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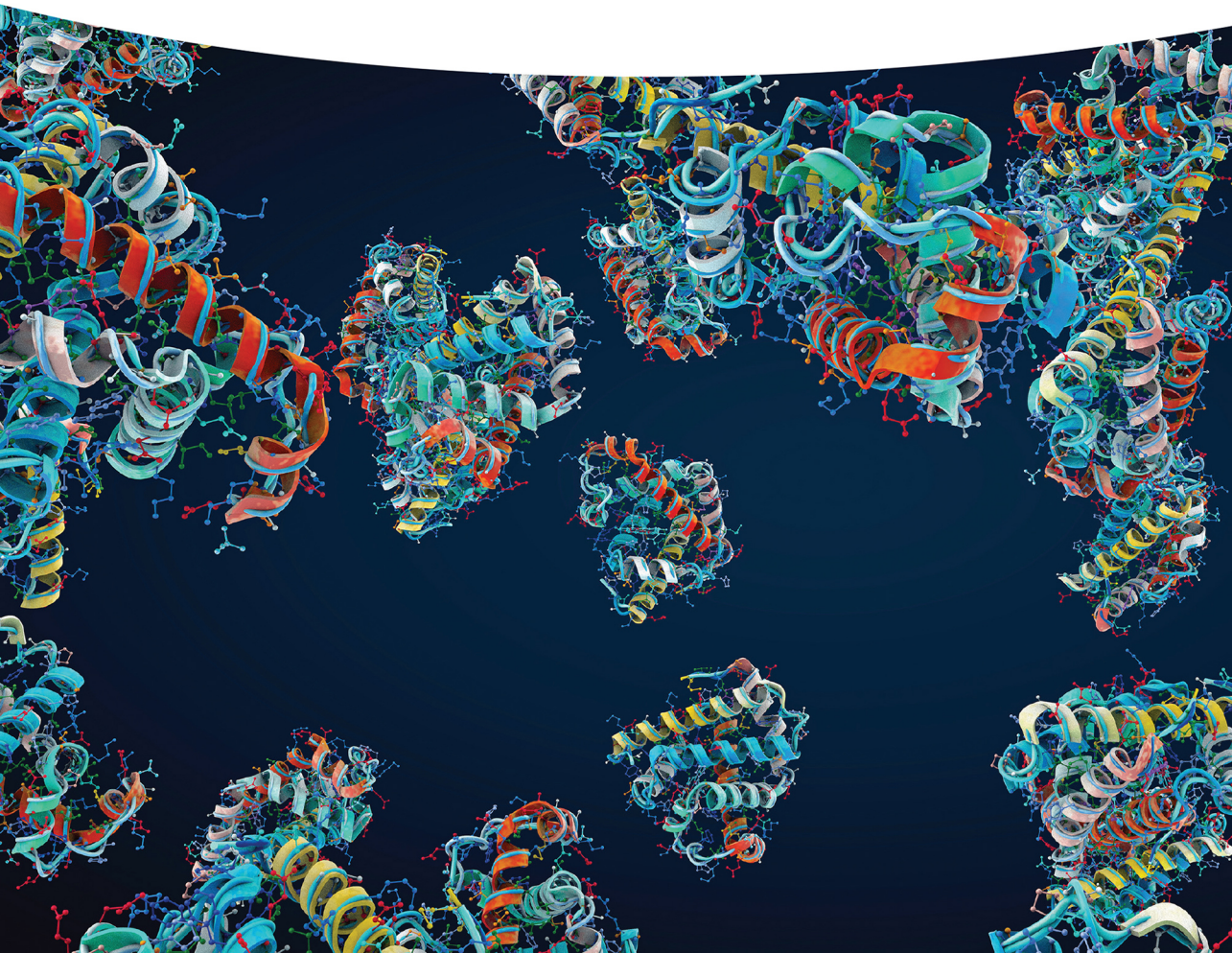
Edited by Dinesh Yadav, Pankaj Chowdhary, Gautam Anand, and Rajarshi Kumar Gaur

