INTRODUCTION

Xylanases [EC 3.2.1.8] are one of the lignocellulases that specifically act on β-1,4-xylosidic linkage, joining the two xylose moieties and hydrolyze the complex xylan structure [1]. Xylan is the second most abundant lignocellulosic component after cellulose. Thus, action of xylanases can effectively carry out hydrolysis of diverse lignocellulosic residues such as wood and forest residues, agricultural residues and landfill wastes; which constitute xylan as one of their structural ingredient. The diverse sources of xylanases constitute bacteria, fungi, arthropods, protozoa, gastropods and actinomycetes. Among various producers, bacteria and fungi are the leading sources that are exploited on commercial scale for xylanase production [2]. Especially, thermostable xylanases have extensive commercial viability with an approximate annual market to reach USD 500 million by 2023 [3].

The thermophilic microfauna thrive in geothermal areas; hot springs are the most enriched areas, bestowed with diversity of microbial thermophiles [4, 5]. So, far researchers have discovered wide variety of potential thermophiles, which have been exploited for xylanase production. Some of the thermophilic producers have been used in their wild form; whereas some have been genetically evolved to meet the desired commercial requirements. SmF and SSF are the two methods being practiced for xylanase production. However, 90% of the total xylanase production is performed through SmF because there is homogenized distribution of microbial biomass, nutrients and oxygen in the liquid medium used in SmF [6]. The production of xylanases may be intracellular or extracellular in nature [7]. Majority of xylanases being extracellular are excreted outside the cell because the large sized substrate cannot penetrate into the microbial cell. However, current belief regarding the production of xylanase is that it is induced by means of their hydrolysis products produced. Also, extracellular xylanases are commercially acceptable due to easy downstream processing. Xylanase acts on xylan via hydrolytic reaction which involves an acid-base catalyst and a nucleophile for the formation of hydrolysis products through single and double displacement mechanism [1].

For attaining maximum xylanase production from any microbial producer under specific conditions, the influencing parameters need to be optimized. Parameters such as temperature, pH, agitation speed rate, nitrogen sources, nitrogen concentration, substrate concentration etc. have influential impact on production of xylanase. Various optimization methods such as OFAT and statistical approach called RSM are opted for optimizing the influencing parameters for

maximum xylanase production. OFAT is a conventional method of optimization that takes one parameter under observation at a single given time. On the contrary, RSM is statistical method of optimization which is used to study the interactive effect of two parameters at a given time. Literature reports depict that optimization is a prerequisite step for achieving maximum enzyme production [8]. Besides enhancing the production titer of an enzyme, minimizing the cost of production process is equally significant. Especially for large scale enzyme production, cost-effective strategies need to be indulged. Using commercial xylan as substrate for large scale xylanase production does not have economical feasibility. For reducing the production cost, commercial xylan is being replaced by cheaper substrates (lignocellulosic residues) such as corn cob, wheat straw, wheat bran etc. The hemicelluloses fraction of these lignocellulosic residues is composed of majorly xylose monomers, along with small amount of accessory sugars such as arabinose, mannose, galactose and glucose. This valorization of lignocellulosic waste for xylanase production is an eco-friendly and economical approach.

For industrial applicability of xylanase, its characteristic features in terms of pH optima, temperature optima, thermostability and pH stability play significant role. For commercialization, characteristic profile of xylanase needs to comply with requirements of particular industry. Besides pH and temperature profile, storage stability, easy recovery process and reusability are other parameters that have vital contribution in deciding the industrial applicability of xylanase. Enzyme immobilization is an approach to enhance the efficiency of enzymes and elevate their commercial value [9, 10].The reported techniques of immobilization (carrier bound or carrier-free) are comprised of certain loopholes. In adsorption method leakage from carrier material is observed, immobilization through binding to solid-carrier via affinity interactions involves use of recombinant tags which is unsuitable for large scale application [11]. Similarly, binding to carrier material through covalent interactions require surface modifications of carrier, which is an expensive approach. With reference to the above mentioned problems, CLEAs offer easy recovery of immobilized enzyme from the reaction medium by centrifuging or filtering the medium, preparation cost is comparatively low and renders efficient reusability, higher stability profile, easy scale-up and allow low cost preparation by directly using crude enzyme; instead of purified [12]. Also, preparation of CLEAs does not requires carrier or immobilization support, thus they can be fed into industrial reactors in higher amounts as extra space is acquired by the carrier or immobilization support used [13]. Scarce literature is available

on preparation of xylanase CLEAs. In recent study by J.S. Hero and coworkers, xylanase CLEAs of *Conhella* sp. AR92 were prepared for their application in lignocellulosic biomass conversion [14]. More research on preparation of xylanase CLEAs is required to explore the potential of this immobilization strategy in lowering the cost and enhancing the applicability xylanase on industrial scale.

Xylanases serve diverse industries; namely food and feed industry, pharmaceutical industry, biofuel industry and paper industry. In food industry, xylanases aid in reducing the viscosity and increasing the clarity of juices, which enhances the juice quality [15]. Addition of xylanases in poultry diet increases the nutritional value and digestibility of feed. Xylooligosaccharides produced by hydrolyzing action of xylanases on xylan; are used as ingredient in many feed products [15]. In biofuel industry, xylanases help in lignocellulosic biomass conversion by hydrolyzing the hemicellulosic component into sugars, which could be further fermented into biofuel [16]. In paper industry, xylanases act on hemicelluloses component of paper pulp and thus breaks the bonds between hemicellulose and lignin. This makes the lignin molecules easily accessible to other bleaching agents for removal [17]. Moreover, it has been reported that use of xylanases for bio-bleaching leads to reduction in consumption of chlorine and also enhance the quality of paper.

The present study was aimed at finding a potential thermo-alkali-stable xylanase for application in paper pulp bleaching. In this context, from literature survey certain research gaps were filtered out:

Research Gaps

- To be applicable in paper industry, xylanases need to be thermo-alkali-stable and cellulase free. Application of xylanases for bio-bleaching in paper industry is limited by their pH stability in narrow range, low thermal stability and high expenditure on production process.
- The xylanases from fungi have associated cellulase activity, with pH optima in neutral or acidic range and are active at mesophilic range temperature. That is why; bacterial xylanases have been in more demand in comparison to fungal xylanases; especially in bleaching of wood pulp.
- Commercial applications require cheaper enzymes. So, cost of the process for enzyme production need to be reduced by developing better fermentation processes and recovery systems.

With respect to the above mentioned research gaps, following objectives were designed for the present study:

Objectives

- Screening of potent thermophilic xylanolytic bacteria from Tattapani hot spring of Himachal Pradesh.
- Production, parametric optimization and characterization of xylanase from *Geobacillus thermodenitrificans* X1.
- Synthesis and characterization of cross-linked enzyme aggregates (CLEAs) of xylanase.
- Application of xylanase for bio-bleaching of wood pulp in paper industry.