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Microbial β-Glucosidases: Screening, Characterization, Cloning and Applications

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Abstract Cellulose is the most abundant biomaterial in the biosphere and the major component of plant biomass. Cellulase is an enzymatic system required for conversion of renewable cellulose biomass into free sugar for subsequent use in different applications. Cellulase system mainly consists of three individual enzymes namely: endoglucanase, exoglucanase and β-glucosidases. β-Glucosidases are ubiquitous enzymes found in all living organisms with great biological significance. β-Glucosidases have also tremendous biotechnological applications such as biofuel production, beverage industry, food industry, cassava detoxification and oligosaccharides synthesis. Microbial β-glucosidases are preferred for industrial uses because of robust activity and novel properties exhibited by them. This review aims at describing the various biochemical methods used for screening and evaluating β-glucosidases activity from microbial sources. Subsequently, it generally highlights techniques used for purification of β-glucosidases. It then elaborates various biochemical and molecular properties of this valuable enzyme such as pH and temperature optima, glucose tolerance, substrate specificity, molecular weight, and multiplicity. Furthermore, it describes molecular cloning and expression of bacterial, fungal and metagenomic β-glucosidases. Finally, it highlights the potential biotechnological applications of β-glucosidases.

Keywords: *lignocellulose biomass, cellulose, cellulase, β-glucosidase, biofuel, transglycosylation*

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1. Introduction

Cellulose is the most abundant biomaterial in the biosphere and the major constituent of plant biomass representing 35-50% of lignocellulose biomass [1]. Cellulase is an enzymatic system that converts renewable cellulose biomass into free sugar for the subsequent use in different application. Cellulase systems consist mainly of three enzymes namely: endoglucanase (1,4-β-D-glucan-4-glucanohydrolase; EC 3.2.1.4) and exoglucanase (1,4-β-D-glucan glucohydrolase; EC 3.2.1.74) and β-glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21) that synergistically degrade cellulose to glucose unit [2,3,4]. First, endoglucanase attacks the internal β(1-4)-linkages of cellulose chains releasing β-glucan chains of different length. Subsequently, exoglucanase acts on β-glucan chains from both reducing and nonreducing end releasing cellobiose, and short oligosaccharides. Finally, β-glucosidase degrades cellobiose and short oligosaccharides into glucose units [5,6,7,8,9]. In addition, β-glucosidase is diverse group of enzymes that catalyze liberation of nonreducing glucosyl terminal residue from different aryl-, alkyl-β-D-glucosides, β-linked disaccharides and short oligosaccharides [10,11]. β-Glucosidase is ubiquitous enzyme found in bacteria, fungi, plant, and animals including noncellulolytic organisms e.g., human [12,13].

Glycoside hydrolases (GHs) is a widespread class of enzymes which catalyzes hydrolysis of glycosidic bonds between carbohydrates or a carbohydrate and a non-carbohydrate moiety. GHs comprise of enzymes GHs encompass 135 enzymes families based on nucleotide sequences identity and hydrophobic cluster analysis as in the last update of CaZy database [14]. β-Glucosidases are placed in GH family 1 and family 3. β-Glucosidases belonging to GH family 1 are from bacteria, plants and mammals whereas those belonging to GH family 3 are from bacteria, fungi and plants [12,15].

β-Glucosidase plays fundamental roles in many biological processes in different organisms. For example, in human, at least five β-glucosidases have been identified which differ in their localization and substrates specificity [16]. Probably the most studied one is the lysosomal acid β-glucosidase (3.2.1.45) which hydrolyzes β-glucosyl linkage of glucosylceramide releasing ceramide and involved in Gaucher's disease pathogenesis [17]. In plants, β-glucosidases are involved in many physiological processes such as cell wall lignification [18], seed germination [19], phytohormones activation [20], indole alkaloids biosynthesis [21], cyanogenesis [22], and defense against biotic stresses by releasing of toxic

compounds from their glycosides precursors [23]. In microorganism, β-glucosidases are localized as intracellular, extracellular and cell wall-associated enzyme and involved in degradation of cellulose and other carbohydrates for nutrients up-take and carbon recycling [24], cellulase gene induction [25], cell wall metabolism, host–pathogen interactions, and symbiotic association [1,10,26].

