UDC 663.12:577.152.321
ISSN 1330-9862

review

Yeasts and their Enzyme Systems Degrading Cellulose, Hemicelluloses and Pectin

Peter Biely and Lubomír Kremnický*

Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava, Slovakia

Received: September 10, 1997.
Accepted: November 11, 1998

Summary

In contrast to most fungi, yeasts are generally unable to utilize insoluble, crystalline cellulose as a carbon source. Some yeasts are capable of utilizing amorphous, matrix polysaccharides of plant cell walls. Xylan, the main hardwood hemicellulose and the second most abundant softwood hemicellulose, serves as a carbon source for strains in the following genera: Aureobasidium, Cryptococcus, Pichia and Candida. Colour variants of Aureobasidium pullulans belong to the best producers of microbial xylanases.

Galactoglucomannan is the major softwood hemicellulose and various β-1,4-mannans occur in plant seeds. Our recent screening for the production of galactomannan-depolymerizing enzymes showed that the ability occurs within 5 different yeast genera, with highest frequency among Aureobasidium pullulans and Stephanoascus. The deterioration of softwood by Aureobasidium pullulans might be associated with its ability to colonize wood due to the production of both xylanolytic and mannanolytic enzyme systems.

A new screening for pectin-depolymerizing yeasts showed that the production of pectin-depolymerizing enzymes is scatterred within 13 genera, most frequently in Aureobasidium, Cryptococcus, Kluyveromyces, Rhodosporidium, Trichosporon and Ustilago.

The enzyme systems are generally inducible with fragments of the corresponding polysaccharides. With disaccharides (xylobiose, mannobiose) serving as inducers, the endoglycanase is secreted out of the cells into the surrounding medium and the corresponding glycosidase remains localized intracellularly. The oligosaccharides formed from the polysaccharides extracellularly are then transported into the cells by an inducible membrane-bound permease, an active transport system. If monosaccharides are the compounds triggerring the enzyme synthesis (e.g. xylose, galacturonic acid), the corresponding exo-acting enzymes, e.g. glycosidases cleaving oligosaccharides liberated from the polymer by endoglycanases, are secreted into the medium.

Keywords: yeasts, enzymes, plant polysaccharides, xylan, mannan, pectin, inducers

Introduction

The investigation of the ability of yeasts and yeast-like microorganisms to utilize plant polysaccharides is important for yeast ecology and taxonomy. It also provides information on substances utilized by yeasts in natural habitats and on the biotechnological potential of strains in the conversion of plant materials into a variety of useful products, such as single cell protein and chemical fuels. Yeasts growing on plant polysaccharides could also be used as the producers of industrially important enzyme systems.

In contrast to most fungi, yeasts are unable to utilize insoluble, crystalline cellulose as a carbon source. Some exceptions are strains of the genera *Trichosporon* and *Geotrichum (1,2)*. Screening for the production of endo-β-1,4-glucanases on soluble cellulose derivatives revealed the presence of genes encoding endo-β-1,4-glucanase even in some non-cellulolytic yeasts, like *Aureobasium pullulans (2–5)*, which could be an evolutionary heritage. However, endo-β-1,4-glucanase-positive strains do not grow even on water-soluble cellulose derivatives, such as carboxymethylcellulose.

Yeasts are capable of utilizing amorphous matrix polysaccharides of plant cell walls. Such yeasts have never attracted much attention as, for instance, amylolytic

* Corresponding author: E-mail: chempbsa@savba.sk

yeasts, and because the level of their enzyme production is generally lower that of fungi, they have also remained on the periphery of interest in connection with complex utilization of lignocellulosic materials. In view of the new trends of using yeasts as probiotic inclusions and biotherapeutic agents in animal food *(6,7)*, yeasts growing on plant polysaccharides and capable of producing a broad spectrum of enzymes depolymerizing a variety of polysaccharides may be important in this regard. In this article we review yeasts growing on or degrading three plant polysaccharides: xylans, mannans and pectin.

Xylanolytic Yeasts

Xylan is the main hardwood hemicellulose and the second most abundant softwood hemicellulose. After cellulose, xylan is the most abundant polysaccharide in nature. Its complete digestion requires a series of different enzymes (Fig. 1). Screening of yeasts for production of endo-β-1,4-xylanase in several laboratories showed that xylan serves as a carbon source for strains in the following genera: *Aureobasidium, Bullera, Candida, Cryptococcus, Pichia,* and *Trichosporon (2–4,8,9)*. The highest frequency of the production of xylanolytic enzymes occurs within the first three genera (Table 1). However, all strains of the pathogenic yeast *Cryptococcus neoformans* were found to be xylanase-negative, which means that based upon this criterion the pathogenic strains are not related to the saprophytic *Cryptococci*. It is interesting that only few of the tested strains of *Candida* and *Pichia stipitis* species showed the ability to grow on xylan. Within the genus *Candida*, xylanolysis was exhibited only by several strains of *Candida shehatae*, the anamorph of *Pichia stipitis*, and by *Candida ergatensis*. These are the only naturally occurring xylanolytic yeasts which also ferment xylose to ethanol, so they have a potential of one-step polysaccharide conversion to ethanol *(4)*. The 60% conversion of the polysaccharide into ethanol by a *Pichia stipitis* strain was achieved in cell recycle experiments done under oxygen limitation *(4)*. Colour variants of *Aureobasidium pullulans* were recognized as hyperproducers of endoxylanase *(3,10)*. This property was one of the reasons why considerable attention was devoted to other polysaccharide-degrading enzyme systems of these strains. Some of the fermentation abilities of xylanolytic yeasts are summarized in Table 2. One of the products of xylan fermentation are triglycerides *(11)*, that can be used as a diesel fuel without major processing *(12)*.

