Biotechnology Advances 59 (2022) 107981

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Cellulose- and xylan-degrading yeasts: Enzymes, applications and biotechnological potential

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ARTICLE INFO

Keywords:
Yeast
Cellulose
Xylan
Cellulase
Xylanase
Consolidated bioprocess
Lignocellulose
Carbohydrate-active enzymes (CAZymes)

ABSTRACT

Microbes and their carbohydrate-active enzymes are central for depolymerization of complex lignocellulosic polysaccharides in the global carbon cycle. Their unique abilities to degrade and ferment carbohydrates are also utilized in many industrial processes such as baking, brewing and production of biofuels and drugs. Effective degradation and utilization of cellulose and hemicelluloses is important for the shift towards green bioeconomy, and requires microbes equipped with proper sets of carbohydrate-active enzymes (CAZymes). Knowledge of cellulolytic and xylanolytic CAZymes has mainly been generated from bacteria and filamentous fungi, while yeasts have been largely overlooked and may represent an untapped resource in natural CAZymes with industrial relevance. Cellulose and xylan-degrading yeasts with the ability to ferment saccharides are also promising candidates for consolidated bioprocesses (CBPs), as they can degrade lignocellulose and utilize its constituents to produce desired products at the same time. Cellulolytic yeasts able to utilize insoluble crystalline cellulose are rare while xylanolytic yeasts are rather widespread in nature. The lack of particular enzymes in yeasts can be remediated by introducing the missing enzymes into strains having outstanding product-forming attributes.

In this review, we provide a comprehensive overview of the cellulose- and xylan-degrading ascomycetous and basidiomycetous yeasts known to date. We describe how these yeasts can be identified through bioprospecting and bioinformatic approaches and summarize available growth and enzymatic assays for strain characterization. Known and predicted CAZymes are extensively analyzed, both in individual species and in a phylogenetic perspective. We also describe the strategies used for construction of recombinant cellulolytic and xylanolytic strains as well as current applications for polysaccharide-degrading yeasts. Finally, we discuss the great potential of these yeasts as industrial cell factories, identify open research questions and provide suggestions for future investigations.

1. Introduction

A cornerstone in the transition from an oil-based to a bio-based economy is the development of economically viable production processes of fuels, chemicals and materials from renewable green carbons. Here, lignocellulosic biomass (i.e., plant cell walls) attracts attention as the largest available renewable source of carbon. Conversion of lignocellulosic biomass to product is typically a multistep process involving pretreatment, enzymatic hydrolysis, microbial conversion of sugars to product and finally product purification. The focus of this review is on cellulose- and xylan-degrading yeasts that provide opportunity to combine the hydrolysis and product formation steps, decrease production costs, solve important process bottlenecks and aid in the development of new bioprocesses.

Abbreviations: CAZymes, Carbohydrate-active enzymes; CBP, consolidated bioprocess; SSF, Simultaneous saccharification and fermentation; GHs, glycoside hydrolases; CEs, carbohydrate esterases; AA, auxiliary activity; CBM, carbohydrate-binding module; CMC, carboxymethyl cellulose; HEC, hydroxyethyl cellulose; LPMO, lytic polysaccharide monooxygenases; CDH, cellobiose dehydrogenase.

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https://doi.org/10.1016/j.biotechadv.2022.107981
Received 19 March 2022; Received in revised form 8 May 2022; Accepted 10 May 2022
Available online 14 May 2022

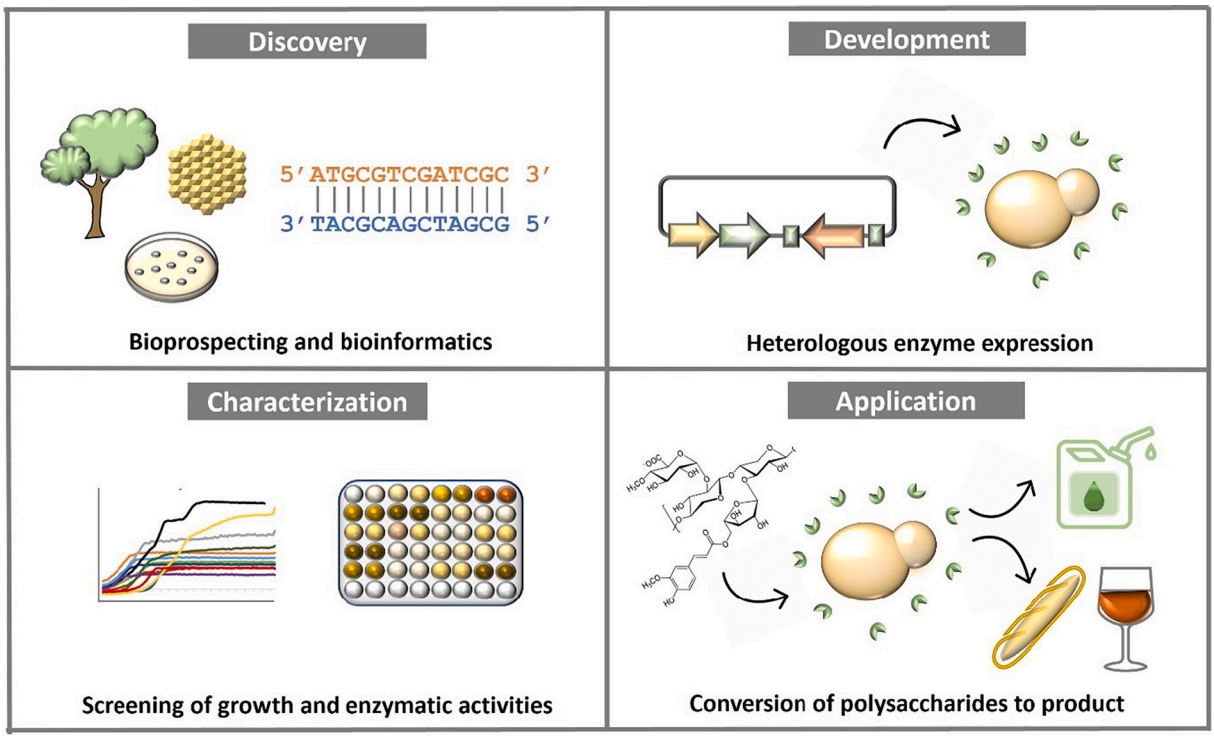

Fig. 1. Overview of the topics covered in this review.

Lignocellulosic biomass is mainly composed of a highly complex network of the homopolysaccharide cellulose (40–60% of dry weight), various hemicellulosic heteropolysaccharides (20–35% of dry weight), and the aromatic polymer lignin (15–40% of dry weight) (Zoghlami and Paës, 2019). Together, cellulose and xylan constitute the main bulk of polysaccharides in plant cell walls with potential for bioconversion. Cellulose is a linear polysaccharide of β-1,4-linked D-glucose units that form crystalline and insoluble microfibrils (Heinze, 2015). Hemicelluloses coat the cellulose fibrils, and their proportions and abundances differ between plant species. In industrially important grasses and hardwoods, xylans are the most abundant hemicellulose types, while in other species galacto-glucomannans, xyloglucans and mixed-linkage β-glucans are more abundant. Xylans comprise a backbone of β-1,4-linked D-xylose residues which are commonly decorated with different sidechains that can crosslink with other xylans and with the hydrophobic lignin polymers in the plant cell wall (Ebringerová and Heinze, 2000). The heterogenous carbohydrate matrix in the plant cell wall is recalcitrant to hydrolysis and deconstruction, which poses one of the main challenges in efficient and rapid conversion of biomass and biowastes to value-added chemicals and fuels (De Buck et al., 2020; Den et al., 2018).

Depolymerization of the diverse polysaccharides present in plant biomass requires a consortium of CAZymes with complementing exo-, endo- and auxiliary activities, which sequentially hydrolyses the polysaccharide side chains and backbones into oligo- and monosaccharides for microbial uptake and consumption (Claes et al., 2020; Minty and Lin, 2015). Current knowledge on cellulolytic and hemicellulolytic enzymes comes almost exclusively from studies on filamentous fungi and bacteria. Ecological studies of filamentous fungi and yeasts during natural decay of wood show that the yeast diversity is high in delignified wood, where fungi act as the main lignocellulose-degrading organisms providing monomeric or oligomeric saccharides that support yeast growth (Jiménez et al., 1991). However, several yeast species have also been shown to degrade and grow on plant polysaccharides (Lara et al., 2014; Otero et al., 2015; Goldbeck, 2012).

Yeasts are unicellular fungi of both ascomycota and basidiomycota origin. They are typically easy to cultivate and manipulate genetically and several species and strains are already extensively used as cell factories for industrial bioproduction (Navarrete and Martínez, 2020; Parapouli et al., 2020). Cellulose- and xylan-degrading yeasts are of high biotechnological interest for use in simultaneous saccharification and fermentation (SSF) processes and consolidated bioprocesses (CBPs), where the hydrolysis and fermentation steps are combined (Chettri et al., 2020). Such yeasts can also act as producers of polysaccharide-degrading enzymes for industrial applications such as paper, food, feed and textile industries and in modern and future biotechnological processes (Christov and Prior, 1993).

Cellulose and xylan-degrading yeasts can be isolated from nature and tested for the ability to cleave polysaccharides and use the hydrolysis products for growth or conversion to interesting products. The advantage of these natural enzyme producers is the inherent presence of all necessities required for a successful expression of the genes coding for hydrolytic enzymes, as well as efficient uptake systems and intracellular catabolism of the hydrolyzed saccharides. Alternatively, polysaccharide-degrading yeasts can be constructed using genome editing tools to express heterologous genes encoding enzymes of interest in laboratory or industrial strains of well characterized yeasts such as *Saccharomyces cerevisiae*, *Komagataella phaffii (Pichia pastoris)* or *Kluyveromyces marxianus* (Anandharaj et al., 2020; Dong et al., 2020; Tian et al., 2019; Yamada et al., 2011). The advantages of this approach are that the yeasts can be tailor-made to degrade only selected parts of the plant polysaccharides and the possibility to use strains that are naturally good producers of the intended end products.

This review summarizes available literature on identification and characterization of yeasts and yeast-like microorganisms that can grow on and ferment cellulose and xylan, as well as the yeasts' repertoires of cellulolytic and hemicellulolytic enzymes. This includes both natural degraders identified through bioprospecting and bioinformatics and recombinant strains tailored to degrade specific polysaccharides. We also list and discuss current and future applications and the biotechnological potential of these yeasts in a circular, green bioeconomy (Fig. 1).

2. Finding and characterizing polysaccharide-degrading yeasts

Through literature searches performed for this review, we have identified approximately 150 different polysaccharide-degrading yeast species. The complete list of species, their confirmed enzymatic activities and referenced articles has been deposited in the Mendeley Data repository: https://data.mendeley.com/datasets/8y5nsjc6kt/draft?a=14d47ca4-6620-4e80-a1fa-7285769e90a1. As the yeast nomenclature changes over time, naming the yeasts in a consistent way has not been trivial. Throughout the review, we have chosen to mainly keep the yeast names from the original publications, but their alternative names are included in the deposited complete list.

A comparison of the level of enzymes produced by these natural polysaccharide-degrading yeasts with enzyme production by fungi or bacteria is certainly not in favor of yeasts. However, there may be significantly better yeasts that are still waiting to be discovered. Less than 3000 yeast species have been identified to date, and estimates suggest that >90% of existing fungal yeast diversity still remains unexplored (Boekhout et al., 2021; Lachance, 2006). Moreover, the vast majority of the already identified yeast species are still poorly characterized, which also means that we largely lack information about their capacities to degrade polysaccharides and overall biotechnological potential.

2.1. Bioprospecting and screening of strain collections

Novel polysaccharide-degrading strains can be found through bioprospecting, the discovery pipeline in which microorganisms and enzymes are sought from different natural and domestic environments. Many yeasts have been found to reside on fruits and the bark of trees (Rao et al., 2008), on flowers and in nectar (Lachance et al., 2001), and in the soil (Mestre et al., 2011). As may be expected, plant polysaccharide-degrading yeasts and in particular xylan-degrading yeasts have often been isolated from environments that are rich in plant-polysaccharides, for example rotting wood, compost, decaying sugarcane bagasse, rain forest soil and intestines of wood-eating insects (Ali et al., 2017; Arcuri et al., 2014; Elahi and Rehman, 2018; Giese et al., 2017; Gomes et al., 2015; Jaiboon et al., 2016; Lara et al., 2014; Morais et al., 2013, 2020; Tiwari et al., 2020; Yun et al., 2015). Moreover, yeasts have been isolated in numerous geographic locations, non the least in areas in the tropical zone that are identified as biodiversity hotspots as they host a very large number of species of plants, animals and microorganisms. However, overall, the habitats that harbor yeasts are poorly understood and many biotopes remain uninvestigated. Thus, future research and bioprospecting campaigns will likely add to the lists of yeast, biodiversity-rich substrates, niches and areas.

During bioprospecting, samples are collected at the site and typically suspended in water and thereafter spread on agar plates to allow single yeast colony formation. Plates can contain an antibiotic to prevent bacterial growth, and possibly also an additive such as sodium propionate to reduce growth of filamentous fungi (Brock and Buckel, 2004). The growth medium can either be composed such that it favors growth of essentially all yeast species, or selective to primarily find species that display sought-after trait(s). For example, plates with glucose, yeast extract and peptone allow growth of most yeasts, whereas exchanging glucose for cellulose or xylan captures mostly yeasts with the inherent capacity to degrade these polysaccharides. The yeasts that grow on the plates after incubation must be restreaked for single colonies or otherwise purified to ensure clonality for each yeast isolate. Characterization can then be carried out in different ways. Clade/family/species identity is usually determined through ITS and D1/D2 sequencing (Vu et al., 2016) and yeast morphology and physiology can be examined for example by methods described by Van der Walt and Yarrow (van der Walt and Yarrow, 1984).

Another option to identify natural polysaccharide-degrading yeasts is to screen yeast culture collections. Numerous public and private yeast culture collections exist, which hold thousands of wild-type species and strains (Boundy-Mills et al., 2016). In contrast to bioprospecting, some prior knowledge of which species are of interest is usually needed before ordering strains from cultural collections, which limits the chance of making unexpected connections between strains and sought-after traits. However, some culture collections now offer screening services where hundreds or even thousands of strains can be screened for relevant traits, thereby opening up new possibilities for culture collection use.

2.2. Bioinformatic approaches

An emerging method to identify polysaccharide-degrading yeasts is to use bioinformatic tools to predict genes encoding proteins involved in degradation of polysaccharides. The amount of available genomic data is growing exponentially as next generation sequencing technology is becoming increasingly cheaper and more accessible. Combined efforts in the scientific community including ambitious projects such as the 1000 fungal genome project (Grigoriev et al., 2014) have recently provided many yeast genomes for genetic exploration.

Moreover, bioinformatic pipelines are continuously being developed for easy access and use. The potential of individual species to degrade different polysaccharides may be predicted by the presence of CAZyme-encoding genes in the yeast genomes. CAZymes are divided into classes and families in the Carbohydrate-Active Enzymes database (CAZy, www.cazy.org; (Lombard et al., 2014). The division is based on the enzymes' sequence similarities, which determine their protein structures and functions (Davies et al., 2018). The different enzyme classes found in CAZy include glycoside hydrolases (GHs), carbohydrate esterases (CEs), polysaccharide lyases (PLs), auxiliary activities (AAs) and non-catalytic carbohydrate-binding modules (CBMs) that are often linked to CAZymes providing substrate-binding capabilities and improved overall enzyme efficiency (Boraston et al., 2004). Since many CAZyme families display specific activity towards certain polysaccharides, their genetic mapping may give good clues to yeasts that have potential for specific polysaccharide degradation. However, it is important to note that bioinformatic prediction of enzyme substrate specificity and efficiency still needs to be confirmed through wet-lab characterization.

