Mini Review

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Pseudozyma hubeiensis, an unexplored yeast: It's potential in biomass conversion to value added products

Abstract

The species of the genus *Pseudozyma* belong to ustilaginales based on the studies on morphology and molecular characterization. There are 11 species reported so far from this genus that differ from one another in the sequences of ITS and D1/D2 regions. Eleven species of *Pseudozyma* reported so far were studied for assimilation reactions which differentiated all the species of the genus *Pseudozyma* from one another. Almost all the species of the *Pseudozyma* are able to assimilate inositol but *P. hubeiensis* shows negative inositol assimilation reaction. There are no reports available for the production of industrially important enzymes and other biological products from *P. hubeiensis* and hence it remained still unexplored. *P. hubeiensis* was first isolated from sandal wood in our laboratory which produced a complete cellulase free xylanolytic system consisting of endoxylanase and β-xylosidase. The purified xylanases produced smaller chain length (X3-X7) xylooligosaccharides (XOS) which have great potential use as prebiotic in functional food. In addition, it is also used in cosmetics, pharmaceuticals or agricultural products and as a plant growth regulator. It also produces high amounts of metal and ethanol tolerant β-xylosidase qualifying its use in lignocellulosic biomass hydrolysis when mixed with enzyme preparations that are deficient in β-xylosidase. *P. hubeiensis* utilizes simultaneously all the sugars present in biomass hydrolysate and convert them to lipids. In this mini-review, the biology and enzymology underlying the biomass degrading enzymes and the production of lipids/biosurfactants especially MELs are described. An approach to developing *P. hubeiensis* strains for production of biomass degrading enzymes and their application is outlined.

Keywords: *pseudozyma hubeiensis*, Endoxylanase, β-xylosidase, Xylooligosaccharides (XOS), Biomass degradation, mannosylerythritol lipids (MELs)

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The genus pseudozyma

The species of the genus *Pseudozyma* belong to ustilaginales based on the morphological studies[1] and molecular characterization.[2,3] There are 11 species reported so far from this genus that are distinguished by analysing the sequences of combined ITS and D1/D2 regions. Boekhout & Fell[4] reported seven species and Sugita et al.[5] described two species isolated from blood of the patients. Two more species of *Pseudozyma* were isolated from wilting leaves of different plants that are named as *Pseudozyma hubeiensis* and *Psedozyma shanxiensis*.[6] These species are distinguished from other reported species by morphological studies and physiological characterization. The novelty of these two species was also confirmed by molecular taxonomic analysis based on sequencing of 26S rRNA gene, D1/D2 domain and internal transcribed spacer (ITS) regions (6). All 11 species of *Pseudozyma* were further studied for assimilation reactions which differentiated all the species of the genus *Pseudozyma* from one another. The ability to grow at 40°C and no assimilation of erythritol are the characteristics that differentiate *P. shanxiensis* from all other species of *Pseudozyma*. However, *P. hubeiensis* does not assimilate inositol and this characteristic differentiates *P. hubeiensis* from all other species.[6] *P. hubeiensis* was first isolated from decaying sandal wood in our laboratory[7] and then it was sent for identification to National Collection of Yeast Cultures (NCYC) in 2008. The sequencing of 26 rDNA D1/D2 domain and standard taxonomic tests confirmed that it is a novel strain of *P. hubeiensis*. It was then deposited in NCIM Resource Center, CSIR-National Chemical Laboratory, Pune, with an accession number NCIM 3574.

Among all species of *Pseudozyma, Pseudzyma antarctica* (Formally known as *Candida* antarctica) has been a most studied. It is well known producer of glycolipid, manosylerythritol (MEL) from vegetable oil including soybean oil, alkanes, glycerol, glucose and xylose[8] and also from cellulosic materials.[9] MELs show excellent surface active properties in addition to versatile biochemical actions. In addition to its bio-surfactant property, MEL possesses antitumor and cell differentiation induction activities.[10] The other species such as *P. aphidis*, *P. rugulosa, P. fusiformata* are also known to produce MEL.[11] Extracellular esterases[12] and biodegradable plastic degrading enzymes[13] have been reported from *P. antarctica*. The plastic degrading enzyme named PaE produced by *P. antarctica* degrades biodegradable plastic films composed of poly (butylele succinate) (PBS), poly (butylene succinate-co-adipate) (PBSA) and poly (lactic acid) (PLA). Xylose induced xylanases were reported from *P. antarctica* and the 33 kDa purified protein was found to produce xylose from xylan indicating that they are endo-xylanases.[14] Genome and transcriptome analysis of *P. antarctica* was recently reported.[15] The another novel yeast species, *P. brasiliensis* produced the xylan induced secretome containing endo-xylanase and β-xylosidase.[16]

