Critical Reviews in Biotechnology, 27:197–216, 2007

ISSN: 0738-8551 print / 1549-7801 online
DOI: 10.1080/07388550701775919

informa
healthcare

Microbial Mannanases: An Overview of Production and Applications

Samriti Dhawan
Department of Biotechnology, GGDSD College, Chandigarh, India

Jagdeep Kaur
Department of Biotechnology, Panjab University, Chandigarh, India

ABSTRACT Microbial mannanases have become biotechnologically important since they target the hydrolysis of complex polysaccharides of plant tissues into simple molecules like manno-oligosaccharides and mannoses. The role of mannanases in the paper and pulp industry is well established and recently they have found application in the food and feed technology, coffee extraction, oil drilling and detergent industry. Mannanses are enzymes produced mainly from microorganisms but mannanases produced from plants and animals have also been reported. Bacterial mannanases are mostly extracellular and can act in a wide range of pH and temperature, though acidic and neutral mannanases are more common. This review will focus on complex mannan structure and the microbial enzyme complex involved in its complete breakdown, mannanase sources, production conditions and their applications in the commercial sector. The reference to plant and animal mannanases has been made to complete the overview. However, the major emphasis of the review is on the microbial mannanases.

KEYWORDS hemicellulases, mannanase, mannooligosaccharides, mannose, locust bean gum, konjac glucomannan

INTRODUCTION

Enzymes are the catalytic keystone of metabolism and are the hub of concentrated worldwide research. The advent of enzymology represents an important breakthrough in the biotechnology industry, with the worldwide increase in usage of enzymes. The latter half of the twentieth century saw an unparalleled expansion in our knowledge of the use of microorganisms, their metabolic products, and enzymes in a broad area of basic research and their potential industrial applications. Hydrolases such as proteases, amylases, amidases, esterases and carbohydrolases such as cellulases, hemicellulases and pectinases occupy the major share of the industrial enzyme market. Cellulases and hemicellulases have numerous applications and biotechnological potential (Bhat, 2000; Sun and Cheng, 2002; Wong and Saddler, 1992; Beauchemin *et al.,* 2001). It is estimated that approximately 20% of the more than one billion US dollars of the world's sale of industrial enzymes consist of cellulases, hemicellulases and pectinases (Bhat, 2000).

Mannan and heteromannans are a part of the hemicellulose fraction in plant cell walls. Structural analysis of plant cell wall polysaccharides has revealed that

Address correspondence to Jagdeep Kaur, Department of Biotechnology, Panjab University, Chandigarh 160014, India. E-mail: jagsekhon@yahoo.com

the cell wall of a dicot sp. contains three major classes of polysaccharides viz. cellulose, hemicellulose and lignin (Dekker *et al.*, 1985). Hemicelluloses are defined as those plant cell wall polysaccharides that are not solubilized by water or chelating agents but are solubilized by aqueous alkali (Selvendran and O'Neill, 1985). According to this definition, hemicelluloses include mannan, xylan, galactan and arabinan. Hemicelluloses are also defined chemically as cell wall polysaccharides that are structurally homologous to cellulose because they have a backbone composed of 1,4-linked β-D-pyranosyl residues (O'Neill and York, 2003). Hemicellulose is abundant in primary walls but is also found in secondary walls (Puls, 1997).

In recent years, hemicellulases have emerged as key enzymes in the rapidly growing biotechnology industry, owing to their multifaceted properties, which find usage in a wide array of industrial applications (Hongpattarkere *et al.*, 2002). Most studies on hemicellulases have focused until now on enzymes that hydrolyse xylan, the primary constituent of hemicellulose in grasses. Enzymes that hydrolyse mannan have been largely neglected, even though it is an abundant hemicellulose, therefore the application of mannanase for catalyzing the random hydrolysis of β-D-1,4 mannopyranoside linkages in β-1,4 mannans is as important as the application of xylanases.

The use of mannanases in the paper and pulp industry increased significantly with the discovery of Gubitz *et al.* (1997). Since then researchers worldwide have focused their attention toward newer microbial isolates for mannanases. Despite the availability of several reports on mannolytic microorganisms, there are only a few reviews available on mannanases. These reviews emphasize mainly production and properties of mannanases and not their biotechnological potential. Thus there is a need to assemble information available on these enzymes from different sources in order to bring to light their importance and direct research into the probable application of these enzymes. The present review will encompass the microbial sources, production, properties and potential industrial applications of mannanases.

MANNAN: OCCURRENCE AND STRUCTURE

Mannans and heteromannans are widely distributed in nature as part of the hemicellulose fraction in hardwoods and softwoods (Capoe *et al.*, 2000), seeds of leguminous plants (Handford *et al.*, 2003; Buckeridge *et al.*, 2000) and in beans (Lundqvist *et al.*, 2002). Hemicelluloses are copolymers of both hexose and pentose sugars. The branched structure allows hemicellulose to exist in an amorphous form that is more susceptible to hydrolysis. Within biomass, mannans or the hemicelluloses are situated between the lignin and the collection of cellulose fibres underneath. Consistent with their structure and side group substitutions, mannans seem to be interspersed and covalently linked with lignins at various points while producing a coat around underlying cellulose strands via hydrogen bonds, but as few H-bonds are involved they are much more easily broken down than cellulose. The mannan layer with its covalent linkage to lignin and its non-covalent interaction with cellulose may be important in maintaining the integrity of the cellulose in situ and in helping protect the fibers against degradation to cellulases (Puls and Schuseil, 1993).

Mannan is the predominant hemicellulosic polysaccharide in softwoods from gymnosperms, but is the minor hemicellulose in hardwood from angiosperms (Puls, 1997). Unsubstituted β-1,4-mannan, composed of a main chain of β-mannopyranose residues, is an important structural component of some marine algae (Yamasaki *et al.*, 1998) and terrestrial plants such as ivory nut (Chanzy *et al.*, 2004) and coffee bean (Nunes *et al.*, 2006). It resembles cellulose in the conformation of the individual polysaccharide chains, and is water insoluble.

Hardwood mannans are composed of β-1,4-linked mannopyranose and glucopyranose units, whereas softwood contains two different types of acetylated galactoglucomannans. These consist of glucose, mannose and galactose in the ratio 1:3:1 and 1:4:0.1 respectively (Lundqvist *et al.*, 2002). In leguminous seeds, water soluble galactomannan is the main storage carbohydrate, comprising up to 20% of the total dry weight (McCleary, 1988). It has a α-galactose linked at the O-6 position of some mannose residues and may also have some β-D-glucose residues incorporated in the backbone. Furthermore, the mannose residues can be acetylated to various degrees at the C-2 and C-3 positions (Figure 1). The galactomannans from different leguminous taxonomic groupings differ in their degrees of galactose substitution and M:G ratios between 1.1:1 (high galactose) and 3.5:1 (low galactose) are encountered. The high degree of galactose substitution of the (1-4) β-D-mannan in galactomannans is clearly sufficient to prevent the chain

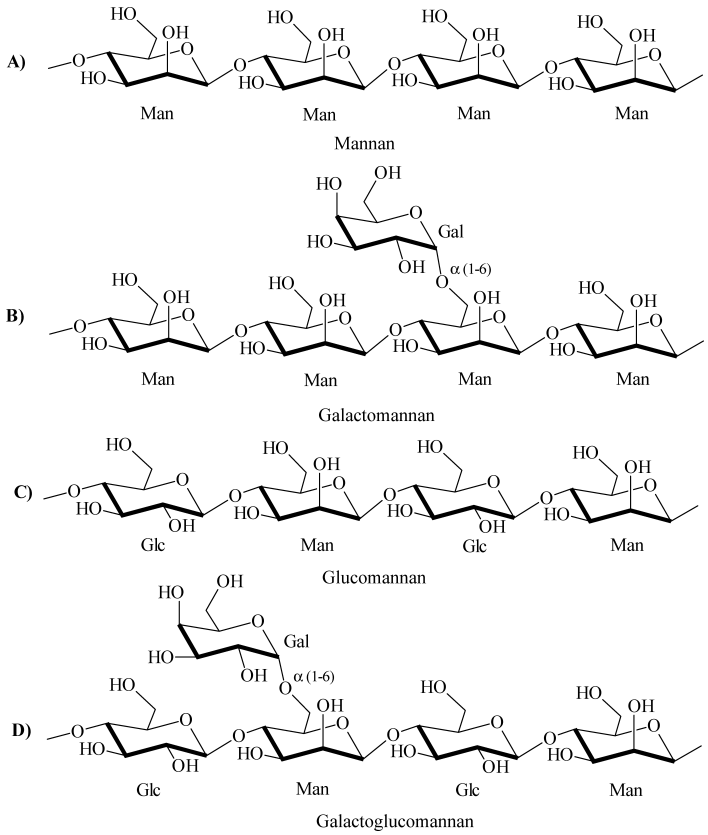

FIGURE 1 **General structure of mannan and heteromannans. A) A typical mannan structure, a main chain of $\beta-1,4$ linked mannose (Man) residues; B) A typical galactomannan structure, a main chain of $\beta-1,4$ linked mannose residues with $\alpha-1,6$ linked galactose (Gal) residues attached to some (Man) residues; C) A typical glucomannan structure, a main chain of $\beta-1,4$ linked mannose (Man) and glucose (Glc) residues; D) A typical galactoglucomannan structure, a main chain of $\beta-1,4$ linked mannose (Man) and glucose (Glc) residues, with $\alpha-1,6$ linked galactose (Gal) residues attached to some (Man) residues.**

aggregation that leads to insolubility and crystalline order in the mannans and glucomannans. The mannose: galactose ratio (M:G) is key in determining the amount of intermolecular association called hyperentanglements. Without any galactose side chains, mannan backbone will aggregate due to intermolecular interaction between the unbranched parts of heteromannans.

The substrate used routinely for the study of mannanases is galactomannan from locust bean gum (*Ceratonia siliqua*) with a mannose: galactose ratio of 4:1 (De Nicolas-Santiago et al., 2006). In addition, ivory nut (*Phytelephas macrocarpa*) mannan, an unbranched β-1,4-linked mannan homopolymer and manno-oligosaccharides (mannobiose, mannotriose, mannotetraose and mannopentoase), can also be used as substrates in the hydrolysis (Stoll et al., 2005).

ENZYMATIC HYDROLYSIS OF MANNAN

The main component of mannan is D-mannose, a six carbon sugar, but due to the heterogeneity and complex chemical nature of plant mannans, its complete breakdown into simple sugars that can be readily used as energy sources by particular microorganisms, the synergistic action of endo-1,4-β-mannanases (E.C 3.2.1.78, mannan endo-1,4-β-mannosidase) and exoacting β-mannosidases (E.C 3.2.1.25) is required (Table 1). Additional enzymes, such as β-glucosidases (EC 3.2.1.21), α-galactosidases (EC 3.2.1.22) and acetyl mannan esterases (Tenkanen, 1998) are required to remove side chain sugars that are attached at various points on mannans. A galactomannan structure whose

TABLE 1 **The major mannanases and their classification**

Enzymes	Substrates	EC number	Family
Endo-β-1,4-mannanase	β-1,4-Mannan	3.2.1.78	GH 5, 26
Exo- β-1,4-mannosidase	β-1,4-Mannooligomers, mannobiose	3.2.1.25	GH 1, 2, 5

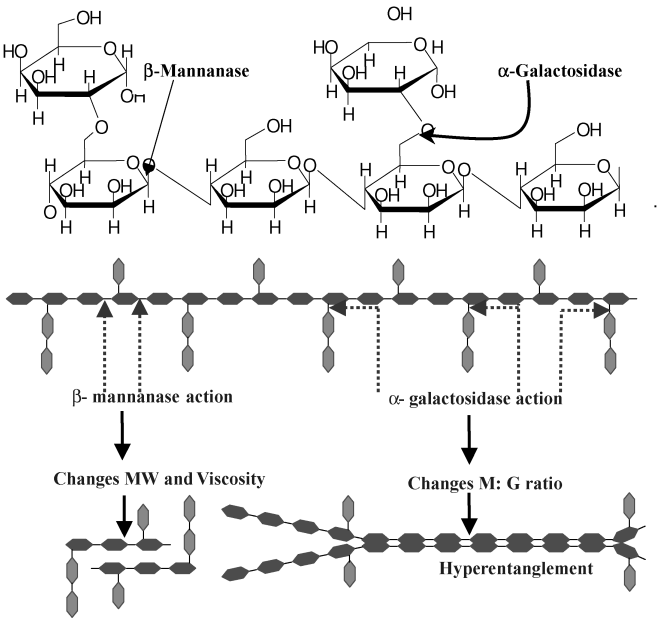

FIGURE 2 **Scheme of Enzymatic Action on Galactomannan. The β-(1-4) linked polymannose chain is substituted with α-(1-6) linked galactose (GAL) residues. The arrows represents the glycosidic links recognized by β-mannanase and α-galactosidase.**

glycosidic bonds are hydrolyzed by two enzymes viz. endo-1,4-β-mannanases and α-galactosidases is shown in Figure 2.

β-1,4-mannanases are endohydrolases that cleave randomly within the 1,4-β-D mannan main chain of galactomannan, glucomannan, galactoglucomannan, and mannan (McCleary and Matheson, 1986). Apart from their ability to hydrolyze different mannans, some β-D-mannanases have also been reported to transglycosylate manno-oligosaccharide substrates (Harjunpää *et al.*, 1995; Schroder *et al.*, 2004).