High-throughput prediction and annotation of CAZymes have recently become feasible using the automated online meta server dbCAN2, which predicts CAZymes based on known protein domains (Zhang et al., 2018). The tool provides researchers with an opportunity to rapidly identify CAZymes from DNA sequences and even provide protein sequences for each CAZyme identified. Other online available bioinformatic tools include CUPP that predicts carbohydrate-active enzymes by conserved unique peptide patterns (Barrett and Lange, 2019) and AUGUSTUS that predicts proteins in eukaryotic genomic sequences (Hoff and Stanke, 2013).

2.3. Screening methods

The identification and characterization of plant polysaccharide-degrading yeasts require sophisticated screening methods which are reviewed below.

2.3.1. Polysaccharide based substrates

The simplest method for screening of cellulolytic and xylanolytic yeasts is to grow them in liquid media on the corresponding polysaccharide and evaluate the growth by counting cells, measuring the turbidity against a polysaccharide-free control growth medium, or to follow the disappearance of the polysaccharide carbon source from the medium. As this approach is applied for individual strains, it is not a high-throughput screening method unless performed in multi-well microplates. The first screening of xylanolytic yeasts deposited in a yeast collection was done in this way (Biely et al., 1978).

More powerful are screening methods performed in solid agar media containing a polysaccharide as the single carbon source. Solid media can

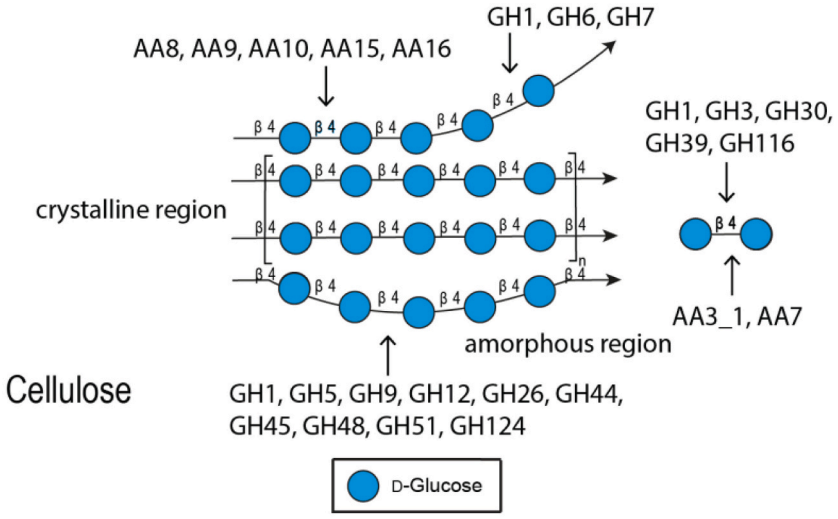

Fig. 2. Chemical structure of cellulose and the CAZymes involved in its deconstruction.

be supplied with fine powder of insoluble or partially soluble polysaccharides, and the hydrolysis is visualized by formation of transparent areas around the growing colony on a cloudy background. Dissolution of oat spelt xylan was for instance a criterion for selection of xylanolytic yeasts (Bhadra et al., 2008; Lara et al., 2014).

More sensitive are the screening methods with soluble polysaccharides which are specific for enzymes cleaving the polysaccharide chain in endo-fashion. The most common screening method for extracellular glycoside hydrolases is the use of Congo Red staining of agar media supplemented with soluble polysaccharides where the monomers are linked by β-glycosidic linkages, such as xylan, β-mannan or soluble cellulose derivatives such as carboxymethyl- or hydroxyethyl cellulose (Teather and Wood, 1982). The principle is the formation of a color complex of Congo Red with unhydrolyzed polysaccharides. After de-staining, colorless areas around the enzyme-secreting colonies will appear. Gram's iodine has also been used for staining of non-hydrolyzed CMC (Johnsen and Krause, 2014; Kim et al., 2019). Another screening method for endoglycanases is based on precipitation of unhydrolyzed polymers in gels by hexadecyltrimethylammonium bromide (CTAB) (Bertheau et al., 1984). The method can be used for polysaccharide containing both α- and β-glycosidic linkages.

An alternative to the Congo Red staining is the application of soluble covalently dyed polysaccharides with a controlled dye content not blocking the action of the enzymes. This method does not require the staining and de-staining steps. The secretion of enzymes leads to decolorization of the areas around colonies due to a radial diffusion of dyed polysaccharide fragments. In contrast to Congo Red, the dyed polysaccharides enable continuous monitoring of enzyme secretion in the growing colonies. In case the hydrolases are confined to the cell surface, the radial diffusion of dyed fragments remains limited to the cell colony and the results are less unambiguous. The first substrates of this type were Remazol Brilliant Blue (RBB)-glucuronoxylan for endo-β-1,4-xylanase and Ostazine Brilliant Red (OBR)-hydroxyethylcellulose for endo-β-1,4-glucanase (cellulase) (Biely et al., 1985; Farkaš et al., 1985). A covalently dyed galactomannan, OBR-galactomannan, was successfully used for screening of endo-β-1,4-mannanase activity (Kremnický et al., 1996). A combination of two differently dyed polysaccharides in the same growth medium enables simultaneous screening for two different endoglycanases (Kremnický et al., 1996). These substrates are available at the Institute of Chemistry, Slovak Academy of Sciences in Bratislava, Slovakia and Biosynth Carbosynth. Other variants of soluble covalently dyed polysaccharides are available at NEOGEN. A new generation of chromogenic polysaccharide hydrogel substrates based on chlorotriazine dyes has been developed for a high-throughput analysis of biomass-degrading enzymes (Kračun et al., 2015). All substrates of this type have the potential to be incorporated into fully or semi-automated robotic enzyme screening systems (Kračun et al., 2015). After screening, xylanase activity is usually confirmed by measuring reducing sugars released from xylan by 2,4-dinitrosalicylic acid (DNS), Somogyi-Nelson or 2,2'-bicinchoninate assays (Miller, 2002; Nelson, 1944; Somogyi, 1937; Waffenschmidt and Jaenicke, 1987).

Table 1
Classification of cellulolytic enzymes in the CAZy database.

Enzymatic activity	CAZy family
endo-β-1,4-glucanases (EC 3.2.1.4)	GH 5, 6, 7, 8[a], 9, 10, 12, 44[a], 45, 48, 51, 124[a]
glucan 1,4-β-glucosidase (EC 3.2.1.74)	GH 1, 3, 5, 9, 39
cellulose 1,4-β-cellobiosidase releasing cellobiose from the non-reducing end (EC 3.2.1.91)	GH 5, 6, 9
cellulose 1,4-β-cellobiosidase releasing cellobiose from the reducing end (EC 3.2.1.176)	GH 7, 48
β-glucosidases (EC 3.2.1.21)	GH 1, 3, 5, 30, 39, 116
LPMO (EC 1.14.99.54, EC 1.14.99.56)	AA 9 (formerly GH61), 10, 15, 16
cellobiose dehydrogenases (EC 1.1.99.18)	AA 3_1, 8
glucooligosaccharide oxidase (EC 1.1.3.-)	AA 7

[a] GH8, GH44 and GH124 endo-β-1,4-glucanases have only been identified in bacterial species.

2.3.2. *Chromogenic and fluorogenic glycosides*

Chromogenic and fluorogenic glycosides are widely used to detect and quantify particularly glycosidases but some of them may be used also for endo-acting enzymes. The most common substrates are 4-nitrophenyl and 4-methylumbelliferyl glycosides. Liberation of 4-nitrophenol is followed photometrically at 405–410 nm after termination of the enzymic reaction with alkaline reagents. Fluorimetry is used to follow the liberation of 4-methylumbelliferone (excitation at 365 nm and emission at 448 nm at pH 10–11) in alkaline conditions (Gee et al., 1999; Robinson and Willcox, 1969). Respective 4-nitrophenyl and 4-methylumbelliferyl glycosides are used for a detection of β-glucosidase, β-xylosidase, α-arabinofuranosidase, β-mannosidase, α-galactosidase and acetyl esterase activities. 4-*O*-Methylumbelliferyl glycosides of cellobiose can serve as screening substrates for cellobiohydrolases and some cellulases, and 4-*O*-methylumbelliferyl glycosides of xylobiose and xylotriose as substrates for endoxylanases (van Tilbeurgh and Claeyssens, 1985; Hrmová et al., 1986). However, the test with glycosides of oligosaccharides is not specific, since the aglycon can be released from the substrate in two or three steps of hydrolysis by the corresponding

glycosidases, such as β-glucosidases and β-xylosidases. Since the fluorogenic substrates offer extremely sensitive and rapid detection of enzyme activities, they are not used in growth media, but their solutions are just poured on the gel with already developed cell colonies. Another option is to overlay developed cell colonies with low-melting point agarose gel containing the substrate to avoid thermal denaturation of the enzymes (Biely et al., 1992). Examples of protocols of some of the plate screening methods can be found in a recent publication (Karnaouri et al., 2021).

3. Yeasts and cellulose degradation

3.1. Enzymes of cellulose hydrolysis

Enzymatic hydrolysis of cellulose to glucose requires cooperation of endo and exo-acting enzymes (Fig. 2, Table 1) (Lynd et al., 2002). Endo-β-1,4-glucanases attack randomly amorphous regions of the polysaccharide, generating cellooligosaccharides of various lengths. Exoglucanases act in a processive manner on the reducing or nonreducing ends of cellulose, liberating either cellobiose or glucose. Exoglucanases can also act on microcrystalline cellulose. Finally, β-glucosidases hydrolyze soluble cellooligosaccharides and cellobiose to glucose. Other important enzymes able to act on highly crystalline cellulose are the copper-dependent lytic polysaccharide monooxygenases (LPMOs) which are classified as Auxiliary Activity (AA) in CAZY database (http://www.cazy.org). LPMOs cleave the β-1,4-glycosidic bonds in crystalline cellulose, leaving the C1 or the C4 carbon oxidized (Beeson et al., 2012; Filiatrault-Chastel et al., 2019; Horn et al., 2012; Lombard et al., 2014; Sabbadin et al., 2018; Vaaje-Kolstad et al., 2010). Furthermore, oxidoreductases acting on cellooligosaccharides are cellobiose dehydrogenases and glucooligosaccharide oxidases.

3.2. Naturally occurring cellulolytic yeasts

While numerous filamentous fungi are known to utilize insoluble, crystalline cellulose (Avicel, filter paper (FP), α-cellulose) as a sole carbon source, yeasts have not been unambiguously reported to have this ability. Most of the cellulases described in yeasts are acting only on soluble derivatives of cellulose such as carboxymethyl cellulose (CMC) or hydroxyethyl cellulose (HEC). The exceptions occur in the genera *Trichosporon*. *Tr. cutaneum* and *Tr. pullulans* exhibited limited growth on ball-milled Whatman no. I filter paper (Dennis, 1972; Stevens and Payne, 1977). However, the levels of extracellular cellulase were found to be much lower in media with highly ordered forms of cellulose, such as α-cellulose and dewaxed cotton yarn (Stevens and Payne, 1977). The ability of several *Trichosporon* and *Cryptococcus* strains to grow on Avicel and to produce enzymes active on Avicel, FP-cellulose as well as CMC was also shown by Giese et al. (2017) and de Souza et al. (2013). The highest cellulase activities were exhibited by *Tr. laibachii*, *Tr. moniliiforme* and *Cr. laurentii*. Other yeasts reported to show activity or ability to grow on crystalline cellulose belong to genera *Candida* (*C. homilentoma*, *C. kashinagacola*, *C. tropicalis*, *C. utilis*), *Meyerozyma (Candida) guilliermondii*, and tropical isolates of *Aureobasidium pullulans* (Kudanga and Mwenje, 2005; Mattam et al., 2016; Villas-Boas et al., 2002; Yun et al., 2015). However, the limited growth yields and extremely low levels of cellulase activity produced suggest that the yeasts are not able to efficiently utilize the insoluble cellulose. Moreover, the commercial cellulose preparations vary in fine structure, and particularly in content of the amorphous regions and content of hemicelluloses as impurities (Lynd et al., 2002). All these properties of cellulose substrates undoubtedly affect the results. Unfortunately, none of the reports claiming yeast growth on insoluble crystalline cellulose analyzed the released products so the ability of the yeast strains to cleave cellulose was not sufficiently proven.

On the contrary, the production of cellulolytic enzymes acting on soluble forms of cellulose including cellooligosaccharides has been described in many yeast species (please see the complete list in the Mendeley Data repository). The use of CMC as an enzymatic substrate has weakened the meaning of the term "cellulolytic," since many organisms that cannot degrade cellulose can hydrolyze CMC or other soluble cellulose derivatives using β-1,3-β-1,4-glucanase (Lynd et al., 2002). Several strains of *Cryptococcus*, *Trichosporon*, *Debaryomyces*, *Aureobasidium, Myriangiale* and *Candida* species (*C. homilentoma*, *C. kashinagacola*, *C. tropicalis*) that showed the activity on CMC were isolated from different environments including the ambrosia beetle and soil samples (Augustín, 2000; Giese et al., 2017; Gomes et al., 2015; Jaiboon et al., 2016; Korhola et al., 2014; Lee et al., 1986; Mattam et al., 2016; Shariq and Sohail, 2019; Yun et al., 2015). A great variety of the yeasts active on CMC was isolated from the integuments of males of leaf-cutting ants (*A. pullulans*, *Cr. flavescens*, *Cr. nemorosus*, *Hannaella kunmingensis*, *Tr. chiarellii*, *Rhodosporidium babjevae*, *Rhodosporidium lusitaniae*, *Rhodotorula mucilaginosa*, *Rhodotorula marina*) (Arcuri et al., 2014). CMC-ase was also produced by psychrotolerant yeast strains from *Rhodotorula*, *Mrakia*, *Cryptoccocus*, *Aureobasidium*, *Leuconeurospora*, and *Tetracladium* species (Carrasco et al., 2012, 2016; Chi et al., 2009a; Krishnan et al., 2011; Pathan et al., 2010). As mentioned above, the activity on CMC does not correlate with the ability of strains to utilize insoluble cellulose as a carbon source. It seems that in nature, the crystalline cellulose is primarily degraded by fungi which provide soluble saccharides for yeast growth.

3.3. Yeast cellulases

3.3.1. Endo-β-1,4-glucanases

The only purified and characterized yeast endo-β-1,4-glucanase active on crystalline cellulose is a thermostable GH45 enzyme from *P. pastoris* GS115 containing five different CBM1 modules at the N-terminus (Couturier et al., 2011). The enzyme is active on variety of soluble substrates such as lichenan, β-1,4-1,3-glucan, CMC, HEC, glucomannan but it also displays activity on Avicel. The removal of CBMs decreases the thermostability of the enzyme, although both binding to crystalline cellulose and hydrolysis of crystalline cellulose and cellohexaose were substantially boosted by the presence of one CBM rather than five.

The other yeast cellulases that have been purified and characterized are active only on the soluble form of cellulose, CMC. A thermostable GH5 CMC-ase of 34 kDa inducible by CMC and cellobiose was purified from the strain *Cr. flavus* MUT-6 isolated from soil (Hatano et al., 1991) and expressed in *S. cerevisiae* (Cui et al., 1992; Hatano et al., 1994). CMC-ases with similar properties were also found in *Cryptococcus* sp. S-2 (Thongekkaew et al., 2008) and psychrotrophic yeast *Rhodotorula glutinis* KUJ 2731 isolated from soil (Oikawa et al., 1998). Moreover, the marine *A. pullulans* 98 isolated from surface seawater of sea saltern at Yellow sea in China was shown to produce CMC-ase of 67 kDa that also belong to the GH5 family (Chi et al., 2009a; Rong et al., 2015).