Apart from physiological functions of β-glucosidases, these enzymes are used in many biotechnological applications such as food and flavors technology, paper recycling, cassava detoxification, and most importantly biofuel production by enhancing the rate of saccharification of lignocellulose biomass [27,28,29,30]. In addition, some β-glucosidase can catalyze synthetic activity under certain circumstances of high substrate or product concentration which shift reaction equilibrium toward synthesis and can therefore be utilized in the synthesis of oligosaccharides, aryl- and alkyl-β-glucosides [12,16,31]. These compounds are chemically stable, environmentally friendly, and have nonionic surfactants and biodegradable properties and have wide range of applications such as therapeutic agents, vaccines, cosmetics, diagnostics tools, and growth promoting agents for probiotic bacteria [32,33,34,35]. Researchers therefore are focusing on characterization of this valuable enzyme from microorganisms for their industrial applications rather than investigating physiological roles.

In this review, various biochemical methods for screening and measuring microbial β-glucosidase activity are described and general procedures for purification of β-glucosidases are highlighted. Subsequently, various biochemical and molecular properties such as pH and temperature optima, substrate specificity, glucose tolerance, transglycosylation, molecular weight, and multiplicity are elaborated. Furthermore, molecular cloning and expression of microbial β-glucosidase from bacteria, fungi and metagenome is discussed. Finally, potential industrial applications of β-glucosidase are highlighted.

2. Screening for β-Glucosidases

β-Glucosidase production by a microorganism grown in agar medium can be detected using esculin, 8-hydroxyquinoline-β-D-glucoside, *cyclohexenoesculetin-β-D-glucoside, or arbutin which upon hydrolysis by β-glucosidase gives rise to glucose and aglycone moiety: esculetin*, hydroxyquinoline, *cyclohexenoesculetin, and* hydroquinone, respectively. Aglycone then chelates ferric ions supplemented to the growth medium (Figure 1), and forms dark brown complex against clear background [36,37]. *The main disadvantage for using esculin is diffusion of esculetin-iron complex throughout medium making it difficult to distinguish β-glucosidase producing colonies from non-producers* [38]. Hydroxyquinoline-β-D-glucoside is known to be less diffusible in comparison to esculin, although it is toxic to gram positive bacteria [39]. Contrarily, *cyclohexenoesculetin-β-glucoside allows the growth of gram-positive bacteria* and does not suffer from the diffusion of the 8-hydroxyquinoline-iron complex throughout the plate [40]. The ferric salt may inhibit the growth of microorganisms and misinterpretation of the results may occur [37,41].

Alternatively, MUG (methylumbelliferyl-β-D-glucoside) can be used to detect β-glucosidase activity on agar medium. MUG is an artificial glucoside that is hydrolyzed by β-glucosidase into methylumbelliferone and glucose. Methylumbelliferone is known to fluoresce when induced by UV light. Appearance of white zone around the colonies indicates that the microorganism is a β-glucosidase producer [42,43].

Indoxyl-β-D-glucosides such as 5-bromo-4-chloro-3-indoxyl-, 5-bromo-6-chloro-3-indoxyl-, 6-chloro-3-indoxyl-, 5-bromo-3 indoxyl-β-D-glucosides may be used to detect β-glucosidase which upon hydrolysis by the enzyme, the indoxyl derivative imparts specific color to the positive clone. For instance, 5-bromo-4-chloro-3-indoxyl forms blue color and 6-chloro-3-indoxyl forms rose color [44,45,46].

Perry *et al* utilized β-glucosides of alizarin (1,2 dihydroxyanthraquinone), 3,4-dihydroxyflavone and 3-hydroxyflavone for the detection of β-glucosidase on plate and found that alizarin glucoside was highly sensitive for bacterial β-glucosidase, and 3,4-dihydroxyflavone and 3-hydroxyflavone were also sensitive for β-glucosidase from *Enterococci* and *Listeria* spp. [46]. Cellobiose was used for combinatorial screening and selection for β-glucosidase producers e.g., microorganism with β-glucosidase production capability can grow on media containing cellobiose as sole carbon source while those without β-glucosidase activity cannot grow [47].