The yeast xylanolytic systems are controlled by induction and catabolic repression. Three constitutents of the xylanolytic system, endoxylanase, β-xylosidase and β-xyloside permease of *Cryptococcus albidus* are produced during growth on xylan or during induction with xylobiose or with a synthetic, almost non-metabolisable inducer, methyl β-D-xylopyranoside *(13)*. In this yeast only endoxylanase is secreted into the culture fluid. β-xylosidase remains purely intracellular and its substrates, xylobiose and xylotriose, are transported into the cell by an active transport system called β-xyloside permease *(14)*. A somewhat different situation is found in *Cryptococcus flavus* and *Aureobasidium pullulans* where xylanase is induced not only by the dimer derived from the polysaccharides, but also by its monomers, D-xylose and L-arabinose *(15–17)*. In *A. pullulans* β-xylosidase is secreted into the medium together with xylanase *(18)*. In *Trichosporon cutaneum*, β-xylosidase, inducible by a variety of compounds structurally related to xylan, was found both outside and inside the cells *(19)*. Another strain of *A. pullulans* (NRRL Y 2311-1) secretes xylanase and acetylesterase, but β-xylosidase and α-L-arabinofuranosidase were reported to be localized both extracellularly and intracellularly *(20,21)*. The induction of xylanase and β-xylosidase in the strain NRRL 2311-1 by xylobiose and xylose, is accompanied by an increase in the rate of xylobiose uptake, which indicates the coin-

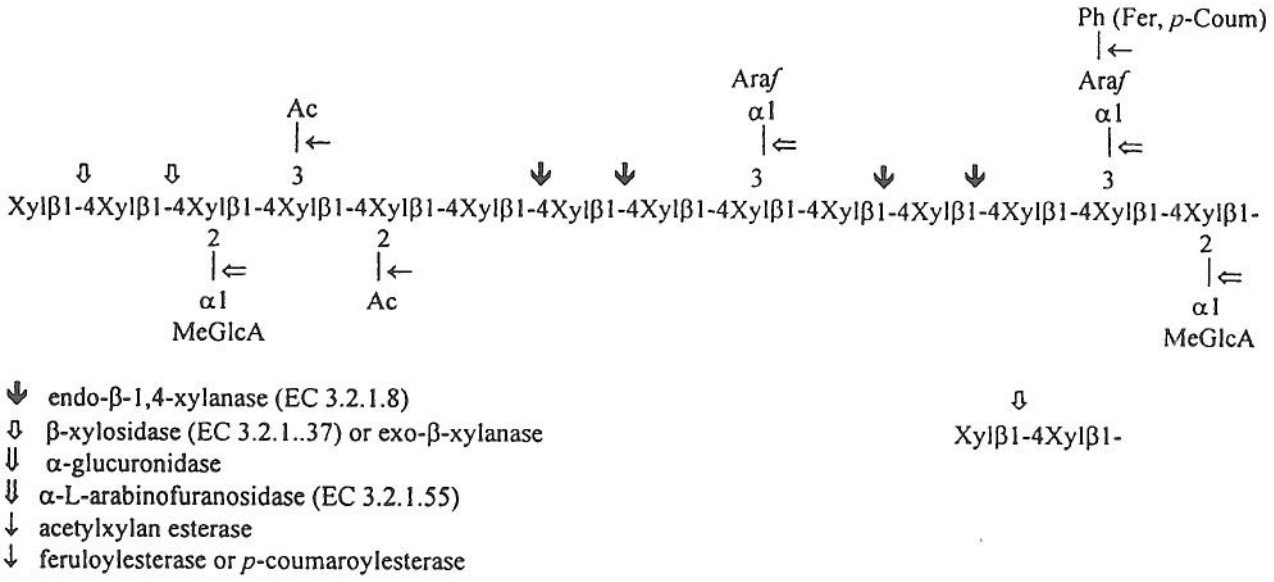

Fig. 1. Fragment of a hypothetical plant xylan and the enzymes required for its complete hydrolysis. Abbreviations: Xyl = D-xylopyranosyl residue; MeGlcA = 4-O-methyl-D- glucuronosyl residue; Ara*f* = L-arabinofuranosyl residue; Ac = acetyl group; Fer = p-Coum, feruloyl and p-coumaroyl groups.

Table 1. Xylanolytic yeasts (screened 35 genera, 350 species) Summary from references *(4)* and *(8)*

Genus	Number of strains	
	tested	positive
Aureobasidium (pullulans)	14	13
Bullera	7	5
Candida	45	3*
Cryptococcus	14	10
Pichia	35	3**
Trichosporon	12	1***

**C. shehatae and C. ergatensis; **P. stipitis; ***T. cutaneum*

Table 2. Fermentation abilities of xylanolytic yeasts

Strain	Fermentation ability	Conversion %	Ref.
Candida shehatae	xylan → ethanol	4*	*(4)*
Pichia stipitis	xylan → ethanol	60*	*(4)*
Cryptococcus albidus	xylan → triglycerides	13.7**	*(10)*
Aureobasidium pullulans	hyperproduction of xylanase		*(9)*

* Calculated from: 3 Xyl → 5 EtOH + 5 CO_2,
** g from 100 g of xylan

duction of a transport system for the disaccharide inducer. The transport system is an energy-dependent process because it is inhibited by 2,4-dinitrophenol or sodium azide *(22)*. A scheme of the known regulatory aspects of the xylanolytic system of *A. pullulans* is depicted in Fig. 2. The main difference between the *C. albidus (14)* and *A. pullulans* system is in the role of xylose and cellular localization of β-xylosidase.