Hydrolysis of these polysaccharides is affected by the degree and pattern of substitution of the main chain by β-D-galactosyl residues in galactomannan and galactoglucomannan (McCleary, 1979) and by the pattern of distribution of D-glucosyl residues within the main chain in glucomannan and galactoglucomannan. In glucomannan, the pattern of distribution of O-acetyl groups may also affect the susceptibility of the polysaccharide to hydrolysis. Like β-glucosidases in the cellulase system, β-mannosidases are essential for the complete hydrolysis of plant heteromannans. They convert the manno-oligosaccharides produced by β-mannanases to mannose (Franco *et al.*, 2004). The galactose release from softwood pulp is enhanced by the presence of mannanase in combination with α-galactosidase (Clark *et al.*, 2000). The main products obtained during the hydrolysis of mannan by β-mannanases are mannobiose and mannotriose. β-mannanases from *Aspergillus tamarii* (Civas *et al.*, 1984), *Trichoderma reesei* (Stålbrand *et al.*, 1993) and *Aspergillus niger* (Ademark *et al.*, 1998) all produced mainly mannobiose and mannotriose and traces of higher oligosaccharides.

The pattern of galactomannan hydrolysis and the apparent subsite-binding requirements have been interpreted from structural analysis by NMR of isolated hydrolysis products from glucomannan and galactomannan incubated with *Aspergillus niger* β-mannanase. It is suggested that the enzyme has five substrate binding subsites-α, β, γ, δ and ε, which is equivalent to -3, -2, -1, $+1$, and $+2$, according to the nomenclature given by Davies *et al.* (1998). Binding to at least four subsites is required for efficient hydrolysis. Substitution of the substrate monomers at two of the subsite positions restricted hydrolysis, most likely by preventing binding (McCleary and Matheson, 1986). The hydrolysis of the pine craft pulp by the *Trichoderma reesei* β-mannanase was also studied (Tenkanen *et al.*, 1997). Judging from this study, *Trichoderma reesei* enzyme also appears to be restricted by galactosyl residues in a similar way to the *Aspergillus niger* enzyme.

SOURCES OF MANNANASES

Mannanases are ubiquitous in nature and are elaborated by a compendia of microorganisms largely isolated from natural environments. A vast variety of bacteria, actinomycetes, yeasts and fungi are known to be mannan degraders (Talbot and Sygusch, 1990; Puchart et al., 2004). The important degraders are listed in Table 2.

Among bacteria, degradation is mostly confined to gram-positives, including various *Bacillus* species (Yanhe *et al.*, 2004; Sun *et al.*, 2003) and *Clostridia* species (Kataoka *et al.*, 1998; Perret *et al.*, 2004; Nakajima and Matsuura, 1997). However, a few strains of gram-negative bacteria, viz. *Vibrio* (Tamaru *et al.*, 1997, *Pseudomonas* (Braithwaite *et al.*, 1995) and *Bacteroides* (Gherardini and Salyers, 1987) have also been reported. In addition, a few thermophiles and extremophiles belonging to genera *Bacillus*, *Caldocellum, Caldibacillus, Rhodothermus,* have also been described (Hatada *et al.*, 2005; Morris *et al.*, 1995; Sunna *et al.*, 2000; Politz *et al.*, 2000). Besides these, actinomycetes from the streptomycetes group, viz. *Streptomyces galbus* (Kansoh and Nagieb, 2004), *Streptomyces lividans* (Arcand *et al.*,

TABLE 2 Sources of mannanases

Organism	Reference
Agaricus bisporus	(Tang *et al*., 2001)
Aspergillu tamarii	(Civas *et al*., 1984)
Aspergillus aculeatus	(Christgau *et al*., 1994)
Aspergillus awamori	(Setati *et al*., 2001)
Aspergillus fumigatus	(Puchart *et al*., 2004)
Aspergillus niger	(Ademark *et al*., 1998).
Aspergillus oryzae NRRL	(Regaldo *et al*., 2000)
Aspergillus sulphureus	(Chen *et al*., 2007)
Aspergillus terrus	(Huang *et al*., 2007)
Bacillus agaradhaerens	(Bettiol and Showell, 2002)
Bacillus AM001	(Akino *et al*., 1989)
Bacillus brevis	(Araujo and Ward, 1990)
Bacillus circulans K-1	(Yosida *et al*.,1998)
Bacillus polymyxa	(Araujo and Ward, 1990)
Bacillus sp JAMB-750	(Hatada *et al*., 2005)
Bacillus sp. 1633	(Kauppinen *et al*., 2003)
Bacillus sp. M50	(Chen *et al*., 2000)
Bacillus sp. N 16-5	(Yanhe *et al*., 2004)
Bacillus stearothermophilus	(Talbot and Sygusch, 1990)
Bacillus subtilis	Mendoza *et al*., 1994
Bacillus subtilis B36	(Li *et al*., 2006)
Bacillus subtilis BM9602	(Cui *et al*., 1999)
Bacillus subtilis SA–22	(Sun *et al*., 2003)
Bacillus subtilis168	(Helow and Khattab,1996)
Bacteroides ovatus	(Gherardini and Salyers, 1987)
Bacteroides ruminicola	(Matsushita *et al*.,1991)
Caldibacillus cellulovorans	(Sunna *et al*., 2000)
Caldocellulosiruptor saccharolyticus	(Morris *et al*., 1995)
Caldocellum saccharolyticum	(Bicho *et al*., 1991)
Cellulomonas fimi	(Stoll *et al*., 1999, 2000)
Clostridium butyricum/ beijerinckii	(Nakajima and Matsuura, 1997)
Clostridium cellulolyticum	(Perret *et al*., 2004)
Clostridium tertium	(Kataoka and Tokiwa,1998)
Clostridium thermocellum	(Halstead *et al*.,1999)
Dictyoglomus thermophilum	(Gibbs *et al*.,1999)
Flavobacterium sp	(Zakaria *et al*., 1998)
Gastropoda pulmonata	(Charrier and Rouland, 2001)
Littorina brevicula	(Yamamura *et al*., 1996)
Lycopersicon esculentum	(Filichkin *et al*., 2000)
Paenibacillus curdlanolyticus	(Pason and Ratanakhanokchai, 2006)
Paenibacillus polymyxa	(Han *et al*., 2006)
Phanerochaete chrysosporium	(Wymelenberg *et al*., 2005)
Piromyces sp.	(Fanutti *et al*.,1995)
Pomacea insulars	(Yamamura *et al*.,1993)
Pseudomonas fluorescens subsp. cellulosa	(Braithwaite *et al*., 1995)
Rhodothermus marinus	(Politz *et al*., 2000)
Sclerotium rolfsii	(Sachslehner *et al*., 2000)
Streptomyces galbus	(Kansoh and Nagieb, 2004)
Streptomyces lividans	(Arcand *et al*., 1993)
Thermoanaerobacterium Polysaccharolyticum	(Cann *et al*., 1999)
Thermomonospora fusca	(Hilge *et al*., 1998)
Thermotoga maritima	(Parker *et al*., 2001)
Thermotoga neapolitana	(Duffaud *et al*., 1997)
Trichoderma harzanium strain T4	(Franco *et al*., 2004)
Trichoderma reesei	(Stalbrand *et al*., 1993,1995)
Vibrio sp.	(Tamaru *et al*.,1997)

1993), actinobacteria group, viz. *Cellulomonas fimi* (Stoll *et al*., 1999) and the actinoplanetes group viz. *Thermomonospora fusca* KW3 (Hilge *et al*., 1996) are described as mannan degraders with an ability to act on a wide variety of mannan substrates. The most mannolytic group among fungi belongs to genera *Aspergillus, Agaricus, Trichoderma*, *Sclerotium* (Huang *et al*., 2007; Chen *et al*., 2007; Tang *et al*., 2001; Franco *et al*., 2004; Sachslehner *et al*., 2000). Thus the property of mannolysis is widespread in the microbial world. Mannanases of microbial origin have been reported to be both induced as well as constitutive enzymes and are usually being secreted extracellularly into the medium in which the microorganism is cultured. The bacterial mannanases produced by *Sporocytophaga coccoids* and *Aerobacter mannolyticus* were found to be intracellular (Dekker and Richards, 1976). Extracellular mannanases are of considerable commercial importance, as their bulk production is much easier. Mannanases are also produced in higher plants (Shimahara *et al*., 1975; Marraccini *et al*., 2001) and animals (Yamaura *et al*., 1993, 1996) Although a number of mannanase-producing bacterial sources are available, only a few are commercially exploited as wild or recombinant strains, of these, the important ones are: *Bacillus sp*., *Streptomyces, Caldibacillus cellulovorans, Caldicellulosiruptor Rt8B, Caldocellum saccharolyticum*. (Zhang *et al*., 2006; Hatada *et al*., 2005; Morris *et al*., 1995; Sunna *et al*., 2000).

ASSAY METHODS

Numerous screening methods exist for detecting mannanase activity in microorganisms. A solid medium

providing rapid assays is useful for the direct measurement and isolation of mannanase-producing organisms from natural substrates and for the isolation of mutants. Common screening techniques devised for the detection of mannan degrading enzymes by microorganisms involve plate assays where the respective polymer, or its derivative, is incorporated into the basal growth medium. The production of corresponding hydrolases is indicated by the clearing of the opaque medium as the substrate is dissolved by the enzymes produced by the growing colonies. In such procedures, the results are often difficult to interpret in case of fungal isolates, because the zones of clearing are not always easy to distinguish from the unaffected medium and activity might not be detected because of the masking effect of the mycelial mass when the rate of fungal growth exceeds enzyme diffusion. A gel-diffusion assay for the quantification of endo-β-mannanase activity has been developed (Downie *et al.*, 1994). The assay is specific and detects activity as low as 0.07 pkatal. The assay was equally effective at acidic and neutral pH. One per cent (w/v) Congo Red dye resulted in fast staining times (15 min) and good contrast. The diameter of the clearing zones decreased linearly with increasing substrate concentration in the range of 0.1–1.0% w/v galactomannan. The assay was specific for this endo-enzyme, with no zone of clearing developing for α-galactosidase, and only a slight decrease in dye intensity with β-mannosidase. The clearing zone diameter was greater in the gel-diffusion assay using Congo Red than using Remazol Brilliant Blue colored Carob substrate at identical concentrations, enzyme activities and assay conditions. This problem was further overcome by inoculating a polycarbonate membrane 0.2 μm, 90 mm diameter, Nucleopore Co (Chang *et al.,* 1992), or a circle of sweet cellophane 400 (De Nicolas-Santiago *et al.*, 2006) with the test fungus. After incubation, the membrane is removed and the enzyme activity recorded. The membranes, which can resist penetration by fungi, are placed between moistened filter papers and sterilized by autoclaving before use.

Solid media are also used to screen cell-free extracts from microorganisms for mannan degrading enzyme activity. Generally, the enzyme solution is placed in a well (cup) cut in an agar medium containing a mannan/heteromannan substrate. The agar surface usually needs to be flooded with a reagent to develop the zones of enzyme activity around the cup. The diameter of the cleared zone is measured with calipers and recorded. Diameters are converted to enzyme activity by running a dilution of the enzyme and plotting zone diameter against the log of the enzyme concentration (Dingle *et al.*, 1953).

An assay based on the quantification of hydrolysis products has been described by McCleary (1988). Dyed polysaccharide substrates (AZO-carob galactomannan, Megazyme) can also be used as a substrate and incubated with enzyme for ten minutes; ethanol is added to stop the reaction and to precipitate high molecular-weight galactomannan. Low molecular-weight fragments stay soluble and can be spectrophotometrically quantified at 590 nm, due to the attached dye (Stalbrand, 2003). To relate to enzyme activity units, a standard curve (supplied by Megazyme for each batch of substrate) with known enzyme amounts can be prepared.

PRODUCTION CONDITIONS

Microbial mannanases are mostly extracellular and are greatly influenced by nutritional and physicochemical factors, such as temperature, pH, nitrogen and carbon sources, inorganic salts, agitation and dissolved oxygen concentration (Mabyalwa and Setati, 2006; Li *et al.,* 2006; Lin *et al.*, 2007).

However their production is significantly influenced by using different mannan substrates such as glucomannan, galactomannan, galactoglucomannan. Galactomannans of guar gum (mannose:galactose) ratio of 2:1 and locust bean gum (mannose:galactose) ratio of 4:1 and glucomannan of konjac (mannose:glucose) ratio of 1.6:1 induced their production (Dong *et al.*, 1991; Yagüe *et al.*, 1997). Besides carbon source, the type of nitrogen source in the medium also influences the mannanase titers in production broth (Kataoka and Tokiwa, 1998). Generally, organic nitrogen is preferred, such as peptone and yeast autolysate (Puchart *et al.*, 2004), which have been used as a nitrogen source for mannanase production by various *Bacillus* strain viz. *Bacillus polymyxa*, *Bacillus subtilis*, *Bacillus brevis* (Zhang *et al.*, 2006; Cui *et al.*, 1999) while beef extract and peptone have been used in the case of *Clostridium tertium* (Kataoka and Tokiwa, 1998). Inorganic nitrogen sources such as ammonium sulfate, diammonium hydrogen phosphate, ammonium dihydrogen phosphate and sodium nitrate have also been reported to be effective in some microbes (Zakaria *et al.*, 1998; Perret *et al.*, 2004; Araujo and Ward, 1990).