3.3.2. Exo-β-1,4-glucanases and β-glucosidases

Production of yeast exo-β-1,4-glucanases (GH7 cellobiohydrolase) has only been reported for the yeast-like fungus *Geotrichum candidum* 3C (Borisova et al., 2015), which was later re-classified as the ascomycetous filamentous fungus *Scytalidium candidum* 3C belonging to the class of *Leotiomycetes* (Pavlov et al., 2018). No other yeast exo-β-1,4-glucanases have been reported. On the other hand, β-glucosidase activity has been described in many yeasts including *Saccharomyces*, *Kluyveromyces*, *Pichia*, *Candida*, *Brettanomyces*, *Rhodotorula, Sporobolomyces, Trichosporon*, *Cryptococcus*, *Aureobasidium*, *Hansenula*, *Kloeckera*, *Metschnikowia*, *Meyerozyma*, *Ambrosiozyma*, *Saccharomycopsis, Debaryomyces* (Giese et al., 2017; Iembo et al., 2002; Leclerc et al., 1987; McMahon et al., 1999; Onishi and Tanaka, 1996; Saha and Bothast, 1996a; Shariq and Sohail, 2019; de Souza et al., 2013; Yun et al., 2015). The production of β-glucosidases by wine yeasts has been recently reviewed by Zhang et al. (2021). The widespread distribution of β-glucosidases in yeast may be caused by a diverse specificity of these enzymes, some of which

Table 2
Cellulolytic yeasts: Cellulolytic CAZymes predicted from sequenced ascomycota and basidiomycota shown to grow on CMC and/or Avicel.

Clade	Species	Activity	LPMO	CDH	β-G	Endo-β-1,4-glucanase/CBH				
			AA9 AA16	AA3_1 AA8	GH1 GH3	GH5	GH6 GH7 GH9	GH10 GH12	GH45 GH51	Total
CUG-Ser1	*Candida homilentoma*	Avicel-ase + CMC-ase			GH3(4)	GH5, GH5_9(3), GH5_22, GH5_49				10
	Candida tropicalis	Avicel-ase			GH3(3)	GH5_9(3), GH5_12, GH5_22, GH5_49				9
	Debaryomyces hansenii	Avicel-ase + CMC-ase		AA3_1 (2)	GH3(4)	GH5_9(3), GH5_12, GH5_22, GH5_49			GH51	13
	Meyerozyma guilliermondii	Avicel-ase + CMC-ase		AA3_1	GH1 GH3(5)	GH5, GH5_9(2), GH5_22, GH5_49				12
Pichiaceae	*Ambrosiozyma kashinagacola*	Avicel-ase + CMC-ase			GH3(2)	GH5_9(3), GH5_12, GH5_49				7
	Pichia kudriavzevii	CMC-ase			GH3(2)	GH5_9(2), GH5_12, GH5_49				6
Dipodascaceae	*Geotrichum candidum*	CMC-ase + Avicel-ase	AA9 (4)		GH3(2)	GH5_9(2), GH5_12(3), GH5_49(2)			GH45 (4)	17
Saccharomycetaceae	*Kazachstania unispora*	CMC-ase				GH5_9(3), GH5_12, GH5_49				5
Phaffomycetaceae	*Wickerhamomyces anomalus*	CMC-ase			GH3(6)	GH5_9(2), GH5_12, GH5_49				10
Saccotheciaceae	*Aureobasidium pullulans*	CMC-ase	AA9 (6) AA16	AA3_1 (3) AA8 (2)	GH1(6) GH3 (21)	GH5_4, GH5_5(3), GH5_7(3), GH5_9(5), GH5_12, GH5_16(2), GH5_22, GH5_23(9), GH5_49	GH6, GH7, GH9, GH10 (4)	GH12 (3)	GH45 GH51 (3)	83
Trichosporonaceae	*Vanrija humicola*	Avicel-ase + CMC-ase		AA3_1	GH1(4) GH3(5)	GH5_9(3), GH5_12, GH5_50	GH9(2)			17
Ustilaginaceae	*Pseudozyma aphidis*	CMC-ase		AA3	GH1 GH3(3)	GH5_7, GH5_9(5), GH5_12(2), GH5_16, GH5_50(2)	GH9	GH10	GH45 (2) GH51 (2)	22
Cryptococcaceae	*Kwoniella heveanensis*	CMCase	AA9		GH1(2) GH3(7)	GH5_5, GH5_9(6), GH5_12(3), GH5_22(2), GH5_50(2), GH5_54	GH9	GH10	GH51	28
	Papiliotrema laurentii	Avicel-ase + CMC-ase			GH1(3) GH3(6)	GH5_9(6), GH5_12(3), GH5_22 (2), GH5_50, GH5_54	GH9	GH10		24

Number of gene copies of each CAZyme in each species is shown in parenthesis after GH family.

hydrolyze other β-glucosidic linkages and not just β-1,4-linkage present in cellobiose and cellooligosaccharides. β-Glucosidases are usually located intracellularly but they can be also cell-attached or secreted. β-Glucosidase induced by cellobiose, cellulose (CMC and Avicel), and lignocellulolytic substrates was found to be secreted in cellulolytic and xylanolytic yeasts *Trichosporon mycotoxinivorans, Tr. laibachii, Tr. mucoides, Cr. laurentii, Debaryomyces hansenii, A. pullulans* (Giese et al., 2017; Leite et al., 2008). Several yeast β-glucosidases have industrially interesting properties. For example, the highly termostable β-glucosidase from *Aureobasidium* sp. is optimally active at 75 °C and pH 4.5 (Saha et al., 1994; Leite et al., 2007b; Leite et al., 2008), and a β-glucosidase from *C. peltata* displays high tolerance to glucose (inhibition constant Ki 1.4 M) (Saha and Bothast, 1996b).

3.3.3. Lytic polysaccharide monooxygenases (LPMOs)

Genomic analysis of the strain *G. candidum* CLIB 918 revealed the presence of four genes encoding AA9 LPMOs (Morel et al., 2015). Three of them were shown to disrupt cellulose fibers and improve the saccharification of woody biomass (Ladevèze et al., 2017). Together with four GH45 endoglucanases, these AA9 LPMO genes have been specifically retained by *G. candidum* after the filamentous fungi–yeasts split (Morel et al., 2015). The AA9 genes were also reported in genomes of four *A. pullulans* varieties (Gostinčar et al., 2014), but not yet characterized.

3.4. Prediction of cellulolytic CAZymes in yeasts

3.4.1. CAZymes in yeasts with confirmed cellulolytic activity

Among the yeasts shown to grow or enzymatically act on CMC and Avicel, several species have been genome sequenced and analyzed for CAZymes by automated protein annotation and dbCAN2 prediction (Ravn et al., 2021). Selected species are listed in Table 2 together with their predicted cellulolytic CAZymes. Interestingly, ascomycetous yeasts contain relatively few predicted endo-acting cellulolytic CAZymes except putative enzymes from Glycoside Hydrolase (GH) families 3 and 5. CMC can be broken down by mixed-linked β-1,3-β-1,4-glucanases (Lynd et al., 2002) classified in GH3 family, which are present in most of these species. In the GH5 family, putative CAZymes of subfamilies GH5_9, GH5_12, GH5_22 or GH5_49 were found in several yeasts with confirmed cellulolytic activities, suggesting that some of these GH5 subfamilies may also hold cellulolytic activity. No GH5_49 enzymes have to date been biochemically characterized (Lombard et al., 2014) and may thus be interesting targets for future research investigations. It is important to note that the dbCAN2 analysis may fail to predict key cellulolytic CAZymes due to incomplete yeast genomes/proteomes or that the enzymes have low domain conservation.

CAZymes from the yeast-like fungus *A. pullulans* and a few basidiomycetous yeasts from different clades with online available proteomes were also analyzed by dbCAN2 to compare and supplement the dataset from Ravn et al. (2021). *A. pullulans* (with 520 predicted CAZymes) is an exception among the cellulolytic ascomycetous yeast having as many as 83 CAZymes that may play a role in cellulose hydrolysis. Besides containing many GH3 and GH5 enzymes, the yeast contains putative endo-acting GH6, GH7, GH9, GH10, GH12, GH45 and GH51 CAZymes of which some are likely secreted (Vieira et al., 2021; Gostinčar et al., 2014). The assessed basidiomycetous yeasts all possess putative GH9 and/or GH10 endoglucanases (with 65%-100% sequence identity to characterized endoglucanases) with *Kwoniella heveanensis* as the only basidiomycete containing a putative AA9 LPMO. Compared to the ascomycetous yeasts (except for *A. pullulans*), the basidiomycetous yeasts contain more putative cellulolytic CAZymes with a wider range of putative GH1, GH3 and GH5 CAZymes, potentially giving them a

Table 3
Overview of bioinformatically predicted cellulolytic CAZymes in 332 Ascomycetous yeasts.

Endo-β-1,4-glucanases	β-glucosidases	glucan 1,4-β-glucosidase	cellulose 1,4-β-cellobiosidase releasing cellobiose (non-reducing end)	cellulose 1,4-β-cellobiosidase releasing cellobiose (reducing end)	Cellobiose dehydrogenase or LPMO
GH5 (326)	GH1 (48)	GH1 (48)	GH5 (264)	GH7 (1) *Blastobotrys peoriensis*	AA3_1 (110) (CBH)
GH6 (0)	GH3 (264)	GH3 (264)	GH6 (0)	GH48 (0)	AA8 (0) (CBH)
GH7 (1) *Blastobotrys peoriensis*	GH5 (326)	GH5 (326)	GH9 (0)		AA9 (15) (LPMO)
GH8 (1) *Ogataea minuta*	GH30[a] (11) *Blastobotrys* species (9) *Spencermartinsiella europaea* *Sugiyamaella lignohabitans*	GH9 (0)			AA10 (0) (LPMO)
GH9 (0)	GH39 (0)	GH39 (0)			AA15 (0) (LPMO)
GH10 (5) *Blastobotrys peoriensis* *Scheffersomyces lignosus* *Scheffersomyces stipitis* *Spencermartinsiella europaea* *Sugiyamaella lignohabitans*	GH116 (0)				AA16 (0) (LPMO)
GH45 (20)					
GH48 (0)					
GH51 (39)					

The number of species containing a particular CAZyme family within the 332 sequenced species is marked by the number in parenthesis. AA = auxiliary activities; CBH = cellobiose dehydrogenase; GH = glycoside hydrolase, LPMO = lytic polysaccharide monooxygenase
[a] GH30 included subfamilies (GH30_3, GH30_5 and GH30_7).

broader polysaccharide degrading ability (Table 2).

3.4.2. Prediction of cellulases in the Ascomycota yeast CAZyme dataset

The CAZyme analysis of 332 genome sequenced yeast species that spread over the full Ascomycota phylum (Ravn et al., 2021; Shen et al., 2018) provides an opportunity to both identify yeasts enriched in cellulolytic CAZymes but also to draw conclusions about the presence and absence of CAZyme families on a phylogenetic level. The full genetic CAZyme prediction and associated protein sequences of the 332 ascomycetous yeasts can be downloaded from Zenodo (https://zenodo.org/record/4548336/export/hx#.YLSzGqgzaUk, https://doi.org/10.5281/zenodo.4548336). Ravn and colleagues identified several species enriched both in cellulolytic and xylanolytic CAZymes. Whereas several of these yeasts displayed growth and enzymatic activities on different xylan substrates (see Section 4 below), they did not show corresponding growth on CMC and Avicel. As the yeasts' cellulase activities were not determined, there is unfortunately no information about the correlation between the presence of putative cellulolytic CAZymes and enzymatic activities on cellulose substrates for these yeasts.

Overall, the cellulolytic CAZymes in ascomycetous yeast from the 332-yeast dataset are mainly attributed to GH1, GH3 and GH5 families. Several species also contain GH45 and GH51 putative CAZymes (Table 2). An overview of the total predicted cellulolytic CAZymes in 332 ascomycetous yeasts is presented in Table 3. The inability of most ascomycetous yeasts to grow on crystalline cellulose may be due to the absence of one or several of the endo- and exo-acting CAZyme families known to include such enzymatic activities, such as GH6 or GH9 endoglucanases (totally absent in the 332 yeasts) and GH7 cellobiohydrolases (only present in our dataset in *Blastobotrys peoriensis*), GH8 endoglucanases (only present in *Ogataea minuta*) and GH12 endoglucanases (only found in *Blastobotrys mokoenaii*). However, BLASTp analysis showed only low sequence identity to characterized fungal endoglucases for the predicted GH8 CAZyme, and the GH12 CAZyme displayed higher (>70%) sequence identity to fungal xyloglucanases than endoglucanases, highlighting the importance of performing detailed enzymatic characterization. Nevertheless, the *Blastobotrys* species listed above all belong to the *Trichomonascaceae* clade in the ascomycetous phylogenetic three, which holds many CAZyme rich species (Ravn et al., 2021) and may be worth-while to screen for cellulolytic activities.

150 out of the 332 ascomycetous yeasts are able to grow on cellobiose (Shen et al., 2018). The high abundance of putative GH1 and GH3 β-glucosidases in ascomycetous yeasts may suggest an opportune preference for oligosaccharide uptake and utilization from cellulose. Thus, even though most yeasts are unable to depolymerize cellulose, many seem to have the potential to utilize solubilized cellulose fragments made available by bacteria or other fungi during plant polysaccharide decay in nature. Finally, the bioinformatic CAZyme prediction also shows presence of putative cellulolytic LPMOs (Table 3). AA9s were observed in 15 species such as *Ascoidea asiatica* (7 genes), *G. candidum* (4 genes) and *Saccharomycopsis malanga* (4 genes) while all *Lipomyces* species included in the dataset possess one putative AA9 gene. The cellobiose dehydrogenase AA3_1 family was more widespread among yeasts and found in 110 out of 332 species and typically present in one or two copies per species, in good correlation with the many ascomycetous yeast that can utilize cellobiose (Shen et al., 2018).