Endoxylanase of *C. albidus* is the first eukaryotic xylanase gene that was isolated and sequenced *(23)*. The second cloned yeast xylanase gene belongs to *A. pullulans (24)*. It is interesting that the products of these two genes represent members of two phylogenetically different families of glycosyl hydrolases *(25)*. According to molecular mass, xylanase of *Pichia stipitis* fits into the same family as the *C. albidus* enzyme *(26)*. Other yeast xylanolytic enzymes, specially the debranching ones (Fig. 1), have not been investigated to such an extent. There is also an acetylxylanesterase activity among extracellular proteins of the non-xylanolytic yeast *Rhodotorula mucilaginosa (27)*.

Mannanolytic Yeasts

β-1,4-mannans are branched heteropolysaccharides found in wood and in plant seeds. The principal hemicellulose of softwood is galactoglucomannan, the backbone of which contains mainly β-1,4-linked mannopyranosyl residues interrupted occasionally with glucopyranosyl residues. The backbone built exclusively from mannopyranosyl residues is characteristic for plant seed β-mannans. Depending on the source, β-mannans and glucomannans are substituted to a different degree with β-1,6-linked D-galactopyranosyl residues. Moreover, softwood galactoglucomannans are partially acetylated. Complete breakdown of such a structure requires concerted action of a variety of polysaccharide hydrolases (Fig. 2). The crucial enzyme for mannan depolymerization is endo-β-1,4-mannanase. Increased interest in mannanolytic enzymes is connected with their possible application in the pulp and paper industry for bleaching softwood pulp *(28–30)*, and in hydrolysis of highly viscous galactomannans used in the food industry, *e.g.* in a coffee extraction process *(31)*. A prospective application of mannanases is their use for production of mannooligosaccharides which will be used as food additives stimulating the growth of *Bifidobacteria (32)*.

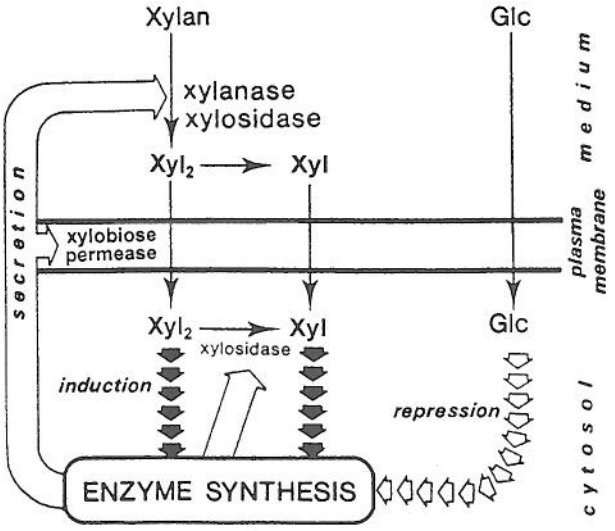

Fig. 2. Schematic representation of the xylanolytic system of *Aureobasidium pullulans*.
Abbreviations: Xyl = D-xylose; Xyl_2 = β-1,4-xylobiose; Glc = D-glucose. Reproduced with permission from Ref. 22.

Reports on occurrence of mannanolytic yeasts are very rare. A preliminary report on the production of mannanase by *Trichosporon beigelii* (*T. cutaneum*) *(33)* was recently confirmed *(34)*. Other yeasts and yeast-like microorganisms were systematically screened for the production of mannanolytic enzymes in our group *(35)*. Covalently dyed galactomannan, Ostazin Brilliant Red-galactomannan, was used as the substrate either alone or in combination with Remazol Brilliant Blue-xylan, so the production of the two types of glycanases could be followed simultaneously. The screening of 530 strains belonging to 73 genera showed that the production of galactomannan-depolymerizing enzymes occurs within 5 genera, with the highest frequency among *Aureobasidium pullulans* and *Stephanoascus* (Table 3). Quite surprising was an almost complete absence of this ability in the xylanolytic genus *Cryptococcus* (1 positive of 15 tested strains), and in the genus *Trichosporon* (no positive strains of the 14 tested) in which one strain was showed to be mannanolytic *(33,34)*. With the exception of two *Stephanoascus* strains all mannanase-positive strains were also xylanolytic.

A comparison of the mannanase-positive strains for the production of the enzyme after growth in a liquid

medium containing locust bean galactomannan as a carbon source showed that the best producers of endo-β--1,4-mannanase were the colour variant strains of *Aureobasidium pullulans* (NRRL Y 2311, Y 2311-1, Y 23121-2) that are known as hyperproducers of xylanase *(3,10)*. However, the differences between the production of mannanase by these strains and other strains of *A. pullulans* were not as great as in the case of xylanase production. In other words, xylanase hyperproducers were not hyperproducers of mannanase. The unusually high production of xylanase as the only enzyme of the two hemicellulolytic systems remains unexplained. It could be related to regulatory aspects or to a high specific activity of xylanase *(10)*.

The ability of the strains of *A. pullulans* to produce both xylanase and mannanase indicates their potential to produce mixed enzyme preparations with possible use in pulp and paper industry. At the same time this ability is an interesting information regarding the wood blackening. The deterioration of softwood by *A. pullulans (36)*, called "black yeast" in this respect, might be associated with its ability to colonize the softwood due to the production of enzyme systems hydrolyzing both softwood hemicelluloses, xylan and galactoglucomannan. Moreover, the strains also showed the ability to produce high levels of acetylesterases (L. Kremnický and P. Biely, unpublished results) which may be involved in the degradation of partially acetylated hemicellulose (Fig. 2).