Divalent cations stimulate or inhibit enzyme production in microorganisms. Nakajima and Matsuura (1997) observed stimulation in mannanase production from *Clostridium sp.* in the presence of Ca^{2+} and Mg^{2+}. However, most other metal ion salts Fe^{3+}, Al^{3+}, EDTA, Hg^{2+} were inhibitory to mannanase production (Chen *et al.*, 2000; Sun *et al.*, 2003). Iron was found to play a critical role in the production of mannanase by most of the microorganisms. In addition to the various chemical constituents of a production medium, physiological parameters such as pH, temperature, agitation, aeration and incubation period also play an important role in influencing production by different microorganisms. The initial pH of the growth medium is important for mannanase production. Largely, bacteria prefer pH around 7.0 for best growth and mannanase production, such as in the case of *Bacillus sp.*, *Clostridium sp.* (Helow and Khattab, 1997; Talbot and Sygusch, 1990) However, maximum activity at higher pH (>7.0) has been observed in some cases (Hatada *et al.*, 2005). The optimum temperature for mannanase production corresponds with the growth temperature of the respective microorganism, for example, the best temperature for growth and mannanase production in the case of *Bacillus sp. M50* was 50°C (Chen *et al.*, 2000). It has been observed that, in general, mannanases are produced in the temperature range 4–85°C (Politz and Borriss, 2000; Kansoh and Nagieb, 2004). Incubation periods ranging from a few hours to several days have been found to be best suited for maximum mannanase production by bacteria. An incubation period of 24 h was optimum for mannanase production by *Bacillus subtilis* 168 (Helow and Khattab, 1996), and 36 h for *Bacillus subtilis BM 9602* (Cui *et al.*, 1999), maximum mannanase was produced after 72 h and 96 h of incubation. Mannanases are often secreted into the culture liquid as multiple enzyme forms as observed for some bacteria (Stoll *et al.*, 1999; Akino *et al.*, 1989), and several fungi including *Schlerotium rolfsii* (Gübitz *et al.*, 1996), *Aspergillus fumigatus* (Puchart *et al.*, 2004) and *Trichoderma reesei* (Stålbrand *et al.*, 1993). The multiplicity of these mannanases is thought to be due to their ability to bind and degrade different substrates (Johnson *et al.*, 1990). These β-1,4-mannanase isoforms may be secreted as products of the same gene differing only in their post-translational modification, such as with *A. fumigatus* (Puchart *et al.*, 2004) or their production may be regulated differently such as *Sclerotium rolfii* isoforms that exhibited different functions on substrates of varying sizes (Großwindhager *et al.*, 1999). Kinetics of mannan depolymerization, i.e. Michaelis-Menten constant (K_m) and the maximal reaction velocity (V_{max}) values, are reported for different fungal and bacterial β-mannanases. K_m and V_{max} values reported for *Aspergillus fumigatus* are 3.07 mg/ml and 1935 μmol min^{-1} mg^{-1} for Man I and 3.12 mg/ml and 2139 μmol min^{-1} mg^{-1} for Man II on locust bean gum (Puchart *et al.*, 2004). For *Aspergillus tamarii* V_{max} and K_m reported are 43.5 μmol min^{-1} mg^{-1} and 2×10^{-2} g/100 ml (Civas *et al.*, 1984) on locust bean gum. Franco *et al.* (2004) reported V_{max} and K_m values as 20.1 units /ml and 6.0 mg/ml, respectively, for *Trichoderma harzanium strain T4* on wheat bran. Among bacterial β-mannanases (Talbot and Sygusch, 1990) reported for *Bacillus stearothermophilus* a V_{max} value of 455 ± 60 U/mg and the estimated Km value of 1.5 ± 0.3 mg/ml on locust bean gum. In *Bacillus subtilis* SA–22 the Michaelis constants (K_m) were measured as 11.30 mg/ml for locust bean gum and 4.76 mg/ml for konjac powder, while V_{max} for these two polysaccharides were 188.68 μmol min^{-1} ml^{-1} and 114.94 μmol min^{-1} ml^{-1} respectively (Sun *et al.*, 2003).

Thus, microbial mannanases are generally produced in the presence of different mannan substrates, viz. guar gum (*Cyamopsis tetragonobola*), locust bean gum (*Ceratonia siliqua*), and konjac glucomannan (*Amorphophallus konjac*) in the presence of any complex nitrogen source. Dye-based substrate ostazin brilliant red mannan and azo-carob galactomannan can also be used for easy screening of mannanase producing microorganisms. The requirement for metal ions varies with the organism. However, physical parameters such as pH, temperature, agitation and aeration influence mannanase production via modulating the growth of the bacterium. Mannanases are produced throughout bacterial growth, with peak production being obtained by the late logarithimic phase. The production period for mannanases varies from a few hours to a few days. A list of various production conditions used with different microorganisms is presented in Table 3.

FAMILY CLASSIFICATION AND MODULARITY OF MANNANASES

Mannanases are glycoside hydrolases that degrade mannans and heteromannans. Glycoside hydrolases (EC 3.2.1.-) are a widespread group of enzymes that hydrolyze the glycosidic bonds in oligo- and polysaccharides. Due to the complicated structures of the

TABLE 3 **Overview of various production conditions (molecular weight, optimum pH, optimum temperature, carbon source, and pI values) of mannanase producing microorganisms**

Organism	Molecular weight	pH opt	Temperature (opt.)	Carbon Source/PI	Reference
Aspergillus awamori	—	3.0	80°C	—	Setati *et al.*, 2001
Aspergillus niger	56	3.0	50°C	–/4.9	Regaldo *et al.*, 2000
Aspergillus oryzae	110	6.0	40°C	Copra waste	Regaldo *et al.,* 2000
Agaricus bisporus	—	—	—	LBG	Tang *et al.,* 2001
Aspergillu tamarii	53	4.5	—	LBG	Civas *et al.,* 1984
Aspergillus aculeatus	45	5.0	60-70°C	–/4.5	Christgau *et al.,* 1994
Aspergillus fumigatus,	60,63	4.5, 4.5	60°C	LBG/5.2, 4.9	Puchart *et al.,* 2004
Aspergillus sulphureus	48	2.4	50°C	LBG/4.8	Chen *et al.*, 2007
Aspergillus terrus	—	7.5	55°C	—	Huang *et al.,* 2007
Bacillus agaradhaerens	38	8-10	60°C	—	Bettiol and Showell, 2002
Bacillus AM001	55, 38	9.0, 8.5	60°C, 65°C	—	Akino *et al.*, 1989
Bacillus circulans K-1	62	6.9	65°C	—	Yosida *et al.*, 1998
Bacillus sp. JAMB-750	—	10.0	—	—	Hatada *et al.*, 2005
Bacillus sp. M50	—	6.0	50°C	KGM	Chen *et al.*, 2000
Bacillus sp. N 16-5	55	9.5	70°C	–/4.3	Yanhe *et al.*, 2004
Bacillus sp.1633	34	—	50°C	—	Kauppinen *et al.*, 2003
Bacillus stearothermophilus	162	6.5	70°C	LBG	Talbot and Sygusch, 1990
Bacillus subtilis	38	5.0	55°C	–/4.8	Mendoza *et al.*, 1994
Bacillus subtilis B36	38	6.4	50°C	LBG	Li *et al.,* 2006
Bacillus subtilis SA –22	38	6.5	70°C	LBG/KGM	Sun *et al.*, 2003
Bacillus subtilis168	—	7.0	37°C	Galman	Helow and Khattab, 1996
Bacillus subtilis BM 9602	—	6.0	50°C	KGM,LBG	Cui *et al.,* 1999
Bacteroides ovatus	190	—		GG/4.8	Gherardini and Salyers, 1987
Caldibacillus cellulovorans	30.7	6.0	85°C	—	Sunna *et al.*, 2000
Caldocellum saccharolyticum	—	6.0	80°C	—	Morris *et al.*, 1995
Cellulomonas fimi	100	5.5	42°C	LBG	Stoll *et al.*, 1999
Clostridium butyricum/beijerinckii	50–53	7.0–8.0	—	KGM, coffee mannan	Nakajima and Matsuura, 1997
Clostridium cellulolyticum	45	—	—	—	Perret *et al.*, 2004
Clostridium tertium	53	7.0	30°C	GG	Kataoka *et al.*, 1998
Clostridium thermocellum d	70	6.5	65°C	—	Halstead *et al.*, 1999
Dictyoglomus thermophilum	40	5.0	80°C	LBG	Gibbs *et al.*, 1999
Flavobacterium sp	—	7.0	35°C	KGM, LBG	Zakaria *et al.*, 1998
Paenibacillus curdlanolyticus	—	4.0	—	GG	Pason and Ratanakhanokchai, 2006
Paenibacillus polymyxa	—	5.0	50°C	—	Han *et al.*, 2006
Piromyces sp.	68	—	—	—	Fanutti *et al.*, 1995
Rhodothermus marinus	113	5.4	85°C	LBG,GG	Politz *et al.*, 2000
Sclerotium rolfsii	61.2, 41.9	2.9, 3.3	74, 72	–/3.5	Gubitz *et al.*, 1996
Streptomyces galbus	—	7.0	35°C	Galman of Palm seeds	Kansoh and Nagieb, 2004
Streptomyces lividans	36	6.8	58°C	Galman /3.5	Arcand *et al.*, 1993
Thermotoga maritima	76.9	7.0	90°C	—	Parker *et al.*, 2001
Trichoderma harzianum T4	36.5	2.6	65° C	Wheat bran	Franco *et al.*, 2004
Trichoderma reesei	53, 51	3.5, 3.5	70°C, 70°C	5.4, 4.6	Stalbrand *et al.*, 1993

LBG: locust bean gum; KGM: konjac glucomannan; GG: guar gum; Galman: galactomannan; GH: Glycoside hydrolase; —: Not reported.

carbohydrates with different combinations in nature, a large number of enzymes with different substrate specificities are required. Glycoside hydrolases from various sources were classified into different families based on their amino acid sequence similarities (Henrissat and Bairoch, 1993, 1996). The basic principle behind the family classification is that the family membership of a particular enzyme can be defined from its sequence

alone and there is a direct relationship between sequence and folding similarities. Structure conservation was shown to be much stronger than amino acid conservation. Families can thus be grouped into "clans" according to their three-dimensional structure (Henrissat, 1990). So far there are a total of 110 families and some of them are well studied. The β-mannanase sequence comparison studies permit assignment of these enzymes to either glycoside hydrolase family 5 or 26. Family 5 comprises several bacterial mannanases including *Caldocellum saccharolyticum, Caldibacillus, Vibrio* species, fungal mannanases from *Aspergillus aculeatus, Trichoderma reesei* and *Agaricus bisporus* and eukaryotic mannanase from *Lycopersicon esculentum* and *Mytilus edulis*. Family 26 comprises mannanases from *Bacilli sp.*, *Cellvibrio japonicus, Pseudomonas fluorescens and Rhodothermus marinus.* Thus both bacterial and eukaryotic mannanases have been annotated to family 5. With the exception of a few anaerobic fungi, the mannanases in family 26 are of bacterial origin. In some cases, mannanases from the same genus have been classified in different families; β-mannanases from different strains of *Caldocellulosiruptor saccharolyticus* have been classified in both families 5 and 26 (Gibbs *et al.*, 1996), and multiple β- mannanases in *Cellvibrio japonicus* have been classified in both families 5 and 26 (Hogg *et al.,* 2003). Besides this, β-mannanases from different *Bacilli* species are also found in both families (Sygusch *et al.,* 1998; Hatada *et al.*, 2005). Some glycoside hydrolases are multifunctional enzymes that contain catalytic domains that belong to different GH families like *Paenibacillus polymyxa. A cel*44C-*man*26A gene was cloned from this endophytic strain. This gene encodes for a protein that contains a glycoside hydrolase family 44 (GH44) catalytic domain and a glycoside hydrolase family 26 (GH26) catalytic domain. The multifunctional enzyme domain GH44 possesses cellulase, xylanase, and lichenase activities, while the enzyme domain GH26 possesses mannanase activity (Han *et al.*, 2006).

Most mannanases often display a modular organization and usually consist of two-domain proteins (Henrissat *et al.*, 1995; Perret *et al.*, 2004). These proteins generally contain structurally discrete catalytic and non-catalytic modules (Warren *et al.,* 1996).

β-mannanases in both families belong to the 3-D structure group $(\beta/\alpha)_8$ fold catalytic module characteristic of clan Glycoside hydrolase-A (Stoll *et al.*, 2005). The most important non-catalytic module consist of carbohydrate binding module (CBM) which facilitate the targeting of the enzyme to the polysaccharide, By analogy to the glycoside hydrolases, CBMs are classified into families based on sequence similarities and three dimensional structures (Boraston *et al.*, 2004). Presently, 49 families of CBMs have been reported and a number of these families are closely related and consequently grouped into superfamilies or clans. A comprehensive classification of mannanases and other hydrolases is available at http://afmb.cnrs-mrs.fr/CAZY. Amongst β-mannanases from aerobic fungi, the enzymes from *T. reesei* and *A. bisporus* are composed of a family 5 catalytic module linked to a family 1 CBM (Tang *et al.*, 2001; Yague *et al.*, 1997). Some bacterial β-mannanases from families 5 and 26 have more complex structures: the *C. fimi* β-mannanase Man26A contains both a mannan binding family 23 CBM, a putative SLH-module and a module of unknown function (Stoll *et al.*, 1999, 2000; Stalbrand *et al.*, 2006). The family 5 β-mannanase from *Thermoanaerobacterium polysaccharolyticum* also contain a SLH-module and, in addition, two internal family 16 CBMs (Cann *et al.*, 1999). Several β-mannanases from anaerobic bacteria and fungi contain dockerin modules, which attach the β-mannanases either to the microbial cell surface or to multienzyme complexes such as the cellulosome (Shallom and Shoham, 2003; Halstead *et al.*, 1999; Tamaru and Doi, 2000; Perret *et al.*, 2004). The family 26 β-mannanases from *Caldocellulosiruptor saccharolyticus* contains two family 27 mannan-binding modules (Sunna *et al.*, 2001) and from *Paenibacillus polymyxa* contain a fibronectin domain type 3, CBM family 3 in addition to two catalytic domains (Han *et al.*, 2006). A comparison of the modular structure of different mannanases reveals CBMs have been predicted for several β-mannanases from family 5 and family 26. However, only in a few cases has the binding been experimentally confirmed. In addition to Man5A, family 1 CBMs have been indicated in the fungal β-mannanases from *Agaricus bisporus* (Tang *et al.*, 2001; Yague *et al.*, 1997). However, the β-mannanases from *Aspergillus aculeatus* and *Aspergillus niger* appear to lack CBMs (Ademark *et al.*, 1998; Christgau *et al.*, 1994). Some other family 5 β-mannanases have CBMs that have been classified into family 3, 16 and family 35. Moreover, some family 26 β-mannanases have family 23, 27 and 35 CBMs, which have been shown to bind to mannan (Stoll *et al.*, 2000; Sunna *et al.*, 2001). However, many β-mannanases from both families 5 and 26 appear to be sole catalytic modules. Thus, at present, it is difficult to establish any obvious patterns in modularity among

β-mannanases. It clearly appears as if several different strategies in mannan-hydrolysis subsist. Henrissat *et al.* (1998) proposed a scheme for designating enzymes that hydrolyze the polysaccharides in cell walls of plants. A written designation of the module organization of recently studied β-mannanases is depicted in Table 4.

