1. INTRODUCTION

Enzymes are functional proteins capable of catalyzing chemical reactions of biological origin without themselves undergoing any change. Enzyme catalysis is highly substrate selective, which is due to a series of highly specific non-covalent enzyme-substrate binding interactions. These molecules are the essential activators in majority of the life processes. All living organisms including microorganisms, higher plants and animals are the sources of enzymes. Microbial enzymes have the advantage over the animal and plant enzymes. Firstly, microbial enzymes are economical and can be produced on large scale within limited space and time. Secondly, there is technical advantage in producing enzymes via microorganisms as they can grow in a wide range of environmental conditions and are capable of producing wide variety of enzymes (Trevan, 1987). Microbial enzymes are of two types, the intracellular and the extracellular enzymes. The intracellular enzyme is synthesized and retained in the cell. The extracellular enzyme is synthesized in the cell and is secreted out into the medium. Enzymes are synthesized in the ribosomes and are transported through the spaces between rough endoplasmic reticulum (RER). Proteins depart from the RER wrapped in the vesicles which get pinched off from the sides of the RER. These vesicles containing proteins fuse with the membranes of the Golgi body and enter the Golgi stacks. As they move through the Golgi body, the proteins are modified in different ways, ready to be used within the cell. Alternatively, in case of extracellular enzymes the vesicles containing the proteins bud off the Golgi membrane, fuse with the cell surface membrane and release the proteins. The intracellular enzymes are difficult to extract as they are not secreted to the external medium of the cell (Riviere, 1977). They can be obtained by breaking the cells by means of a homogenizer or a bead mill.

The unique catalytic properties of enzymes make them desirable in many chemical processes. However, poor thermostability, short operational lifetimes and impossibility for reuse restrict their wide range of applications. Enzyme immobilization onto or within solid support has been accepted as one of the most successful methods in eliminating these limitations of the free enzyme (Woodly, 1992).
Apart from their vital role in living bodies, the enzymes are important agents in maintaining soil health and its environment. The enzymatic activity in the soil is mainly of microbial origin. The enzymes catalyze several reactions necessary for the life processes of micro-organisms in soils and stabilize soil structure, the decomposition of organic wastes, organic matter formation and nutrient cycling. Enzymes play an important role in agriculture (Dick et al., 1994; Dick, 1997). The enzyme levels in soil systems vary in amounts primarily due to the fact that each soil type has different amounts of organic matter content, composition and activity of its living organisms and intensity of biological processes (Kiss et al., 1978). These enzymes may include amylase, arylsulphatases, β-glucoSIDase, cellulase, chitinase, dehydrogenase, phosphatase, protease and urease released from animals (Kanfer et al., 1974), organic compounds and microorganisms (Shawale and Sadana, 1981; James et al., 1991; Richmond, 1991).

Urease is one of the important enzymes secreted by microorganisms in soil. Urease (EC 3.5.1.5), belongs to the super family of amidohydrolases and phosphotriesterases (Holm and Sander, 1997). The best-studied urease is that from jack bean (Canavalia ensiformis) (Blakeley and Zerner, 1984) which was the first enzyme to be crystallized (Sumner, 1926). Urease is a nickel-dependent metalloenzyme and is synthesized by plants, some bacteria and fungi. Urease enzyme is responsible for the hydrolysis of urea fertilizers applied to the soil into NH₃ and CO₂ with the concomitant rise in soil pH (Andrews et al., 1989; Byrnes and Amberger, 1989). Due to this role, urease enzyme from soil microflora have received a lot of attention, since it was first reported by Rotini (1935), a process considered essential in the regulation of N supply to plants after urea fertilization. Urease enzyme in plants is predominantly intracellular (Polacco, 1977) and in microorganisms, found as both intra- and extra-cellular (Burns, 1986; Mobley and Hausinger, 1989). On the other hand, urease extracted from plants or microorganisms is rapidly degraded in soil by proteolytic enzymes (Pettit et al., 1976; Zantua and Bremner, 1977). This suggests that a significant fraction of ureolytic activity in the soil is carried out by extracellular urease, which is stabilized by immobilization on organic and mineral soil colloids. Urease activity in soils is influenced by many factors. These include cropping history, organic matter content of the soil, soil depth, soil amendments, heavy metals and environmental factors such as temperature and moisture (Tabatabai, 1977; Yang et al., 2006). For
example, studies have shown that urease was very sensitive to toxic concentrations of heavy metals (Yang et al., 2006).

The presence of urease has been detected in numerous organisms, including plants, bacteria, algae, fungi and invertebrates. The plant and fungal ureases are mostly homohexamers $\alpha_6$, whereas bacterial ureases typically are heterotrimers $(\alpha\beta\gamma)_3$. Bacterial ureases are composed of three distinct subunits, one large ($\alpha$ 60-76 kDa) and two small ($\beta$ 8-21 kDa, $\gamma$ 6-14 kDa) subunits commonly forming $(\alpha\beta\gamma)_3$ trimers. Fungal and plant ureases are made up of identical subunits (~90 kDa each) and most commonly assembled as trimers and hexamers. It is important to note, that although composed of different types of subunits, ureases from different sources extending from bacteria to plants and fungi exhibit high homology of amino acid sequences with the subunit of jack bean urease (Krajewska, 2009).

Urease producing soil microorganisms have been studied from a variety of bacteria and fungi. Urease producing soil microorganisms have been identified as *Aspergillus niger* (Ghasemi et al., 2004), *Rhizopus Oryzae* (Geweely, 2006), *Bacillus thuringiensis* (Chatterjee et al., 2007), *Actinomycete* species (Gopinath et al., 2011), *Fusarium culmorum*, *Penicillium spinulosum*, *Citrobacter freundii*, *Enterobacter ludwigii* and *Pseudomonas chlororaphis* (Alizadeh et al., 2014), *Bacillus* sp. CR2 (Achal and Pan, 2014), *Sporosarcina* sp., *Bacillus* sp. and *Brevundimonas* sp. (Wei et al., 2015). Since extracellular urease plays a key role in the hydrolysis of urea, it is important to study about urease enzyme. Hence, this study was designed to identify bacterial and fungal strains isolated from soil of farm fields of Shoolini University, Solan, H.P. In this area urea based fertilizers were regularly used which might have encouraged development of newer microbial strains capable of producing extracellular urease activity and work under varied conditions of the environment. Biochemical characterization and their identification would shed light on type of microorganism secreting urease.
1.1 Aim and objectives of the research

Keeping the above in view, the present study was proposed to accomplish the following aim and objectives.

Aim

Comparative characterization of urease secreted by bacterial and fungal isolates from soil samples of farm fields.

Objectives

1. Isolation of urease producing bacterial and fungal strains from soil samples.
2. Characterization of the isolates for their genetic distances with other similar strains.
3. Characterization of the isolates for maximization of urease production under varied conditions.
4. Comparison of urease activity in free and immobilized state of enzyme between bacterial and fungal isolates.