Microbial Enzymes

Production, Purification, and Industrial Applications

Volumes 1–2



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Volume 1

Edited by Dinesh Yadav, Pankaj Chowdhary, Gautam Anand, and Rajarshi Kumar Gaur

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About the Editors

Dr. Dinesh Yadav is Professor of the Department of Biotechnology and currently the Director of the Research and Development Cell at DDU Gorakhpur University. He has served as the Dean of the Faculty of Rural Sciences, Head of the Department of Biotechnology, Nodal Officer of the IPR Cell and Coordinator of Centre for Genomics and Bioinformatics at D.D.U. Gorakhpur University. He has also served as Associate Professor of the Department of Molecular Biology and Genetic Engineering at G.B. Pant University of Agriculture and Technology, Pantnagar, from 2006 to 2009. He completed his master's degree in biotechnology from Devi Ahilya University, Indore, in 1996 and PhD from G.B. Pant University of Agriculture and Technology, Pantnagar, in 2002. He has more than 23 years of teaching and research experience.

He has availed the DST-BOYSCAST Fellowship at the Australian Centre for Plant Functional Genomics (ACPFG), University of Adelaide, South Australia, from 3 May 2012 to 12 April 2013. He was awarded for Excellence in Teaching and Research in Higher Education by the University on Teacher's Day, 5 September 2021. He received Dr. Pushpendra Kumar Gupta Vishisht Krishi Vaigyanik Puraskar (2015) in the field of agricultural sciences by Uttar Pradesh Academy of Agricultural Sciences (UPAAS), Lucknow, and the Young Scientist Award (2008) by the Uttarakhand State Council of Science & Technology in the discipline of biotechnology, biochemistry, and microbiology.

His areas of specialization include molecular biology, bioinformatics, plant biotechnology, and enzyme technology. He has published more than 150 research papers, including reviews, books, book chapters, and conference proceedings, and has more than 300 GenBank accession numbers. A total of 48 indigenously isolated microbial strains (fungal and bacterial) have been deposited in several culture collection centers in India. He has carried out nine projects with support from funding agencies such as DBT, UGC, UP Council of Agricultural Research, Lucknow, and DST as PI/Co-PI and Mentor. Presently, he is involved in three projects funded by CST (UP) and UPCAR (Lucknow).

He has guided 12 students to PhD in biotechnology, and four students have submitted PhD thesis. He has also supervised more than 114 students for MSc dissertations

and short project works in biotechnology. He has been a mentor for five postdoctoral fellowships, namely UGC-Dr. D.S. Kothari (twice), DST-Women Scientist-A, DST-Women Scientist-B, and SERB National PDF.

He is a life member of various scientific societies such as BRSI, Trivandrum; SBC(I), Bangalore; Indian Science Congress Association, Calcutta; Society of Plant Biochemistry and Biotechnology, IARI, New Delhi, Association of Microbiologists of India (AMI), New Delhi; and UPAAS, Lucknow.

He has delivered more than 100 invited talks and lectures at conferences and symposiums and served as Mentor for the DST-INSPIRE Science Internship Camp and Inspire Awards. Currently, he is working on plant-specific transcription factor-DOF (DNA binding with one finger) and nuclear factor-Y (NF-Y) for developing biotic and abiotic stress tolerance crops and pectinases group of enzymes with potential applications in different industries.

His research works have been published in journals of national and international repute, including *Journal of Experimental Botany, Cellulose, World Journal of Microbiology and Biotechnology, Environmental Science and Pollution Research, Theoretical and Applied Genetics, Cellulose, Frontiers in Microbiology, Planta, World Journal of Microbiology and Biotechnology, Process Biochemistry, Molecular Biology Reports, Plant Systematics and Evolution, Molecular Biotechnology, Applied Biochemistry and Biotechnology, Annals of Microbiology, Journal of Basic Microbiology, 3 Biotech, Biologia, Journal of Cereal Sciences, Physiology and Molecular Biology of Plants, Biochemistry (Moscow), Current Proteomics, Interdisciplinary Sciences: Computational Life Sciences, Biocatalysis and Agricultural Biotechnology, Cell Biochemistry and Biophysics, Online Journal of Bioinformatics, Chemistry and Ecology, African Journal of Biotechnology, Applied Biochemistry and Microbiology, Sugar Tech,* and *Enzyme Research*.

