CHAPTER - 1
Introduction
Microbial polysaccharides are one of the major products of microbial metabolism which are water soluble polymers and may be ionic or nonionic (Razack, 2013) and among them exopolysaccharides (EPS) are extracellular, rich sources of carbohydrate containing molecules, chemically well defined biopolymers produced by a wide variety of microorganisms which have attracted worldwide attention due to their novel and unique physical properties (Sutherland, 1990). They are long chain molecules, high molecular mass, branched (containing α- and β- linkages) or straight, composed of simple sugar units linked in a definite fashion (Cuddihy et al., 2003). EPS are either homopolysaccharides (made up of the same type of monomeric units e.g., pullulan, levan, curdlan or bacterial cellulose) or heteropolysaccharides (made up of different types of monomeric units e.g., gellan or xanthan) in nature (De Vuyst and Degeest, 1999). These exopolysaccharides find multifarious industrial applications in foods, pharmaceuticals and other industries as emulsifiers, stabilizers, binders, gelling agents, lubricants, biopolymer film and thickening agents (Yuen, 1974; Israilides et al., 1998; Leathers, 2003; Poli et al., 2010; Satpute et al., 2010). Microbial EPS is rapidly emerging as a new and industrially important source of metabolites, which are gradually becoming economically competitive (Joshi et al., 2013). Microbial polysaccharides serve different functions in the microbial cells and are distinguished into three main types, intracellular polysaccharides, which provide mechanisms for storing carbon or energy for the cell; structural polysaccharides, which are components of the cell structure or are integral parts of the cell walls. Extracellular polysaccharides or exopolysaccharides, depending on the microbial system, (i) form capsules outside the cell, there by becoming a part of the cell wall, or (ii) form slimes that accumulate outside the cell wall and which subsequently
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diffuse in the liquid phase during the fermentation (Cerning et al., 1995; Banik et al., 2000; Ruas-Madiedo et al., 2002).

Today the exopolysaccharides used at the industrial level are almost entirely of plant, seaweed and bacterial origin such as starch, alginate, Arabic gum, agar and guar gum, which are widely employed in the food, pharmaceutical and cosmetic industries (Roller and Dea, 1992; Sutherland, 1996; Wang and McNeil, 1996; Manzi and Pizzpferrato, 2000). But the industrial production of these EPS from plant and algal sources is having certain problem like, 1) polysaccharides production from plant and algal source is achieved in 3–6 months and highly affected from geographical and seasonal variations and ever increasing concerns about the sustainable use of agricultural lands (Oner, 2013), 2) Moreover, production is not only independent of solar energy which is indispensable for production from microalgae but also suitable for utilizing different organic resources as fermentation substrates (Donot et al., 2012), 3) the chemical composition of polysaccharides may vary in response to change from season to season or ageing cycle of plants, 4) The exopolysaccharides are prone to modification and degradation during the harsh processing procedures like strong acid extraction and precipitation, 5) It is difficult to maintain the quality of EPS due to climatic condition, 6) The cost of final marketed EPS product is very much high (Oner, 2013), 7) In spite of having novel functionality, reproducible physicochemical properties, stable cost and supply, EPS of microbial origin have received very little attention and that too restricted to EPS produced by bacterial species (Selbmann et al., 2002). However, during the past few decades much interest has been generated in the subject of polysaccharides produced by different microorganisms. The advantages of microbial production of EPS are that it can be achieved in very short time, it is also independent of seasonal variations and its recovery and purification does not create any particular problems (Sutherland, 1996). Besides, rheology and high thermostability with a broad pH range make microbial EPS, a great biomacromolecule with immense industrial applicability (Lachman and Shelth, 1968; Sutherland, 1996). Fungi have been explored and
screened out mainly for the production efficiencies of industrial enzymes, antibiotics and food products (for eg., mycoproteins, SCP etc.) but a very less emphasis has been given on the production of exopolysaccharides. Fungi with respect to research on its EPS producing potential have been received limited attention. The variety of fungal species in nature is tremendous, according to more recent data which showed that there is as many as 5.1 million fungal species exist and approximately 75,000 scientifically identified species of fungi have only been described and the production efficiencies of only hundreds of them have been assessed and rest yet remain to explore (Hawksworth, 1995; Blackwell, 2011. Fungi have gain interest of many researchers in this field during last three decades due to many advantages of fungal EPS over other microbial groups. The exopolysaccharides produced by fungi are structurally more diverse than bacterial reported exopolysaccharides. Fungal EPS may have six to ten different monomers in their structure hence most of them are heteropolysaccharides. A structure which gives different structural orientation to its EPS which may find multifaceted biotechnological applications. This may be due to inadequate research especially on correlation of physiology of potential fungal strains and EPS production (Sutherland, 1996). The fungal polysaccharides have property to alter the rheological properties of water which make them to act as biostabilizer and bioemulsifiers. Fungi are better suited than macroalgae or higher plants, since they exhibit high growth rate and are more amenable to manipulation of condition for enhancing growth or EPS production. Numerous fungal polysaccharides are potentially available, known to be involved in pathogenesis, symbiosis, biofilm formation, protection from phagocytic predation and stress resistance in microorganisms, but relatively only few have been commercially established (Sutherland, 2001; Vanhaverbeke et al., 2003).