4. Yeasts and xylan degradation

4.1. Enzymes of xylan hydrolysis

Xylan polysaccharides are the most abundant of the hemicelluloses in the plant cell wall (Wierzbicki et al., 2019). Depending on plant cultivar, the xylan main chain is built of β-1,4-linked xylose and may be substituted by α-1,2- or α-1,3-linked arabinosyl units and/or α-1,2-linked (methyl)-glucuronic acid residues. Arabinosyl side residues are occasionally esterified with phenolic acids, mainly with ferulic acid which provides hydroxyl groups for the formation of cross-links with other feruloylated xylans or with the hydrophobic lignin polymers

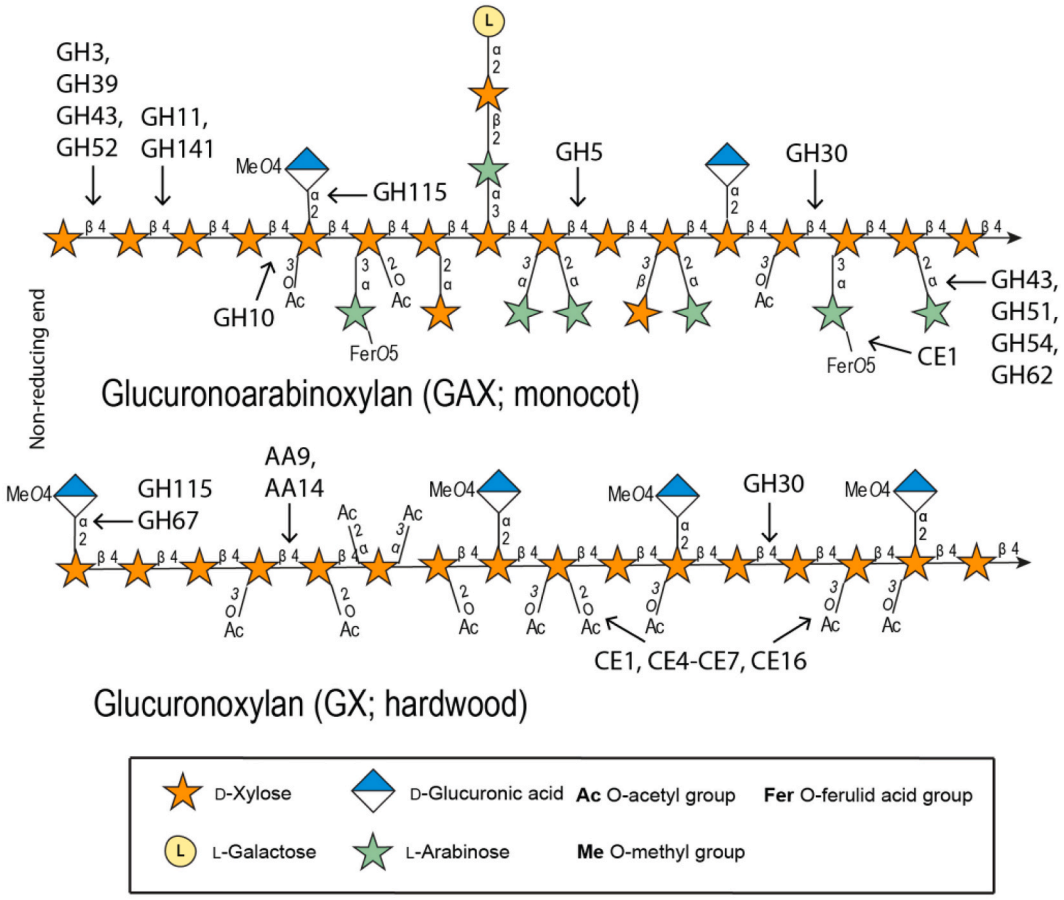

Fig. 3. Chemical structure of glucuronoxylan and glucuronoarabinoxylan and the main CAZymes involved in deconstruction of these polysaccharides.

Table 4
Classification of xylanolytic enzymes in the CAZy database.

Enzymatic activity	CAZy family
endo-β-1,4-xylanase (EC 3.2.1.8)	GH 5, 8[a], 10, 11, 30, 43, 98[a], 141
β-xylosidase (EC 3.2.1.37)	GH 3, 39, 43, 52
reducing-end-xylose releasing exo-oligoxylanase (EC 3.2.1.156)	GH 8, 30
α-glucuronidase (EC 3.2.1.139)	GH 67, 115
α-L-arabinofuranosidase (EC 3.2.1.55)	GH 43, 51, 54, 62
acetylxylan esterase (EC 3.1.1.72)	CE 1, 4, 5, 6, 7, 16
feruloyl esterase (EC 3.1.1.73)	CE 1
LPMO	AA 9, 14

AA = auxiliary activities; CE = carbohydrate esterase; GH = glycoside hydrolase; LPMO = lytic polysaccharide monooxygenases.

[a] GH8 and GH98 endo-β-1,4-xylanases have only been identified in bacterial species.

(Mnich et al., 2020). Most plant xylans are partially *O*-acylated giving rise to acetylated arabinoxylan (AX), glucuronoxylan (GX) and glucuronoarabinoxylan (GAX) (Ebringerová and Heinze, 2000). Alkali extracted xylans are deprived of acetic and phenolic acids. To degrade xylan, the cleavage of the main chain by endo-acting xylanases must be accompanied by the action of a range of xylanolytic debranching enzymes including carbohydrate esterases (CEs) removing the acetyl and feruloyl groups (see Fig. 3 and Table 4).

4.2. Naturally occurring xylanolytic yeasts

In contrast to the limited number of yeast and yeast-like microorganisms capable of growing on cellulose, the ability of yeasts to utilize xylan as a sole carbon source is relatively wide-spread. The first screenings of yeasts for production of xylan-degrading enzymes were done more than 40 years ago. Biely et al. (1978) tested 95 strains of 35 genera of yeasts and yeast-like organisms and only 13 (from genera *Aureobasidium*, *Cryptococcus* and *Trichosporon*) utilized xylan as a carbon source. Xylanase production was also described for *Trichosporon cutaneum* and *Tr. pullulans* grown on ball-milled filter paper, and particularly on xylan (Dennis, 1972; Stevens and Payne, 1977; Liu et al., 1999). Of 250 yeast strains able to grow on D-xylose, only 19 strains mostly belonging to the genus *Cryptococcus*, *Scheffersomyces (Pichia) stipitis* and its anamorph *Sc. (Candida) shehatae* degraded xylan and only two strains of *Sc. stipitis* converted xylan into ethanol under oxygen limitation (Lee et al., 1986). The ability to produce extracellular xylanases is especially widespread among *Cryptococcus* and *Aureobasidium* species (Arcuri et al., 2014; Augustín, 2000; Bhadra et al., 2008; Gomes et al., 2015; Korhola et al., 2014; Manitchotpisit et al., 2009), but also newly discovered yeasts such as *B. mokoenaii* display this trait (Ravn et al., 2021).

Xylanase producing strains isolated from different environments (soil, rotting wood, decaying sugarcane bagasse, tropical peat swamp forest, guts of insect feeding on plants) mainly belong to genera *Aureobasidium*, *Candida*, *Cryptococcus*, *Hannaella, Kodamaea, Meyerozyma, Pseudozyma, Rhodotorula, Saitozyma*, *Scheffersomyces*, *Sugiyamaella*, *Trichosporon* (*Apiotrichum*) (Ali et al., 2017; Arcuri et al., 2014; Elahi and Rehman, 2018; Giese et al., 2017; Gomes et al., 2015; Jaiboon et al., 2016; Lara et al., 2014; Morais et al., 2013, 2020; Tiwari et al., 2020; Yun et al., 2015). Interestingly, several species were shown to produce xylanase as well as to ferment D-xylose (*Candida boidinii*, *Candida cellulosicola*, *Candida pseudorhagii*, *Hamamotoa lignophila*, *M. guilliermondii Scheffersomyces queiroziae*, *Sc. shehatae*, *Sc. stipitis*, *Wickerhamomyces*

Table 5
Characterized xylanolytic enzymes.

Yeast	Strain	Enzyme	GH family	Mw (kDa)	pI	Optimal pH	Optimal temperature (°C)	Reference
	Y-2311-1	xylanase APX-I	11	20				(Leathers, 1986)
	Y-2311-1	xylanase APX-II	11	25	9.4	4.8	54	(Li et al., 1993)
	ATCC 42023	xylanase	11	21		3.0 - 4.5	35	(Vadi et al., 1996)
	Y-2311-1	xylanase	11	21.6		4.0	30 - 50	(Yegin, 2017)
	ATCC 20524	xylanase XynI	11	24	6.7	2.0	50	(Ohta et al., 2001)
	ATCC 20524	xylanase XynII	10	39	8.9	6.0	70	(Tanaka et al., 2006)
	CBS 135684	xylanase		72		6.0	70	(Bankeeree et al., 2014)
	ER-16	crude xylanase				5.0	50	(Leite et al., 2007a)
	NRRL Y-2311-1	crude xylanase				4	45-50	(Myburgh et al., 1991a, 1991b)
	NRRL Y-2311-1	crude β-xylosidase				4-7	45-50	(Myburgh et al., 1991a, 1991b)
Aureobasidium pullulans	CBS 58475	β-xylosidase		224		4.5	80	(Dobberstein and Emeis, 1991)
	ATCC 20524	β-xylosidase		411		3.5	80	(Hayashi et al., 2001)
	ATCC 20524	β-xylosidase	3	88.5	4.57	3.5	70	(Ohta et al., 2010)
	CBS 135684	β-xylosidase		172		6.0	70	(Bankeeree et al., 2018)
	NRRL Y-12974	α-L-arabinofuranosidase		210		4.0-4.5	75	(Saha and Bothast, 1998a)
	NRRL Y-2311-1	α-L-arabinofuranosidase	54	49		3.5-4.0	55	(de Wet et al., 2008)
	ATCC 20524	α-L-arabinofuranosidase	51	110	4.95	4.5	75	(Ohta et al., 2013)
	NRRL Y 2311-1	feruloyl esterase		210	6.5	6.7	60	(Rumbold et al., 2003a)
	NRRL Y 2311-1	α-glucuronidase	67	157		5.0-6.0	65	(de Wet et al., 2006)
Cryptococcus albidus	CCY 17-4-1	xylanase	10	26		5.4	30	(Biely et al., 1980b)
Cryptococcus albidus	CCY 17-4-4	xylanase	10	48	5.7			(Morosoli et al., 1986)
Cryptococcus sp.	S-2	xylanase	11	22	7.4	2	40	(Iefuji et al., 1996)
Cryptococcus adeliae	ATCC 201412	xylanase	10	41.4		5.0-5.5	45-50	(Petrescu et al., 2000)
Cryptococcus flavus	I-11	xylanase	11	21.2	7.0	3.0	50	(Parachin et al., 2009)
Cryptococcus sp.	LEB-AY10	crude xylanase				4.1-4.8	80	(Lopes et al., 2011)
Pichia stipitis	NRRL Y-11543	xylanase	11	43.7		5.5	40	(Basaran et al., 2001)
Pichia stipitis	m4	xylanase		31.6		6.0	50	(Ding et al., 2018)
Pichia stipitis	NP54376	β-xylosidase		37		5.0	50	(Basaran and Ozcan, 2008)
Pichia stipitis	CBS 6054	α-glucuronidase	115	120	4.0	4.4	60	(Ryabova et al., 2009)
Pichia membranifaciens	C105	β-xylosidase		50		6.0	35	(Romero et al., 2012)
Pichia capsulata	X91	α-L-arabinofuranosidase		250	5.1	6.0	50	(Yanai and Sato, 2000)
Pseudozyma hubeiensis	NCIM 3574	xylanase		20.1		4.2	60	(Adsul et al., 2009)
Pseudozyma hubeiensis	NCIM 3574	xylanase		33.3		4.7-6.0	65	(Adsul et al., 2009)
Pseudozyma hubeiensis	NCIM 3574	β-xylosidase		110		4.5	60	(Mhetras et al., 2016)
Pseudozyma brasiliensis	GHG001	xylanase PbXynA		24		4.0	55	(Borges et al., 2014)
Pseudozyma sp.	EBV97-87	xylanase		19.9		4.8	50	(Botto et al., 2019)
Pseudozyma antarctica	T-34 and GB-4 (0)	xylanase	10	33		5.2	50	(Watanabe et al., 2015)
Candida utilis	IFO 0639	β-xylosidase		92	5.6	6.0	40	(Yanai and Sato, 2001)
Candida guilliermondii	NRRL Y-17257	acetyl esterase		67	7.6	7.5	50-60	(Basaran and Hang, 2000)
Blastobotrys mokoenaii	NRRL Y-27120	xylanase	11	23.1			60	(Ravn et al., 2021; du Preez et al., 2009)
Saccharomycopsis fibuligera	KJJ81	α-L-arabinofuranosidase SfABF51A	51	53		7.0	40-45	(Park et al., 2021)
Saccharomycopsis fibuligera	KJJ81	α-L-arabinofuranosidase SfABF51B	51	53		6.0	50	(Park et al., 2021)
Sugiyamaella lignohabitans	CBS 10342	xylanase	30	58		3.5	50	(Šuchová et al., 2022)

pijperi and several *Sugiyamaella* species) (Ali et al., 2017; Morais et al., 2013; Sena et al., 2017). Of sixteen yeast isolates belonging to the genus *Sugiyamaella,* the strains of *Su. lignohabitans*, *Su. xylanicola*, and *Su. valenteae* were the best xylanase producers (Sena et al., 2017). Xylanase activities were detected for several strains of *Tr. laibachi*, *Tr. moniliiforme*, and *Tr. mucoides* isolated from a decayed wood and the environment of ants (Arcuri et al., 2014; Giese et al., 2017; Jaiboon et al., 2016), and several xylanolytic strains of *C. tropicalis* were isolated from the soil or different plants in India, Iran and Pakistan (Mattam et al., 2016; Nouri et al., 2017; Shariq and Sohail, 2019, 2020). Extracellular glycoside hydrolases were examined also among psychrotolerant yeasts isolated from different marine and terrestrial samples collected in Antarctic regions (Carrasco et al., 2012, 2016; Duarte et al., 2013; Faria et al., 2019; Pathan et al., 2010). Xylanase activity was demonstrated by *Moesziomyces antarcticus, Moesziomyces aphidis, Dioszegia fristingensis*, *Cycladophora davisiana*, *Cryptococcus adeliensis*, *Guehomyces (Trichosporon) pullulans*. Finally, novel and highly xylanolytic ascomycetous yeasts such as *B. mokoenaii, Blastobotrys illinoisensis*, *Blastobotrys malaysiensis*, *Scheffersomyces lignosus* and *Wickerhamomyces canadensis* were recently identified using bioinformatics followed by wet lab characterization in terms of growth and enzymatic activities on different xylans (Ravn et al., 2021).

It is difficult to compare the performances of all the xylanolytic strains identified to date, since values reported in the numerous

publications are not unified (some values are presented in U.ml^{-1}, some in U.mg^{-1} of protein or U.mg^{-1} of substrate). Furthermore, the authors used different substrates (beechwood, birchwood, oat spelt xylan from different suppliers) and the xylanolytic activities were measured by different methods (DNS vs arsenomolybdate; for discrepancy of the assays see Jeffries et al. (1998)) or at different conditions (temperature, pH). However, we can conclude that yeasts from genera *Aureobasidium*, *Blastobotrys, Candida*, *Cryptococcus*, *Meyerozyma, Scheffersomyces, Sugiyamaella* and *Trichosporon* repeatedly display high xylanolytic activities.

4.3. Yeast xylanases

4.3.1. Endo-β-1,4-xylanases and β-xylosidases

The most widely studied hemicellulose- (and cellulose-) degrading yeast-like microorganism is *Aureobasidium pullulans* (Chi et al., 2009b), however, its production of xylanases is very strain specific (Leathers, 1986). The color variants produce high levels of xylanase when growing on xylan (47-373 U.ml^{-1}) while typically pigmented strains yielded lower xylanase activity (2.4-7.3 U.ml^{-1}). In the color variants, extracellular monomeric proteins of 20 to 21 kDa represented nearly half the total extracellular protein. The specific activity of partially purified xylanase (APX-I) from *A. pullulans* Y-2311-1 exceeded 2,000 U.mg^{-1}. Low molecular weight GH11 xylanase (20 - 25 kDa) was purified from different *A. pullulans* strains in several laboratories (Table 5) (Li and Ljungdahl, 1994, 1996; Li et al., 1993; Ohta et al., 2001; Tanaka et al., 2004; Vadi et al., 1996; Yegin, 2017; Yegin et al., 2017). Also GH10 xylanase from *A. pullulans* (XynII) was discovered in culture supernatant of the strain ATCC 20524 grown on xylan (Tanaka et al., 2006). Production of xylanases was also described in other *A. pullulans* strains (Table 5) (Bankeeree et al., 2014; Christov et al., 1997; Christov and Prior, 1993; Leite et al., 2007a; Victoria Gautério et al., 2018; Yu and Gu, 2014). Xylose, xylobiose and arabinose were identified as natural inducers of xylanase from a color variant strain of *A. pullulans* (Leathers et al., 1986). β-Xylosidase was co-produced with β-xylanase (Myburgh et al., 1991a, 1991b) and extracellular as well as cell-associated β-xylosidases were purified from different strains of *A. pullulans* (Table 5) (Bankeeree et al., 2018; Dobberstein and Emeis, 1991; Hayashi et al., 2001; Ohta et al., 2010). Co-induction of xylobiose-permease with the two above mentioned hydrolases has also been reported (Lubomír and Peter, 1998).