Table 3. Mannanolytic yeasts (screened 73 genera, 530 species) Summary from reference *(37)*.

Genus	Number of strains	
	tested	positive
Aureobasidium (pullulans)	14	14
Stephanoascus	2	2
Cryptococcus	15	1
Pichia	35	1
Geotrichum	6	1

The mannanolytic system of the strain *A. pullulans* Y 2311-1 was subjected to a more detailed investigation. The strain was found to produce all enzymes required for complete degradation of galactomannan or galactoglucomannan. The enzymes differ in function and cellular localization (Fig. 3). Mannanase is secreted into the culture fluid, β-mannosidase is strictly intracellular, and β-galactosidase and β-glucosidase are found both extra- and intracellularly. Among these enzymes, only β-mannanase and β-mannosidase are inducible. The production of these two enzymes was 10 – 100 times higher in a galactomannan medium than on other carbon sources. Both enzymes were induced in glucose-grown cells by different β-mannans, β-1,4-mannooligosaccharides and partially also by xylan *(37)*. The natural inducer of both enzymes appears to be β-1,4-mannobiose. The synthesis of both enzymes is completely repressed by glucose, galactose and mannose. The synthetic glycoside, methyl β-D-mannopyranoside served as a less efficient, but non-utilizable inducer of only β-mannanase and β-mannosidase *(37)*. A comparison of the uptake rates of mannobiose in the cells grown on glucose (repressed cells) and grown on galactomannan suggested that the induction of the two hydrolases is accompanied by induction of a transport system for mannobiose, the inducer (22, *38*). The transport system, called mannobiose-permease, could be blocked by inhibitors of the energy metabolism, 2,4-dinitrophenol and sodium azide. This implies that the mannobiose permease is an active, energy-dependent transport system *(38)*. The mannanolytic system of *Trichosporon cutaneum* (JCM 2947) differs from that *of A. pullulans* by extracellular localization of both mannanase and β-mannosidase *(34)*.

The relation between the production of xylanases and mannanases was investigated in several strains of *A. pullulans* including the color variant strains *(39)*. The

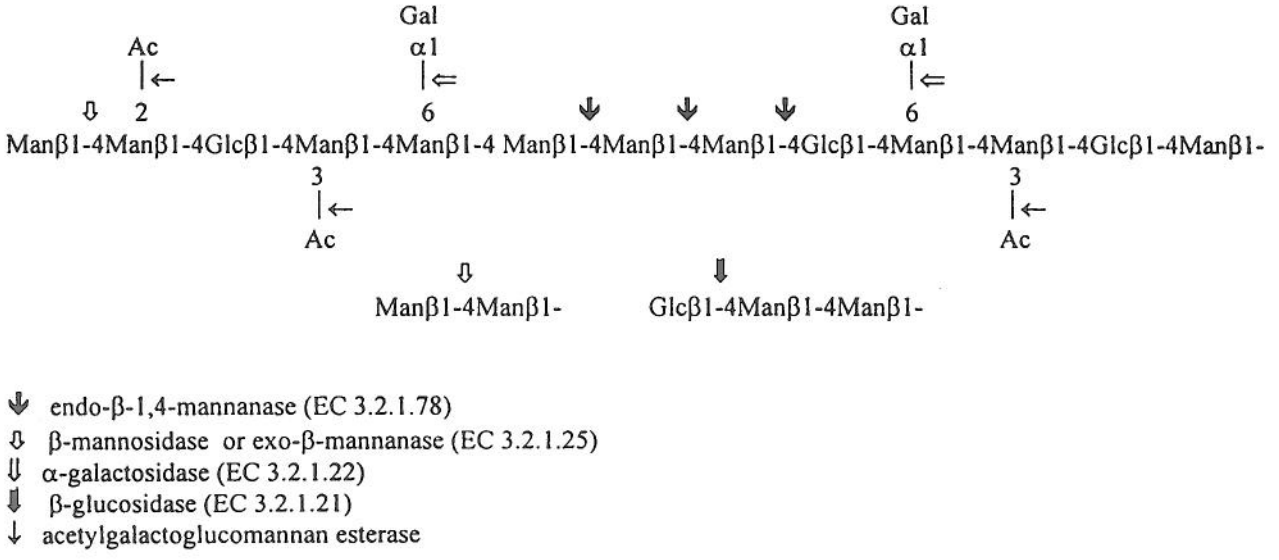

Fig. 3. Hypothetical plant galactoglucomannan and the enzymes required for its complete hydrolysis. Man = D-mannopyranosyl residue; Glc = D-glucopyranosyl residue; Gal = D-galactopyranosyl residue; Ac = acetyl group.

synthesis of xylanase and mannanase was found to be under separate control, though the two hemicelluloses never occur separately in nature. The strain produced xylanase on xylan and mannananse on galactomannan *(39)*. A constituent of the xylanolytic system of this strain is an active transport system for xylobiose, the inducer, called xylobiose permease *(22,38)*.

Pectolytic Yeasts

Pectin is a gel-forming acidic polysaccharide that occurs in the middle lamella and the primary cell wall of higher plants. Its structure is rather complicated. In Fig. 4 we present only the most characteristic features of the polysaccharide as well as the corresponding enzymes required for complete degradation of pectin. Pectolytic enzymes are important enzymes in food industry.