Dr. Pankaj Chowdhary is President of the Society for Green Environment (SGE), New Delhi, India. He completed his PhD in 2018 in the area of microbiology from the Department of Environmental Microbiology at Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India. His doctorate work was mainly focused on the role of ligninolytic-enzyme-producing bacterial strains in the decolorization and degradation of coloring compounds from distillery wastewater. His main research areas include microbial biotechnology, biodegradation and bioremediation of environmental contaminants in industrial wastewaters, metagenomics, and lignocellulosic waste valorization. He has edited eight international books and authored two books. He has published more than 60 research and review papers in national and international peer-reviewed journals with a high impact factor, published by Springer, Elsevier, Royal Society of Chemistry (RSC), Taylor & Francis Group, and Frontiers. He has also published many national and international book chapters and magazine articles on the biodegradation and bioremediation of environmental pollutants. He has presented many posters and papers relevant to

research areas in national and international conferences. He has actively worked as a potential reviewer for various SCI journals published by reputed international publishers (Springer, Elsevier, RSC, Taylor & Francis Group, Wiley, and Frontiers). He is a life member of the Association of Microbiologists of India (AMI) and the Indian Science Congress Association (ISCA), Kolkata, India".

Dr. Gautam Anand is a postdoctoral fellow at the Department of Plant Pathology and Weed Research, Agricultural Research Organization (ARO) – Volcani Institute, Israel. He obtained his MSc and PhD degrees in biotechnology from Deen Dayal Upadhyaya Gorakhpur University, India. During his PhD, he worked on an industrially important microbial enzyme of the pectinases group, namely polygalacturonase. He was selected for the Agricultural Research Organization – Postdoctoral Fellowship, Israel, for the year 2020–2021 and the Valazzy Pikovsky Fellowship for postdoctoral research at the Hebrew University of Jerusalem, Israel, for the year 2018–2019. He has also worked as a postdoctoral fellow at the Robert H. Smith Faculty of Agriculture, Food and Environment, Hebrew University of Jerusalem, Israel. He has worked extensively on plant–fungus interactions. His main area of research is characterizing the importance of trafficking pathways in fungal virulence using deletion mutagenesis and cell biology techniques. Dr. Anand has also worked on fungal pectinases, particularly polygalacturonases and pectin lyases. He has published more than 30 papers in reputable international journals and 14 book chapters.

Dr. Rajarshi Kumar Gaur earned his PhD in 2005. Currently, he is Professor at the Department of Biotechnology, Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur, Uttar Pradesh, India. His PhD focused on the molecular characterization of sugarcane viruses, namely, mosaic, streak mosaic, and yellow luteovirus. He received the MA SHAV Fellowship from the Israel government for his postdoctoral studies and joined the Volcani Centre, Israel, and Ben-Gurion University, Negev, Israel. In 2007, he received a Visiting Scientist Fellowship from the Swedish Institute Fellowship, Sweden, to work at Umeå University, Umeå, Sweden. He received a Postdoctoral Fellowship from ICGEB, Italy, in 2008. He has made significant contributions to the study on sugarcane viruses and has published 135 national and international papers, authored 27 edited books, and presented about 50 papers in national and international conferences. He is an honored Fellow of Linnean Society, Royal Society of Biology, Society of Plant Research, Society of Applied Biology (FSAB), International Society of Biotechnology (FISBT), and Indian Virological Society (FIVS). He has received awards such as Prof. B.M. Johri Memorial Award, Society of Plant Research (SPR); Excellent Teaching Award by Astha Foundation, Meerut; UGC-Research Teacher Award; Young Scientist Award-2012 in Biotechnology by Society of Plant Research

(SPR), Meerut; and Scientific & Applied Research Centre Gold Medal Award (2011) for outstanding contribution in the field of biotechnology. He has visited several laboratories in the United States, Canada, New Zealand, the United Kingdom, Thailand, Sweden, and Italy. Currently, he is handling national and international grants and international collaborative projects on plant viruses and disease management.

