INTRODUCTION

Biotechnology is defined generally as the use of living organisms to produce products beneficial to mankind. It is the application of biological organisms to technical and industrial processes for development of an industrial process. It is better to replace the usual protein source in microbiological media (peptone beef extract, yeast extract etc.) with natural proteins as cheaper sources of nitrogen as well as carbon for fermentation. It involves the use of novel microbes, which have been altered or manipulated by humans techniques of genetic engineering, **(Glazer and Nikaido, 1998).**

Alkaliphilic microorganisms (particularly prokaryotes) are widely distributed and can be found in almost on environment **(Denizci *et al* 2004),** even in environments where the overall pH may not be particularly alkaline. Obligate alkaliphiles are in capable of growth around neutrality.

Genetic and phenotypic diversity among bacteria strains **(Gutell, *et al*., 2002 and Seham, 2009)** are well known, but ecological and systemic assessments have been problematic because readily used morphological, biochemical or molecular markers have largely been locking. Analysis of the nucleotide sequence of RNA from the small ribosomal subunit (i.e. 16S rRNA) has gained rapid and wide acceptance in systematic evolutionary and ecological studies of various microorganisms **(Hari *et al*., 1989).** The comparison of rRNA sequences is a powerful tool for deducing phylogenetic and evolutionary relationships among bacteria, arehae-bacteria and eucaryotic organisms. There sequences have been derived previously by methods including oligonucleotide cataloging **(Fox *et al*., 1980),** sequencing of clones, direct sequencing of RNA by using reverse transcriptase **(Lane *et***

***al*., 1985)** and sequencing of material amplified by polymerase chain reaction (PCR) **(Edwards *et al*., 1989)** reported that, diversity in nucleotide sequences of selected regions of 16S RNA from the two *Frankia* isolates. These regions were chosen because they had bean found to very among other regions were chosen because they had been found to very among other actinomycetes. **Nagesh *et al*. (2005)** found that, similarly, the 800 bp 16S-23 rRNA ITS region was amplified from *Ps. fluorescens* strain Gpf01 and its sequence aligned with the nucleotides of the 16S-23S rRNA ITS region of the other *Pseudomonas* spp. which showed up to 93.5, 75.1, 89.8, 75.1, 81.8 and 92.3% identifies to *Ps. fluorescens* I, *Ps. fluorescens* II, *Ps. syringae, Ps. agarici* and *Ps. toloasii* respectively. The phylogenetic analysis based on the nucleotide sequences of this region also revealed the strain GpfO1 to be closer of *Ps. fluorescens*.

Lipase of the *Pseudomonas* species are secreted by a least two different pathways. The two-step pathway, which requires at least 12 x cp gene products, is used by the signal sequence-containing lipases of *A. aeruginosa* **(Tommaseen *et al*., 1992)** and *Ps. glumae* **(Franken *et al*., (1993).**

For development of an lipase and protease production, it is better to replace the usual protein source in microbiological media (peptone, beef extract, yeast extract … etc.) with natural proteins as cheaper sources of nitrogen, for the fermentation **(Sodhi *et al*., 2005).** The solid substrate natural is the most important factors in state fermentation. The solid state fermentation is receiving a viewed surge of interest, primarily because increased productivity and prospect of using a wide lipase and protease agro industrial residues as substrates **(Park *et al*., 2002).** In order to production of

low cost of lipase and protease those bacterial isolates under study were allowed to grow an natural substrate such as slaughter house waste under submerged fermentation conditions. However, the selection of the previously mentioned substrates for the process of lipase and protease biosynthesis were based on the following factors viz. (1). It represent the most cheapest agro-industrial wastes in Egypt; (2) It is available at any time in the year, (3) It storage represents no problem in comparison with other substrates and (4) It resist any drastic effect due to the exposure to other environmental conditions e.g. temperature, variation in the weather from season to season and or from day to might. This trial resulted in the fact that the bacterial isolates *Pseudomonas* and *Bacillus* were considered to be the best for lipase and protease production by growing on animal blood (slaughter house wastes). The environmental conditions in solid state fermentation conditions can stimulate the microbe to product the extracellular enzymes with different properties other than those of enzymes produced by the same organism under the condition performed in submerged fermentation **(Pandey *et al*., 2000).**

The largest application of industrial enzymes is an additives in detergents **(Kawada, 1998 and El-Sayed, 2006).** Many detergent brands contain combination of protease lipase (EC 3.1.1.3), cellulases (EC 3.2.1.4) and other degradation enzymes. Generally proteases and lipases are added to detergent to digest proteins and lipase from human sweat, foods, and soils in cloth fibers.

Proteases are the most widely used enzymes in detergent industry. They remove protein based strains such as blood, eggs, grass, meat sauce and body secretions, these detergent enzymes (serine proteases) are

produced by the fermentation of *Bacillus* sp. lipases facilitate the removal of fatty strains such as lipsticks, frying fasts, butter, salad oil, sauces and tough stains on collars and cuffs **(Prist, 2000).**

A combination of amylases, protease and lipases is an important components of detergents for automatic dish washing machines **(Oberol *et al*., 2001).** One commercial application of microbial enzymes has been the removal of stains from clothing. Many substances that strains clothes either re made of lipids, proteins or starches or are held in a fibric by these types of compounds. Needless to say, stains can caused by a large variety of substances, and appropriate enzymes may not be available or if available may not be incorporated into detergent or presoak compounds used in laundering. Thus it is not surprising that all stains are not removed by commercial products **(Wistreich and Lechtman, 1984).** Currently, the detergent industry occupies about 25-30% of the entire industrial enzymes market.

Fibrinogen is a plasma protein that can be transformed into an insoluble fibrin network by the action of thrombin and factor XIIIa (fibrin-stabilizing factor) in the final stages of the blood coagulation cascade. Fibrinogen is a dimmer consisting of two identical halves that contain three polypeptide chains each, designated Aα, Bβ and γ, which are interlinked by disulfide bands **(Blombäck and Yamashina, 1958).** Two fibrinopeptides A and two fibrinopeptides B are sequentially cleaved from the Aα and Bβ chains, respectively, by thrombin. This event alters the charge and conformation of the molecule leading to the formation of fibrin monomers that eventually polymerize to form a three dimensional insoluble fibrin network. Factor XIII, after activation into factor XIIIa by thrombin in the

presence of Ca^{2+} ions, further strengthens the fibrin network by forming covalent bands between glutamyl residues in one fibrin molecule and lysyl residue in another **(Lorand and Ong, 1966; Fuller and Doolittle, 1966).** In general, fibrinogen has been isolated from whole blood using centrifugation in combination with cryoprecipittion or precipitation agents like ethanol, ammonium sulfate or polyethylene glycol **(Silver *et al*., 1995).** Human fibrinogen has also been purfied by affinity chromatography **(Kuyas *et al*., 1999).**

The objectives of this study are :

1. Isolation and identification of some bacteria association animal blood using molecular biological techniques.
2. Industrial enzymes production and stability.
3. Application of enzymes in detergent industry.
4. Purification of animal blood fibrinogen and characterize fibrinogen and studying clottability.