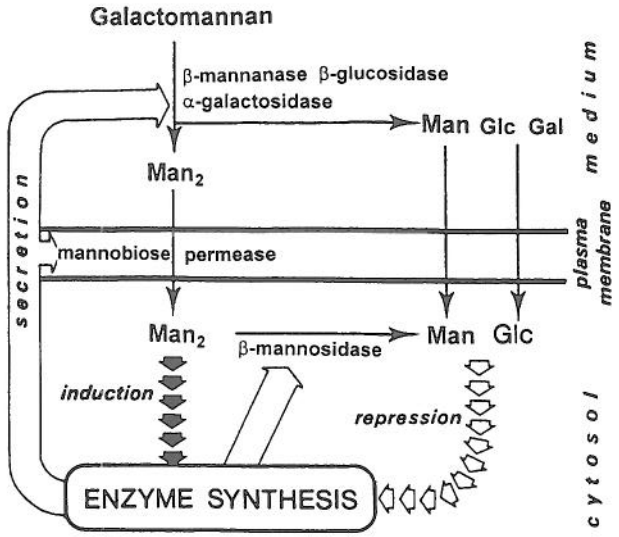

Fig. 4. Schematic representation of the mannanolytic sytem of *Aureobasidium pullulans*.
Abbreviations: Man = D-mannose; Man_2 = β-1,4-mannobiose; Glc = D-glucose; Gal = D-galactose. Reprinted with permission from Ref. 22.

Their major use is in fruit and vegetable processing, maceration, and fruit juice clarification and extraction *(40)*. Considerable amounts of pectic substances are present in waste materials from fruit processing and some agricultural wastes, and they represent a possible carbon source for microbial growth. The interest in pectolytic yeasts is associated with conversions of pectin-rich materials into stock fodder and for use as a prebiotic inclusion in animal diet *(6)*. Of particular interest in this connection would be yeasts that produce a broad spectrum of polysaccharide-degrading enzymes.

Yeasts as a group have long been known to be capable of producing pectin-degrading enzymes and utilizing pectin as a carbon source. Earlier screening surveys of yeasts for pectolytic activity showed that the property is not generally distributed and is limited to several genera only. Luh and Phaff *(41)* were the first to report the ability of *Saccharomyces fragilis* (synonim for *Kluyveromyces marxianus*) and *Candida tropicalis* to clarify liquid media containing citrus pectin. Other pectolytic yeasts identified later included strains of the genera *Candida, Pichia, Zygosaccharomyces Kluyveromyces, Rhodotorula, Cryptococcus, Trichosporon (42–53)* and, surprisingly, also

Table 4. Pectolytic yeasts (screened 52 genera, 300 species) Summary from ref. *(60)*

Genus	Number of strains	
	tested	positive
Ambrosiozyma	3	1
Aureobasidium (pullulans)	6	4
Candida	125	13
Cryptococcus	38	29
Geotrichum	9	4
Kluyveromyces	5	5
Leucosporidium	2	2
Rhodosporidium	2	2
Saccharomycopsis	6	2
Stephanoascus	1	1
Trichosporon	6	3
Ustilago	2	2

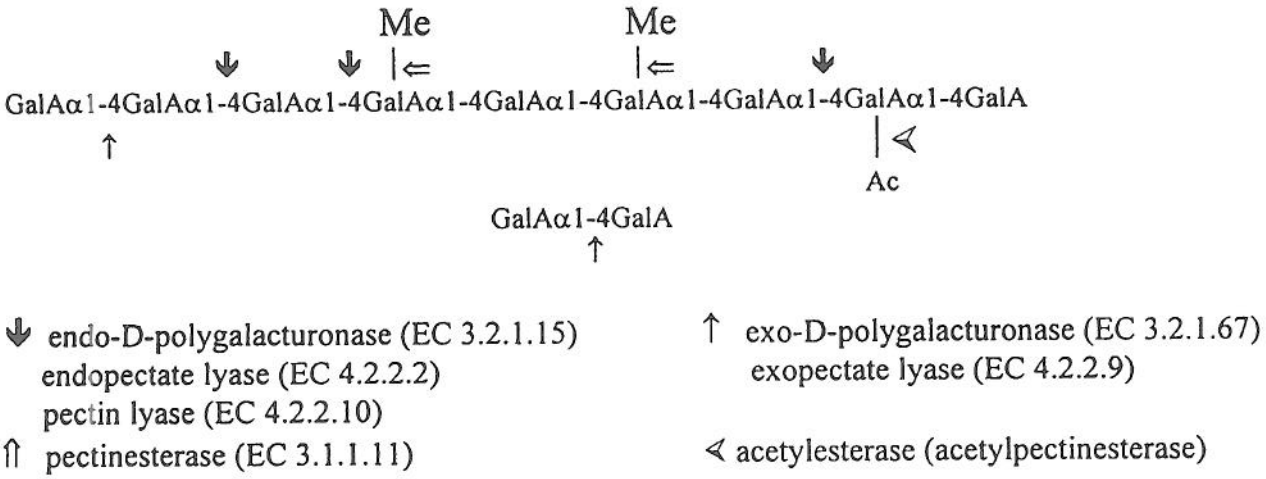

Fig. 5. A simplified structure of pectin and the enzymes required for its degradation.
Abbreviations: GalA = D-galacturonosyl residue; Me = methyl group; Ac = acetyl group.

Saccharomyces cerevisiae (54,55). In this list we intentionally omitted the strains *of Aureobasidium pullulans* which, together with strains of *Kluyveromyces marxianus*, can be considered the most extensively investigated pectolytic yeasts. Numerous papers deal with *A. pullulans* pectolytic enzymes, their production and application *(56–59)*.