Preface

The book *Microbial Enzymes: Production, Purification, and Industrial Applications* provides an insight into diverse aspects of microbial enzymes, highlighting strategies for their production, purification, manipulation, and elucidating multifarious industrial applications. Microbial enzymes have played a pivotal role in several industries, and over the years, substantial efforts have been made to reveal the hidden potential of untapped microbial diversity in search of a repertoire of enzymes. A plethora of microbial enzymes have been reported so far, and efforts have been made to incorporate many of these enzymes in this book authored by experts. An emphasis has also been placed on discussing the recent technological interventions in microbial enzyme technology, such as metagenomics, system biology, molecular biology, genomics, directed evolution, and bioinformatics, in this book. The important microbial enzymes highlighted in this book include xylanases, ureases, methane monooxygenase, polyhydroxyalkanoates, pectinases, peroxidases, α-L-rhamnosidase, alkane hydroxylases, laccases, proteases, gallic acid decarboxylase, chitinases, beta-glucosidase, lipases, inulinases, tannase, mycozyme, ACC deaminase, and ligninolytic enzymes, among others. Few chapters are exclusively focused on microbial enzyme intervention as an eco-friendly approach in diverse industrial applications.

From an environmental point of view, all the recent and classic microbial treatment technologies should be amplified to make them more viable and feasible. Contaminate mitigation or removal using enzyme technology has become an attractive and potential alternative in recent days. Further, recent developments in the fields of biotechnology, molecular biology, ecology, and microbiology have been applied to develop different novel treatment methods involving novel strains of microorganisms with desirable properties that would be applicable in the process of bioremediation. Various types of beneficial microbes are present in the ecosystem, and they can play a key role in mitigating climate concern, improving green production technology, enhancing agriculture productivity, and providing a means of earning a livelihood. A few chapters have highlighted the omics-driven research in microbial enzyme technology.

This book is a good collection of chapters reflecting multidimensional aspects of microbial enzyme technology, and it will be of immense importance for students, scientists, biotechnologists, microbiologists, and policymakers working in

environmental microbiology, biotechnology, and environmental sciences with the basics and advanced enzyme technology. Moreover, readers can also get state-of-the-art or background information on existing technologies, their challenges, and future prospects.

The editors express sincere thanks to the contributors for submitting their work in a timely and proper manner. The editors are also thankful to national and international reviewers for their evaluation and valuable suggestions and comments to heighten the book's quality for readers. Further, editors also acknowledge the cooperation received from the Wiley team for their guidance in finalizing this book.

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1

Xylanases: Sources, Production, and Purification Strategies

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1.1 Introduction

The cell wall of the plant is composed of different lignocellulosic compounds, being the xylan the main compound of hemicellulose. This structure consists of xylose united by β-1,4-glycosidic bonds and different branches of α-D-glucuronide, arabinose, galactose, acetate, methyl glucuronic acid, and other simple sugars [1, 2]. Xylanase is a group of hydrolytic enzymes involved in the hydrolysis of xylan to convert it into monosaccharides and xylooligosaccharides. The xylanase system is constituted by glycosyl hydrolases (*endo*-xylanases, *exo*-xylanases, β-D-xylosidases, α-glucuronidase, and α-L-arabinofuranosidases) and esterases [3].

The heterogeneous composition of hemicellulose hampers the complete depolymerization by a single enzyme, requiring the action of both glycosyl hydrolases and esterases [4]. Each enzyme of the xylanase group contributes to xylan degradation in a specific way: *endo*-xylanase randomly cleaves the xylan; *exo* and *endo* xylanases acting on the xylan backbone and producing short-chain oligomers; β-D-xylosidases cleaves xylose monomers, α-L-arabinofuranosidases removes the side groups, α-D-glucuronidases, and acetylxylan esterases remove acetyl and phenolic side branches and act synergistically on the complex polymer [4, 5]. The most common natural sources of xylanases are produced by different biological systems such as bacteria, protozoans, fungi, plants, and mollusks. Actually, it has been reported that xylanases have been identified from lignocellulose-degrading microbiota from cow rumen and, the termite hindgut. There are two strategies applied to date for microbial xylanase production, either using native microorganisms or genetic engineering modified microorganisms [3, 4].

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Xylanase production from nonmodified fungi and bacteria must use proper microorganisms, which should produce acceptable yields and should not produce toxins or any other unsought products [6]. Xylanases can be produced by hydrolysis of xylan by the microorganisms that express the enzyme gene. Nonetheless, due to the xylan complexity, the production of this enzyme from different microorganisms on a large-scale is hard because one of the main problems is the presence of other enzymes. This problem is also present during the purification steps, increasing costs. Hence, one alternative is the use of modified strains for large-scale xylanase production [7, 8].

