A Review of XET Enzymes, Current Applications and Future Trends

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Abstract:- **Xyloglucanis a well-known plant polymer and has been used in the textile industry as a sizing agent. The enzyme xyloglucanendotransglycosylase/hydrolase (XET) has become important in the last 25 years for understanding the plant growth system. The discovery of various XET enzymes and understanding their role in cell wall metabolism in plants lead towards its application in various fields. Cloning and expression of many different XET enzymes in industrially important microbes paved way for producing sufficient quantities of the enzyme that was essential for further studies. The uniqueness of XET enzymes in terms of introducing groups through xyloglu can eventualized their usage in surface modification of cellulosic substrates. This review article provides a compilation of most of the XET enzymes reported and the associated applications. The article also provides a complete list of patents listed till date that reported the XGO-XET system for an application involving surface modification. The possibility of future trends in wider areas where XET enzymes could be used is also discussed.**

I. INTRODUCTION

Ever since the first documentation of the xyloglucanendotransglucosylase/hydrolase (XTH/XET) enzymes about three decades ago [1], [2] they have been an important class of enzymes with respect to understanding several mechanisms in plants. The unique mechanism of XET enzymes enables them to hydrolyse and re-ligate the xyloglucan oligosaccharide to other xyloglucanoligosachharide/polysachharide chains [3]. The xyloglucan molecules have strong tendency to bind naturally with cellulose chains via hydrogen bonds and hydrophobic interactions *in vivo* [4] and *in vitro* the xyloglucan molecules bind almost instantaneously with cellulose. The interaction of xyloglucan with cellulose plays a key role in controlling the growth of plant cells, fruit softening, response to pathogens and vascular differentiation [5]. Different isozymes of the XTH enzymes are expressed at different stages of plant/fruit growth [6].

There are about 2000 genes belonging to the XTH family that have been reported in the plant genome database [7]. Several enzymes from the XTH family have been cloned and expressed most notably from Arabidopsis, Persimmon Vignaangularisand more than 50 others which have been expressed and purified [8]. The structure and enzymology of hybrid aspen XET has been studied extensively [9]. Apart from understanding the molecular mechanisms, the biochemistry and enzymology of XTH/XET family of enzymes there has been considerable amount of work done in establishing their use in commercial applications. The most commonly used XET enzyme in applications has been cloned from hybrid aspen [10].

In applied chemistry XET enzyme is used to transfer a functional group of choice to a cellulosic surface. The xyloglucan polysaccharide, usually from tamarind kernel is first treated with xyloglucanase enzyme to yield xyloglucan oligosaccharides (XGO). The XGO can be chemically modified to incorporate a functional group of choice. Using the XET enzymes, chemically modified xyloglucan oligosaccharides with a functional group of choice attached to it can be easily transferred into a xyloglucan polysaccharide. Due to its natural affinity for cellulose, the modified xyloglucan polysaccharide can be used to incorporate desired surface chemistry on a cellulosic surface [11].

There are at least four patents reported for surface modification of cellulose for instance in textiles, in paper, in fibers, bio composites etc. This review highlights all applications patented so far with respect to XET enzymes and the difficulties associated thereof with implementing these applications. The review also tries to look into various other areas where XET enzymes can have potential applications.

II. XET ENZYMES

There are XET enzymes reported from various sources, including dicotyledons, monocotyledons and even microbes.

A. Chronological order of findings

The first XET activity was reported by Smith and Fry in the Spinacia and other plants [2]. A year later Nishitani and Tominaga published the purification of XET enzyme activity from Vignaangularis [1]. In the year 1993, Potter and Fry reported XET activity in Pisum Sativum [12] and Fanutti et al reported the XET enzyme in Nasturtium seeds [13]. This was followed by molecular characterization of the XET enzyme in Nasturtium seeds and the molecule was found to be a 31 kDa protein [14]. Arrowsmith and de Silva produced a recombinant XET enzyme from tomato expressed in E. coli [15]. Rose et al in 1996 found that a divergent XET was expressed in nasturtium seed epicotyl. The group had earlier reported XET from nasturtium seeds at the germinating stage [16]. This divergence was with respect to enzyme expression, the genes were found to be highly homologous, thereby suggesting that different XET enzymes are expressed at different stage of plant/fruit development.