The induction of xylan-degrading enzymes has also been intensively studied in the yeast *Cryptococcus albidus* CCY 17-4-1 (Biely et al., 1980b; Biely et al., 1980a). Both extracellular β-xylanase and intracellular β-xylosidase were induced when the cells grew on xylan, xylooligosaccharides and methyl β-D-xylopyranoside (Biely et al., 1980a). The cell-bound β-xylosidase was detected in glucose-grown cells of *Cr. albidus* var. *aerius* that also produced a cell-bound β-xylanase in a constitutive manner (Notario et al., 1976, 1979). A valuable finding of the regulation studies was the co-induction of a plasma membrane transport system for xylan fragments generated by extracellular endoxylanase (Krátký and Biely, 1980). This study suggested that the expression of the xylanolytic enzymes in xylose-fermenting yeasts may not be sufficient for efficient utilization of the polysaccharide without simultaneous introduction of xylooligosaccharide transporters. Properties of β-xylanase from *Cr. albidus* were studied in detail (Biely et al., 1981; Biely and Vršanská, 1983, 1988; Vršanská et al., 1990). The first nucleotide sequence of a yeast xylanase gene was published for *Cr. albidus* GH10 endo-1,4-β-xylanase (Boucher et al., 1988; Morosoli et al., 1986). The gene was cloned and expressed in *Escherichia coli*, *S. cerevisiae* and *Sc. stipitis* (Moreau et al., 1992; Morosoli et al., 1992, 1993). Structural characterization of a xylanase from a psychrophilic strain of *Cr. albidus* was focused on its glycosylation pattern and the disulphide bridges presence (Amoresano et al., 2000). Xylanases were characterized also from other strains of *Cryptococcus* genus (Table 5) (Gomes et al., 2000; Iefuji et al., 1996; Lopes et al., 2011; Parachin et al., 2009; Petrescu et al., 2000).

Pseudozyma hubeiensis NCIM 3574 isolated from decaying sandal wood was the first species of this genus reported as a xylanase producer (Bastawde et al., 1994). Extracellular β-xylosidase and α-arabinofuranosidase were also produced in low levels, while no activity towards cellulosic substrates was observed. Two distinct xylanases and one β-xylosidase were purified to homogeneity from this strain (Adsul et al., 2009; Mhetras et al., 2016). Xylanases from the *Pseudozyma* strains *Ps. brasiliensis*, *Pseudozyma* sp. EBV97-87 and *Ps. antarctica*) were also characterized (Table 5) (Borges et al., 2014; de Oliveira et al., 2014; Botto et al., 2019; Watanabe et al., 2015). In *Ps. antarctica* PYCC 5048T and *Ps. aphidis* PYCC 5535T grown on xylan, the production of xylanase was accompanied by high levels of β-xylosidase which was wall-bound as well as extracellular (Faria et al., 2015). The secretome analysis of *Ps. brasiliensis* GHG001 grown on different carbon sources resulted in identification of 4 oxidative enzymes (one from AA3 and three from AA7 family), 3 carbohydrate esterases (two from CE4 and one from CE5 family) and 13 glycoside hydrolases (families GH 3, 5, 11, 15, 16, 18, 25, 51, 62, 128) (Kaupert Neto et al., 2016). Xylan and xylose induced all hydrolytic enzymes.

Xylanases and β-xylosidase were purified and characterized also from xylose-fermenting strains of *Sc. stipitis* (Basaran et al., 2001; Basaran and Ozcan, 2008; Ding et al., 2018) as well as from *Pichia membranifaciens* (Romero et al., 2012) (Table 5). A GH11 (23.10 kDa) xylanase from *B. mokoenii* showed activity (3-4 U.ml^{-1}) towards different xylans (Ravn et al., 2021) with high thermotolerance and activity comparable to pigmented strains of *A. pullulans* (du Preez et al., 2009).

4.3.2. Accessory xylanolytic enzymes

Even though endo-β-1,4-xylanases and β-xylosidases have been extensively studied in yeasts, only little is known about accessory enzymes which are necessary for complete degradation of xylan. The first yeast α-L-arabinofuranosidase was characterized from a color-variant strain of *A. pullulans* NRRL Y-12974 grown on oat spelt xylan (Saha and Bothast, 1998a). α-L-Arabinofuranosidase reached the highest production levels on L-arabinose followed by L-arabitol (Saha and Bothast, 1998b). The genes of GH54 and GH51 α-L-arabinofuranosidases from different strains of *A. pullulans* were cloned, expressed and characterized (de Wet et al., 2008; Ohta et al., 2013) (Table 5). *A. pullulans* NRRL Y-2311-1 was also found to produce a GH67 α-glucuronidase (de Wet et al., 2006) while the xylose-fermenting yeast *Sc. stipitis* CBS 6054 produced α-glucuronidase from GH115 family (Ryabova et al., 2009).

Very little is known about yeast esterases acting on plant polysaccharides. Screening of about 350 yeast strains of 34 genera for extracellular acetyl esterase activity on glucose β-D-pentaacetate showed 13 positive strains of *A. pullulans*, *Candida* sp., *Cr. albidus*, *Cr. laurentii*, *Cr. luteolus*, *P. abadieae*, *P. lindnerii*, *Rhodosporidium toruloides*, *Rhodotorula mucilaginosa*, *Tr. cutaneum*, *Tr. pullulans* (Lee et al., 1987). In *Rhodotorula mucilaginosa* triacetin induced the production of the extracellular esterase which exhibited the highest activity against acetylxylan and glucose β-D-pentaacetate (Lee et al., 1987). An extracellular acetyl esterase (EC 3.1.1.6) was purified from *M. guilliermondii* NRRL Y-17257 (Basaran and Hang, 2000). Production of feruloyl esterase by *A. pullulans* was reported by Rumbold et al. (2003b, 2003a) and Rich et al. (2016). Information on yeasts producing glucuronoyl esterase which cleaves linkages between glucuronic acid of glucuronoxylan and lignin (Arnling Bååth et al., 2016; Biely, 2016) has not appeared yet, although production of this enzyme classified in carbohydrate esterase family CE15 can be anticipated on the basis of *Aureobasidium* species gene assignments (Gostinčar et al., 2014) as well as in *Spencermartinsiella europae* and *Su. lignohabitans* (Ravn et al., 2021).

4.4. Prediction of xylanolytic CAZymes in yeasts

Yeasts with sequenced genome and confirmed capacity to grow on xylan that are part of the 332-yeast dataset (Ravn et al., 2021) include:

Table 6
Xylanolytic yeasts: Xylanolytic CAZymes predicted from whole-genome sequenced xylanolytic yeasts.

Clade	Species	CE	GH3	GH5	GH10 GH11	GH30	GH43	GH51 GH54 GH62	GH67 GH115	Total
Trichomonascaceae	*Spencermartinsiella europaea*	CE1, CE4, CE15	GH3 (11)	GH5_5(2), GH5_9(2), GH5_12(2), GH5_22(4), GH5_49	GH10 (2)	GH30_5, GH30_7	GH43_14, GH43_24	GH51	GH67 GH115 (3)	36
	Sugiyamaella lignohabitans	CE1(2), CE4(3), CE15	GH3 (5)	GH5_9(2), GH5_12(2), GH5_22(2), GH5_49	GH10 (2)	GH30_7		GH51	GH115	23
	Blastobotrys peoriensis	CE1, CE4	GH3 (15)	GH5_9(3), GH5_12(2), GH5_22(5), GH5_49	GH10	GH30_3(3)		GH51		33
	Blastobotrys mokoenaii	CE1, CE4	GH3 (8)	GH5, GH5_5, GH5_7(2), GH5_9(2), GH5_12, GH5_22 (2), GH5_49	GH11	GH30_5, GH30_7	GH43_6, GH43_24	GH51(3) GH62	GH67 GH115 (2)	32
CUG-Ser1	*Meyerozyma guilliermondii*	CE1, CE4	GH3 (5)	GH5, GH5_9(2), GH5_22, GH5_49						12
	Candida tropicalis	CE1, CE4	GH3 (3)	GH5_9(3), GH5_12, GH5_22, GH5_49						11
	Candida intermedia	CE1, CE4	GH3 (8)	GH5_9(2), GH5_12, GH5_22, GH5_49						15
	Scheffersomyces lignosus	CE1, CE4,	GH3 (7)	GH5_5, GH5_9(3), GH5_12, GH5_22(2), GH5_49	GH10 (2)				GH115	20
	Scheffersomyces stipitis	CE1, CE4,	GH3 (7)	GH5_9(2), GH5_12, GH5_22 (3), GH5_49	GH10				GH115	17
Phaffomycetaceae	*Wickerhamomyces canadensis*	CE1(3), CE4	GH3 (5)	GH5_9(2), GH5_12, GH5_22, GH5_49						14
	Wickerhamomyces anomalus	CE1(5), CE4(2)	GH3 (6)	GH5_9(2), GH5_12, GH5_49						17
	Cyberlindnera xylosilytica	CE1(3), CE4	GH3 (12)	GH5_9(2), GH5_12, GH5_22 (4), GH5_49					GH115	25
Pichiaceae	*Candida boidinii*	CE1(2), CE4(2)	GH3	GH5_9(2), GH5_12, GH5_49						9
	Ambrosiozyma kashinagacola	CE1, CE4	GH3 (2)	GH5_9(2), GH5_12, GH5_49						8
	Saturnispora silvae	CE1, CE4	GH3	GH5_9(2), GH5_12, GH5_49						7
Saccotheciaceae	*Aureobasidium pullulans*	CE1(8), CE4(7), CE5(9), CE16(4)	GH3 (21)	GH5_4, GH5_5(3), GH5_7 (3), GH5_9(5), GH5_12, GH5_16(2), GH5_22, GH5_23(9), GH5_49	GH10 (4) GH11 (3)	GH30_7	GH43(30)	GH51(3) GH54 GH62	GH67 GH115	120
Trichosporonaceae	*Trichosporon asahii*	CE1, CE4 (4)	GH3 (4)	GH5_5, GH5_9(3), GH5_12 (2), GH5_22(2), GH5_50			GH43_1	GH51		20
	Vanrija humicola	CE1, CE4 (4)	GH3 (5)	GH5_9(3), GH5_12, GH5_50						15
Ustilaginaceae	*Moesziomyces aphidis*	CE1(2), CE4(5), CE5(3)	GH3 (3)	GH5_7, GH5_9(5), GH5_12 (2), GH5_16, GH5_50(2)	GH10 (2) GH11	GH30_3(2)	GH43(7)	GH51(2), GH54(2) GH62	GH115	42
	Pseudozyma hubeiensis	CE1(3), CE4(7), CE5(5)	GH3 (4)	GH5_7, GH5_9(6), GH5_12 (2), GH5_16, GH5_50(2)	GH10 (2) GH11	GH30_3(2)	GH43(3)	GH51(3), GH54 GH62	GH115	45
Cryptococcaceae	*Kwoniella heveanensis*	CE1(2), CE4(5), CE16	GH3 (7)	GH5_5, GH5_9(6), GH5_12 (3), GH5_22(2), GH5_50 (2), GH5_54	GH10		GH43_13, GH43_26	GH51	GH115 (2)	36
	Papiliotrema laurentii	CE1, CE4 (5), CE5	GH3 (6)	GH5_9(6), GH5_12(3), GH5_22(2), GH5_50, GH5_54	GH10		GH43_13(4)		GH115 (2)	33

Number of gene copies of each CAZyme in each species is shown in parenthesis after GH family.

Barnettozyma californica, C. boidinii, C. gotoi, C. intermedia, Am. kashinagacola, Sa. silvae, C. tropicalis, D. hansenii, K. ohmeri, M. guilliermondii, Su. lignohabitans, Sc. stipitis and *W. anomalus*. The CAZymes linked to xylanolytic activity of a selection of these species as well as *A. pullulans* and representative basidiomycetous species are listed in Table 6. Strikingly, all the ascomycetous yeasts, except for *Sc. lignosus, Sc. stipitis* and the species listed from the *Trichomonascaceae* clade, lack genes for putative endo-xylanases from the GH10 and GH11 and GH30_7 families, suggesting that these yeasts may employ GH5 or even novel endo-xylanases. Moreover, they share very similar predicted CAZyme profiles with GH5_22 and GH5_49 subfamilies as putative endo-acting xylanases and GH3 as putative β-xylosidases. Except *Vanrija humicola* and *Trichosporon asahii* from the *Trichosporonacea* clade, all of the assessed basidiomycetous yeasts contain a GH10 or GH11. *A. pullulans* is an exception to all the analyzed yeasts, having a broad fungi-like repertoire of both confirmed (as described above) and putative xylanolytic CAZymes (120).

Regarding classical endo-acting xylanases (Lombard et al., 2014), only 6 ascomycetous yeast species contain GH10 enzymes (*Spencermartinsiella europaea*, *B. peoriensis*, *Su. lignohabitans*, *Sc. lignosus*, *Sc. stipitis* and *A. pullulans*) while a GH11 xylanase was a unique trait in *B. mokoenaii* and *A. pullulans*. The GH11 xylanase previously reported for *Sc. stipitis* strain NRRL Y-11543 (Basaran et al., 2001) was not identified in the CAZyme database where *Sc. stipitis* is represented by strain NRRL Y-7124, but instead a GH10 was found (Ravn et al., 2021). The presence of a gene coding for GH10 xylanase and absence of GH11 coding gene was also described in the genome of *P. stipitis* strain NRRL Y-11545 (= ATCC 58785 = CBS 6054 = IFO 10063) (Jeffries et al., 2007). BLASTp analysis shows that the strain specific GH11 xylanase in *Sc. stipitis* NRRL Y-11543 is closely related to bacterial GH11 xylanases, and

Table 7
Direct ethanol production from cellulosic materials using recombinant yeasts (selected examples).