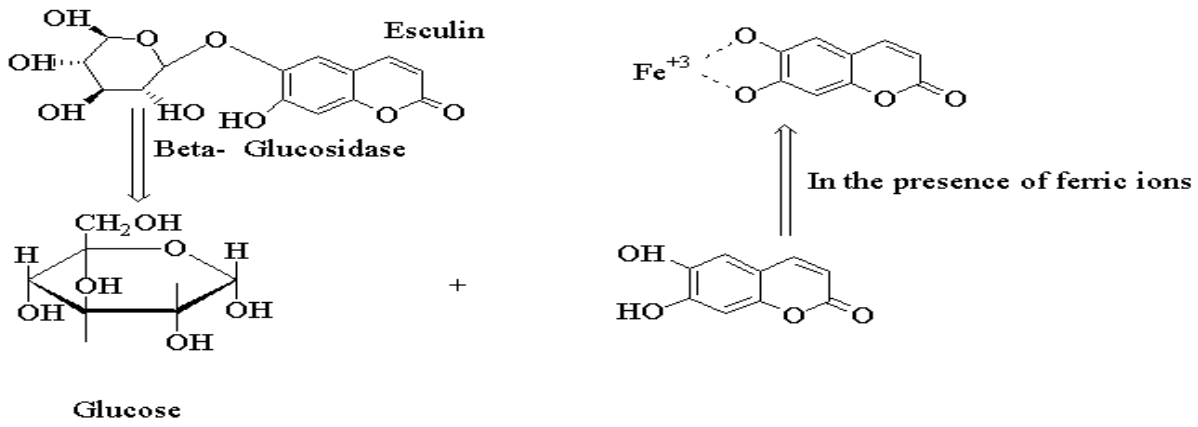

Figure 1. Principle for β-glucosidase screening using esculin as substrate

3. Determination of β-Glucosidase Activity

β-Glucosidase activity is most commonly measured using *p*-nitrophenyl-β-D-glucopyranoside (p-NPG) as substrate. β-Glucosidase hydrolyzes p-NPG to glucose and *p*-nitrophenol. *p*-Nitrophenol, under alkaline conditions, turns yellow with optimal absorbance at 405 nm which can be measured using spectrophotometer. Generally β-glucosidase has a high affinity for this artificial substrate making it the most widely used substrate for measuring β-glucosidase activity [48,49,50,51].

β-Glucosidase activity can also be measured using cellobiose which is a natural disaccharide produced from cellulose by the action of endo- and exo-glucanase. Cellobiose is hydrolyzed by β-glucosidase into two glucose units. The liberated glucose is measured using glucose specific test such as glucose oxidase peroxidase (GOD/POD) kit, hexokinase kit, or High Pressure Liquid Chromatography (HPLC). Since cellobiose is the natural substrate for β-glucosidase, it is preferred to be utilized for evaluating its activity [52,53,54]. Other substrates such as salicin and arbutin have been used to evaluate the enzyme activity. The enzyme hydrolyzes salicin or arbutin to glucose and aglycone moiety either saligenen or hydroquinone, respectively, and the released glucose is measured using standard method such as DNS, or GOD/POD kit [29,55,56]. MUG may also be used for measuring β-glucosidase activity. The enzyme hydrolyzed MUG into β–methylumbelliferone and glucose. The amount of methylumbelliferone released by the enzymes is measured by fluorimeter for excitation and emission at 380 nm and 448 nm, respectively [57,58].

Furthermore, cellotriose, cellotetraose, cellopentose and cellohexoses are used to evaluate β-glucosidase hydrolytic activity on short oligosaccharides [59]. Daidzin, and genistin are used to measure β-glucosidase capability to hydrolyze isoflavone glycosides [60,61]. Some β-glucosidase showed β-galactosidase side activity, and/ β-xylosidase side activity which can be measured using lactose, *p*-nitrophenol-β-D-galactoside, or *p*-nitrophenyl-β-D-xylopyranoside, respectively [62,63,64].