The discovery of new enzyme and it's mechanism in plant growth lead to further investigation and molecular

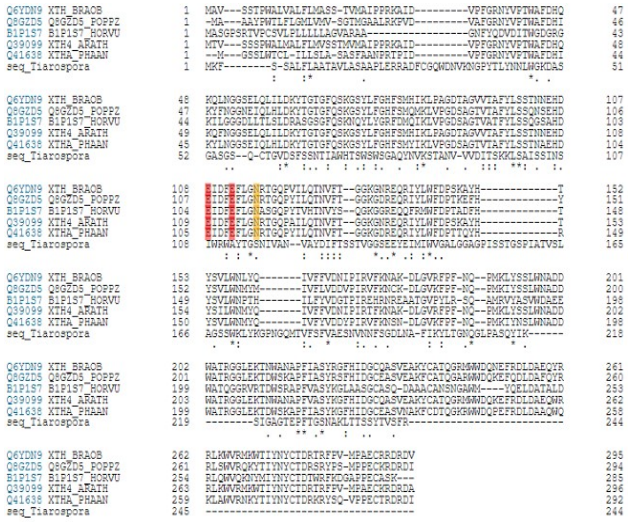
characterization. In the year 1997, Purugannan MM et al expressed XET enzyme from Arabidopsis thaliana in E. coli and characterized the enzyme [17]. Schroder R et al isolated XET enzyme from ripe Kiwifruit and reported the molecular characterization of the same [18].Four XET enzymes were expressed from Arabidopsis thaliana using the baculovirus system. All the four enzymes were characterized and found to exhibit transglycosylase activities [19]. Steele and Fry demonstrated that the catalytic properties of isoenzymes of XET differ suggesting the role these isozymes play in different tissues [20]. Steele et al produced a very interesting fact about the XET enzymes in the year 2001, that the isozymes of XET from cauliflower, mung bean, lentil and nasturtium seeds catalyze their substrates independently of size of the substrate chain [21]. The gene sequence analysis of several of the XET enzymes had shown a conserved catalytic domain of the GH16 family of enzymes. Henricksson et al conducted LC/MS MS/MS analysis of the cauliflower XET to show that a glycosylation site was situated close to the predicted catalytic domain and the difference in glycosylation of the recombinant enzyme did not influence the XET catalytic activity significantly [22].Vissenberg et al in 2005 published that the expression profiles of different XET genes diversify within the roots in order to cater different physiological roles in cell wall dynamics [23]. Bollock et al expressed poplar XET in yeast [24], followed by Kallas et al who reported that the expressed poplar XET enzyme in yeast has a specific activity that is not dependent on the N glycosylation site in the enzyme [25].

B. Xet enzyme in applied sciences

Series of developments with respect to deciphering the role of the enzyme, its characterization, expression and molecular studies lead to the quest of applying the XET enzymes in industry related to cellulosic materials. Cellulose modification has been an interesting area in textile, paper, biocomposite industry. The native cellulose strength depends upon the hydrogen bond network between the hydroxyl groups of the glucan chains. Chemical derivatization of these hydroxyl groups in order to introduce specific groups on the glucan chains weakens the microfibril and fiber superstructures. In the past, attempts have been made to modify the surface properties of cellulose using plant polysachharides such as mannans, xylans and xyloglucans [4]. In 2004, for the first time, Brumer et al demonstrated that XET enzymes can be used to modify the surface chemistry of cellulose using filter papers. Zhou et al also demonstrated technology for attachment of various functional groups on cellulosic surface using the XGO-XET enzyme system [26]. A year later, Gustavsson et al reported the acylation of cellulose fibers using XET and lipase [11]. The XET enzyme from hybrid aspen aka*populustremulaxmuloides*(pttXET16A) expressed in *pichiapastoris* was used in these studies. The group presented a proof of concept by attaching various functional groups to the cellulose surface via chemo-enzymatic approach using XGO-XET system [27]. The pttXET16A is unique as it was shown to have a conserved GH16 catalytic sequence motif and a conserved N-glycosylation site. Removal of the glycosylation site did not influence the enzyme activity. Also, the three-dimensional enzyme structural analysis [9] indicates that this enzyme has an active site cleft with the potential to tolerate a diversity of stearically bulky XGO-R functional groups.

