such as Carbon source, Nitrogen source, size of the inoculum, pH, temperature, incubation time. The partial purification of the crude keratinase by ammonium sulphate precipitation and dialysis method, standard keratinase assay by Folin Lowery method. This chapter also deals with the biochemical characterization of a keratinase enzyme such as determination of molecular molecular weight, the effect of different pH, temperature, substrate concentration, inhibitors on keratinase enzyme activity.

The list of observations and results made throughout the study as mentioned above are elaborated in the thesis under chronology; preface, introduction, material and methods, results, discussion, summary, conclusion, and references. All these efforts are made to illustrate matter with the help of maps, photographs, figures, tables, and graphs. For a compilation of the thesis, APA system was opted along with the guidelines recommended by the university. However, it is expected that the present investigation may add part of the knowledge in the field of Biotechnology.