A new screening for pectin-depolymerizing yeasts based on the precipitation of non-hydrolyzed pectin in non-agar solid media *(60)* essentially confirmed the distribution of pectolytic activities in yeasts as quoted above. In a group of 300 strains belonging to 52 genera the production of pectin-depolymerizing enzymes was found to be scatterred within 13 genera, with the highest frequency in the genera *Aureobasium, Cryptococcus Kluyveromyces, Rhodosporidium, Trichosporon* and *Ustilago* (Table 4). In the same screening it was shown that the pathogenic strains *Cryptococcus neoformans* do not belong to the pectolytic family of *Cryptococcus* strains. The best pectin utilization was observed with the strains of the first two genera, which are known to utilize well the main hemicellulose polysaccharides. *Kluyveromyces* strains did not show such good growth as the strains of *Aureobasidium pullulans* and some of the *Cryptococci*. Some of the pectolytic *Kluyveromyces* do not grow on pectic substances and produce pectolytic enzymes constitutively, *e.g.* on glucose *(61–63)*.

None of the yeasts were shown to produce all pectolytic enzymes listed in Fig. 5. *Kluyveromyces marxianus* and *Aureobasidium pullulans* were reported to produce polygalacturonase and pectinesterase *(5,64,65)*. Pectin lyases were reported to be produced in *A. pullulans* LV10, however, only at higher pH values of the growth media *(59,66)*. Polygalacturonases were purified and characterized from both *K. marxianus (61,63,51)* and *A. pullulans (67,59)*.

As we have indicated, yeasts exhibit a certain versatility in the response to the carbon source and the production of pectolytic enzymes. In contrast to the constitutive production of polygalacturonase in *Klyuveromyces (61–63)*, inducible polygalacturonases were reported in *Saccharomycopsis fibuligera (68), Cryptococcus albidus (48)* and *A. pullulans (57, 69)*. In addition to pectin and pectate, oligogalacturonic acids and their unsaturated derivatives which are formed upon the action of pectate or pectin lyases, were found to be the suitable carbon sources *(70,71)*. The nature of low-molecular inducers was investigated in one of the pectolytic *A. pullulans* strains which is known as a hyperproducer of xylanase (*A. pullulans* NRLL Y-2311) *(72)*. The strain secretes into the culture fluid endopolygalacturonase and exopolygalacturonase, but no pectin lyase or pectate lyase. Endo- and exo-polygalacturonase are induced in glucose-grown cells by D-galacturonic acid and its oligomers. These results suggest that the monomer derived from the polysaccharide serves as natural inducer or as precursor of an inducer of pectolytic enzymes in the studied strain of *A. pullulans*. Here we observe again that if a monosaccharide is the compound triggering the synthesis of a polysaccharide-degrading system the corresponding exo-acting enzymes are secreted into the medium.

Conclusions

The ability of yeasts and yeast-like microorganisms to utilize three plant polysaccharides, xylan, galactomannan and pectin, is restricted to a limited number of yeast genera or species. Some yeasts are only xylanolytic, *e.g.* some *Candida* and *Pichia* strains, some are only pectolytic, *e.g. Kluyveromyces* and *Saccharomycopsis*, some are both xylanolytic and pectolytic, *e.g. Cryptococci*, and *Stephanoascus* (*Candida ciferii*) strains appear to be both mannanolytic and pectolytic. Only the representatives of *Aureobasidium pullulans* possess the enzyme machinery for utilizing all three tested polysaccharides. The production of enzyme systems degrading main wood hemicelluloses, xylan and galactoglucomannan by *A. pullulans* is compatible with the deteriorating effect of this black yeast on wood.

Hemicellulolytic and pectolytic enzymes of yeasts are in most cases inducible by fragments of the corresponding polysaccharide. An exception is the constitutive production of pectolytic enzymes in *Kluyveromyces marxianus*. There are pectolytic strains of this species which do not utilize pectic substances as a carbon source. This fact is a challenge to investigate the real physiological role of pectolytic enzymes in this species. One interesting generalization emerged from studies on the regulation of the synthesis of polysaccharide-hydrolyzing enzymes. When a monosaccharide serves as inducer, the corresponding exoglycanase or glycosidase cleaving short oligosaccharides is secreted out of the cells together with endo-acting glycanases. When a disaccharide derived from the polysaccharide serves as inducer, the corresponding exoglycanase or glycosidase remains intracellular and the dimer is transported into the cell by an inducible transport system. Although xylan, mannan and pectin occur in plant cell walls together, the formation of enzyme systems for their degradation seems to be under separate control, at least in *A. pullulans*.

Acknowledgments

Our thanks are due to all our colleagues who contributed experimentally to work which made this article possible. These were M. Vršanská, Z. Krátký, M. Hrmová, M. Cziszárová, E. Slávíková and D. Urmaničová-Logan. The latest support of this work comes from the Slovak Grant Agency VEGA through grant 2/4147/98.

References

1. C. Dennis, *J. Gen. Microbiol. 71* (1972) 409.
2. B. J. H. Stevens, J. Payne, *J. Gen. Microbiol. 100* (1977) 381.
3. T. D. Leathers, C. P. Kurzman, R. W. Detroy, *Biotechnol. Bioeng. Symp. 14* (1984) 225.
4. H. Lee, P. Biely, R. K. Latta, M. F. S. Barbosa, H. Schneider, *Appl. Environ. Microbiol. 52* (1986) 320.
5. M. S. Deshpande, V. B. Rale, J. M. Lynch, *Enzyme Microbiol. Technol. 14* (1992) 514.
6. I. Zelenák, D. Jalč, V. Kmet', P. Siroka, *Anim. Feed Sci. Technol. 49* (1994) 211–221.
7. L. V. McFarland, *9th International Symposium on Yeasts*, Sydney, Australia (1996) p. 5.