Strain	Expression strategy	Enzymes	Raw material	Ethanol titer (reaction time)	Reference
Attempts on purified cellulosic substrates					
Saccharomyces cerevisiae	Secretion	EG of *T. reesei* and BGL of *Sa. fibuligera*	PASC (10 g/L)	1 g/L (192 h)	den Haan et al. (2007)
Saccharomyces cerevisiae	Secretion	BGL of *As. aculeatus*, EG of *T. reesei*, CBH of *Ta. emersonii*	CMC (20 g/L), PASC (20 g/L), and Avicel (10 g/L)	5.4 g/L, 4.7 g/L, and 3.8 g/L (96 h)	Song et al. (2018)
Saccharomyces cerevisiae	Secretion	EG of *Cl. cellulovorans,* BGL of *Sa. fibuligera*	Barley β-glucan (20 g/L)	9.2 g/L (50 h)	Jeon et al. (2009)
Saccharomyces cerevisiae	Direct surface display of single enzyme	EG of *Ba. pumilus*	CMC (10 g/L)	3.9 g/L (55 h)	Yang et al., 2013
Saccharomyces cerevisiae	Direct surface display of single enzymes by yeast consortium	Strain 1 - EG of *Th. aurantiacus*, Strain 2 - CBH of *T. reesei,* Strain 3 - BGL of *As. aculeatus*	PASC (10 g/L)	2.1 g/L (60 h)	Baek et al. (2012)
Saccharomyces cerevisiae	Direct surface display of multiple enzymes	EG and CBH of *T. reesei,* BGL of *As. aculeatus*	PASC (10 g/L)	2.1 g/L (60 h)	Yanase et al. (2010b)
Saccharomyces cerevisiae	Direct surface display of multiple enzymes	BGL of *As. aculeatus,* EG of *T. reesei,* CBH of *Ta. emersonii*	PASC (10 g/L)	2.9 g/L (96 h)	Liu et al. (2015)
Saccharomyces cerevisiae	Direct surface display of multiple enzymes	EG and CBH of *T. reesei,*BGL of *As. aculeatus,*expansin-like protein of *As. oryzae* or *T. reesei*	PASC (20 g/L)	3.4 g/L (96 h)	Nakatani et al. (2013)
Saccharomyces cerevisiae	Direct surface display of multiple enzymes	EG of *T. reesei,*BGL of *As. aculeatus*	Barley β-glucan (45 g/L)	16.5 g/L (50 h)	Fujita et al. (2002)
Saccharomyces cerevisiae	Direct surface display of multiple enzymes	EG and CBH of *T. reesei,* BGL of *As. aculeatus*	PASC (10 g/L)	3 g/L (40 h)	Fujita et al. (2004)
Saccharomyces cerevisiae	Cellulosome (bifunctional) by yeast consortium	Strain 1 - scaffoldin of *Cl. thermocellum*, Strain 2 - EG (secretion) of *Cl. thermocellum*, Strain 3 - CBH (secretion) of *T. reesei*, Strain 4 - BGL (display) of *As. aculeatus*	PASC (1%)	1.8 g/L (94 h)	Kim et al. (2013)
Saccharomyces cerevisiae	Cellulosome (trifunctional)	EG and CBH of *T. reesei*, BGL of *As. aculeatus*, scaffoldin of *Cl. thermocellum*	PASC (1%)	1.8 g/L (70 h)	Wen et al. (2010)
Saccharomyces cerevisiae	Cellulosome (pentafunctional)	EG and CBH of *T. reesei*, BGL of *As. aculeatus*, LPMO of *Th. aurantiacus*, CDH of *H. insolens*, scaffoldin of *Cl. thermocellum*	PASC (1%)	2.7 g/L (96 h)	Liang et al. (2014)
Kluyveromyces marxianus	Secretion	EG and CBH of *T. reesei*, BGL of *N. patriciarum*	β-glucan (2%)	5.4 g/L (168 h)	Chang et al. (2012)
Kluyveromyces marxianus	Direct surface display of multiple enzymes	EG of *T. reeseiand* BGL of *As. aculeatus*	β-glucan (10 g/L)	4.2 g/L (24 h)	Yanase et al. (2010a)
Kluyveromyces marxianus	Cellulosome (pentafunctional) by yeast consortium	Strain 1 - scaffoldins of *Cl. thermocellum*, Strain 2 - EG of *T. reesei*, CBH (synthetic gene), BGL of *N. patriciarum*, LPMO of *Th. aurantiacus*, and CDH of *M. thermophila*	Avicel (1%) and PASC (1%)	3.1 g/L and 8.6 g/L (144 h)	Anandharaj et al. (2020)
Pichia pastoris	Cellulosome (trifunctional) by using *E. coli* for recombinant enzyme production	scaffoldin from IM7 of *E.coli* (*E. Coli* - CBH of *Y. lipolytica*, EG of *Cl. Thermocellum*, BGL of *Thermoanaerobacterium thermosaccharolyticum*)	Avicel, PASC, and CMC (1%)	2.5 g/L, 1.2 g/L, and 5.1 g/L. (72 h)	(Dong et al., 2020)
Attempts on lignocellulosic substrates					
Saccharomyces cerevisiae	Secretion by yeast consortium	Strain 1 - CBH of *Cha. thermophilum,* Strain 2 - CBH of *Ch. lucknowense,* Strain 3 - EG of *T. reesei,* Strain 4 - BGL of *Sa. fibuligera*	NaOH pretreated rice straw (3%) *	14 g/L (46 h)	Lee et al. (2017)
Saccharomyces cerevisiae	Secretion	BGL of *As. aculeatus*, EG and CBH of *T. reesei*	dilute sulphuric acid pretreated corn stover (10%)	2.6% v/v (≈20.5 g/L) (96 h)	Khramtsov et al. (2011)
Saccharomyces cerevisiae	Direct surface display of single enzyme	BGL of *As. acleatus*	Wood chip hydrolysate	30 g/L (36 h)	Katahira et al. (2006)
Saccharomyces cerevisiae	Direct surface display of single enzyme	EG of *T. reeseii*	hydrothermally processed rice straw (100 g/L) *	13.6 g/L (96 h)	Inokuma et al. (2014)
Saccharomyces cerevisiae	Direct surface display of multiple enzymes	EG of *T. reesei*, CBHs of *Ta. emersonii* and *Ch. lucknowense*, and BGL of *As. aculeatus*	hot water pretreated, milled, rice straw (100 g/L) *	18 g/L (96 h)	Liu et al. (2016)
Saccharomyces cerevisiae	Direct surface display of multiple enzymes	EG and CBH of *T. reesei,* BGL of *As. aculeatus*	hot water pretreated rice straw (100 g/L)	7.5 g/L (72 h)	Yamada et al. (2011)
Saccharomyces cerevisiae (modified to utilize xylose)	Direct surface display of multiple enzymes by yeast consortium	Strain 1 - BGL of *As. aculeatus,* CBH and EG of *T. reesei*, Strain 2 - BXYL of *T. reesei,* EX of *As. oryzae*	steam-exploded corn stover (20 g/L)	1.6 g/L (144 h)	Chen et al. (2018)
Saccharomyces cerevisiae (modified to utilize xylose)	Cellulosome (pentafunctional) by yeast consortium	Strain 1 - EG and CBH of *T. reesei*, BGL of *As. aculeatus*, Strain 2 - EX of *As. oryzae*, BXYL of *T. reesei*, Strain 3 - scaffoldins of *Cl. thermocellum*, *Cl. Cellulolyticum* and *Ru. flavefaciens*	steam-exploded corn stover (20 g/L)	0.9 g/L (96 h)	Tian et al. (2019)

EG – endoglucanase, BGL – β-glucosidase, EX – endo-xylanase, BXYL – β-xylosidase, PASC – phosphoric acid swollen cellulose, *Commercial cellulase enzyme mixture was added during the fermentation.

Table 8
Recombinant and wild-type hemicellulolytic yeasts tested in CBPs and other applications (selected examples).

Product/ application	Strain	Wild/ recombinant	Method	Enzymes	Raw material	Results	Reference
Ethanol	*Pichia pastoris*	Wild	Secretion	Xylanases	Beechwood xylan (1%)	0.2% w/v ethanol (72 h)	Lee et al. (1986)
Ethanol	*Hansenula polymorpha*	Recombinant	Secretion	EX of *T. reesei*, BXYL of *As. niger*	Birchwood xylan (9%)	0.3 g/L ethanol (24 h)	Voronovsky et al. (2009)
Ethanol	*Saccharomyces cerevisiae* (intracellular XR and XDH of *Sc. stipitis)*	Recombinant	Direct surface display of multiple enzymes	EX of *T. reesei*, BXYL of *As. oryzae*	Birchwood xylan (≈88 g/L)	7.1 g/L ethanol (62 h)	Katahira et al. (2004)
Ethanol	*Saccharomyces cerevisiae* (xylose-utilizing pathway of *Sc. stipitis*)	Recombinant	Hemicellulosome (bifunctional)	scaffoldin of *Cl. thermocellum*, EX of *T. reesei*, BXYL of *As. niger*	Birchwood xylan (10 g/ L)	0.95 g/L ethanol (80 h)	Sun et al. (2012)
Xylitol	*Candida tropicalis*	Recombinant	Secretion	EX and BXYL of *As. terreus*	Xylan (5g/L) and corncob powder (15g/L)	3.78 g/L and 3.01g/L xylitol (60h and 80h)	(Guo et al., 2013)
Xylitol	*Saccharomyces cerevisiae* (intracellular XR of *Sc. stipitis*)	Recombinant	Direct surface display of multiple enzymes	BGL of *As. aculeatus*, BXYL of *As. oryzae*, EX of *T. reesei*	Rice straw hydrolysate (obtained from hydrothermal pretreatment) and nanofiltered rice straw hydrolysate (70% (v/v))	5.8 g/L and 37.9 g/L xylitol (96 h)	Guirimand et al. (2016)
Xyltiol	*Saccharomyces cerevisiae* (intracellular XR of *Sc. stipitis*)	Recombinant	Direct surface display of multiple enzymes	BGL of *As. aculeatus*, BXYL of *As. oryza*, EX of *T. reesei*	Pretreated (by using hemicellulases) Kraft pulp (50% (v/v))	3.7 g/L xylitol (96 h)	Guirimand et al. (2019)
Triglycerides	*Cryptococcus albidus*	Wild	Secretion	Xylanase	Oat xylan (2%)	0.15 g/g xylan (5 days)	Fall et al. (1984)
Biosurfactant production	*Pseudozyma antarctica*	Wild	Secretion	Xylanase (secreted) (cell-wall associated β-xylosidase)	Beechwood xylan (40 g/ L) and with xylan feed (+40 g/L)	1.3 g/L and 2 g/L mannosylerythritol lipids (10 days)	Faria et al. (2015)
Vine production	*Saccharomyces cerevisiae*	Recombinant	Secretion	ABF of *As. niger*	Grape juice	Increased levels of some volatile compounds	Sánchez-Torres et al. (1996)
Vine production	*Saccharomyces cerevisiae*	Recombinant	Secretion	ABF of *As. awamori*, BGL of *Sa. fibuligera* or *As. kawachii*	Grape juice (*Gewürztraminer* grapes)	Increased free monoterpenes, enhanced aroma profile, eliminated need for the addition of commercial enzyme preparations	Zietsman et al. (2011)
Bread making	*Saccharomyces cerevisiae*	Recombinant	Secretion	EX of *As. nidulans*	Commercial wheat bread flour	Increased bread volume (by 5%) and lower densitiy	Monfort et al. (1997)
Plant protection	*Saccharomyces cerevisiae*	Recombinant	Direct surface display of multiple enzymes	Two xylanases of *Se. turcica*	Mazie	Four defense genes (PR-1, PR-4, SOD, and CAT) of maize were induced.	Liang et al. (2020)

EX – endoxylanase, BXYL – β-xylosidase, BGL – β-glucosidase, ABF – arabinofuranosidase, XR – xylose reductase, XDH – xylitol dehydrogenase.

thus may be a result of horizontal gene transfer from bacteria or an accidental bacterial contamination in the yeast culture. Besides GH11, *B. mokoenaii* also has a GH30_7 enzyme (putative endo- or exo-β-1,4-xylanase or glucuronoxylanase), a feature shared with only *Su. lignohabitans, Sp. europaea* and *A. pullulans*.

β-Xylosidase activity may be found in the GH3 family that is highly abundant in ascomycetous yeasts (263/332 yeasts), and in the GH43 family that is much less represented (22/332 yeasts) (Ravn et al., 2021; Shen et al., 2018). In terms of accessory enzymes, CAZymes from the GH43 or GH51, GH54 or GH62 families may also function as a α-L-arabinofuranosidases, while GH67 and GH115 may function as α-glucuronidases. The α-arabinofuranosidase activity in yeasts is likely represented by GH51 (39/332 yeasts) or GH43 (22/332 yeasts) enzymes and mainly found in yeast from the *Trichomonascaceae* clade and in Basidiomycetous yeasts. Interestingly, no GH54 and only one GH62 α-arabinofuranosidase was predicted in the yeasts (in *B. mokoenaii*). Yeast GH115 α-glucuronidases (28/332 yeasts) are more abundant than those from GH67 family (only 4 predicted).

All CAZyme screened yeasts contain at least one CE with most CEs families targeting de-acylation. Yeasts from *Trichomonascaceae* clade seems to possess most of known enzymes required for hydrolysis of complex GAX. On the other hand, clades *CUG-Ser1*, *Phaffomycetaceae* and *Pichiaceae* seem to lack the enzymes for arabinoxylan hydrolysis. These yeasts may be specialized for hydrolysis of glucuronoxylan, or they may contain new unexplored enzymes. The most striking finding is that for species within the *Pichiaceae* clade, no obvious xylanases, α-arabinofuranosidases or α-glucuronidases were found. It is possible that the hydrolysis of xylan is not a general ability of *Pichiaceae* yeasts, or that these yeasts are a source of unknown enzymes.

5. Recombinant yeasts with cellulose- and xylan-degrading activities

Along with the identification and characterization of natural cellulose- and xylan-degrading yeasts, large research efforts have been made to construct recombinant (hemi)cellulolytic yeast strains intended for industrial lignocellulose-based bioprocesses. To date, several cellulases and hemicellulases from bacteria (e.g. *Clostridium cellulovorans*, *Cl. thermocellum*, *Bacillus pumilus*) and fungi (e.g. *Trichoderma reesei*, *Talaromyces emersonii, Chaetomium thermophilum, Chrysosporium lucknowense, Thermoascus aurantiacus, Saccharomycopsis fibuligera, Aspergillus aculeatus, As. oryzae, As. niger, As. terreus, As. awamori, As. kawachii, As. nidulans, Setosphaeria turcica, Neocallimastix patriciarum*) have been successfully expressed in recombinant yeasts (Tables 7 and 8).

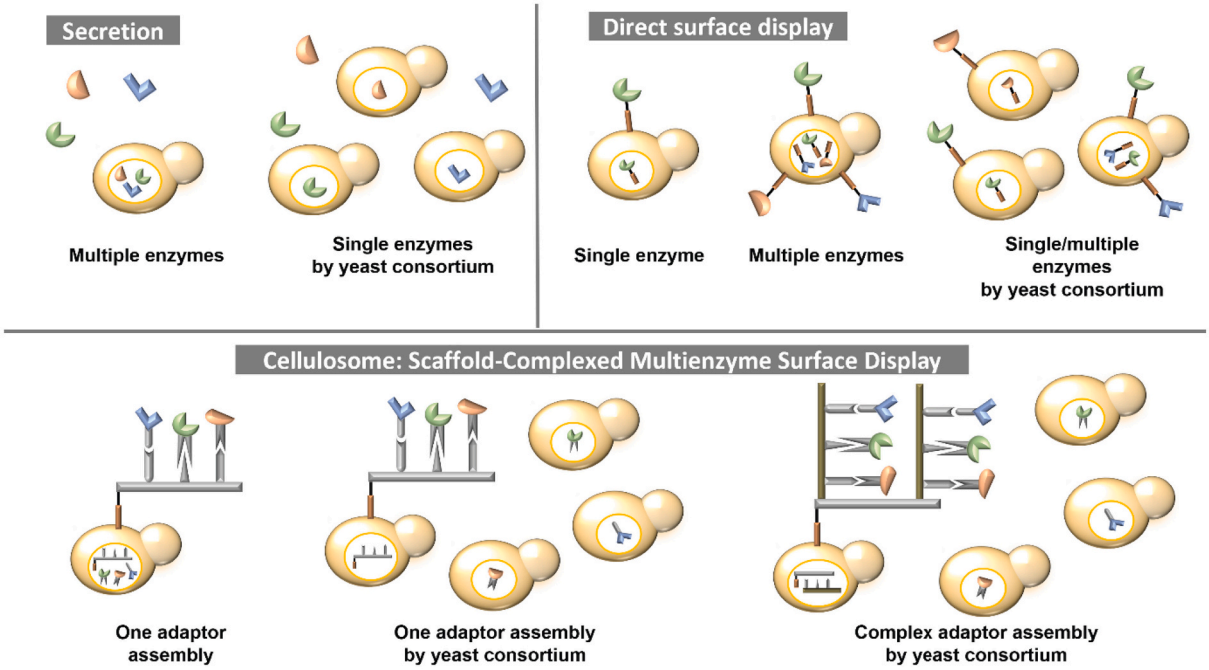

Fig. 4. Different enzyme-producing strategies by recombinant yeasts for lignocellulosic bioprocesses.