4. Purification of β-Glucosidase

Majority of industrial applications of β-glucosidase do not demand a homogeneous preparation of the enzyme. However, a highly pure enzyme is required for biochemical and molecular characterization, 3-D structure elucidation, and the structure–function relationships. β-Glucosidase therefore has been purified from different sources including fungi, bacteria and yeast and various strategies have been employed for β-glucosidase purifications [65,66,67]. The pre-purification step usually involves protein precipitation/fractionation from microbial culture using ammonium sulfate at 75% [68], 80% [69,70,71,72], and 90% saturation [55,73]. Other workers have used ultrafiltration [66,74], acetone precipitation [75], and ethanol precipitation [76]. The next step usually involves the use of dialysis to remove ammonium sulfate traces and other impurities present in the culture medium [77,78]. Further purification is done by chromatographic technique such as gel filtration chromatography, ions exchange chromatography, adsorption chromatography, hydrophobic interaction chromatography or HPLC [68,71,79]. For example, β-glucosidase has been purified up to 9.47 fold from culture supernatant of *Tolypocladium cylindrosporum* Syzx4 using ammonium sulfate (75% saturation), dialysis, (DEAE)–cellulose column, and Sephadex G-100 column [68]. β-Glucosidase has also been purified from *Aureobasidium pullulans* using ammonium sulfate precipitation, CM Bio-Gel A agaraose, and sephacryl S-200 gel filtration chromatography up to 129 fold [80] and that from the human pathogenic fungus *Sporothrix schenckii*, was purified to homogeneity using hydroxyapatite (HAp) adsorption chromatography and Sephacryl S200-HR size exclusion chromatography [81]. Affinity chromatography methods such as Immobilized metal-affinity chromatography (IMAC) has been utilized for purification of recombinant enzymes containing short affinity tag of polyhistdine residues which show strong interactions with transition metal ions such as Ni^{2+} immobilized in matrix [82]. For instance, β-glucosidase from *Anoxybacillus* sp. DT3-1 [83], *Exiguobacterium antarcticum* B7 [84], and *Thermoanaerobacterium thermosaccharolyticum* DSM 571 [85] has been expressed in *E. coli* as fusion protein with six His tag at N-terminal and purified through Nickel-Nitrilotriacetic acid (Ni-NTA) chromatography [85,86,87]. Glutathione-S-Transferase (GST) affinity has been used for purification of recombinant β-glucosidase from *Exiguobacterium oxidotolerans* A011 expressed as fusion protein with GST which provides additional advantage by acting as a chaperone protein to facilitate protein folding, and provide a mean for their purification by immobilized glutathione column [88,89]. In general, β-glucosidase purification is quite troublesome, time-consuming and results in low purification yield. Therefore, novel purification strategies have yet to be adopted to reduce the number of purification steps and enhance the purification yield.

5. Biochemical Properties of β-Glucosidase

Subsequently after purification, β-glucosidases are characterized for their biochemical properties such as pH and temperature optima, substrate specificity, glucose tolerance, transglycosylation to determine potential biotechnological applications of the enzyme [90,91].

5.1. pH and Temperature Optima

β-Glucosidases differ in pH optima depending on their origin, and sources. Generally fungal β-glucosidase exhibits optimal activity at pH of 4.0-6.0 [59,69,86,92,93], nevertheless β-glucosidase with optimal pH of 2.4 has been reported from *T. cylindrosporum* Syzx4 [68] and that with optimal pH of 9 and 10 has been reported from *Acremonium murorun* LPSC 927 and *Chaetomium globosum*, respectively [94,95]. Bacterial β-glucosidase exhibits optimal activity at pH of 6-7 [44,87,96,97], although those with pH optima of 5, 8 and 10 has been reported from *Caldicellulosiruptor saccharolyticus* [98],

Bacillus halodurans [99], *Klebsiella pneumoniae* [100], respectively. Yeast β-glucosidase reported from *A. pullulans* and *Candida peltata* exhibited optimal activity at pH 5 [80,101] and that from *Kluyveromyces marxianus* showed optimal activity at pH 5.5 [102]. The yeast-like *Aureobasidium* sp. was found to secrete highly acidophilic extracellular β-glucosidase with optimal activity at pH 2.5 [103]. Metagenomic β-glucosidases exhibiting optimal activity at pH 7 [104], 6 [105,106], 6.5 [45,49,107], 5.5 [108,109], 4.0 [110], 8 and 10 [111,112] has been identified reflecting the great diversity of unculturable microbial world and its potential for production of industrially novel enzymes.

