Production of Enzymes with Industrial Interest

Subjects: Biotechnology & Applied Microbiology

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Industrial enzymes are enzymes that are commercially used in a variety of industries such as pharmaceuticals, chemical production, biofuels, food & beverage, and consumer products.

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1. Lytic Polysaccharide Monooxygenases (LPMOs)

Increasing demands of transportation fuel consumption (i.e., oil and biofuels), have prompted industries to invest in potentially renewable sources of energy production, such as plant biomass [1]. The main components of plant cell walls are cellulose, some non-cellulosic polysaccharides, and lignin. Plant biomass-derived lignocellulosic residues are considered great sources of fermentable sugars, the processing of which may lead to the production of renewable liquid transport fuels [2][3][4][5]. Morel and coworkers [6] identified the presence of genes encoding lignocellulolytic enzymes in the genome of *G. candidum* strain CLIB 918 (ATCC 204307). These enzymes included lytic polysaccharide monooxygenases (LPMOs) of the AA9 family, GH45 endoglucanases, which are strong oxidative enzymes that are important for the degradation of cellulose and hemicelluloses, such as xyloglucan and glucomannan [7], and endo-polygalacturonases. Similarly, *G. candidum* 3C was found to encode functional lytic LPMOs of the AA9 family [8][9]. LPMOs produced by *G. candidum* are promising candidates for enzymatic cocktails applied in biorefineries enrichment. Moreover, a cellulase isolated from *G. candidum* GAD1 was found to be promising for the degradation of carboxymethylcellulose salt from agricultural waste (rice straw), the fermentation of which resulted in bioethanol production [10].

Due to their improved catalytic activity against cellulose and hemicelluloses, some *G. candidum* strains were used for filter paper and cotton degradation. For instance, *G. candidum* strain 3C was discovered to encode a glycoside hydrolase (GH) of the family 7 cellobiohydrolases (CBHs), named Cel7A [11]. This strain's cellulase complex exhibited more effective activity than that of *Hypocrea jecorina*, the most commonly used species for cellulase production [12]. An enzymatic cocktail from *G. candidum* strain 3C named 'Cellokandin G10x' has been applied for industrial pulp and wastepaper utilization [11]. Similarly, enzymatic isolates from *G. candidum* strain Dec 1 were found to improve kraft pulp bleaching [13]. Noteworthy, ITS, 18S rDNA, 28S rDNA, and RPB2 gene sequence comparisons and multiple sequencing analysis of *G. candidum* strain 3C indicated that the strain should be included within the genus *Scytalidium* (Pezizomycotina, Leotiomycetes) and renamed *Scytalidium candidum* 3C comb. nov. [14].

2. Lipases

G. candidum can produce extracellular lipases, especially when cultured in the presence of an inducer, such as triglycerides or olive oil, in the culture medium [15][16][17][18][19]. Lipases have numerous industrial applications, including the manufacture of enantiomerically pure pharmaceuticals, cosmetics, agrochemicals, surfactants, biolubricants, construction and destruction of biopolymers, waste-water-treatment, influence on food products' sensorial characteristics, detergent industries, etc. [20][21][22][23]. Ferreira and coworkers [24] used a lipase secreted by *G. candidum* (GCL-I) to produce free fatty acids from olive, palm kernel, and cottonseed oils. Glycerol and free fatty acids are used by oleochemical industries to produce several products, including personal care products, such as shampoos, coatings, adhesives, surfactants, fatty alcohols, and lubricating oils [25][26][27]. *G. candidum* (ATCC 34614) was found to produce four lipases with different substrate specificities [28]. Specifically, lipases I and II indicated non-regional specificity with triolein, while lipases III and IV catalyzed the hydrolysis of triolein oleoyl esters at the sn-2 position with greater specificity compared to the sn-1(3) position, indicating an sn-2-regioselectivity. Laguerre and coworkers [29] indicated that the lipases isolated from *G. candidum* NRRL Y-552 were able to hydrolyze triolein and six edible oils, producing high amounts of diacylglycerol (DAG)-1,3 and lower amounts of DAG-1,2(2,3). The reduction in DAG-1,3 in oils with increased DAG-1,3 concentration is of great importance for the production of foods containing nutraceutical diacylglycerols able to reduce the levels of triacylglycerol in the plasma. Moreover, *G. candidum* lipase (LGC-I) was administered to produce decyl oleate ester from

rice husks (phenyl-silica), which were chemically modified [30]. Furthermore, GCL-I and -II were applied for the hydrolysis or ethanolysis of Crambe and Camelina oils, producing high concentrations of erucic acid and gondoic acid [31].

To be applied as a biocatalyst, the lipases produced during fermentation by *G. candidum* need to be immobilized. Ferreira and coworkers [32] analyzed different approaches for purified lipase immobilization, to reveal that the most efficient protocol was ionic adsorption on MANAE-agarose. The molecular masses of the currently identified and purified lipases produced by *Geotrichum* sp. range from 32 kDa to 75 kDa [21][33][34][35][36][37][38]. Moreover, administration of endo-β-N-acetylglucosaminidase showed that these lipases are glycosylated, and beyond their high homology, they have different substrate specificity [39]. Furthermore, chemically modified to contain more amino groups, surface lipase produced by *G. candidum* (GCL) indicated faster and easiest immobilization on carboxymethyl and sulfopropyl agarose-based supports [39][40]. Administration of the ionic derivatives into fish oil resulted in increased hydrolysis and production of a high yield of Omega-3 polyunsaturated fatty acids (6.65 U and 7.85 U per gram of support of carboxymethyl derivative and sulfopropyl derivative, respectively) [39].