The following strategies of producing (hemi)cellulolytic enzymes by recombinant yeasts can be distinguished: *i)* secretion of free enzymes into the fermentation broth, *ii)* production of enzymes directly displayed on the cell surface (direct surface display), and *iii)* production of tethered enzymes assembled in a scaffoldin to form cellulosomes (scaffold-complexed multienzyme surface display) (Guirimand et al., 2016; Oh and Jin, 2020; Smith et al., 2015; Song et al., 2018; Tian et al., 2019).

Secretion of free enzymes by recombinant yeasts is a common strategy mimicking the cellulose-degrading strategy of most studied fungal systems (e.g. *T. reesei*). Efficient production and secretion of recombinant glycoside hydrolases requires multiple metabolic and secretory pathway engineering efforts (Song et al., 2018; Tanaka and Kondo, 2015a, 2015b). Here, one of the most important factors is to select the appropriate secretion signal peptide to fuse to the enzyme of interest. A high-throughput screening method for identifying the optimal enzyme secretion signals for specific target proteins in *S. cerevisiae* has been developed (Bae et al., 2015a) and successfully applied for hydrolytic enzymes (Lee et al., 2017).

Cell surface display technology has been developed to localize heterologously produced, functional proteins or peptides to the cell surface of different microorganisms. It is a powerful technique for diverse applications such as whole-cell biocatalysts, bioremediation, biosensors, health-related processes, and protein engineering (Ueda, 2016; Ye et al., 2021). Yeasts are attractive hosts for cell surface display technology due to their ability to accomplish proper glycosylation and folding of eukaryotic proteins, and the technology has proven successful for attaching CAZymes to yeast cells (Bamba et al., 2021; Liu et al., 2015; Yanase et al., 2010a). Cell surface displayed enzymes not only retain their catalytic activity, but their structural stability can also be increased due to the immobilisation (Kuroda and Ueda, 2013; Liu et al., 2016). One of the key elements for successful display of heterologous proteins on the cell surface is the anchor protein motif which enables stable immobilisation of the target protein through covalent linkages to the cell wall. Glycosylphosphatidylinositol anchor proteins like agglutinins and flocculins have been studied and used extensively for this purpose (Chen, 2017; Duquesne et al., 2014; Smith et al., 2015; Van der Vaart et al., 1997). Currently explored strategies of yeast cell surface display can be distinguished as direct surface display of single or multiple enzymes and scaffold-complexed multienzyme surface display (Ye et al., 2021).

In direct cell surface display, the target protein is genetically fused to the anchor protein, and the chimera is localized to the cell surface by the guidance of an appropriate signal sequence (Fujita et al., 2002; Smith et al., 2015). The activity of the displayed protein is highly dependent on the construct design, where ideally the enzyme's active site is exposed to the target polysaccharides rather than buried in the yeast cell wall. Along these lines, using a spacer with optimised length between the anchor and target protein has been shown beneficial in some instances (Tanaka and Kondo, 2015a; Washida et al., 2001).

Scaffold-complexed multienzyme surface display technology allows construction of recombinant yeasts bearing artificially designed cellulosomes attached to the cell surface. Cellulosomes are multi-enzyme complexes that are naturally produced by anaerobic bacteria to efficiently deconstruct complex substrates containing cellulose and hemicellulose (Tabañag et al., 2018). In order to create a recombinant cellulosome, different enzyme activities contributing to the deconstruction of lignocelluloses are assembled on a non-catalytic scaffoldin protein that is anchored to the yeast cell surface. The scaffoldin proteins contain cohesin units and usually also carbohydrate binding modules (CBM) that mediate adsorption of the cellulosome onto the (hemi) cellulosic substrate. The (hemi)cellulases, bearing specific dockerin units, are connected with the scaffoldin unit via highly specific and high-affinity cohesin-dockerin interactions, and form the multifunctional cellulosome (Anandharaj et al., 2020; Liang et al., 2014; Smith et al., 2015; Tang et al., 2018; Ye et al., 2021). The most commonly used scaffoldin elements are derived from the *Clostridium* genera, while the cellulases and hemicellulases are obtained from different cellulolytic bacteria or fungi (Tables 7 and 8).

Currently explored strategies for producing polysaccharide-hydrolyzing enzymes by recombinant yeasts are illustrated in Fig. 4. However, different combinations or variations of the presented main strategies are also possible. Identification of the most suitable strategy for a given bioprocess is still challenging. Bioprocess design can differ significantly in terms of the type and amount of substrate, the cultivation/fermentation conditions and the yeast inoculum size applied, which affect the yeast's performance and the product titers and yields

and make it difficult to quantitatively compare results between studies.

One advantage of secreting free enzymes into the extracellular medium is that the amount of secreted proteins is not limited by area of the yeast cell surface (as is the case for cell surface immobilized enzymes) (den Haan et al., 2015; Lee et al., 2017). Free enzymes can also diffuse into the lignocellulose structure, enhancing the rate of the hydrolysis reaction (Rohrbach and Luterbacher, 2021). However, diffusion of secreted enzymes can result in lignocellulose degradation far from the production organism, which may not benefit from the released saccharides. This can become problematic if the enzyme production causes a metabolic burden on the cells, as it then promotes faster growth of cells in the population that produce no or only low levels of enzymes.

In contrast to the secreted enzymes, cell surface immobilized enzymes can easily be re-used in repeated batch processes, reducing the additional cost of cell propagation and enzyme production or addition of exogenous enzymes (Liu et al., 2015). In addition, hydrolytic products are generated close to the cell surface, which could improve the substrate utilisation and thus products formation. Enzyme-enzyme synergisms, enzyme proximity effects and tailored enzyme-substrate interactions are considerably enhanced by synthetizing artificial cellulosome complexes (Bae et al., 2015b; Wen et al., 2010). Those complexes can contain multiple cellulases and hemicellulases in suitable ratios and proximities, resulting in improved hydrolyzing performance of the displaying host. However, efficient production of heterologous cellulosome complexes is usually limited by the high number of different enzymes, scaffoldin proteins, and cohesin-dockerin units needed to be synthetized and secreted simultaneously, which easily cause a metabolic burden in the recombinant yeasts (Baek et al., 2012; Wen et al., 2010). In another approach, the different constituents of the cellulosome are produced and secreted by different strains of a mixed yeast consortium, enabling the construction of a variable and highly complex, artificial cellulosome on the scaffoldin-bearing strain through a self-assembling process (Kim et al., 2013; Tian et al., 2019). However, adjusting and maintaining the optimal ratio of the different strains of such a consortium is challenging, likely making this strategy impractical in industrial scale processes.

6. Industrial applications of cellulose- and xylan-degrading yeasts

Cellulose- and xylan-degrading yeasts provide a unique opportunity to combine production of hydrolyzing enzymes with conversion of released sugars into products. The yeasts can be useful in a broad range of industrial sectors, including chemical and pharmaceutical, human and animal nutrition, pulp and paper, textiles, energy, materials and polymers.

Lignocellulose is a highly abundant raw material that can be converted to a range of different products in a biorefinery setting. However, conversion processes of lignocellulose are associated with high production costs, much due to the complex and recalcitrant structure of plant biomass (Zoghlami and Paës, 2019). To convert lignocellulose to di- and monosaccharides that microbial cell factories such as yeasts can utilize, a pretreatment step is necessary to make the polysaccharides within the plant cell walls amenable to the subsequent enzymatic depolymerization step. Addition of saccharifying enzymes accounts for a substantial part of the total process cost (Ferreira et al., 2021). A strategy to improve the cost-efficiency and feasibility of lignocellulosic biorefineries is the previously mentioned CBP, where enzyme production, enzymatic hydrolysis and co-fermentation of the liberated sugars into product are combined in a one-step process. CBP requires a microbial cell factory that can both degrade (hemi)cellulosic polysaccharides and convert the resulting di- and monosaccharides into desired products (Salehi Jouzani and Taherzadeh, 2015; Tabañag et al., 2018). Thus, yeasts having the ability of producing plant polysaccharide-degrading enzymes are ideal candidates for CBPs implemented in a lignocellulosic biorefinery.

Cellulose- and xylan-degrading yeasts also have potential as cell factories for valorization of currently underutilized sugar-rich side products and waste streams formed during industrial processes. The pulp and paper industries are major sources of such lignocellulose-derived polysaccharide-containing waste streams including black liquor, pulp and paper sludge and wastewater (Haile et al., 2021). Wheat or rice straw, bagasse, husks from oat and corn, corn stover and peanut shells are other examples.

6.1. Products

6.1.1. Cellulosic bioethanol

Yeasts are widely used as microbial cell factories for first- and second-generation bioethanol production. *S. cerevisiae* is world famous for its high capacity to ferment hexose sugars to ethanol (Olsson and Hahn-Hagerdal, 1996; Oh and Jin, 2020), but also other species produce satisfactory amounts of ethanol (Navarrete and Martínez, 2020). Thus, there is little surprise that ethanol has been and still is the primary target metabolite for cellulose-degrading yeasts. So far, only recombinant strains have been used for this purpose, likely due to lack of natural cellulolytic yeast species.

For *S. cerevisiae* to degrade cellulose, heterologous expression of endoglucanases, cellobiohydrolases and β-glucosidases is generally needed. If the β-glucosidases are intracellularly localized, expression of cellobiose/cellodextrin transporters is also required (Yamada et al., 2013). As summarized in Table 7, ethanol production from purified cellulosic substrates (CMC, Avicel, phosphoric acid swollen cellulose) by recombinant *S. cerevisiae* has been demonstrated using several different strategies, including secretion of enzymes, direct cell surface display of single enzymes, direct co-display of two or three cellulases and construction of bi-, tri- or penta-functional cellulosomes. The enzymes have either been expressed in a single host or in a consortium of enzyme-displaying yeast strains. Moreover, to increase the cellulose degrading efficiency of recombinant *S. cerevisiae*, artificially designed cellulosomes have been constructed where cellulases are co-expressed with LPMOs and cellobiose dehydrogenases (CDHs) (Liang et al., 2014).

Also *K. marxianus* has been engineered to express cellulases for direct ethanol production from lignocelluloses. The advantages of *K. marxianus* include its thermotolerance, high growth rate and capacity to utilize xylose (Anandharaj et al., 2020). Similar to *S. cerevisiae*, multiple engineering strategies including secretion, direct cell surface display and construction of cellulosomes have been successfully demonstrated in *K. marxianus* (Table 7). Moreover, one of the largest cellulosomes has been expressed in *K. marxianus*, containing up to 63 cellulolytic enzymes including glycoside hydrolases, LPMOs and CDHs (Anandharaj et al., 2020).

Genetic modifications of *P. pastoris* to display small cellulosomes on its cell surface have also been demonstrated (Dong et al., 2020; Jingshen and Yicheng, 2014). *P. pastoris* is a well-studied yeast system for heterologous enzyme production and application in many bioprocesses. As an advantage over *S. cerevisiae*, exceptionally high cell densities can be achieved during cultivations of *P. pastoris*. Engineered *P. pastoris* displaying cellulosomes has also been successfully tested in direct ethanol production from model cellulosic substrates (Dong et al., 2020).

Most studies on recombinant strains of *S. cerevisiae, P. pastoris,* and *K. marxianus* have focused on the development of new strategies to achieve stable genome integration and increased levels and activities of the produced enzymes. The resulting strains display direct ethanol fermentations on model cellulosic substrates, but generally producing only low ethanol titers (1-16.5 g/L) and requiring long reaction times (24-192 h). Thus, the recombinant strains provide proof of concept for their usefulness for bioethanol production, but process development and optimization of product titers, rates and yields must be improved.

A limited number of investigations demonstrate the feasibility of recombinant cellulolytic yeasts for bioethanol production using real lignocellulosic substrate, which is a crucial step towards industrial implementation. These attempts exclusively employed recombinant

S. cerevisiae strains but using a variety of different enzyme-producing strategies (Table 7). For example, direct ethanol production from pretreated corn stover and rice straw or wood chips hydrolysate have been successfully demonstrated, with and without supplementation of commercial enzyme preparations to speed up the saccharification process (Inokuma et al., 2014; Lee et al., 2017; Liu et al., 2016). A yeast consortium containing two types of recombinant strains displaying cellulases or xylanases was also demonstrated to produce ethanol from pretreated corn stover in a CBP setting (Chen et al., 2018). A recombinant *S. cerevisiae* that displays complex cellulosomes containing cellulases as well as xylanases was also constructed and tested in direct ethanol production from pretreated biomass, demonstrating the potential of yeasts with dual action against both cellulose and xylan (Tian et al., 2019). Here, the achieved ethanol titers varied between 0.9-30 g/L.

Another important aspect in terms of the industrial realization would be a more widespread utilization of industrial S. *cerevisiae* strains instead of the commonly employed laboratory strains. Industrial *S. cerevisiae* strains are generally characterized by higher ploidy, higher cell growth rates and yields and higher tolerances to various stresses compared to their haploid laboratory counterparts (Yamada et al., 2011). Unfortunately, still, only limited information is available on modification of industrial *S. cerevisiae* strains for cellulolytic activity and its application in ethanol production (Saitoh et al., 2010; Yamada et al., 2011).

Collectively, we can conclude that many innovative strategies have been developed and promising results were achieved in the field of direct conversion of cellulosic substrates to bioethanol in CBP setups. However, the obtained low ethanol titers and long fermentation times are far from the requirements of a viable industrial process.

6.1.2. Xylan-derived ethanol and xylitol

For lignocellulosic-based production, the yields and titers can be efficiently increased by using not only cellulose but also the hemicellulose/xylan fraction. In this respect, yeasts that can convert xylan to product are of biotechnological interest.

As *S. cerevisiae* cannot naturally metabolize xylose, extensive research efforts have been directed towards developing xylose-fermenting strains, mainly for the purpose of producing ethanol. This can be achieved through heterologous expression of either the xylose oxidoreductive pathway (xylose reductases (XR) and xylitol dehydrogenases (XDH)) donated from various xylose-utilizing yeasts, or the xylose isomerase (XI) pathway of either bacterial or fungal origin (Kwak and Jin, 2017; Moysés et al., 2016). *S. cerevisiae* xylose-utilizing recombinant strains have been further engineered to co-display xylanase enzymes or display hemicellulosomes on the cell surface, and the resulting strains has been shown to produce ethanol from purified xylan substrates or pretreated lignocelluloses (Tables 7 and 8). Recombinant strains of *Hansenula polymorpha* expressing fungal xylanases have also been used for the conversion of purified xylan into ethanol (Lee et al., 1986; Voronovsky et al., 2009) (Table 8). Moreover, several wild-type xylanolytic species and strains have been identified as possible candidates for direct ethanol production from xylan-containing materials due their fermentative and xylanase secreting capabilities (see chapter 4.2). However, in all cases reported so far, only relatively low ethanol concentrations have been achieved (Tables 7 and 8).

Alongside ethanol, xylitol is an industrially interesting metabolite that can be produced in yeast-mediated CBP. Xylitol is widely used in the food, pharmaceutical, cosmetic and dental care industries. Many microorganisms can produce xylitol from xylose through the action of xylose reductases, but the most efficient producers are found among yeasts. Direct production of xylitol from purified xylan, xylan-containing biomass or hydrolysates of partly decomposed xylan has so far only been reported for recombinant strains of *S. cerevisiae* (Guirimand et al., 2019; Guirimand et al., 2016) and *C. tropicalis*. *C. tropicalis* is a native, xylose-assimilating yeast with outstanding capability for xylitol formation, and heterologous expression of xylanases made it suitable for direct xylitol production from lignocellulose (Guo et al., 2013). Moreover, a native strain of *C. tropicalis* was reported to secrete cellulases and xylanases and produce ethanol and xylitol from glucose and xylose obtained from lignocellulosic hydrolysates, respectively (Mattam et al., 2016). Similarly, several *Sugiyamaella* species secreting xylanases were also found to be promising candidates for xylitol and ethanol production (Sena et al., 2017). In the case of solid substrates, only low xylitol concentrations (3-3.8 g/L) were achieved. However, using concentrated hydrolysates, xylitol concentration was significantly enhanced (37.9 g/L), making this a promising strategy for industrial implementation (Table 8).