CLONING AND EXPRESSION OF THE MANNANASE GENE

Gene cloning is a rapidly progressing technology that has been instrumental in improving our understanding of the structure-function relationship of genetic systems. It provides an excellent method for the manipulation and control of genes. More than 50% of the industrially important enzymes are now produced from genetically engineered microorganisms. Recombinant DNA techniques offer a plethora of opportunities for construction of genetically modified microbial strains with selected enzyme machinery. To ensure the commercial utilization of hemicellulosic residues in paper and pulp industries, the production of higher mannanase yields at low capital cost is required. Microbial genes encoding proteins associated with sugar metabolism are clustered which indicates that their expression is coordinated. The sequence of some of the genes encoding β-1,4-mannanases have been determined and analyzed to deduce the primary structure of mannanase enzymes. In this respect, isolation and cloning of the mannanse gene represents an essential step in the engineering of the most efficient microorganism. Several reports have been published in the past decade (Table 4) on the isolation and manipulation of microbial mannanase genes with the aim of enzyme overproduction, studying the primary structure of the protein, its role in the secreting microorganism, and protein engineering to locate the active-site residues and/or to alter the enzyme properties to suit its commercial applications. Henrissat (1990) recognized two families encoding β-mannanases on the basis of amino acid sequence similarities. Only two β-mannanases genes were available for comparison at the time his enzyme classification was made. ManA from *Caldicellulosiruptor saccharolyticus* (Luthi *et al.*, 1991) and ManA from a *Bacillus* sp. (Akino *et al.*, 1989) were compared with 301 glycosyl hydrolases and classified respectively as type 5 and type 26 glycosyl hydrolases. A number of mannanase genes had been sequenced since the classification of mannanases by Henrissat (1990) and all share homology with either manA from *Caldicellulosiruptor saccharolyticus* or the *Bacillus* mannanase gene (Gibbs *et al.*, 1999). Attempts have been made to clone and express mannanase from bacteria such as *Bacillus stearothermophilus, Caldibacillus cellulovorans, Caldicellulosiruptor Rt8B, Caldocellum saccharolyticum* (Sygusch *et al.*, 1998; Morris *et al.*, 1995; Sunna *et al.*, 2000) into a non mannanase producing strain of *Escherichia coli*. As alkaline β-mannanases provide obvious advantage for the application in the manufacture of kraft pulp and in the detergent industry, where high pH processes are common. Few reports describe the molecular cloning, sequencing and expression of mannanase genes from alkaliphilic *Bacillus sp.* (Takeda *et al.*, 2004a; Takeda *et al.*, 2004b; Hatada *et al.*, 2005), *Bacillus subtilis* B36 (Li *et al.*, 2006), *Bacillus* N16-5 (Yanhe Ma *et al.*, 2004), *Bacillus agaradhaerens* (Bettiol and Showell, 2002), *Bacillus sp. 1633* (Kauppinen *et al.*, 2003) and *Thermoanaerobacterium polysaccharolyticum* (Cann *et al.*, 1999). The sizes of mannanase genes and their protein products are highly divergent (Table 4). This indicates that mannanases form a group of proteins that possess similar enzyme activities even though they have highly different primary protein structure. Amino acid sequence alignments of members of family 5 rarely reveal levels of sequence identity greater than 20%, As the amino acid sequence of members of family 5 were divergent, members of family 5 have been classified in eight subfamilies, subfamilies A1–A8 (Hilge *et al.*, 1998). Subfamilies A7 (eukaryotic mannanases) and A8 (bacterial mannanases) have recently been included. Members of subfamily A8 exhibit levels of sequence identity greater than 43%, while the levels of sequence identity for members of subfamily A7 and members of subfamily A8 are less than 20%. Three dimensional structures of two β-1,4-mannanases from family 5 in complex with oligosaccharide products have been determined by X-ray crystallography (Hilge *et al.*, 1998; Sabini *et al.*, 2000). Recently, the preliminary structure determination of a bacterial family 5 β-1,4-mannanase (Akita *et al.*, 2004) has been reported. Eight conserved residues of all family 5 enzymes were demonstrated to be responsible for the catalytic activity of the enzyme including two catalytic glutamates (Sakon *et al.*, 1996).

Yanhe *et al.* (2004) reported the eight essential conserved active site residues of β-mannanase in strain *Bacillus* N16-5 as Arg-83, His-119, Asn-157, Glu-158, His-224, Tyr-226, Glu-254, and Trp-283. Computer analysis of the deduced amino acid sequence of ManA

TABLE 4 **Occurrence of the main mannanase genes and their products**

Microbial species	Protein	Size of gene (bp) and enzyme (aa /kDa)	Module Arrangement	Reference
Aspergillus sulphureus	ManN	1345 bp/48 kDa (*P. pastoris* expressed)	CD5	Chen *et al*., 2007
Bacillus subtilis B36	Man 36B	1104 bp /367 aa, 38 kDa *(E. coli* expressed)	—	Li *et al*., 2006
Bacillus subtilis B36	Man	1080 bp /38 kDa (*E. coli* expressed)	—	Zhang *et al*.,, 2006
Bacillus sp. JAMB-750	Man26A	2994 bp /997 aa (*B. subtilis* expressed)	CD26/CBM23/MBM/ CTIX/MAR	Hatada *et al*., 2005
Clostridium cellulolyticum	Man5K	424 aa, 45 kDa (*E. coli* expressed)	LP/DM/CD5	Perret *et al*., 2004
Bacillus sp. N16-5	Man A	1479 bp /461 aa, 50.7 kDa	CD5	Yanhe Ma *et al*., 2004
Cellvibrio japonicus	Man5A Man5B Man5C	—	CD5/CBM2a CD5/CBD5 CD5/CBD10/?	Hogg *et al*., 2003
Cellvibrio japonicus	Man26B	—	CD26	Hogg *et al*., 2003
Dictyoglomus thermophilum Rt46B.!	ManA	—	CD26	
Trichoderma reesei	Man5A	—	CD5/LP/CBM1	Hagguland *et al*., 2003
Thermotoga maritima	Man5	2,007 bp, 669 aa, 76.9 kDa (*E. coli* expressed)		Chhabra *et al*., 2002
Agaricus bisporus	Cel4	(*S. cerevisiae, P. pastoris* expressed)	CD/CBM1	Tang *et al*., 2001
Clostridium thermocellum	Man26B	1773 bp/ 591 aa, 67.04 kDa (*E. coli* expressed)	CD26	Kurokawa *et al*., 2001
Clostridium cellulovorans	ManA	1275 bp/ 425 aa, 47.15 kDa (*E. coli* expressed	LP/DM/CD5	Tamaru and Doi, 2000
Caldibacillus cellulovorans	ManA	4567 bp (*E. coli* expressed)	ORF1/?/CBM3B/CD5/ CBM3b/ORF3	Sunna *et al*., 2000
Rhodothermus marinus	ManA	997 aa /113 kDa	CD 26	Politz *et al*., 2000
Cellulomonas fimi	Man26A	951 aa (*E. coli* expressed)	CD26/LP/CBM23	Stoll *et al*., 2005
Clostridium thermocellum	Man26A	1767 bp /66.81kDa (*E. coli* expressed)	CD26/LP/DM	Halstead *et al*., 1999
Thermoanaerobacterium polysaccharolyticum	ManA	3291 bp/119.6 kDa (*E. coli* expressed)	LP/CD/CBM16/SLH	Cann *et al*., 1999
Bacillus stearothermophilus	ManF	2085 bp/76 kDa (*E. coli* expressed)	CD5/CBM27	Sygusch ett al., 1998
Vibrio sp.	ManA	—	CD5	Tamaru *et al*., 1997
Piromyces sp.	ManA	—	CD/DM	Millward *et al*., 1996
Paenibacillus polymyxa	Man26A	4056 bp/1352 aa (*E. coli* expressed)	CD44/FN3/CD26/CBM3	Han *et al*., 2006
Caldocellum saccharolyticum Rt8B.4	ManA	—	CD26/?	Gibbs *et al*., 1996
Caldocellulosiruptor saccharolyticus	ManB in CelC	4098 bp	EG/CBM27/CD5	Morris *et al*., 1995
*Pseudomonas fluorescens*GH26	MANA	1257 bp /46.9 kDa (*E. coli* expressed)	CD26	Braithwaite *et al*., 1995

CD: catalytic domain with family number; CBM: Carbohydrate binding module with family number; CTIX: collagen type IX alpha I chain; DM: dockerin module; EG: endoglucanase; FN3: fibronectin type III like repeat; LP: leader peptide; MAR: Membrane anchor region of Gram positive surface protein; MBM: mannan binding module; ORF: open reading frame; SLH: Surface layer like protein region; ?: domain of unknown function; —: not available.

from *Bacillus* sp. N16-5 with sequences in the GenBank and UniProt databases showed that ManA consists of a single catalytic domain. The amino acid sequence of ManA showed high homology to family 5 mannanases: 96.8% sequence similarity to ManA of *B. agaradhaerens* (GenBank accession number: AAN27517), 58.2% to ManG of *B. circulans* (BAA25878), 52.3% to ManA of *Bacillus* sp. I633 (AAQ31835), 39.4% to ManA of *Thermobifida fusca* (CAA06924), 34.4% to ManA of *Vibrio* sp. MA-138 (BAA25188), and 33.1% to ManA of *Streptomyces lividans* 66 (S30386).

Yanhe *et al.* (2004) compared the sequences of mannanases from *Bacillus* sp. N16-5 and *Bacillus* sp. AM001, the only two mannanases extensively characterized among alkaline mannanases reported to date. A higher level of similarity (48%) was observed for the C terminal region (about 150 amino acid residues long) of these two enzymes. Hatada *et al.* (2005) reported a novel alkaline mannanase Man26A in the culture of an alkaliphilic *Bacillus* sp. strain JAMB-750. The N-terminal half (Glu27–Val486) of the enzyme exhibited moderate similarities to other mannanases belonging to glycoside hydrolase family 26, such as the enzymes from *Cellvibrio japonicus* (37% identity), *Cellulomonas fimi* (33% identity), and *Bacillus* sp. strain AM-001 (28% identity).

Another family 26 β-mannanase Man A of *D. thermophilum* Rt46B.1 on alignment with related enzymes shows that it shares considerable sequence similarity (58% sequence identity) with the catalytic domain of ManA from *Caldicellulosiruptor* sp. strain Rt8B.4 (Gibbs *et al.*, 1996), and at least 25% sequence identity with the other family 26 mannanases listed in Table 2. Matsushita *et al.* (1991) published a sequence coding for a β-1,4-endoglucanase from *Bacteroides ruminicola*. However, comparison with the mannanase domains from *D. thermophilum* Rt46B.1 and *Caldicellulosiruptor* Rt8B.4 ManA suggests that the *B. ruminicola* enzyme is a multidomain enzyme possessing both a mannanase and an endoglucanase domain. Currently, the reported three-dimensional structures for GH26 members are for the *Cellvibrio japonicus* mannanase CjMan26A, and for the *Cellulomonas fimi* mannanase CfMan26A. These enzymes possess a conserved motif in which two glutamates have been identified as the putative catalytic residues; at least two additional residues are conserved (Bolam *et al.*, 1996; Le Nours *et al.*, 2005). Notably, the location of the catalytic apparatus is completely conserved, with the catalytic residues positioned on strands β-4 and β-7 (Jenkins *et al.*, 1995).

Amongst the fungal mannanases, The *T. reesei* man 1 gene was the first fungal mannanase gene characterized (Stalbrand *et al.*, 1995). Tang *et al.* (2001) demonstrated that the catalytic domain of CEL4 from *Agaricus bisporus* had the most amino acid sequence similarity with Ascomycete mannanases from *Aspergillus aculeatus* and *Trichoderma reesei*, which belong to glycosyl hydrolase family 5 (43 and 42%, respectively).

The availability of a number of family 26 mannanase sequences provides the opportunity for the design of PCR primers to highly conserved regions. It has allowed the identification of strains that carry mannanase genes from this family as well as providing the necessary sequence information for the isolation of the entire genes by genomic walking. These consensus primers have been successfully tested against the genes known to be present in genomic DNA from *D. thermophilum Rt46B.1* and *Caldicellulosiruptor Rt8B.4* and have revealed several other family 26 mannanases present in culturable and unculturable hemicellulolytic thermophilic bacteria (Bergquist *et al.*, 1996). Further sequence information on family 5 mannanases is required before consensus primers can be designed to allow the identification and isolation of these genes.

APPLICATIONS

There are many applications for mannanases in the industrial processes. The worldwide requirement for enzymes for individual applications varies considerably. Mannanases are used mainly for improving the quality of food, feed and aiding in enzymatic bleaching of softwood pulps in the paper and pulp industries. There is a paucity of knowledge about the roles that govern the diverse specificity of these enzymes. Deciphering these secrets would enable us to exploit mannanases for their applications in biotechnology. The following section will discuss details of some of the most promising applications of mannanases (Howard *et al.*, 2003; Galbe *et al.*, 2002; Comfort *et al.*, 2004).