8. B. Flannigan, *Trans. Brit. Mycol. Soc. 55* (1970) 277.
9. P. Biely, Z. Krátký, A. Kocková-Kratochvílová, Š. Bauer, *Folia Microbiol. 23* (1978) 366.
10. T. Leathers, *Appl. Environ. Microbiol. 52* (1986) 1026.
11. R. Fall, P. Phelps, D. Spindler, *Appl. Environ. Microbiol. 47* (1984) 1130.
12. C. Ratledge, *Chem. Soc. Rev. 8* (1979) 283.
13. P. Biely, Z. Krátký, M. Vršanská, D. Urmaničová, *Eur. J. Biochem. 108* (1980) 323.
14. P. Biely, Biochemical aspects of the production of microbial hemicellulases. In: *Hemicellulose and Hemicellulases*, M. P. Coughlan, G. P. Hazlewood (Eds.), Portland Press, London (1993) pp. 29–51.
15. T. D. Leathers, R. W. Detroy, R. J. Bothast, *Biotechnol. Lett. 8* (1986) 867.
16. T. Yasui, B. T. Nguyen, K. Nakanishi, *J. Ferment. Technol. 62* (1984) 353.
17. J. Pou-Llinas, H. Driguez, *Appl. Microbiol. Biotechnol. 27* (1987) 134.
18. J. Dobberstein, C. C. Emeis, *Appl. Microbiol. Biotechnol. 35* (1991) 210.
19. M. Hrmová, P. Biely, M. Vršanská, E. Petráková, *Arch. Microbiol. 138* (1984) 371.
20. J. Myburgh, B. A. Prior, S. G. Kilian, *Process Biochem. 26* (1991) 343.
21. J. Myburgh, B. A. Prior, S. G. Kilian, *J. Ferment. Bioeng. 72* (1991) 135.
22. L. Kremnický, P. Biely, *Biochim. Biophys. Acta*, in press.
23. R. Morosoli, S. Durand, A. Moreau, *Gene, 117* (1992) 145.
24. X.-L. Li, L. G. Ljungdahl, *Appl. Environ. Microbiol. 60* (1994) 3160.
25. N. R. Gilkes, B. Henrissat, D. G. Kilburn, R. C. Miller Jr., A. J. Warren, *Microbiol. Rev. 55* (1991) 303.
26. S. Özcan, P. Kötter, M. Ciriacy, *Appl. Microbiol. Biotechnol. 36* (1991) 190.
27. H. Lee, R. J. B. To, R. K. Latta, P. Biely, H. Schneider, *Appl. Environ. Microbiol. 53* (1987) 2831.
28. J. Buchert, J. Salminen, M. Siika-Aho, M. Ranua, L. Viikari, *Holzforschung, 49* (1993) 473.
29. M. Paice, N. Garnagul, D. H. Page, L. Jurasek, *Enzyme Microbiol. Technol. 14* (1992) 272.
30. A. Suurnäkki, A. Kantelinen, J. Buchert, L. Viikari, *Tappi, 11* (1994) 111.
31. B. V. McCleary, *ACS Symp. Ser. 460* (1991) 437.
32. Y. Kobayashi, R. Echizen, M. Mada, M. Mutai, Effect of hydrolysate of Konjac mannan and soybean oligosaccharides on intestinal flora in man and rats. In: *Intestinal Flora and Dietary Factors*, T. Mitsuoka (Ed.), Japan Scientific Societies Press, Tokyo (1983) pp. 69–90.
33. M. Zimmermann, C. C. Emeis, *Yeast, 5* (1989) S131.
34. Y. Oda, K. Tonomura, *Lett. Appl. Microbiol. 22* (1996) 173.
35. L. Kremnický, E. Sláviková, D. Mislovičová, P. Biely, *Folia Microbiol. 41* (1996) 43.
36. R. S. Horvath, M. M. Brent, D. G. Cropper, *Appl. Environ. Microbiol. 32* (1976) 505.
37. L. Kremnický, P. Biely, *Arch. Microbiol. 167* (1997) 350.
38. L. Kremnický, P. Biely, *9th International Symposium on Yeasts*, Sydney, Australia (1996) p. 49.
39. P. Biely, M. Vršanská, L. Kremnický, *Folia Microbiol. 41* (1996) 278.
40. W. Pilnik, F. M. Rombouts, Pectic enzymes. In: *Polysaccharides in Food*, J. M. V. Blanshard, J. R. Mitchell (Eds.), Butterworth, London, (1979) pp. 7–109.
41. B. S. Luh, H. J. Phaff, *Arch. Biochem. Biophys. 33* (1951) 212.
42. P. A. Roelofsen, *Biochim. Biophys. Acta, 10* (1953) 410.
43. M. H. Bilimoria, J. V. Bhat, *J. Indian. Inst. Sci. 44* (1962) 15.
44. R. H. Vaughn, T. Jakubczyl, J. D. MacMillan, T. E. Higgins, B. A. Dave, V. M. Crampton, *Appl. Microbiol. 18* (1969) 771.
45. T. Sakai, M. Okushima, *Agr. Biol. Chem. 42* (1978) 2427.
46. M. P. Wimborne, P. A. D. Rickard, *Biotechnol. Bioeng. 20* (1978) 231.
47. F. Federici, *Antonie van Leeuwenhoek, 51* (1985) 139.
48. H. Tanabe, H. Kamishima, *Agr. Biol. Chem. 52* (1988) 611.
49. P. J. Fellows, J. T. Worgan, *Enzyme Microbiol. Technol. 9* (1987) 430.
50. H. A. Murad, M. S. Toda, *Bioresource Technol. 41* (1992) 247.
51. J. Lim, Y. Yamasaki, Y. Suzuki, J. Ozawa, *Agr. Biol. Chem. 44* (1980) 473.
52. R. F. Schwan, R. M. Cooper, A. E. Wheals, In: *Pectins and Pectinases*, J. Visser, A. G. J. Voragen, (Eds.) Elsevier, Amsterdam (1996) pp. 861–867.
53. E. Stratilová, E. Breierová, R. Vadkertiová, In: *Pectins and Pectinases*, J. Visser, A. G. J. Voragen, (Eds.) Elsevier, Amsterdam (1996) pp. 899–906.
54. P. Blanco, C. Sieiro. A. Diaz, T. G. Villa, *Can. J. Microbiol. 40* (1994) 974.
55. A. Gainvors, V. Frezier, H. Lemarquier, C. Lequard, M. Aigle, A. Belardi, *Yeast, 106* (1994) 1311.
56. H. Berndt, W. Liese, *Arch. Microbiol. 79* (1971) 140.
57. T. Sakai, A. Takaoka, *Agr. Biol. Chem. 49* (1985) 449.
58. M. Moresi, M. Petruccioli, F. Frederici, *Appl. Microbiol. Biotechnol. 34* (1991) 742.
59. P. L. Manachini, C. Parini, M. G. Fortina, *Enzyme Microbiol. Technol. 10* (1988) 682.
60. P. Biely, E. Sláviková, *Folia Microbiol. 39* (1994) 485.
61. B. S. Luh, H. J. Phaff, *Arch. Biochem. Biophys. 48* (1954) 23.
62. T. Sakai, M. Okushima, S. Yoshitake, *Agr. Biol. Chem. 48* (1984) 1951.
63. F. M. Barnby, F. F. Morpeth, D. L. Pyle, *Enzyme Microbiol. Technol. 12* (1992) 891.
64. A. D. Agate, J. V. Bhat, *Appl. Microbiol. 14* (1966) 256.
65. H. P. Call, C. C. Emeis, *Chem. Microbiol. Technol. Lebensm. 4* (1978) 137.
66. C. Parini, M. G. Fortina, P. L. Manachini, *J. Appl. Bacteriol. 65* (1988) 682.
67. S. A. Archer, A. H. Fielding, *J. Sci. Food Agric. 30* (1979) 711.
68. P. J. Fellows, J. T. Worgan, *Enzyme Microbiol. Technol. 6* (1984) 405.
69. M. Petruccioli, M. Monteleone, F. Fererici, *Yeast, 5* (1989) 157.
70. H. P. Call, B. Wendt, C. C. Emeis, *Chem. Microbiol. Technol. Lebensm. 8* (1984) 175.
71. H. P. Call, P. Decker, C. C. Emeis, *Chem. Microbiol. Technol. Lebensm. 8* (1984) 182.
72. P. Biely, K. Heinrichová, M. Kružiková, *Curr. Microbiol. 33* (1996) 6.