In the case of bacteria, the alkaline-thermostable xylanase-producing trait is useful in most industrial applications since it reduces the steps due to the higher pH level required for the optimal growth and activity of the microorganism [9]. Xylanases require *N*-glycosylation as one of the most important posttranslational modifications; therefore, not all bacterial expression hosts are suitable, such as *Escherichia coli*, which lacks the *pgl* gene, to produce this modification. Because of this, other alternative expression hosts are *Bacillus subtilis* and *Lactobacillus* sp. [10, 11].

Filamentous fungi are an important option to produce high amounts of xylanases in comparison to yeast and bacteria [12]. A problem associated with fungal xylanases is cellulase excretion; therefore, an operational process to obtain xylanolytic systems free of cellulases is very important in this case [13]. Another major problem associated with fungi is the reduced xylanase yield in fermenter studies, principally for the agitation that promotes fungal disruption, leading to low productivity [14].

Some examples of xylanases-producing fungus used in industry are *Penicillium canescens, Streptomyces* sp. P12–137, *Thermomyces lanuginosus* SD-21, *Penicillium fellutanum, Penicillium sclerotiorum, Acremonium furcatum, Aspergillus niger* PPI, *Neocallimastix* sp. Strain L2, *Cochliobolus sativus* Cs6, *Bacillus circulans* D1, *Streptomyces* sp. strain Ib 24D, and *Paecilomyces themophila* J18. The substrate used by these microorganisms for fermentation is derived from cereals as soya, wheat, corn, and oat [15–25]. On the other hand, yeasts are good expression hosts due to their ability to perform eukaryotic posttranslational modifications, high cell density growth, and secretion of proteins into fermentation media [26, 27]. Some yeasts used for xylanase production are *Saccharomyces cerevisiae* and *Pichia pastoris* [28, 29]

Plants are also used for xylanases production, using bio-farming. The requirements for this objective are (i) high-level expression, (ii) stability and functionality of enzymes to be expressed, and (iii) easy purification. *In planta* expression of lignocellulose-digesting enzymes from mesophilic bacteria and fungi can compromise plant biomass production because of autohydrolysis of cell walls and others such as growth, yield, germination, fertility and susceptibility of the host to disease [30]. There are enzymes that can be used during the lignocellulose pretreatment without losing their enzymatic activity for their hypo-thermophilic capacity [31].

Recently, there has been much industrial interest on xylanases, from native microorganisms and recombinant hosts for different applications. For example, in the baking industry, *endo*-1,4-β-xylanase from *Aspergillus oryzae, B. subtilis*, and *Trichoderma longibrachiatum* is used for bread making, the production of maize starch and alcohol through fermentation. Particularly, in the bread industry, the

uses of xylanase are intended for flexibility and stabilization of dough (breaking down polysaccharides) and improve gluten strength. This impacts the sensory perception of bread [32].

In the animal nutrition industry, xylanases from *Acidothermus cellulolyticus* and *Neocallimastix patriciarum* are used to reduce feed conversion rate and enhance the digestibility of cereal feeds in poultry and ruminant [33, 34]. *Lactobacillus* xylanases depolymerize hemicellulose, making silage more stable and digestible by cattle [35]. The most common uses of xylanases have been used in the paper and pulp industry for the benefits of the quality of the products as purity, bright, and more permeability of fiber surface and diffusion during the bleaching processes [36–38]. Due to the current crisis of energy, the utilization of lignocellulosic agents is considered as sustainable biomass to produce nonfossil fuels. These biomasses should be hydrolyzed for bioethanol production from agricultural waste such as corncob, chili residue, rice straw, banana peel, apple pomace, and others [3, 39–42].

Xylanases have an important role in hydrolyzing the xylan and generate value-added products, such as xylitol. Xylitol is a sweetener used in soft drinks, candies, ice cream, chewing gum, and various pharmaceutical products as a natural sweetener in toothpaste [43]. Other uses have been explored, e.g., extracellular xylanase from a culture of *Aspergillus carneus* M34 and used to treat xylooligosaccharide. Feruloyl xylooligosaccharides showed antioxidative capacity in a cell model of ultraviolet B (UVB)-induced oxidative damage, demonstrating the potential of xylanases use in photo-protectant preparation [44].