Paper and Pulp Industry

The most potent application of mannanase is consistent with its potential use in enzymatic bleaching of softwood pulps. The extraction of lignin from wood fibers is an essential step in bleaching of dissolving

pulps. Pulp pretreatment under alkaline conditions hydrolyzes hemicelluloses covalently bound to lignin and thus facilitates subsequent removal of lignin. There is a drawback to alkaline treatment of wood pulps, however, in that it creates an environmental pollution problem (Hongpattarakere, 2002). The alternate use of mannanases equally facilitates lignin removal in pulp bleaching and yields results comparable to alkaline pretreatment (Cuevas *et al.*, 1996). Consequently, to substitute within a pulp-bleaching sequence an enzymatic pretreatment for the ultrahot alkaline extraction stage (Clarke *et al.*, 2000) offers the possibility of significant reduction in environmental pollution and thus is of considerable interest to the pulp and paper industry. To be feasible, however, enzymatic bleaching requires that hemicellulase treatment not impair pulp quality by attacking cellulose fibers. Softwoods from which the majority of pulps are derived contain as much as 15 to 20% hemicellulose in the form of galactomannan (Suurnäkki *et al.*, 1996). Mannanases having substrate specificities for galactomannan constituents would make excellent candidates for use in enzymatic bleaching of softwood pulps (Gubitz *et al.*, 1997). Moreover, pulping is best carried out at elevated temperatures, thermophilic mannanases could offer significant advantages over mesophilic mannanases in terms of their higher intrinsic stability and catalytic efficiencies at such elevated temperatures (Yanhe *et al.*, 2004). Mannanase is useful in chlorine-free bleaching processes for paper pulp (chemical pulps, semichemical pulps, mechanical pulps or kraft pulps) in order to increase the brightness, thus decreasing or eliminating the need for hydrogen peroxide in the bleaching process (Tenkanen *et al.*, 1997).

Hydrolysis of Coffee Extract

Different mannanase preparations are used for the hydrolysis of coffee mannan, thus reducing significantly the viscosity of coffee extracts. Mannan is the main polysaccharide component of these extracts and is responsible for their high viscosity, which negatively affects the technological processing of instant coffee. Mannanase may also be used for hydrolyzing galactomannans present in a liquid coffee extract, preferably in order to inhibit gel formation during freeze drying of the (instant) coffee. Coffee mannan is isolated from green defatted beans by delignification, acid wash and subsequent alkali extraction with a yield of 12.8%. Additionally, coffee extract polysaccharides are separated by alcohol precipitation and are found to form nearly half of the coffee extract dry weight. These isolated mannans as well as the mannans in the coffee extract are efficiently hydrolyzed by the mannanase, which resulted in significant viscosity reductions (Sachslehner *et al.*, 2000; Nicolas *et al.*, 1998). Concurrently, the reducing sugar content increased continuously due to the release of various mannooligosaccharides including mannotetraose, mannotriose, and mannobiose. Both a partially purified, immobilized and a soluble, crude mannanase preparation are successfully employed for the degradation of coffee mannan (Nunes *et al.*, 1998, 2006).

Use in Detergent Industry

Application of carbohydrases in detergents is well known. Amylases and cellulases being the most common enzymes. Recently, alkaline mannanases stable to constituents of detergents have found application in certain laundry segments as stain removal boosters. Mannanases hydrolyze mannan containing materials like gums (galactomannans, glucomannans and guar gum). These gums are used worldwide as a thickener or stabiliser in many types of household products and foods including ice-creams, BBQ sauces, hair styling gels, shampoos, conditioners and toothpaste (Wong and Saddler, 1992). Stains containing mannan are generally difficult to remove because mannans have a tendency to adsorb to cellulose fibers and therefore bind to cotton textiles. Further, mannan also has a "glue effect." This gluing effect means that particulate soils released during the wash cycle or from subsequent wear can bind to invisible residual mannan. In other words, not only many mannan stains reappear, but mannan can also be transferred to otherwise clean fabrics during washing and result in fabric greying. Mannanases cleave the β-1,4-linkages of mannans through hydrolysis, thus breaking down the gum polymer into smaller carbohydrate fragments. These smaller, more water-soluble polysaccharide fragments remain free from the fabric and are siphoned out of the wash. Mannanases can thus prevent redeposition of soil released during washing.

The cleaning compositions must contain at least one additional detergent component. These compositions can also be formulated as sanitization products, contact lens cleansers, dishwashing, hard surface cleaner,

and health and beauty care products (Bettiol *et al.*, 2000). Such additive products are intended to supplement the performance of conventional compositions and can be added at any stage of the cleaning process (McCoy, 2001). Treatment with cleaning or detergent compositions comprising the mannanase can improve whiteness as well as prevent binding of certain soils to the cellulosic material. Accordingly, mannanase are used in cleaning compositions, including laundry, personal cleansing and oral/dental compositions, Such cleaning compositions comprising a mannanase and an enzyme selected from cellulases, proteases, lipases, amylases, pectin degrading enzymes and xyloglucanases, are prepared to provide superior cleaning performance.

To Improve the Nutritional Value of Poultry Feeds

Reduction in weight gain and feed conversion efficiency in poultry birds has been associated with intestinal viscosity due to different feeds. β-Mannan is a polysaccharide commonly found in feed ingredients such as soybean meal (SBM), palm kernel meal (PKM), copra meal (CM), guar gum meal and sesame meal. There is almost universal use of soybean meal and full fat soy as protein sources in poultry feeds, PKM and CM as ruminant feed (Moss and Givens, 1994; Chandrasekariah *et al.*, 2001) pig diets (Pettey *et al.*, 2002) and rabbit diets (Aganga *et al.*, 1991). All these meals have some common properties: high fiber content, low palatability, lack of several essential amino acids and high viscosity coupled with several antinutritional properties such as mannan, galactomannan, xylan and arabinoxylan, their utilization in the intestine is very limited. β-Mannans have been found to be highly deleterious to animal performance, severely compromising weight gain and feed conversion as well as glucose and water absorption. Incorporation of β-mannanase into these diets results in decreased intestinal viscosity, thus improving both the weight gain of chicks and their feed conversion efficiency. More recently, the beneficial effect of enzymatic degradation of β-mannan by addition of β-mannanase to diets containing SBM has been documented in broilers (Jackson *et al.*, 2004; Daskiran *et al.*, 2004; Lee *et al.*, 2003), layers (Wu *et al.*, 2005), turkeys (Odetallah *et al.*, 2002) and swine (Pettey *et al.*, 2002). Using endo-mannanase alone may only produce a small proportion of mannose and thus only a small amount of mannan is likely to be absorbed in the intestine of broilers (Saki *et al.*, 2005). A combination of endo-mannanase from different strains may have a complementary ability to cleave sugars, the use of a combination of endo-mannanase from different strains of organisms may be helpful and that is more possible in the poultry industry. A study of endo-mannanases has been done by Tamaru *et al.* (1997), who found that endo-mannanase from *Vibrio sp.* could not hydrolyse mannotriose but produced mannotriose, while endo-mannanase from *Streptomyces* could hydrolyse mannotriose to form mannose and mannobiose (Kusakabe and Takashi, 1988). Due to the fact that most of mannanase in the market is in form of endo β-mannanase (e.g. gamanase and hemicell mannanase), it can be speculated that manno-oligosaccharides, mannotriose and mannobiose as well as a small amount of mannose are generated when this type of enzyme is included in the diet. Since only mannose can be absorbed in the intestine, the production of mannobiose and mannooligosaccharides are, to some extent, useless. So these components of carbohydrates may not be absorbed and are therefore unable to supply energy to the host. However, in the sense of the health of the poultry, the production of mannooligosaccharides can improve a chicken's health (Laere *et al.*, 1999), either by increasing the population of specific bacteria such as *Bifidiobacteria* because the supply of these types of carbohydrates are a source of feed for bacteria in the caeca and thus suppressing the pathogenic ones, or by flushing out the pathogenic bacteria which attach to the mannooligosaccharides. In fact, mannooligosaccharides are added to the poultry diets for this effect (Lyons and Jacques, 2002). The use of mannanase in poultry research has been widespread and proven to be successful (Sundu *et al.*, 2006).

Use in Oil Drilling

Mannanases can also be used to enhance the flow of oil or gas in drilling operations. Practically, product flow to the well bore is stimulated by forcing out open crevices in the surrounding bedrock, which is done by flooding the well with a natural polymer (guar gum) solution and sand particles, capping the well and then pressurizing the bedrock until it fractures. The viscous polymer solution carries the sand through the fractures, propping open cracks for oil and gas flow. To facilitate product flow, the polymer solution is thinned (Adams

et al., 1995; Christoffersen, 2004). As mannanases are able to hydrolyze guar gum at elevated temperatures (Politz *et al.*, 2000), so implementation of oil production by these enzymes could be feasible.

In Oil Extraction of Coconut Meats

Mannanases can be used in enzymatic oil extraction of coconut meat. The main components of the structural cell wall of coconut meat are mannan and galactomannan (Saittagaroon *et al.*, 1983). A high oil yield is achieved in this process. In the traditional method of oil extraction by expeller, unsanitary handling, drastic processing of copra for oil extraction, refining of the product, the presence of polycylic aromatic hydrocarbons (PAH) retained in the coconut oil not only lessen the quality of the by-product like protein but also render the product susceptible to aflatoxin contamination and oxidative rancidity. The enzymatic oil extraction process not only eliminates such problems but also improves the sensory qualities of the products. In addition, the improvement of the quality of coconut oil minimizes the need for refining the oil as it is already comparable to the semi-refined coconut oil. This enzymatic process gives three valuable products: coconut oil, an aromatic protein containing liquid that may be used as a beverage base and a coconut protein isolate (Chen *et al.*, 2003).

In the Textile and Cellulosic Fiber Processing Industries

The mannanase can be used for preparation of fibers or for cleaning of fibers in combination with detergents in the textile and cellulosic processing industry. The processing of cellulosic material for the textile industry into a material ready for garment manufacture involves several steps: spinning of the fiber into a yarn, construction of woven or knit fabric from the yarn and subsequent preparation, dyeing and finishing operations. Woven goods are constructed by weaving a filling yarn between a series of warp yarns; the yarns could be of two different types. Mannanase is useful in an enzymatic scouring process and in desizing (removal of mannan size) during the preparation of cellulosic material for proper response in subsequent dyeing operations (Palackal *et al.*, 2006).

For Degradation of Thickening Agents

Galactomannans such as guar gum and locust bean gum are widely used as thickening agents, e.g. in food and print paste for textile printing such as prints on T-shirts. Mannanase can be used for reducing the viscosity of residual food in processing equipment and thereby facilitate cleaning after processing. The enzyme or enzyme preparation is useful for reducing viscosity of print paste, thereby facilitating wash out of surplus print paste after textile printings (Ademark *et al.*, 1998).

As Non-Nutritional Food Additives

Mannanases also contribute to the human health as they degrade mannans, which otherwise are resistant to mammalian digestive enzymes in the small intestine but are readily fermented in the large intestine, particularly by probiotic bacteria belonging to the genera *Bifidobacteria* and *Lactobacillus*. Prebiotic oligosaccharides including mannooligosaccharides, i.e. hydrolysis products of mannan degradation, are believed to promote the selective growth and proliferation of human beneficial intestinal microflora (Kobayashi *et al.*, 1984, 1987).

Thus, mannanases only recently attracted increased scientific and commercial attention due to potential applications in several industries. A few commercial products have been launched successfully worldwide in the past few years (Table 5).

CONCLUSION

The benefit of employing novel enzymes for specific industrial processes is well recognized with the discovery of β-mannanases. β-Mannanases (3.2.1.78) hydrolyze mannan based hemicelluloses and liberate short β-1,4 manno-oligomers, which can be further hydrolyzed to mannose by β-mannosidases (EC 3.2.1.25). There are currently about 50 β-mannanase gene sequences in GH families 5 and 26.The increasing number of new microbial genomes is revealing new mannolytic systems. Major challenges in this field include the design of efficient enzyme system for commercial applications. Mannanases occur ubiquitously in animals, plants, and microbes. However, microbes are most potent producers of mannanases and represent the preferred source of enzymes in view of their rapid growth, limited space required for cultivation, and

TABLE 5 List of commercial mannanases and their suppliers

Product Trade Name	Supplier	Source Microorganism	Recommended pH/Temperature	Action Pattern	Applications
Hemicell	ChemGen, USA	*Trichoderma longibrachiatum* and *B. lentus*	—	endo-mannanase	Animal feed supplement
Gamanase	Novo Nordisk, Denmark	*Aspergillus niger*	3–6/20–80°C	endo-mannanase	Coconut oil extraction
Mannaway	P&G (Novozyme)	*Bacillus* sp.	—	endo-mannanase	Detergent
Purabrite, Mannastar	Genencor, USA	Fungal mannanase	—	endo-mannanase	Detergent
Pyrolase 160, Pyrolase 200	Diversa (NASDAQ-DVSA)	Organism from hydrothermal vents	5–10/37–93°C	endo-mannanase and exo-mannanase	Oil recovery and well drilling

Sourced from individual company web sites, annual reports and literature. See www.diversa.com, http://www.biteomics.com, http://www.chemgen.com, http://www.novozymes.com, www.novonordisk.com, http://www.genencor.com.

ready accessibility to genetic manipulation. Microbial mannanases have been used recently in the food, feed and detergent industries. Advances in genetic manipulation of microorganisms have opened new possibilities for the introduction of predesigned changes, resulting in the production of tailor-made mannanases with novel and desirable properties. The development of recombinant mannanases and their commercialization by P&G, ChemGen and Genencor is an excellent example of the successful application of modern biology to biotechnology.