During recent times, much interest has been on the subject of exopolysaccharides producing fungi, due to various biological and pharmacological activities. There are reports of works on fungal polysaccharides but these works mainly concentrates on
medicinal uses and despite having equal industrial potential, EPS from fungi have not been given deserved attention. A recent literature reports, only few commercial EPSs, namely, xanthan, dextran, pullulan, curdlan, and levan, with outstanding potential for various industrial application (Donot et al., 2012). The maximum of the existing exopolysaccharides are of bacterial origin and fungal forms are very few like pullulan, shizophylan and lentinan which have reached up to commercial level and that too for medicinal applications only. Therefore there is need to isolate more fungal species capable of producing EPS especially the indigenous isolates which may prove a prominent source of EPS and find potential industrial application that could be utilized at commercial level. Another important feature of fungal polysaccharide is that their production, quality and characteristics are greatly influenced by various physical and biochemical variables applied during fermentation process (Sutherland, 2007; Nicolaus et al., 2010; Oner et al., 2013). Moreover, the diversity of fungal strain is also responsible for affecting the chemical structure, monomer composition, physicochemical and rheological properties of the final product. Thus, the production of fungal polysaccharides with desired specifications can be done via controlling the fermentation conditions which in turn directly impact the EPS yield and applications at the industrial level (Oner, 2013). Therefore, the present investigation was performed which aimed an exhaustive study to explore potential indigenous fungal isolates capable of producing EPS and to evaluate their potential biotechnological applications especially for the formation of bio-emulsions, biopolymer films and antioxidant compounds. The present work was carried out with the following objectives:

1. Isolation of indigenous fungal organisms from different bio-diverse natural habitats of Chandigarh and near by regions/districts.

2. Maintenance and preservation of pure cultures of successfully isolated fungal organisms.
3. Screening of EPS producing efficiencies of successfully isolated fungal isolates.

4. Identification of the most potential screened out fungal isolate capable of giving the maximum EPS yield.

5. Optimization of production medium and suitable fermentation time period along with other physical and biochemical parameters for enhancing the EPS yield obtained by the most potential isolate.

6. Scale up of the EPS production process under optimized fermentation conditions, first at shake flask level and its validation in 3 litres laboratory fermenter.

7. Purification of the crude EPS.

8. Characterization of the purified EPS for its rheological, physical and molecular and chemical properties.

9. Evaluation of biotechnological applicability of the EPS for the following potential applications:
   - Biopolymer film forming property
   - Bio-emulsifying property
   - Antioxidant property