1.2 Sources, Production, and Purification Strategies

Xylanases can be obtained in a large number of biologic systems such as fungi, bacteria (*Bacillus pumilus, B. subtilis, Bacillus amyloliquefaciens, Bacillus cereus, B. circulans, Bacillus megatorium, Bacillotherus licheniformis, Bacillotherus* sp.*, Streptomyces roseiscleroticus, Streptomyces cuspidosporus, Streptomyces actuosus, Pseudonomas* sp.*, Clostridium absonum,* and *Thermoactinomyces thalophilus*), yeasts, and seaweed [45]. Some other organisms such as mycorrhizae, actinomycetes, protozoa, insects, crustaceans, snails, and some plant seeds during the germination phase have been identified as xylanase sources [46]. Filamentous fungi being the main producers of xylanolytic enzymes, compared to other microorganisms [47]. In this way, xylanases have different applications, according to the source of production and some studies have focused on optimizing enzyme production, mainly from more powerful fungal and bacterial strains or through mutant strains for higher enzyme production [26, 48–50].

Fungal strains are important producers of xylanases due to their high yield and extracellular release of enzymes. They also show greater xylanase activity than yeast and bacteria. However, they present some characteristics that make them little available for use in industry. Fungal xylanases cannot be used in the pulp and paper industry because they need an alkaline pH and a temperature higher than 60 °C [45]. These xylanases are efficient at temperatures below 50 °C and a

pH range of 4–6. The fungal sources of xylanases are *A. niger, Aspergillus fetidus, Aspergillus brasiliensis, Aspergillus flavus, Aspergillus nidulans, Aspergillus terreus Penicillium* sp., *Trichoderma reesei, T. longibrachiatum, Trichoderma harzianum, Trichoderma viride, Trichoderma atroviride, Fusarium oxysporum, T. lanuginosus, Alternaria* sp., *Talaromyces emersonii, Schizophyllum commune*, and *Piromyces* sp. [47]. Although many of the reports focus on studies of xylanolytic systems from filamentous fungi mainly, and by bacteria, there are some reports on obtaining xylanases from yeasts [51, 52]. Two *Cryptococcus* yeast strains had been identified as producers of xylanases with a thermostable behavior [52]. Other reports are on the identification of yeast strains able to produce cellulase-free xylanases to solve the most common problem during the search of biologic systems for xylanase production [53].

From the great variety of xylanase-producing microorganisms, some thermophilic microorganisms have been isolated, which grow at an optimal temperature between 50 and 80 °C, and extremophiles or hyperthermophiles, which grow at temperatures above 80 °C [54]. Thermophilic microorganisms are sources of enzymes with greater activity at high temperatures [55]; these sources are important because xylanolytic enzymes are required to be able to withstand aggressive working conditions, such as acidic or alkaline environments and high temperatures, this due to its various industrial applications. For this reason, xylanases have been isolated from extremophilic bacteria and fungi. According to the analyses of genomic and transcriptomic profiles of xylanase-producing extremophilic fungi, it is argued that the discovery of new sources of thermostable xylanases, using molecular tools such as directed evolution, can satisfy the growing demand for thermostable xylanases. Table 1.1 shows a list of xylanases that come from thermophilic and hyperthermophilic microorganisms [61, 62].

Recent studies have focused on xylanase production optimization, for which they have used new strains of endophytic fungi, which were good producers of xylanases when solid-state fermentation (SSF) is used [63]. In the past, about 80–90% of commercial xylanases have been produced by submerged fermentation (SmF); however, SSF has a great option, such as less space requirement, low cost, and the

Table 1.1 Thermophilic and hyper-thermophilic microorganisms producing thermostable xylanases.

Source	Specie	GH family	References
Bacteria	*Caldicellulosiruptor* sp.	10	[56]
Bacteria	*Caldicoprobacter algeriensis*	11	[56]
Bacteria	*Dictyoglomus thermophilum*	11	[57]
Bacteria	*Microcella alkaliphila*	10	[58]
Hongo	*Aspergillus niger*	11	[59]
Hongo	*Malbranchea cinnamomea*	11	[44]
Hongo	*Thermomyces lanuginosus*	11	[60]