Industrial applications of mannanases have posed several problems and challenges for their further improvements. The biodiversity represents an invaluable resource for biotechnological innovations and plays an important role in the search for improved strains of microorganisms used in the industry. A recent trend has involved conducting industrial reactions with enzymes reaped from exotic microorganisms that inhabit hot waters, freezing Arctic waters, saline waters, or extremely acidic or alkaline habitats. The mannanases isolated from extremophilic organisms are likely to mimic some of the unnatural properties of the enzymes that are desirable for their commercial applications. Exploitation of biodiversity to provide microorganisms that produce mannanases well suited for their diverse applications is considered to be one of the most promising future alternatives. The existing knowledge about the structure-function relationship of mannanases, coupled with novel techniques, promises a fair chance of success, in the near future, in evolving mannanases that were never made in nature and that would meet the requirements of the multitude of mannanase applications.

REFERENCES

Adams, M. W., and Kelly, R. M. 1995. Enzymes from microorganisms in extreme environment. *Chem. Eng. News* 18: 32–42.

Ademark, P., Varga, A., Medve, J., Harjunpaa, V., Drakenberg, T., Tjerneld, F., and Stålbrand, H. 1998. Softwood hemicellulose-degrading enzymes from *Aspergillus niger*: purification and properties of a β-mannanase. *J. Biotechnol*. 63: 199–210.

Aganga, A. A., Aduku, A. O., Abdulmalik, M., and Sekoni, A. 1991. The effect of different protein sources and their levels on the production of breeding rabbits. *J. Appl. Rabbit Research* 14: 30–33.

Akino, T., Kato, C., and Horikoshi, K. 1989. The cloned β–mannanase gene from alkalophilic *Bacillus sp. AM-001* produces two β-mannanases in *Escherichia coli*. *Arch. Microbiol.* 152: 10–15.

Akita, M., Takeda, N., Hirasawa, K., Sakai, H., Kawamoto, M., Yamamoto, M., Grant, W. D., Hatada, Y., Ito, S., and Horikoshi, K. 2004. Crystallization and preliminary X-ray study of alkaline mannanase from an alkaliphilic *Bacillus* isolate. *Acta Crystallogr.* 60: 1490–1492.

Araujo, A., and Ward, O. P. 1990. Hemicellulases of *Bacillus* species: preliminary comparative studies on production and properties of mannanases and galactanases. *J. Appl. Bacteriol.* 68: 253–261.

Arcand, N., Kluepfel, D., Paradis, F. W., Morosoli, R., and Sharek, F. 1993. β-Mannanase of *Streptomyces lividans* 66: cloning and DNA sequencing of the *manA* gene and characterization of the enzyme. *J. Biochem.* 290: 857–863.

Beauchemin, K. A., Morgavi, D. P., Mcallister, T. A., Yang, W. Z., and Rode, L. M. 2001. The use of enzymes in ruminant diets. pp 296–322. In Wiseman, J., Garnsworthy, P.C. (eds.), Recent Advances in Animal Nutrition. Nottingham University Press, Nottingham, UK.

Bettiol, J. L. P., Boutique, J. P., Gualco, L. M. P., and Johnston, J. P. 2000. Nonaqueous liquid detergent compositions comprising a borate releasing compound and a mannanase. EP1059351.

Bettiol, J. P., and Showell, M. S. 2002. Detergent compositions comprising a mannanase and a protease. US Patent 6376445.

Bhat, M. K. 2000. Cellulases and related enzymes in biotechnology. *Biotechnol. Adv*. 18: 355–383.

Bicho, P. A., Clark, T. A., Mackie, K., Morgan, H. W., and Daniel, R. M. 1991. The characterisation of a thermostable endo-β–mannanase cloned from *Caldocellum saccharolyticum*. *Appl. Microbiol. Biotechnol.* 36: 337–343.

Bolam, D. N., Hughes, N., Virden, R., Lakey, J. H., Hazlewood, G. P., Henrissat, B., Braithwaite, K. L., and Gilbert, H. J. 1996. Mannanase A from *Pseudomonas fluorescens sp. Cellulosa* is a retaining glycosyl hydrolase in which E212 and E230 are the putative catalytic residues. *Biochem.* 35: 16195–16204.

Boraston, A. B., Bolam, D. N., Gilbert, H. J., and G. J. Davies. 2004. Carbohydrate-binding modules: fine tuning polysaccharide recognition. *Biochem. J.* 382: 769–781.

Braithwaite, K. L., Black, G. W., Hazlewood, G. P., Ali, B. R., Gilbert, and H. J. 1995. A non-modular endo-beta-1,4-mannanase from *Pseudomonas fluorescens* subspecies *cellulosa. Biochem J*. 305: 1005–1010.

Buckeridge, M. S., Pessoa, dos Santos, H., and Tiné, M. A. S. 2000. Mobilisation of storage cell wall polysaccharides in seeds. *Plant Physiol. Biochem.* 38: 141–156.

Cann, I. K., Kocherginskaya, S., King, M. R., White, B. A., and Mackie, R. I. 1999. Molecular cloning, sequencing, and expression of a novel multidomain mannanase gene from *Thermoanaerobacterium polysaccharolyticum. J. Bacteriol.* 181: 1643–1651.

Capoe, P., Kubackova, M., Alfoldi, J., *et al*. 2000. Galactoglucomannan from the secondary cell wall of *Picea abies*.L.Krast. *Carbohydr. Res.* 329: 635–645.

Chandrasekariah, M., Sampath, K. T., Thulasi, A., and Anandan, S. 2001. Insitu protein degradability of certain feedstuffs in cattle. *Ind. J. Ani. Sci.* 71: 261–264.

Chang, T. T., Yang, X. Y., and Ko, W. H. 1992. A sensitive method for detecting producion of extracellular enzymes by fungi on solid media. *Mycologia.* 84: 923–925.

Chanzy, H., Dube, M., Marchessault, R. H., *et al*. 2004. Single crystals and oriented crystallization of ivory nut mannan. *Biopoly.* 18: 887–898.

Charrier, M., and Rouland, C. 2001. Mannan-degrading enzymes purified from the crop of the brown garden snail *Helix aspersa* Müller (*Gastropoda Pulmonata*). *J. Expt. Zool.* 290: 125–135.

Chen, B. K., and Diosady, L. L. 2003. Enzymatic aqueous processing of coconuts. *Int. J. Appl. Sci. Engg.* 1: 55–61.

Chen, X., Cao, Y., Ding, Y., Lu, W., and Li, D. 2007. Cloning, functional expression and characterization of *Aspergillus sulphureus* beta-mannanase in *Pichia pastoris*. *J. Biotechnol.* 128(3): 452–461.

Chen, Y., Long, J., Liao, L., *et al*. 2000. Study on the production of beta-mannanase by *Bacillus* M50. *Wei Sheng Wu Xue Bao.* 40: 62–68.

Chhabra, S. R., Shockley, K. R., Ward, D. E., and Kelly, R. M. 2002. Regulation of endo-acting glycosyl hydrolases in the hyperthermophilic bacterium *Thermotoga maritima* grown on glucan and mannan based polysaccharides. *Appl. Environ. Microbiol.* 68: 545–554.

Christgau, S., Kauppinen, S., Vind, J., Kofod, L. V., and Dalbøge, H. 1994. Expression, cloning, purification and characterization of a beta-1,4-mannanase from *Aspergillus aculeatus. Biochem. Mol. Biol. Int*. 33: 917–925.

Christoffersen, L. 2004. Products and applications that promote sustainability. In *REBIO 2004 (DIVERSA)*, Boca Chica, Dominican Republic.

Civas, A., Eberhard, R., le Dizet, P., and Petek, F. 1984. Glycosidases induced in *Aspergillus tamarii* secreted α-D-galactosidase and β-D-mannanase. *Biochem. J.* 219: 857–863.

Clarke, J. H., Davidson, K., Rixon, J. E., Halstead, J. R., Fransen, M. P., and Gilbert, H. J. 2000. A comparison of enzyme aided bleaching of softwood paper pulp using combinations of xylanase, mannanase and alpha-galactosidase. *Appl. Microbiol. Biotechnol.* 53: 661–667.

Comfort, D. A., Chhabra, S. R., Conners, S. B., Chou, C.-J., Epting, K. L., Johnson, M.R., Jones, K.L., Sehgal, A.C., and Kelly, R.M. 2004. Strategic biocatalysis with hyperthermophilic enzymes. *Green Chemistry* 6: 459–465.

Cuevas, W. A., Kantelinen, A., Tanner, P., Bodie, B., and Leskinen, S. 1996. Purification and characterization of novel mannanases used in pulp bleaching. pp. 123–126. In: Srebotnik E., Messner K., Srebotnik, E., Messner, K., eds. *Biotechnology in the Pulp and Paper Industry*. Vienna, Austria: Facultas-Universitätsverlag.

Cui, F., Shi, J., and Lu, Z. 1999. Production of neutral beta-mannanase by *Bacillus subtilis* and its properties. *Wei Sheng Wu Xue Bao.* 39(1): 60–63.

Daskiran, M., Teeter, R. G., Fodge, D., and Hsiao, H. Y. 2004. An evaluation of endo-β-D-mannanase (Hemicell) effects on broiler performance and energy use in diets varying in β-mannan content. *Poultry Sci.* 83: 662–668.

Davies, G., Sinnott, M. L., and Withers, S. G. 1998. Glycosyl Transfer. *Comp. Biol. Catal.* 1: 119–209.

Dekker, R. F. H. 1985. Biodegradation of hemicelluloses. pp. 505–533. In J. Higuchi (ed.), *Biosynthesis and Biodegradation of Wood Components*, Academic Press Inc., Orlando, FL.

Dekker, R. F. H., and Richards, G. N. 1976. Hemicelluases: their occurrence, purification, properties and mode of actions. *Adv. Carbohydr. Chem. Biochem.* 32: 277–352.

De-Nicolás-Santiago, S., Regalado-González, C., García-Almendárez, B., Fernández, F. J., Téllez-Jurado, A., and Huerta-Ochoa, S. 2006. Physiological, morphological, and mannanase production studies on *Aspergillus niger* uam-gs1 mutants. *Electron. J. Biotechnol.* 9 Epub Jan. 2006.

Dingle, J., Reid, W. W., and Solamons, G. L. 1953. The enzymatic degradation of Pectin and other polysaccahrides II, Application of the "Cup Plate" Assay to the estimation of enzymes. *J. Sci. Food Agric.* 4: 149.

Dong, X. Z., Schyns, P. J., and Stams, A. J. 1991. Degradation of galactomannan by a *Clostridium butyricum* strain. *Antonie Van Leeuwenhoek*. 60: 109–114.

Downie, B., Hilhorst, W. M., and Bewley, J. D. 1994. A new assay for quantifying endo-β-mannanase activity using congo red dye. *Phytochemistry* 36: 829–835.

Duffaud, G. D., McCutchen, C. M., Leduc, P., Parker, K. N., and Kelly, R. M. 1997. Purification and characterization of extremely thermostable β-mannanase, β-mannosidase, and α-galactosidase from the hyperthermophilic eubacterium *Thermotoga neopolitana* 5068. *Appl. Environ. Microbiol.* 63: 169–177.

Fanutti, C., Ponyi, T., Black, G. W., Hazlewood, G. P., and Gilbert, H. J. 1995. The conserved noncatalytic 40-residue sequence in cellulases and hemicellulases from anaerobic fungi functions as a protein docking domain. *J. Biol. Chem*. 270(49): 29314–29322.

Filichkin, S. A., Leonard, J. M., Liu, P. P., and Nonogaki, H. 2000. A novel endo-mannanase gene in tomato LeMAN5 is associated with anther and pollen development. *Plant Physiol.* 134:1080–1087.

Franco, P. F., Ferreira, H. M., and Filho, E. X. 2004. Production and characterization of hemicellulase activities from *Trichoderma harzianum* strain *T4*. *Biotechnol Appl. Biochem.* 40: 255–259.

Galbe, M., and Zacchi, G. 2002. A review of the production of ethanol from softwood. *Appl. Microbiol. Biotechnol.* 59: 618–628.

Gherardini, F., Babcock, M., and Salyers, A. 1987. Purification and characterization of a cell-associated, soluble mannanase from *Bacteroides ovatus*. *J. Bacteriol.* 169: 2038–2043.

Gibbs, M. D., Reeves, R. A., Sunna, A., and Bergquist, P. L. 1999. Sequencing and expression of a β-mannanase gene from the extreme thermophile *Dictyoglomus thermophilum* Rt46B.1, and characteristics of the recombinant enzyme. *Curr. Microbiol*. 39(6): 351–357.

Gibbs, M. D., Elinder, A. U., Reeves, R. A., and Bergquist, P. L. 1996. Sequencing, cloning and expression of the β-1,4-mannanase gene *manA* from the extremely thermophilic anaerobic bacterium *Caldicellulosiruptor* Rt8B.4. *FEMS Microbiol. Lett.* 141: 37–43.

Großwindhager, C., Sachslehner, A., Nidetzky, B., and Haltrich, D. 1999. Endo-β-1,4 mannanase is efficiently produced by *Sclerotium (Athelia) rolfsii* under derepressed conditions. *J Biotechnol* 67: 189–203.

Gübitz, G. M., Hayn, M., Sommerauer, M., and Steiner, W. 1996. Mannan-degrading enzymes from *Sclerotium rolfsii*: Characterization and synergism of two endo β-mannanases and a β-mannosidase. *Bioresource Technol.* 58: 127–135.

Gübitz, G. M., Lischnig, T., Stebbing, D., and Saddler, J. N. 1997. Enzymatic removal of hemicellulose from dissolving pulps. *Biotechnol. Lett.* 19: 491–495.

Hagglund, P., Eriksson, T., Collen, A., Nerinckx, W., Claeyssens, M., and Stalbrand, H. A. 2003. Cellulose-binding module of the *Trichoderma reesei* beta-mannanase Man5A increases the mannan-hydrolysis of complex substrates. *J. Biotechnol.* 101: 37–48.