Kvasci i njihovi enzimski sustavi koji razgrađuju celulozu, hemicelulozu i pektin

Sažetak

Za razliku od većine gljiva, kvasci su općenito nesposobni koristiti kristalnu celulozu kao izvor ugljika. Neki su kvasci sposobni iskoristiti polisaharide koji su sastavni dio stijenki biljnih stanica. Ksilan, glavni sastojak hemiceluloze tvrdog drveta i druga najraširenija hemiceluloza mekog drveta, služi kao izvor ugljika sljedećim rodovima: Aureobasidium, Cryptococcus, Pichia i Candida. Obojene su varijante Aureobasidium pullulans među najboljim proizvođačima mikrobnih ksilanaza.

Glavna hemiceluloza mekog drveta je galaktoglukomanan, a različiti β-1,4-manani nalaze se u sjemenkama. Nedavnim ispitivanjem autori su pronašli da između pet različitih rodova kvasaca, sposobnost proizvodnje enzima za depolimerizaciju galaktomanana imaju najčešće Aureobasidium pullulans i Stephanoascus. Razgradnja mekog drveta s Aureobasidium pullulans povezana je vjerojatno s njegovom sposobnosti da se naseli jer može proizvesti ksilanolitske i mananolitske enzimske sustave.

Ustanovljeno je da se sposobnost kvasaca koji depolimeriziraju pektin očituje u 13 rodova, najčešće u Aureobasidium, Cryptococcus, Kluyveromyces, Rhodosporidium, Trichosporon i Ustilago.

Enzimski se sustavi najčešće induciraju fragmentima odgovarajućih polisaharida. Kada disaharidi (ksilobioza, manobioza) služe kao induktori, tada se endoglikanaza izlučuje iz stanica u okolni medij, a odgovarajuća glikozidaza ostaje lokalizirana intracelularno. Oligosaharidi nastali iz polisaharida, koji su bili izvan stanice, transportiraju se u stanicu s pomoću inducibilne permeaze vezane na membranu, što je aktivni transportni sustav. Ako su monosaharidi spojevi koji induciraju sintezu enzima (npr. ksiloza, galakturonska kiselina), tada se odgovarajući egzoenzimi (npr. glikozidaze koje cijepaju oligosaharide dobivene iz polimera djelovanjem endoglikanaza) izlučuju iz stanice u okolni medij.