Similarly, β-glucosidases vary in optimum temperature with majority of the reported microbial β-glucosidases are thermophilic enzymes. For example, β-glucosidases exhibiting optimal activity at temperature of 50°C have been identified from *Flammulina velutipes [113]*, *Daldinia eschscholzii [65]*, *Penicillium purpurogenum* [114] and those with optimal activity at 60°C have been reported from *Ceriporiopsis subvermispora [115]*, and *Halothermothrix orenii [116]*. β-Glucosidase with optimal activity at 70°C has been reported from *A. niger* KCCM 11239 [117], and *T. aurantiacus* IFO9748 [43]. Moreover, hyperthermophilic β-glucosidase has been identified from *Pyrococcus furiosus* with optimal temperature of 102-105°C [63] and β-glucosidase with optimal activity at 90°C has also been identified from hot spring metagenome and termite gut metagenome [105,107]. Mesophilic β-glucosidase has been reported from *Neocallimastix patriciarum* W5 [118], *Neosartorya fischeri* NRRL181 [86], and *P. purpurogenum* KJS506 [119] with optimal temperature of 40, and 32°C, respectively. Cold active β-glucosidase have been characterized from *Paenibacillus* sp. Strain C7 [87], and *Shewanella* sp. G5 [120], and *E. oxidotolerans* A011 [89]. This diversity in pH and temperature optima of microbial β-glucosidase reflects the wide distribution and fundamental roles played by this enzyme in all living organisms. Further studies are needed to determine the mechanisms by which this enzyme can work under high or low temperature and various pH. Understanding of these mechanisms will eventually help in designing a better catalyst through protein engineering.

5.2. Substrate Specificity

β-Glucosidases are classified based on substrate specificity into three classes: 1) Cellobiase which catalyzes hydrolysis of cellobiose and short oligosaccharides, 2) Aryl-β-D-glucosidase which hydrolyzes only aryl-β-D-glucosides such as p-NPG and 3) Broad substrate specificity β-glucosidase which catalyze hydrolysis of wide range of substrates with β(1-4), β(1-2), β(1-3), α(1-4), α(1-2), α(1-6) bonds etc. [10]. β-Glucosidase with broad substrate specificity has been identified from *F. oxysporum* [121], *P. thermophile* J18 [59], *P. italicum* [74], *Penicillium simplicissimum* [55], *A. oryzae* [122], *Thermobifida fusca* [123], and environmental DNA [49]. Cellobiase has been identified from *Cellulomonas biazotea* [124], and *A. niger* [52]. Aryl-β-D-glucosidase has been reported in *N. fischeri* NRRL181 [86] and *A. oryzae* [125]. Further studies, including molecular docking studies, are needed to determine interactions between amino acids residues with substrates which may provide an insight into these substrate specificities and allow better use of this multifunctional biocatalyst [126,127].

5.3. Glucose Sensitivity and Tolerance

Microbial β-glucosidases are inhibited by their own product i.e., glucose [128]. For instance, β-glucosidase reported in *F. oxysporum* was inhibited competitively by glucose with inhibitory constant (K*i*) value of 2.05 mM [121], and β-glucosidase reported in *P. italicum* was inhibited non-competitively with K*i* of 8.9 mM [74]. Other β-glucosidases characterized from *Termitomyces cyleaputs* [129], *Fomitopsis palustris* [66], *Monascus purpureus* NRRL1992 [93], *D. eschscholzii* [65], *Gongronella butleri* [130], and *Leuoconstic Mesentenoide* [29] were also sensitive to glucose with K*i* value range of 0.35-14.3 mM.