Halstead, J. R., Vercoe, P. E., Gilbert, H. J., Davidson, K., and Hazlewood, G. P. 1999. A family 26 mannanase produced by *Clostridium thermocellum* as a component of the cellulosome contains a domain which is conserved in mannanases from anaerobic fungi. *Microbiol.* 145: 3101–3108.

Han, Y., Kye, C., Hong, S., Sun, L., Yong, K., Goon, K., and Hoon, K. 2006. A cel44C-man26A gene of endophytic *Paenibacillus polymyxa* GS01 has multi-glycosyl hydrolases in two catalytic domains. *Appl. Microbiol Biotechnol*. 73(3): 618–630.

Handford, M. G., Baldwin, T. C., Goubet, F., *et al*. 2003. Localization and characterization of cell wall mannan polysaccharides in *Arabidopsis thaliana*. *Planta.* 218: 27–36.

Harjunpaa, V., Teleman, A., Siika-Aho, M., and Drakenberg, T. 1995. Kinetic and stereochemical studies of mannooligosaccharide hydrolysis catalyzed by β-mannanases from *Trichoderma reesei*. *Eur. J. Biochem.* 234: 278–283.

Hatada, Y., Takeda, N., Hirasawa, K., Ohta, Y., Usami, R., Yoshida, Y., Ito, S., and Horikoshi, K. 2005. Sequence of the gene for a high-alkaline mannanase from an alkaliphilic *Bacillus sp.* strain JAMB-750, its expression in *B. subtilis* and characterization of the recombinant enzyme. *Extremophiles.* 9: 497–500.

Helow, E. R., and Khattab, A. A. 1996. The development of a *B. subtilis* 168 culture condition for enhanced and accelerated beta-mannanase production. *Acta Microbiol. Immunol. Hung.* 43: 289–299.

Henrissat, B. 1990. A classification of glycosyl hydrolases based on amino acid sequence similarities. *Biochem. J.* 280: 304–316.

Henrissat, B., and Bairoch, A. 1993. New families in the classification of glycosyl hydrolases based on amino acid sequence similarities. *Biochem. J.* 293: 781–788.

Henrissat, B., and Bairoch, A. 1996. Updating the sequence based classification of glycosyl hydrolases. *Biochem. J.* 316: 695–696.

Henrissat, B., Callebaut, I., Fabrega, S., Lehn, P., Mornon, J. P., and Davies, G. 1995. Conserved catalytic machinery and the prediction of a common fold for several families of glycosyl hydrolases. *Proc. Natl. Acad. Sci. U.S.A.* 92: 7090–7094.

Henrissat, B., Teeri, T., and Warren, R. A. J. 1998. A scheme for designating enzymes that hydrolyse the polysaccharides in the cell walls of plants. *FEBS Lett.* 425 (2): 352–354.

Hilge, M., Gloor, S. M., Rypniewski, W., Sauer, O., Heightman, T. D., Zimmerman, W. *et al*. 1998. High-resolution native and complex structures of thermostable β-mannanase from *Thermomonospora fusca*-substrate specificity in glycosyl hydrolase family 5. *Structure* 6: 1433–1444.

Hogg, D., Pell, G., Dupree, P., Goubet, F., Martin, M., Armand, S., and Gilbert, H. J. 2003. The modular architecture of *Cellvibrio japonicus* mannanases in glycoside hydrolase families 5 and 26 points to differences in their role in mannan degradation. *Biochem. J.* 371: 1027–1043.

Hongpattarakere, T. 2002. Hyperthermostable cellulolytic and hemicellulolytic enzymes and their biotechnological applications. *Songklanakarin J. Sci. Technol.* 24: 481–491.

Horikoshi, K. 1996. Alkaliphiles—from an industrial point of view. *FEMS Microbiol. Rev.* 18: 259–270.

Howard, R. L., Abotsi, E., Jansen van Rensburg, E. L., and Howard, S. 2003. Lignocellulose biotechnology: issues of bioconversion and enzyme production. *African J. Biotechnol.* 2 (12): 602–619.

Huang, S. P., Wang, C. L., Zhang, G. M., and Ma, L. X. 2007. Construction of a double functional recombinant strain of *Pichia pastoris* co-expressing phytase and mannanase and the enzymatic analysis. *Wei Sheng Wu Xue Bao.* 47(2): 280–284.

Jackson, M. E., Geronian, K., Knox, A., McNab, J., and McCartney, E. 2004. A dose-response study with the feed enzyme β-Mannanase in broilers provided with corn-soybean meal based diets in the absence of antibiotic growth promoters. *Poultry Sci*. 83: 1992–1996.

Jenkins, J., Leggio, L., Harris, G., and Pickersgill, R. 1995. Beta-glucosidase, beta-galactosidase, family A cellulases, family F xylanases and two barley glycanases form a superfamily of enzymes with $(\beta/\alpha)_8$ fold architecture and with two conserved glutamates near the carboxy terminal ends of β-strands four and seven. *FEBS Lett.* 362: 281–285.

Johnson, K. G., and Ross, N. W. 1990. Enzymatic properties of β-mannanase from *Polyporus versicolor*. *Enzyme Microb. Technol.* 12: 960–964.

Kansoh, A. L., and Nagieb, Z. A. 2004. Xylanase and mannanase enzymes from *Streptomyces galbus* NR and their use in biobleaching of softwood kraft pulp. *Anton. van. Leeuwonhoek*. 85: 103–114.

Kataoka, N., and Tokiwa, Y. 1998. Isolation and characterization of an active mannanase-producing anaerobic bacterium, *C. tertium* KT-5A from lotus soil. *J. Appl. Microbiol.* 84: 357–367.

Kauppinen, M. S., Schulein, M., Schnorr, K., Andersen, L. N., and Bjørnvad, M. E. 2003. Mannanases. US Patent 6,566,114.

Kobayashi, Y., Echigen, R., Mada, M., and Mutai, M. 1984. Effect of hydrolyzate of konjac mannan and soyabean oligisaccharides on intestinal flora in man and rats. pp. 69–90. In: T. Mitsuoka (Ed.), Intestinal flora and dietary factors. *Proceedings of 4th RIKEN Symposium on Intestinal Flora*, Japan Scientific Socities Press, Tokyo.

Kobayashi, Y., Echigen, R., Mada, M., and Mutai, M. 1987. Effect of hydrolyzate of konjac mannan and soyabean oligisaccharides on intestinal flora in man and rats. pp. 79–97 In: T. Mitsuoka (Ed.), *Intestinal Flora and Food Factors*. Gakkai Shuppan Centre, Tokyo.

Kurokawa, J., Hemjinda, E., Arai, T., Karita, S., Kimura, T., and Sakka, K. 2001. Sequence of the *Clostridium thermocellum* mannanase gene man26B and characterization of the translated product. *Biosci Biotechnol. Biochem.* 65: 548–554.

Kusakabe, I., and Takashi, R. 1988. Enzymatic preparation β-1,4-mannooligosaccharides and β-1,4-glucomannooligosaccharides. *Methods in Enzymol.* 160: 518–523.

Laere, K. M., Hartemink, J. V., Beldman, R., Pitson, G., Dijkema, S., Schols, C. *et al*. 1999. Transglycosidase activity of *Bifidiobacterium adolescentis* DSM 20083 galactosidase. *Appl. Microbiol. Biotechnol.* 52: 681–688.

Le Nours, J., Anderson, L., Stoll, D., Stålbrand, H., and Leggio, L. L. 2005. The structure and characterization of a modular endo-β-1,4-mannanase from *Cellulomonas fim*i. *Biochem.* 44: 12700–12708.

Lee, J. T., Bailey, C. A., and Cartwright, A. L. 2003. β-mannanase ameliorates viscosity-associated depression of growth in broiler chickens fed guar germ and hull fractions. *Poultry Sci.* 82:1925–1931.

Li, Y. N., Meng, K., Wangm Y. R., and Yao, B. 2006. A β-mannanase from *B. subtilis* B36: purification, properties sequencing, gene cloning and expression in *E. coli. Z. Naturforsch (C*). 61: 840–846.

Lin, S. S., Dou, W. F., Xu, H., Li, H. Z., Xu, Z. H., and Ma, Y. 2007. Optimization of medium composition for the production of alkaline β-mannanase by alkaliphilic *Bacillus* sp. N16-5 using response surface methodology. *Appl. Microbiol. Biotechnol.* 75 (5): 1015–1022.

Luthi, E., Bhana, J. N., Grayling, R. A., Love, D. R., and Bergquist, P. L. 1991. Cloning, sequence analysis and expression in *Escherichia coli* of a gene coding for a β-mannanase from the extremely thermophilic bacterium *Caldocellum saccharolyticum*. *App. Environ. Microbiol.* 57: 694–700.

Lundqvist, J., Teleman, A., Junel, L., Zacchi, G., Dahlman, O., Tjerneld, F., and Stalbrand, H. 2002. Isolation and characterization of galactoglucomannan from spruce (*Picea abies*). *Carbohydr. Polym.* 48: 29–39.

Lyons, T. P., and Jacques, K. A. (eds). 2002. *Nutritional Biotechnology in the Feed and Food Industries*. Nottingham University Press, Nottingham, UK.

Mabyalwa, M. M., and Setati, M. E. 2006. Screening and identification of endomannanase-producing microfungi from hypersaline environments. *Current Microbiol.* 52 (6): 477–481.

Marraccini, P., Rogers, W. J., Allard, C., Andre, M. L., Caillet, V., Lacoste, N., Lausanne, F., and Michaux, S. 2001. Molecular and biochemical characterization of endo-beta-mannanases from germinating coffee (*Coffea arabica*) grains. *Planta.* 213: 296–308.

Matsushita, O., Russell, J. B., and Wilson, D. B. 1991. A *Bacteroides ruminicola* β-1,4-endoglucanase is encoded in two reading frames. *J. Bacteriol.* 173: 6919–6926.

McCleary, B. V. 1979. Modes of action of β-mannanase enzymes of diverse origin on legume seed galactomannans. *Phytochemistry* 18: 757–763.

McCleary, B. V. 1988. β-D-mannanase. *Methods Enzymol*. 160: 596–610.

McCleary, B. V., and Matheson, N. K. 1986. Enzymic analysis of polysaccharide structure. *Adv. Carbohydr. Chem. Biochem.* 44: 147–176.

McCoy, M. 2001. An update on the latest developments within the detergent industry, also introducing the latest new detergent enzyme: a mannanase. *Chem. Eng. News* 20: 19–32.

Mendoza, N. S., Arai, M., Kawaguchi, T., Yoshida, T., and Joson, L. M. 1994. Isolation of mannan utilizing bacteria and culture conditions for mannanase production. *World J. Microbiol. Biotechnol*. 10: 51–54.

Millward-Sadler, S. J., Hall, J., Black, G. W., Hazlewood, G. P., and Gilbert, H. J. 1996. Evidence that the *Piromyces* gene family encoding endo-1,4-mannanase arose through gene duplication. *FEMS Microbiol. Lett.* 141: 183–188.

Morris, D. D., Reeves, R. A., Gibbs, M. D., Saul, D. J., and Bergquist, P. 1995. Correction of the the β-mannanase domain of the *celC* pseudogene from *Caldocellulosiruptor saccharolyticus* and activity of the gene product on kraft pulp. *Appl. Environ. Microbiol.* 61: 2262–2269.

Moss, A. R., and Givens. D. I. 1994. The chemical composition, digestibility, metabolisable energy content and nitrogen degradability of some protein concentrates. *Animal Feed Sci. Technol.* 47: 335–351.

Nakajima, N., and Matsuura, Y. 1997. Purification and characterization of konjac glucomannan degrading enzyme from anaerobic human intestinal bacterium, *Clostridium butyricum-Clostridium beijerinckii* group. *Biosci. Biotechnol. Biochem*. 61: 1739–1742.

Nicolas, P., Raetz, E., Reymond, S., and Sauvegeat, J. L. 1998. Hydrolysis of the galactomannans of coffee extract with immobilized β-mannanase. Patent US 5714183.

Nunes, F. M., and Coimbra, M. A. 1998. Influence of polysaccharide composition in foam stability of espresso coffee. *Carbohydr. Polym.* 37: 283–285.

Nunes, F. M., Reis, A., Domingues, M. R., and Coimbra, M. A. 2006. Characterization of galactomannan derivatives in roasted coffee beverages. *J. Agric. Food Chem.* 54(9): 3428–3439.

Odetallah, N. H., Ferket, P. R., Grimes, J. L., and McNaughton, J. L. 2002. Effect of mannan-endo-1,4-β-mannosidase on the growth performance of turkeys fed diets containing 44% and 48% crude protein soybean meal. *Poultry Sci.* 81: 1322–1331.

O'Neill, York. 2003. The composition and structure of plant primary walls. pp. 1-54. in *The Plant Cell Wall*, J.K.C. Rose (ed.), Blackwell, London.

Palackal, N., Christopher, L., Zaidi, S., Luginbuhl, P., Dupree, P., Goubet, F. *et al*. 2007. A multifunctional hybrid glycosyl hydrolase discovered in an uncultured microbial consortium from ruminant gut. *Appl. Microbiol. Biotechnol*. 74 (1): 113–124.

Parker, K. N., Chhabra, S. R., Lam, D., Callen, W., Duffaud, G. D., Snead, M. A., Short, J. M., Mathur, E. J., and Kelly, R. M. 2001. Galactomannanases Man2 and Man5 from *Thermotoga* species: growth physiology on galactomannans, gene sequence analysis, and biochemical properties of recombinant enzymes. *Biotechnol. Bioeng*. 75(3): 322–333.

Pason, P., Kyu, K. L., and Ratanakhanokchai, K. 2006. *Paenibacillus curdlanolyticus* Strain B-6 xylanolytic-cellulolytic enzyme system that degrades insoluble polysaccharides. *Appl. Environ. Microbiol.* 72: 2483–2490.