Enzymes and lipids produced by *Pseudozyma hubeiensis*

Though Basidiomycetes yeasts are essential for carbon cycle, the production of biomass degrading enzymes by basidiomycetes yeasts still remained unexplored compared to Ascomycetes. Wide range of enzymes produced by a microbial strain displays its ability to adapt to natural habitats and hence new species may emerge through such adaptations. Such adapted microorganisms have the ability to produce altogether new enzymes of industrial importance. The Basidiomycetes yeasts belonging to the genus *Pseudozyma* have immense potential in industrial processes but they are not still properly explored. The knowledge of the ability of *Pseudozyma* species to degrade biomass polysaccharides is very limited. *P. hubeiensis* was first isolated from sandal wood in our laboratory in 1992 which was found to produce a complete xylanolytic system consisting of cellulase free xylanase[7] and β-xylosidase.[17] The crude xylanase produced by *P. hubeiensis* degraded xylan from different agricultural waste materials to produce xylose which can be the starting material for production of value added chemicals.[18] Such cellulase free xylanases can be used in paper and pulp industries to remove xylan in paper and pulp in place of chlorine which is being used in bio-bleaching process.

Xylo-oligosaccharides (XOS) acting as prebiotics are the most preferred dietary fibres or functional foods. The commercial importance of non-digestible XOS is based on their beneficial properties. XOS stimulate the growth and activity of limited number of bacteria such as *Bifidobacterium* and *Lactobacillus* present in the colon and also suppress the activity of entero-putretive and pathogenic bacteria. In addition, they are moderately sweet with organoleptic characteristics suitable for use as supplements in foods. In addition, they show stability at wide pH range and temperature which make them most suitable dietary supplementation.[19–21] Apart from prebiotic and bulking agents, XOS are used as stabilisers in cosmetics, immune-stimulating agents, and antioxidant in pharmaceuticals. XOS is also known to decrease the blood lipids, protect the liver functions and decrease the blood pressure. It was found that the supplementation of XOS was successful in inhibiting the precancerous lesions and also in lowering the colonel pH value.[22,23] Therefore, diets supplemented with XOS have beneficial effect in improving gastrointestinal health. Furthermore, XOS were found to be more efficient than other prebiotics such as fructo-oligosaccharides in dietary supplementation. The preventive effect of XOS against contact hypersensitivity was also investigated in mice.[24] *P. hubeiensis* NCIM 3574 produces two distinct xylanases which were purified to homogeneity by DEAE cellulose chromatography followed by Sephadex G-50 column chromatography. Molecular masses of two native xylanases were 33.3 kDa (PhX33) and 20.1 kDa (PhX20) which are confirmed by MALDI-TOF mass spectrometry and also SDS-PAGE. The CD spectra analysis revealed that PhX33 has predominantly α-helix and PhX20 contained predominantly β-sheets. The chemical modification studies revealed that the PhX33 has the active site consisting of three tryptophan and one carboxyl residues. The active site of PhX20 is comprised of tryptophan, carboxyl and histidine with one residue each. Carboxyl residue is mainly involved in catalysis and tryptophane residues are solely involved in substrate binding. Histidine residue present at the active site of PhX20 appeared to have a role in substrate binding. LC-MS/MS ion search of tryptic digestion of these xylanases revealed that there is no significant homology of peptides with known fungal xylanase sequences which indicate that these xylanases appear to be new PhX33 hydrolyzed xylan into xylotriose, xylotetraose and xylopentaose and PhX20 hydrolyzed xylan into xylotriose, xylotetraose, xylopentaose, xylohexaose and xyloheptaose. No xylose and xylobiose were detected in the hydrolyzates. Both the xylanases produced only xylooligosaccharides (XOS) with degree of polymerization (DP) 3 to 7.[25] The complete utilization of biomass to obtain bulk chemicals and XOS makes these enzymes very interesting from industrial perspective.

The β-xylosidase from an unexplored yeast, *Pseudozyma hubeinsis* was induced by birchwood xylan when the yeast was grown at 27°C. The enzyme was purified to homogeneity and it was found to be a glycoprotein with 23% glycosylation. The purification protocol involved ammonium sulphate precipitation, QAE-sephadex A50 ion exchange chromatography and sephacryl-200 column chromatography which resulted in 8.3 fold purification with 53.12% final recovery. It is a monomeric protein of 110 kDa molecular weight confirmed by both SDS-PAGE and MALDI-TOF mass spectrometry. The enzyme was active at 60°C and pH 4.5 and stable at pH range (4-9) and at 50°C for 4 h. Its active site is comprised of carboxyl, tyrosine and tryptophan residues. The carboxyl residue is involved in catalysis and tryptophan residue is solely involved in substrate binding. The best match from the search of the NCBInr database was with gi|808364558 glycoside hydrolase of *Pseudozyma hubeiensis* SY62 with 26% sequence coverage confirming that it is a glycoside hydrolase/beta-glucosidase. From the search of customized SWISSPROT database, it was revealed that SWISSPROT does not contain any entries that are similar to the purified enzyme. The properties such as high catalytic performance, significant stability and activity at acidic pH and high temperatures, high metal and ethanol tolerance qualify this enzyme for use in the hydrolysis of lignocellulosic biomass when mixed with efficient and suitable enzyme complexes since most of the commercial enzyme preparations are deficient in β-xylosidases.[17]