Out of the many xyloglucanendotransglucosylase enzymes that have been studied from various sources few have been expressed and used in the surface modification of cellulose. The XET enzyme from Hordeumvulgareseq ID B1P1S7), Arabidopsis thaliana (seq ID Q39099), Brassica oleraceavar (seq ID Q6YDN9), Vignaangularis (seq ID Q41638) and Populustremula x populous tremuloides (seq ID Q8GZD5) have been cloned, expressed and used in surface modification of cellulose [28]. Interestingly, apart from the dicotyledons and monocotyledons plants, XET enzyme has also been found in microorganisms such as fungi and bacteria [29]. The XET from a fungi Tiarosporaphasiolinahas been used in applications such as baking and textiles[29]. Figure 1 compares the similarity between the active site and glycosylation site of all the XET enzymes that have been cloned, expressed and shown to be useful in some or the other application. The figure also shows the taxonomic relationship between the XET enzymes. According to the alignment results from Uniprot, all the plant XET enzymes share a 38.87% of identity except the microbial XET. The XET from fungus *Tiarosporellaphaseolina* is only 5% identical to the other plant XET enzymes. On the other hand, the dicots viz; A. thaliana and Cauliflower share a 94.25% similarity in their sequence whereas, the monocotsviz; V. angularis and hybrid aspen share 80.61% similarity.

[a]



[b]

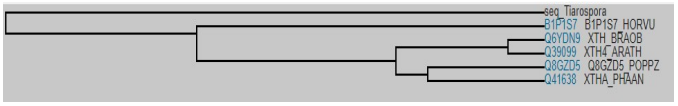


Fig 1 [a] Alignment results of the sequences of XET from plants and microbial source. [b] Taxonomic relationship between the enzyme sequences.

III. SUMMARY OF THE PATENTS ON APPLICATIONS USING XET ENZYMES

In this section, all patents filed for XET enzymes are discussed. The table below lists all the patents, application and the source of XET enzyme used.

TABLE 1

LIST of PATENTS FOR XET ENZYMES USED In VARIOUS APPLICATIONS

Patent number	Application	Enzyme source	Assignee
WO2003033813 A	Modification of polymeric carbohydrate materials	Hybrid aspen and cauliflower	SweeTree Technologies
WO1999062343 A1	Baking	Microbial: Tiarosporellapha seolina expressed in Aspergillus	Novozymes
WO2005075633 A1	Removal of print thickener from textile	Microbial: Tiarosporellapha seolina expressed in Aspergillus	Novozymes
EP3114276 B1	Methods for functionalizing and linking materials	Vignaangularis	Novozymes
WO1997023683 A1	Fabric shape retention/anti wrinkling	Green tomatoes	Novozymes

The very early patents on applications for XET enzymes came from Novozymes, Bagsvaerd in the year 1996 and 1999 for WO1997023683 A1 and WO1999062343 A1 respectively. Table 1 shows the applications and the XET enzyme used. Interestingly, just a year before publishing the use of microbial XET in baking, a patent was filed by Novozymes for discovery of XET enzymes in microbes (WO 1998038288 A1). In the year 2005, SweeTree technologies patented the methods of modification of polymeric carbohydrate materials using XET enzymes. Thus there are only few patents filed so far with respect to the applications. The XET enzyme and the XGO-XET enzyme system is relatively new thereby giving lots of new areas of applications to be explored.

IV. DISCUSSION

In this review article, the chronology of discovery of XET enzymes to the applications in which these enzymes have been used is discussed. The applications using XET enzymes are just the beginning of exploring the use of these enzymes. Although it has not been covered in this article, there are reports where the modified XET genes are introduced in transgenic plants in order to increase the ripening time of leaves and fruits. Xyloglucan is also known to bind to surface of leaves thereby opening up an area of modifying the surface of leaf using XGO-XET system. Xyloglucan can bind to surfaces apart from cellulose as well for example, polyester and polypropylene, rayon, nylon etc. thereby giving many opportunities of trying the XGO-XET system for surface modifications in textile industry. XG also binds very strongly to clay and other types of soil thus, providing room in the agricultural field for improving the soil run off. Thus, the plethora of applications where the XGO-XET modification system can be used is endless. Genetic modification of the XET enzyme with respect to their active site and glycosylation site in order to suit the application conditions should be looked into further.

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