6.1.3. Foods and beverages

The most common and prevalent applications of yeasts and especially *S. cerevisiae* are related to food and beverage production, and yeasts secreting xylanases or arabinofuranosidases have been tested in both bread making and wine fermentation processes (Table 8).

Continuous, controlled application of different glycoside hydrolyses in bread baking (e.g. dough making) is becoming an attractive way to improve bread quality. The positive effect of using recombinant *S. cerevisiae* secreting endoxylanases on the obtained bread volume has been reported (Monfort et al., 1997). Despite this result, application of recombinant yeasts has not been studied intensively, in contrast to applications using heterologously produced xylanases (De Queiroz Brito Cunha et al., 2018; Elgharbi et al., 2015; Zhan et al., 2014).

To improve the aroma profile of wines, liberation of certain aroma components during the wine making process is crucial. It is now well established that certain monoterpene alcohols are important contributors to the flavour and aroma of wines (Sánchez-Torres et al., 1996), however, they are often present as aroma precursor glycosides. The glycosides can be hydrolysed by different glycoside hydrolyses such as α-L-arabinofuranosidases and β-glucosidases, releasing free monoterpene alcohols (Zietsman et al., 2011). Commercial enzyme mixtures can also be used to release monoterpenes although they commonly consist of crude extracts that often have unwanted and unpredictable side-effects on wine aroma (Zietsman et al., 2011). To avoid this, recombinant *S. cerevisiae* strains expressing arabinofuranosidases have been successfully tested in microvinification experiments (Table 8). *P. capsulata* has also been found to produce a novel arabinofuranosidase that is suitable for the liberation of monoterpenols from aroma precursors and hydrolysed arabinan and arabinogalactan (Yanai and Sato, 2000). Other yeasts such as *P. membranifaciens*, *Hanseniaspora vineae*, *Hanseniaspora uvarum*, and *W. anomalus* with β-glucosidase and β-xylosidase activities have also been used in wine making to increase the level of terpenes (Mateo and Maicas, 2016). Direct surface display of β-glucosidase by recombinant *S. cerevisiae* has also been successfully applied for enhancing wine aroma (Zhang et al., 2019).

Moreover, production of xylooligosaccharides (XOS) from cheap lignocellulosic materials is another extensively studied area. XOS are produced by partial hydrolysis of the xylan fraction of lignocelluloses by enzymatic or acid-catalyzed reaction. Enzymatic production of XOS is usually preferable because the chain lengths of the liberated XOS, and their ratio in the final product is controllable due to the specificity of enzymes. In order to avoid complete hydrolysis of xylan into xylose, xylanase enzyme preparations free from β-xylosidase activity are usually required (Pinales-Márquez et al., 2021). XOS can be used as prebiotics in functional foods or in the pharmaceutical industry. Production of XOS has been studied in *Ps. hubeiensis*, *Cr. laurentii* and *Sc. stipitis* (Otero et al., 2015), but other xylanolytic yeasts could potentially also be used for this purpose.

6.1.4. Lipids and biosurfactants

Microbial lipids are interesting alternatives to plant oil for production of biodiesel, drop-in fuels and chemicals. A series of lipid-accumulating yeasts has been examined for their potential to saccharify xylan and accumulate triglycerides, where *Cryptococcus* and *Trichosporon* showed most potential. Strains of *Cr. albidus* were found to

be especially useful for a one-step saccharification of xylan coupled to triglyceride synthesis (Fall et al., 1984). Moreover, a new oleaginous yeast consortium consisting of *Yarrowia* sp., *Ba. californica* and *Sterigmatomyces halophilus* strains has been shown to accumulate triacylglycerols and produce lipases and xylanases. This yeast consortium is claimed to be suitable for biodiesel production, degrading recalcitrant textile reactive azo dyes and lignin valorization (Ali et al., 2020).

Y. lipolytica, one of the most prominent oleaginous yeasts known to date, cannot efficiently utilize lignocellulosic substrates due to the lack of efficient cellulase and hemicellulase systems. This species has a potent production and secretion machinery for both native and heterologous proteins, and it has been equipped with several xylanases to enable production of lipids from xylan (Wang et al., 2014). In addition, an engineered strain of *Y. lipolytica* co-expressing different β-glucosidases, cellobiohydrolases, and endo-glucanases was also constructed. The recombinant strain could grow both on model cellulose substrates and industrial cellulose pulp, demonstrating its potential for lignocellulosic CBPs (Guo et al., 2017).

Biosurfactant production from lignocelluloses using hemicellulolytic yeast is another promising bioprocess with increasing industrial interest. Biosurfactants are surface-active agents that contain both hydrophilic and hydrophobic moieties and can be applied in the production of food, cosmetics, and pharmaceutics as well as in removal of different toxic agents such as heavy metals (Faria et al., 2015). A wild-type strain of *Ps. antarctica* has been found appropriate for production of one type of biosurfactants - mannosylerythritol lipids - from purified xylan in a one-step bioprocess (Faria et al., 2015).

6.1.5. Xylanases

Since many yeasts are ideal hosts for heterologous enzyme production, they can also be used for production of recombinant enzymes for various innovative applications. For some biotechnological applications, it is a clear advantage to use xylanolytic yeasts that lack effective cellulases. For example, *A. pullulans* strains Y-2311-1 and Y-2311 that display very high levels of xylanase activity and low levels of cellulases have been used for production of patented enzyme preparation for pulp bleaching (Farrel and Skerker, 1990; Christov and Prior, 1993). Moreover, an armed strain of *S. cerevisiae* co-displaying xylanases on its cell surface has been reported to induce pathogen defence genes in corn (Liang et al., 2020).

7. Conclusions and future outlook

In this review, we have gathered, compared and contrasted the natural and recombinant cellulose- and xylan-degrading yeasts known to date. From this work, a comprehensive picture of these yeasts, their associated CAZymes and industrial applications has emerged. The work has also revealed important knowledge gaps and open research questions that still awaits to be answered.

In terms of natural cellulolytic yeast, so far only a few degraders of crystalline cellulose have been identified. These include basidiomycetous yeasts from the *Trichosporon* and *Cryptococcus* genera, which possess several putative GH9 endoglucases, AA9 LPMOs and a diverse set of GH1, GH3 and GH5 enzymes that may be important for utilizing cellulose. Also, the ascomycetous yeast-like fungus *A. pullulans* displays a broad range of cellulolytic CAZymes. However, the vast majority of characterized yeasts cannot degrade crystalline cellulose. Bioinformatic analysis of 332 ascomycetous yeasts, including several species with demonstrated CMC-ase activity, revealed that most of them lack apparent endo- and exo-β-1,4-glucanases including GH6, GH7 and GH9 as well as AA9 LPMOs (Ravn et al., 2021). Thus, it is evident that after the split from the common ancestor, most yeasts have lost the ability to degrade cellulose while the function has been retained in many filamentous fungi.

Compared to the relatively short list of identified cellulolytic yeasts, we find more than a hundred different species that can utilize the major plant hemicellulose xylan as a carbon source. The list contains some overlaps with the identified cellulolytic species, including *A. pullulans* and several *Cryptococcus* and *Trichosporon* species, which all show a broad setup of xylanolytic enzymes. It also contains several other species from a range of different genera. Most notable are *Blastobotrys* sp. and *Spencermartinsiella* sp. from the *Trichomonascaceae* clade that display relatively high xylanase activities (Ravn et al., 2021). CAZyme analysis in confirmed xylanolytic and genome sequenced species revealed that only a few of the yeasts, including those mentioned above, possess typical endoxylanase genes (GH10, GH11, GH30_7). In opposite, species in for example *Wickerhamomyces*, *Candida and Meyerozyma* genera all lack apparent endoxylanases, and may instead possess unique strategies and unknown endoxylanases to assimilate xylan. Although we hypothesize that GH5 subfamily members may be the missing link, it is clear that we still don't have a complete picture of how these yeasts hydrolyze xylan. The CAZyme analysis also points to the occurrence of numerous carbohydrate esterases in yeast and yeast-like microorganisms, which have so far received little attention. Future work to answer the remaining questions and to increase our understanding of function, diversity and mechanisms of yeasts and their enzymes will involve detailed yeast growth screening, heterologous expression and enzymatic activity measurements and structural biology. Moreover, characterization of novel yeast CAZymes may provide new enzyme features in terms of fold, stability and specificity with potential to improve current processes and enable new applications.

Regarding the level of production of major cellulases and hemicellulases, yeasts (expect perhaps *A. pullulans*) can hardly compete with fungi and bacteria. However, yeasts living in symbiosis with fungi may produce high levels of accessory enzymes that are lacking or are insufficiently produced in fungi, and thereby can compete for the hemicellulosic carbons in the native consortia of microorganisms. This may also be the case for yeasts that do not contain the whole set of enzymes required for growing on cellulose and xylan. For example, many yeasts express β-glucosidases for hydrolyzing β-glucosidic linkages including soluble cellooligosaccharides and cellobiose to glucose. Also xylose, the monomeric degradation-product of the xylan backbone, is readily metabolized by multiple yeast species that have been sampled in plant biomass-rich biotopes (Shen et al., 2018). Additional fundamental investigations are needed to shed light on how yeast partake in plant biomass degradation in nature by competing or collaborating with bacteria and filamentous fungi.

In parallel to the work of identifying and characterizing natural (hemi)cellulolytic yeasts, multiple efforts have been made to engineer strains intended of biotechnological processes. To date, the starting strain is most often of *S. cerevisiae* or *K. marxianus* origin, both species being well characterized and efficient producers. Independent on if the enzymes are secreted into the supernatant or attached to the cell surface as single enzymes or as part of cellulosomes, many steps in the engineering process still needs careful finetuning and optimization, as the enzymatic activity levels in the recombinant strains are low compared to filamentous fungi and superior natural yeasts. This is likely due to both insufficient and unbalanced expression levels of the enzymes and suboptimal secretion capacity (Chang et al., 2012; Song et al., 2018). So far mainly enzymes from filamentous fungi and bacteria have been heterologously expressed in the recombinant yeasts. However, mimicking how natural (hemi)cellulolytic yeasts do it may be a successful, alternative way forward. It seems that natural yeasts primarily secrete the major depolymerizing enzymes into the surrounding, while the glycosidases hydrolyzing oligosaccharides to monomers are frequently located intracellularly. Consequently, cellulose- and xylan-degrading yeasts must produce plasma membrane-bound transport systems for uptake of oligosaccharides, and such transporters are also of large biotechnological interest (Krátký and Biely, 1980). In addition to yeast-specific enzymes and transporters, promoters, secretion signals and anchors may prove beneficial for heterologous expression in other yeasts.

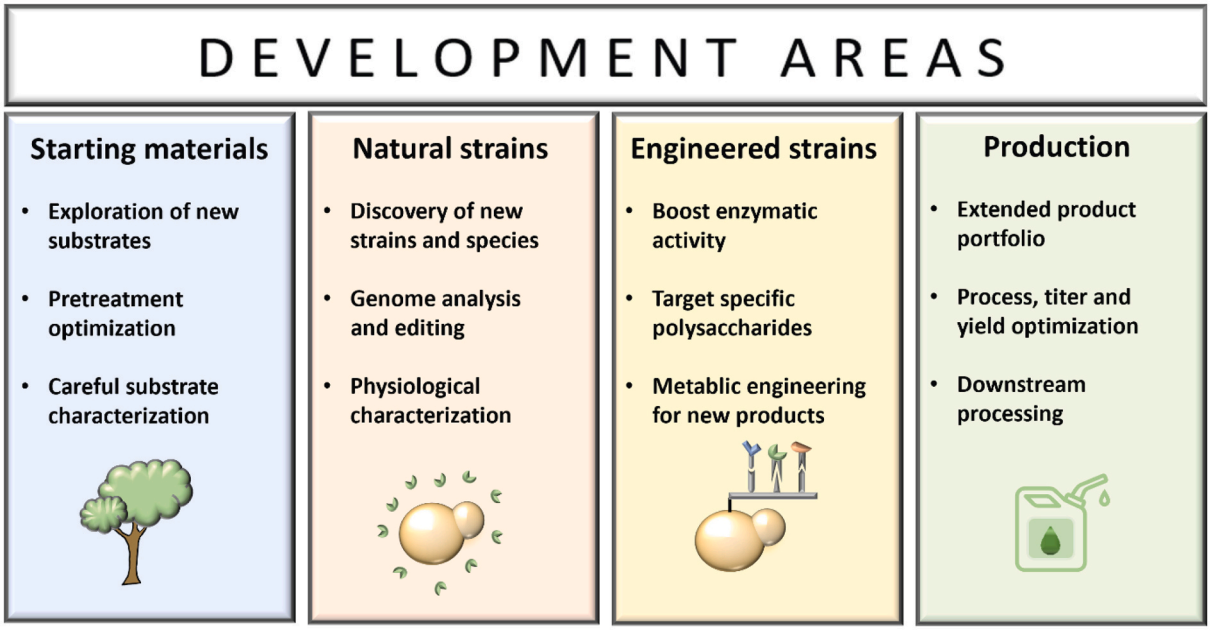

Fig. 5. Development areas for improved biotechnology production processes in which polysaccharide-degrading yeasts are used.

The large potential of (hemi)cellulolytic yeasts in biotechnology is highlighted by ample examples in literature. So far, recombinant cellulolytic strains have been developed with the main purpose of producing bioethanol, whereas mostly natural hemicellulosic yeasts have been used to generate biochemicals, biosurfactants, aroma compounds in wine and bread with improved texture and volume. The list of possible future applications is considerably longer, as yeast cell factories can generate many different biomolecules from cellulose and xylan. To exploit these yeasts more efficiently, process development in several ways is required. As summarized in Fig. 5, this will likely involve *i)* discovery of new strains and exploration of new natural products, *ii)* recombinant strain development, both to boost polysaccharide hydrolysis and to enhance product titers and yields, *iii)* optimization of biomass pretreatment to ensure that the substrate perfectly fits the polysaccharide-degrading yeast of choice, and finally *iv)* process development including downstream processing.

To summarize, the knowledge generated so far on cellulose- and xylan-degrading yeasts is of high importance for the optimization of lignocellulosic biomass conversion processes. The list of xylan- (and cellulose-) degrading yeasts will most likely continue to grow in the years to come, as more species are continuously being identified and characterized. High-throughput growth screening apparatus, sampling of new biotopes and more thorough investigations of strains in existing yeast collections will likely contribute to a rapid expansion of knowledge in this field. As more and more species are being genome sequenced, bioinformatic screening is also becoming a valuable tool that complements conventional bioprospecting and yeast growth screenings for identification of polysaccharide-degrading yeasts. Future research will include careful physiological characterization of the yeast species to determine their precise growth requirements, full substrate ranges and product portfolios.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgements

For JLR and CG, this work has received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No 964430, and Carl Trygger Foundation (Grant nr. CTS 18:118). For KŠ and PB, this work was supported by Scientific Grant Agency under the contract No. 2/0171/22, Slovak Research and Development Agency under the Contract no. APVV-20-0591. For CF and SB, this work was funded by the National Research, Development, and Innovation Fund of Hungary under Grant TKP2021-EGA-02.

Moreover, this article is based upon work from the COST Action Yeast4Bio, supported by COST (European Cooperation in Science and Technology).

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