β-Glucosidase with an excellent glucose tolerance has also been identified from different species such as *C. peltata* [131], *A. unguis* NII-08123 [132], *A. niger* CCRC 31494 [133], *A. oryzae* [122] and *Penicillium funiculosum* NCL1 [134] with K*i* value of 1.4, 0.8, 0.5 and 1.36 mM, respectively. Moreover, β-glucosidases belonging to GH family 1 was found to be stimulated by lower glucose concentration and be tolerant to higher concentration. For examples, β-glucosidase from *Microbispora bispora* was activated in the presence of 300 mM glucose [135] and that reported in *T. thermosaccharolyticum* DSM 571 and *T. aotearoense* was activated by glucose concentration < 200 and 250 mM, respectively [85,136]. Similarly, recombinant GH 1 β-glucosidase from *Exiguobacterium antarcticum* B7 [84], *Fervidobacterium islandicum* [137], *Thermotoga thermarum* DSM 5069T [138] and *Anoxybacillus* DT3-1 [83] was tolerant to high glucose concentration e.g., K*i* of 0.54-15 M. On the other hand, glucose tolerant β-glucosidase with K*i* of 1.0-4.8 M has been identified from different environments using metagenomic approaches [45,53,106,111,139,140].

Glucose inhibition of β-glucosidases is the major obstacle in bioconversion of biomass and biofuel production. Understanding the mechanism of glucose inhibition/tolerance is of crucial importance for biofuel production. Recently, replacement of two amino acids (Leu 167 and Pro 172) at the entrance of the active site of intracellular β-glucosidase of *T. reesei* (Bgl II) with Trp and Leu, respectively, significantly enhanced glucose tolerance e.g., K*i* of 650 mM [141]. Further studies for identification of new β-glucosidase with high glucose tolerance are required. Bioinformatics analysis may be used for identification of amino acid residues playing central role in glucose tolerance and *in silico* mutagenesis and docking studies can be used to modulate this enzyme for better performance as preliminary experiment before protein engineering is employed.

5.4. Transglycosylation Activity

Transglycosylation is transfer of sugar moiety from one compound (donor) to another compound (accepter). Transglycosylation fundamentally is an important reaction for production of many compounds such as aryl/alkyl-,

poly glycosides, and synthetic oligosaccharides e.g., galacto-oligosaccharides and gentio-oligosaccharides [142]. Both glycosyltransferases and glycosidases can be utilized for catalyzing transglycosylation. Glycosyltransferases require an input of energy in form of nucleotide triphosphate and have very narrow substrate specificity. Glycosidases, on the other hand, do not require an input of energy, use cheap donor substrates, and has relaxed substrate specificity for acceptors and are wide spread enzymes. Although glycosidases suffer from limitations such as product hydrolysis and low transglycosylation activity [143,144]. In this context, some β-glucosidase can catalyze transglycosylation under high substrate or product concentration [31]. In this reaction, the nucleophilic residue at active site attacks the glycosidic bond of nonreducing glucosyl terminal unit forming glucosyl-enzyme intermediate. The leaving group e.g., aglycone subtracts a proton from general acid/base residue before leaving the active site. Another acceptor molecule competes with the water molecule to attack enzyme-glucosyl intermediate displacing nucleophilic residue and catalytic acid/base accepts a proton from the hydroxyl group of the acceptor [31,145,146]. Accepter molecule can be glucose, cellobiose, cellotriose, methanol, ethanol or propanol forming cellobiose, cellotriose, cellotetraose, methyl, ethyl, and propyl-β-glucosides, respectively [147].

β-Glucosidase with an excellent transglycosylation activity has been identified from different sources such as *P. thermophile* [59], *Myceliophthora thermophile* [79], *T. aurantiacus* [147], *F. oxysporum* [121], *Melanocarpus* sp. MTCC 3922 [148], *T. clypeatus* [149], and compost microbial metagenome [108]. Understanding mechanism of transglycosylation and identification of amino acid residues playing central role in transglycosylation reactions hold a great importance in protein engineering for the development of β-glucosidase with high transglycosylation reaction. Replacement of asparagine residue to phenylalanine at position 220 of GH 1 β-glucosidase of *Thermotoga neapolitana* resulted in 7-fold increase in the transglycosylation reactions [142].