Paulsen, H. 1982. Advances in selective chemical syntheses of complex oligosaccharides. *Angew. Chem. Int. Ed. Engl.* 21(3): 155–224.

Perret, S., Belaich, A., Fierobe, H. P., Belaich, J. P., and Tardif, C. 2004. Towards designer cellulosomes in *Clostridia*: mannanase enrichment of the cellulosomes produced by *Clostridium cellulolyticum*. *Biotechnol. Appl. Biochem.* 40: 255–259.

Pettey, L. A., Carter, S. D., Senne, B. W., and Shriver, J. A. 2002. Effects of β-mannanase addition to corn-soybean meal diets on growth performance, carcass traits, and nutrient digestibility of weaning and growing-finishing pigs. *Anim. Sci.* 80: 1012–1019.

Politz, O., Krah, M., Thomsen, K. K., and Borriss, R. R. 2000. A highly thermostable endo-1,4-beta-mannanase from the marine bacterium *Rhodothermus marinus*. *Appl. Microbiol. Biotechnol.* 53 (6): 715–721.

Puchart, V., Vrsanska, M., Svoboda, P., Pohl, J., and Biely, P. 2004. Purification and characterization of two forms of endo- β-1,4-mannanase from a thermotolerant fungus, *Aspergillus fumigatus* IMI 385708 (formerly *T. lanuginosus* IMI 158749). *Biochimica et biophysica Acta.* 1674: 239–250.

Puls, J. 1997. Chemistry and biochemistry of hemicelluloses—relationship between hemicellulose structure and enzymes required for hydrolysis. *Macromol. Symp.* 120: 183–196.

Puls, J., and Schuseil, J. 1993. Technical and naturally occuring hemicelluloses. *Proceedings of 2nd Tricel Symposium on Trichoderma cellulases and other hydrolases*. 8: 51–60.

Regalado, C., Garcia-Almendarez, B. E., Venegas-Barrera, L. M., Tellez-Jurado, A., Rodriguez-Serrano, G., Huerta-Ochoa, S., and Whitaker, J. R. 2000. Production, partial purification and properties of β-mannanases obtained by solid substrate fermentation of spent soluble coffee wastes and copra paste using *Aspergillus oryzae* and *Aspergillus niger*. *J. Sci. Food Agric.* 80: 1343–1350.

Sabini, E., Schubert, H., Murshudov, G., Wilson, K. S., Siika-Aho, M., and Penttila, M. 2000. The three-dimensional structure of a *Trichoderma reesei* β -mannanase from glycoside hydrolase family 5. *Acta Crystallogr.* 56: 3–13.

Sachslehner, A., Foidl, G., Foidl, N., Gubitz, G., and Haltrich, D. 2000. Hydrolysis of isolated coffee mannan and coffee extract by mannanases of *Sclerotium rolfsii*. *J. Biotechnol.* 80:127–134.

Saittagaroon, S., Kawakishi, S., and Namiki, M. 1983. Characterization of polysaccharides of copra meal. *J. Sci. Food Agric*. 34: 855–860.

Saki, A. A., Mazugi, M. T., and Kamyab, A. 2005. Effect of mannanase on broiler performance, lleal and *in vitro* protein digestibility, uric acid and litter moisture in broiler feeding. *Int. J. Poultry Sci*. 4: 21–26.

Sakon, J., Adney, W. S., Himmel, M. E., Thomas, S. R., and Karplus, P. A. 1996. Crystal structure of thermostable family 5 endocellulase E1 from *Acidothermus cellulolyticus* in complex with cellotetraose. *Biochemistry* 35: 10648–10660.

Schroder, R., Wegrzyn, T. F., Bolitho, K. M., and Redgwell, R. J. 2004. Mannan transglycolase: A novel enzyme activity in cell walls of higher plants. *Planta* 219: 590–600.

Selvendran, O'Neill. 1985. Isolation and analysis of cell walls from plant material, pp. 25–123. In *Methods of Biochemical Analysis*, vol. 32, D. Glick (ed.), John Wiley & Sons, New York.

Setati, M. E., Ademark, P., Vanzyl, W. H., Hahn-Hagerdal, B., and Stålbrand H. 2001. Expression of the *Aspergillus aculeatus* endo-β-1,4-mannanase encoding gene (*man1*) in *Saccharomyces cerevisiae* and characterization of the recombinant enzyme. *Protein Express Purif*. 21: 105–114.

Shallom, D., and Shoham, Y. 2003. Microbial hemicellulases. *Curr. Opin. Microbiol.* 6: 219–228.

Shimahara, H., Suzuki, H., Sugiyama, N., and Nisizawa, K. 1975. Partial purification of β-mannanases from the konjac tubers and their substrate specificity in relation to the structure of konjac glucomannan. *Agri. Biol. Chem*. 39: 301–312.

Stålbrand, H. 2003. Enzymology of endo- β-1,4-mannanase. Handbook for Food-Enzymology, J. R. Whitaker, A. G. J. Voragen, D. W. S. Wong (eds.), Marcel Dekker, New York, Chapter 75, pp 961–969.

Stalbrand, H., Antov, M., Anderson, L., Andersson, A., and Tjerneld, F. 2006. Affinity partitioning of a *Cellulomonas fimi* beta-mannanase with a mannan-binding module in galactomannan/starch aqueous two-phase system. *J. Chromatogr.* 1123 (1): 53–59.

Stålbrand, H., Saloheimo, A., Vehmaanpera, J., Henrissat, B., and Penttila, M. 1995. Cloning and expression in *Saccharomyces cerevisiae* of a *Trichoderma reesei* β-mannanase gene containing a cellulose-binding domain. *Appl. Environ. Microbiol.* 61: 1090–1097.

Stålbrand, H., Siika-Aho, M., Tenkanen, M., and Viikari, L. 1993. Purification and characterisation of two β-mannanases from *Trichoderma reesei*. *J. Biotechnol.* 29: 229–242.

Stoll, D., Boraston, A., Stalbrand, H., McLean, B. W., Kilburn, D. G., and Warren, R. A. 2000. Mannanase Man26A from *Cellulomonas fimi* has a mannan-binding module. *FEMS Microbiol. Lett.* 183(2): 265–269.

Stoll, D., LeNours, J., Anderson, L., Stalbrand, H., and LoLeggio, L. 2005. The structure and characterization of a modular endo-β-1,4-mannanase from *Cellulomonas fimi*. *Biochem.* 44(38): 12700–12708.

Stoll, D., Stålbrand, H., and Warren, R. A. J. 2000. Hydrolysis of mannans by *Cellulomons fimi*, pp. 91–98. In M. E. Himmel, J. O. Baker, and J. N. Saddler (eds.), *Glycosyl Hydrolases for Biomass Conversion*, vol. 769, American Chemical Society, Washington, D.C.

Stoll, D., Stålbrand, H., and Warren, R. A. 1999. Mannan-degrading enzymes from *Cellulomonas fimi*. *Appl. Environ. Microbiol.* 65(6): 2598–2605.

Sun Y. M., Yu, H. Y., Wang, W. J., Yang, Y. S., and Yang, Y. H. 2003. Purification and properties of *Bacillus subtilis SA–22* endo-1,4-beta-D-mannanase. *Sheng Wu Gong Cheng Xue Bao.* 19(3): 327–330.

Sun, Y., and Cheng, J. 2002. Hydrolysis of lignocellulosic material from ethanol production: A review. *Biores. Technol*. 83: 1–11.

Sundu, B., Kumar, A., and Dingle, J. 2006. Response of broiler chicks fed increasing levels of copra meal and enzymes. *Int. J. Poul. Sci*. 5: 13–18.

Sunna, A., Gibbs, M., Chin, C. W., Nelson, J., and Bergquist, P. J. 2000. A gene encoding a novel multidomain β-1,4-mannanase from *Caldibacillus cellulovorans* and action of the recombinant enzyme on kraft pulp. *Appl. Environ. Microbiol.* 66: 664–670.

Sunna, A., M. D. Gibbs, and P. L. Bergquist. 2001. Identification of novel β-mannan- and β-glucanbinding modules: evidence for a superfamily of carbohydrate-binding modules. *Biochem. J.* 356 (3): 791–798.

Suurnakki, A. T., Clark, A., Allison, R. W., Buchert, J., and Viikari, L. 1996. Mannanase-aided bleaching of softwood kraft pulps. pp. 67-74. In *Proceedings of the 6th International Conference on Biotechnology in the Pulp and Paper Industry.* Facultas-Universitatsverlag, Vienna, Austria.

Sygusch, J., Ethier, N., and Talbot, G. 1998. Gene cloning, DNA sequencing, and expression of thermostable β-mannanase from *Bacillus stearothermophilus. Appl. Environ. Microbiol.* 64: 4428–4432.

Takeda, N., Hirasawa, K., Uchimura, K., Nogi, Y., Hatada, Y., Akita, M. *et al*. 2004a. Alkaline mannanase from a novel species of alkaliphilic *Bacillus. J. Appl. Glycosci.* 51: 229–236.

Takeda, N., Hirasawa, K., Uchimura, K., Nogi, Y., Hatada, Y., Akita, M. *et al*. 2004b. Purification and enzymatic properties of a highly alkaline mannanase from alkaliphilic *Bacillus* sp.strain JAMB-750. *J. Biol. Macromol*. 4: 67–74.

Talbot, G., and Sygusch, J. 1990. Purification and characterization of thermostable β-mannanase and α-galactosidase from *Bacillus stearothermophilus*. *Appl. Environ. Microbiol.* 56: 3505–3510.

Tamaru, Y., and Doi, R. H. 2000. The engL gene cluster of *Clostridium cellulovorans* contains a gene for cellulosomal ManA. *J. Bacteriol.* 182: 244–247.

Tamaru, Y., Araki, T., Morishita, T., Kimura, T., Sakka, K., and Ohmiya, K. 1997. Cloning, DNA sequencing, and expression of the beta-1,4-mannanase gene from a marine, *Vibrio* sp. strain MA-138. *J. Ferment. Bioeng*. 83: 201–205.

Tang, C.M., Waterman, L. D., Smith, M. H., and Thurston, C. F. 2001. The cel4 gene of *Agaricus bisporus* encodes a β-mannanase. *Appl. Environ. Microbiol.* 67: 2298–2303.

Tenkanen, M. 1998. Action of *Trichoderma reesei* and *Aspergillus oryzae* esterases in the deacetylation of hemicelluloses. *Biotechnol. Appl. Biochem.* 27: 19–24.

Tenkanen, M., Makkonen, M., Perttula, M., Viikari, L., and Teleman, A. 1997. Action of *Trichoderma reesei* on galactoglucomannan in pine kraft pulp. *J. Biotechnol*. 57: 191–204.

Warren, R. A. 1996. Microbial hydrolysis of polysaccharides. *Annu. Rev. Microbiol.* 50: 183–212.

Wong, K. K. Y., and Saddler, J. N. 1992. Applications of hemicellulases in the food, feed and pulp and paper industries. pp. 127–143. In Coughlan, P.P., Hazlewood, G.P. (eds.), *Hemicellulose and Hemicellulases*. Portland Press, London.

Wu, G., Bryant, M. M., Voitle, R. A., and Roland, D. A. 2005. Effects of β-Mannanase in corn-soy diets on commercial leghorns in second-cycle hens. *Poultry Sci.* 84: 894–897.

Wymelenberg, A. V., Sabat, G., Martinez, D., Rajangam, A. S., Teeri, T. T., Gaskell, J., Kersten P. J., and Cullen, D. 2005. The *Phanerochaete chrysosporium* secretome: Database predictions and initial mass spectrometry peptide identifications in cellulose-grown medium. *J Biotechnol.* 118: 17–34.

Yagüe, E., Mehak-Zunic, M., Morgan, L., Wood, D. A., and Thurston, C. F. 1997. Expression of CEL2 and CEL4, two proteins from *Agaricus bisporus* with similarity to fungal cellobiohydrolase I and β-mannanase, respectively, is regulated by carbon source. *Microbiol.* 143: 239–244.

Yamamura, I., and Matsumoto, T. 1993. Purification and some properties of endo-β-1,4 mannanase from a mud snail *Pomacea insulars. Biosci. Biotechnol. Biochem.* 7: 1316–1319.

Yamamura, I., Nozaki, Y., Matsumoto, T., and Kato, T. 1996. Purification and ome Properties of endo-β-1,4 mannanase from a marine mollusk *Littorina brevicula. Biosci. Biotechnol. Biochem*. 60: 674–676.

Yamasaki, T., Miyazaki, Y., and Kamei, Y. 1998. Isolation of bacteria that decompose major polysaccharides in the cell wall of the marine red alga *Porphyra yezoensis* and their application for protoplast production. *Can. J. Microbiol. Rev.* 44: 789–794.

Yanhe, Ma., Xue, Y., Dou, Y., Xu, Z., Tao, W., and Zhou, P. 2004. Characterization and gene cloning of a novel beta-mannanase from alkaliphilic *Bacillus sp*. N16-5. *Extremophiles* 8: 447–454.

Yoshida, S., Sako, Y., and Uchida A. 1998. Cloning, sequence analysis, and expression in *Escherichia coli* of a gene coding for an enzyme from *Bacillus circulans* K-1 that degrades guar gum. *Biosci. Biotechnol. Biochem.* 62(3): 514–520.

Zakaria, M. M., Ashiuchi, M., Yamamoto, S., and Yagi, T. 1998. Optimization for beta-mannanase production of a psychrophilic bacterium, *Flavobacterium sp. Biosci. Biotechnol. Biochem.* 62: 655–660.

Zhang, Q., Yan, X., Zhang, L., and Tang, W. 2006. Cloning, sequence analysis, and heterologous expression of a β-mannanase gene from *Bacillus subtilis* Z-2. *Mol. Biol.* 40: 368–374.