3. Alkaline Proteases

Investigation into the production of proteases from 30 *G. candidum* strains revealed that the proteolytic activity differs among the different strains [41]. More recently, 12 strains were tested for their ability to secrete proteases, from which one strain, *G. candidum* GCQAU01 isolated from the fermented milk product Dahiwas, was found to produce an ideal protease for industrial application [42]. Specifically, the identified serine-type protease indicated thermostability, had stable activity at temperatures ranging from 25 to 45 °C and pH 8–9, possessed increased hydrolytic activity against casein and bovine serum albumin (BSA), and its activity was prevented by PMSF (7.5%). Alkaline proteases are of great biotechnological importance due to their numerous applications in food, pharmaceutical and tannery industries, silver recovery, detergents and waste treatment, and amino acid resolution [43].

4. Pectinases

Pectinases produced by *Geotrichum* spp., such as *G. candidum* AA15, are considered good candidates to be applied by the fruit juice industry for clarification of juices [44][45]. The presence of pectin and several other components of fruits makes the produced juice cloudy [46], degrading its qualitative characteristics and affecting consumers' preferences. Additionally, increased levels of pectin lead to an unwanted colloid texture formation [46]. The application of filtration can reduce the presence of pectin [47]. However, the increased presence of fiber-like molecules in pectin's structure makes filtration inefficient for eliminating its existence in juice. Therefore, the addition of pectinolytic enzymes before the filtration process has been applied for the depectinization of several juices [48]. As a result, the process efficiency is increased [49]. The majority of pectinases are isolated from filamentous fungi, including *Aspergillus niger* [50]. However, the administration of pectinases from yeasts, such as *Geotrichum*, offers the advantages that: (a) some species are considered as generally recognized as safe (GRAS) [51]; (b) they produce an efficient type of pectinase for juice clarification [45]; and (c) they produce considerable amounts of pectinases in a shorter fermentation period compared to filamentous fungi [52]. Ahmed and Sohail [44] applied a response surface approach to reveal that the pectinase isolated from *G. candidum* AA15 was efficient for orange juice clarification, with the highest enzymatic activity in incubation time of 25 min at 35 °C, in pH 5. To increase the *G. candidum* AA15 pectinase yield, the strain was immobilized using corncob [45]. Additionally, simple sugars, such as xylose, galacturonic acid, galactose, and pectin, but not glucose, positively affected pectinase production in immobilized yeast cells [53].

5. Aldehyde Dehydrogenases, Glutamate Dehydrogenases, and Baeyer–Villiger Monooxygenases

Several enzymes associated with oxidation reactions have been applied by pharmaceutical and chemical industries for the oxidation of alcohols, sulfides, aldehydes, Baeyer–Villiger oxidation, and hydroxylation [54][55][56][57][58][59]. *G. candidum* was discovered to produce dehydrogenases with broad applications in organic synthesis [57][60][61][62][63][64][65][66]. For instance, *G. candidum* NBRC 4597 (GcAPRD) was discovered to produce a novel alcohol dehydrogenase (ALDH), acetophenone reductase, an enzyme with broad spectrum activity regarding the oxidation of aldehydes to carboxylic acids and selective activity for the oxidation of dialdehydes to aldehydic acids [61][67][68]. This process has important agrochemical and pharmaceutical applications, but there are still limitations on its use due to its limited recyclability [69]. The specific ALDH was found to have broad-spectrum activity, thermostability, and resistance to non-aqueous solvents [65][66][70][71][72][73]. The application of an organic–inorganic nanocrystal formation method successfully immobilized the isolated GcALDH nanocrystal, which retained its enzymatic activity and proved more thermostable compared to the free GcALDH [74]. The same team used graphene oxide and reduced graphene oxide, which are chemically produced oxidized

forms of graphene, to immobilize GcAPRD, enabling its recycling [75]. As a result, a great number of ketones, such as the aliphatic ketone 3-hexanone, were decreased with 99% efficacy.

G. candidum S12 was shown to produce a novel glutamate dehydrogenase, which was highly active against glutamate, hexanol, α-ketoglutarate, and isoamyl alcohol (Km values of 41.74, 4.01, 20.37, and 19.37 mM, respectively) [76]. The catalytic activity against hexanol was enhanced by the addition of ADP, K^+, Fe^{2+}, and Zn^{2+} and reduced by EDTA, Mn^{2+}, Pb^{2+}, ATP, and DTT.

Furthermore, immobilization of the whole *G. candidum* CCT 1205 cell on functionalized silica resulted in ε-caprolactone creation, an advanced polymer with several biomedical applications [77]. E-caprolactone was also produced by whole *G. candidum* CCT 1205 cells using cyclohexenone, cyclohexanone, and cyclohexanol as substrates [78].

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