Researchers are therefore focusing on identification of new β-glucosidases with strong transglycosylation activity. Further mechanistic studies may give an insight to the mechanism of transglycosylation and amino acid residues playing central role in this valuable reaction. Understanding transglycosylation mechanism will eventually help in designing enzymes with efficient transglycosylation activity for better glycoside and oligosaccharides synthesis.

5.5. Organic Solvents and Metal Ions Effect

For β-glucosidase to be used in biofuel production and beverage industries, it has to be tolerant to ethanol and methanol, butanol, acetic acids, the main fermentation products [122,130]. Furthermore synthetic reactions e.g., transglycosylation uses organic solvents to shift reaction equilibrium from hydrolysis to synthesis [150]. As a result, organic solvent-tolerant β-glucosidases are of great significance to these biotechnological applications. A number of β-glucosidase has been identified with tolerance to organic solvent. β-Glucosidase activity from *A. niger* was increased by 30% and 80% in the presence of 30% ethanol and methanol, respectively [73]. Thermostable β-glucosidase from *F. islandicum* was activated in the presence of 99% of hexadecane, n-hexane, heptane, isooctane, amylalcohol, n-decyl alcohol by 2, 5, 28, 10, 28 and 23%, respectively, and was slightly inhibited in the presence of 99% of tert-butanol, ethanol, acetonitrile, isopropanol, pyridine, DMSO, acetone, dimethylformamide, and methanol [137]. High glucose-tolerant β-glucosidase from *A. oryzae* was stimulated by 30% in the presence of 15% ethanol [122]. Methanol and ethanol (50%) stimulated β-glucosidase from *F. velutipes* by 5 and 23%, respectively [113]. β-Glucosidase activity of *Melanocarpus* sp. was enhanced by 1.5 fold in the presence of 70% methanol and ethanol whereas in the presence of 70% propanol it retained complete activity [148]. β-Glucosidase of *F. oxysporum* was stimulated by 0.5 M butanol (2.2-fold) and 1M methanol (1.4 Fold) [121]. β-Glucosidase from *M. thermophile* was highly activated by low molecular weight alcohol e.g., 1.4 fold increase in activity was achieved in the presence of 10% propanol, 15% ethanol and 20% methanol [79]. *G. butleri* produced a β-glucosidase which was activated in the presence of 5% ethanol up to 40% [130] and *Rhizomucor miehei* NRRL 5282 secreted β-glucosidase which was activated by 40% in the presence of 15% ethanol.