Lipids accumulated by oleaginous yeasts is viewed as promising source for second generation biodiesel since the fatty acid compositions produced by these yeast is suitable for biodiesel production. These yeasts grow faster compared to other oleaginous microorganisms and have the potential to convert various carbon sources such as glucose, xylose, starch, Cellobiose to lipids.[26–28] Exhaustive screening of large number of yeast isolates led to the discovery of *Pseudozyma hubeiensis* IPM1-10 that converts sugar mixtures consisting of glucose, xylose and arabinose to lipids.[29] Oleaginous microbes utilise xylose exclusively by phosphoketolase pathway resulting in lower yields of lipids.[30] Due to the absence of glucose repression in *P. hubeiensis*, this yeast will turn out to be a potential candidate for second generation biodiesel production from hydrolysate of cellulosic biomass. The mechanism of absence of glucose repression still remained unknown and further study is essential to unravel the sugar assimilation mechanism in *P. hubeiensis*. Sari et al.[31] isolated *P. hubeiensis* Y10BS025 that produced high levels (115g/L) of mannosylerythritol lipids (MELs) during growth in a medium containing yeast extract and soybean oil after 8 days of fermentation with improved shaking conditions. *P. hubeiensis* produces lipase by assimilating oils and secretes bio-surfactants.[32,33] *P. hubeiensis* also can convert sugars such as glucose, xylose and arabinose to lipids and hence it has a great potential for utilization of

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unused biomass sugars and low cost raw materials. The potential of this novel yeast strain for production of value added products from renewable biomass is given in Figure 1.

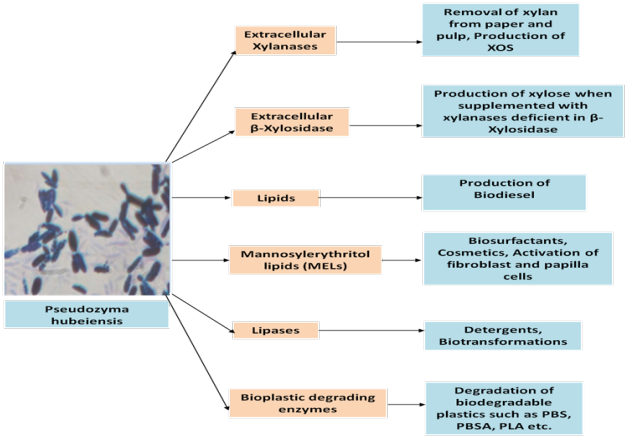

Figure 1 The potential of *Pseudozyma hubeiensis* for production of value added products from renewable biomass.

Conclusion and future perspective

P. hubeiensis produces a complete cellulase free xylanolytic enzyme complex consisting of both xylanases and β-xylosidase. Such cellulase free xylanases have been efficiently used in paper and pulp industries in place of chlorine to remove xylan for improving bio-bleaching processes. The purified xylanases produced smaller chain length (X3-X7) XOS which have great potential use as ingredients of functional food, cosmetics, pharmaceuticals or agricultural products and as a plant growth regulator. It also produces high amounts of metal and ethanol tolerant β-xylosidase qualifying its use in lignocellulosic biomass hydrolysis when mixed with enzyme preparations that are deficient in β-xylosidase. *P. hubeiensis* utilizes simultaneously all the sugars present in biomass hydrolysate and convert them to lipids thus posing to be a potential candidate for second generation biodiesel production. Competitive production of chemicals and fuels from biomass requires generation of revenue from each component (cellulose, hemicellulose and lignin) of lignocellulosic biomass. Cellulose, hemicellulose and lignin have different structures and chemistries and hence the effective fractionation to separate individual components in to their native state is very critical. Alonso et al.[34] used γ-valerolactone (GVL)/water/acid mixture to dissolve hemicellulose and lignin leaving behind high purity cellulose. Under the condition, lignin can be separated with high purity and hemicellulose can be converted to furfural. This hemicellulose fraction can be used for the production of xylanases to be used for XOS production. In addition, the hemicellulose fraction can be used to grow *P. hubeiensis* to get microbial biomass with high lipid content which can be diverted for second generation biodiesel production. It is essential to look for other enzymes such as lipases, esterases which may prove to be novel and efficient in performing chemically difficult transformations of organic compounds to drugs and drugs intermediates. Work is in progress in our laboratory in search of such enzymes in *P. hubeiensis* and assessment of their ability to perform unconventional bio-transformations and to degrade biodegradable plastics such as PBS, PBSA or PLA.

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Conflict of interest

There is no conflict to publish our article in this Journal.

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