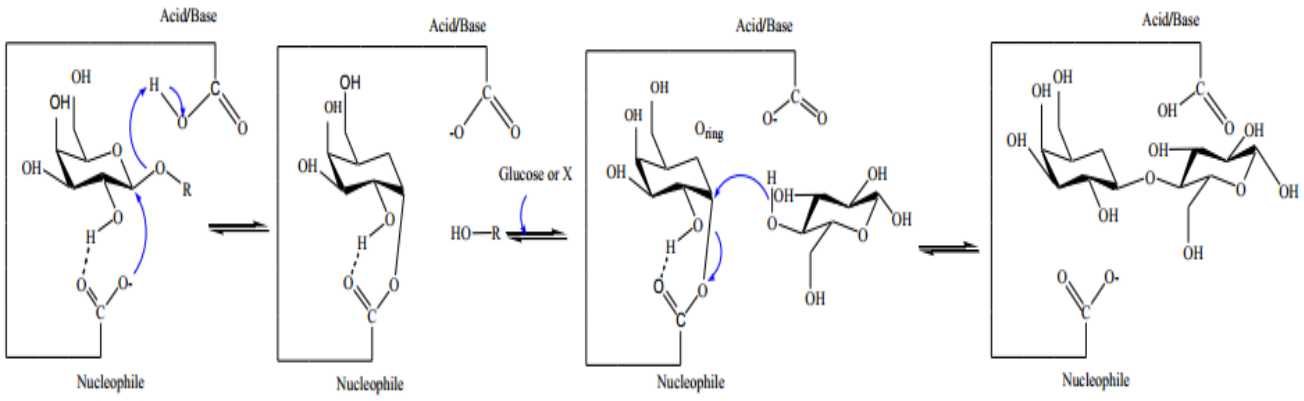

Figure 2. Transglycosylation mechanism of β-glucosidase

β-Glucosidase is a metal-independent enzyme that it does not require metal ion for its activity. Nevertheless, it has been demonstrated that β-glucosidase is deferentially affected by different metal ions [55,72,151,152]. For instance, a cold-adapted β-glucosidase from *E. antarcticum* B7 was activated by Mg^{2+}, Na^{+}, K^{+}, and Li^{3+}, and inhibited by Co^{2+}, Mn^{2+}, Ca^{2+}, Zn^{2+}, Cu^{2+}, Fe^{3+} [84] whereas the recombinant β-glucosidase from *M. thermophile* was highly activated by Zn^{2+}, Cu^{2+}, Mg^{2+}, and Mn^{2+} and inhibited by Ca^{2+} and Co^{2+} [79]. β-Glucosidase from *Ceriporiopsis subvermispora* was stimulated by Mn^{2+}, Fe^{3+} and inhibited by Cu^{2+}, Zn^{2+}, Mg^{2+}, and K^{+}. β-Glucosidase from *Pseudonocardia* sp. Gsoil 1536 was stimulated by Ca^{2+}, Mg^{2+}, Mn^{2+}, K^{2+}, Co^{2+}, Na^{+}, and Zn^{2+} and completely inhibited by Hg^{2+} [44]. The exact mechanism by which these metal ions exert their effect on β-glucosidase either stimulation or inhibition is not understood. Nevertheless, it is generally known that the inactivation caused by Hg^{2+} indicates that thiol groups are required for function of the enzyme or for maintaining 3D structure of the protein [122]. Inhibition by Cu^{2+}, Co^{2+} and Zn^{2+} suggests that basic (Arg, Lys, His) and acidic (Asp, Glu) amino acids may have important roles in the active site and stimulation of enzyme activity by the cations Ca^{2+}, K^{+}, Co^{2+}, Mn^{2+}, Mg^{2+} and Na^{+} suggest that it may enhance the structural stability of the enzyme [153,154].

6. Molecular Characterization of β-Glucosidase

Molecular characterization of enzymes is of great importance for exploring the potential biotechnological applications. Microbial β-glucosidases from different sources have been characterized from different sources for molecular properties [155].

6.1. Molecular Weight

β-Glucosidase varies in their molecular weight depending on number of amino acids and post-translational modifications e.g., glycosylation. Generally β-glucosidases belonging to GH 1 have 400-550 amino acids in length with molecular weight ranges 40-60 kDa [64,156]. Similarly, β-glucosidases belonging to GH family 3 contain 600-900 amino acids in length with molecular weight of 65-90 kDa per subunit, but because these group of enzymes are usually glycosylated, their molecular mass ranges 110-130 kDa [44,157,158]. β-Glucosidase varies in quaternary structure arrangement, for example, monomeric [159], dimeric [160], trimeric [147], tetrameric [151] enzymes have been reported. Native molecular weight therefore may be higher than identified by SDS-PAGE [161]. For instance, β-glucosidase reported from P. italicum had a native molecular weight of 354 kDa as determined by gel filtrations and 88.5 kDa as shown by SDS-PAGE suggesting that the native protein is a tetramer [74]. Similarly, the predicted molecular weight from amino acids sequences may differ from that determined by SDS-PAGE due to post-translational modification [162]. For instance, Chen *et al* reported β-glucosidase from *P. decumben* with predicted molecular weight of 96 kDa and 120 kDa as determined by SDS-PAGE [163]. Two short alkaline β-glucosidases have been identified with 172 and 151 amino acids length and molecular weight of 27 and 26 kDa, respectively [112]. A novel intracellular β-glucosidase from *T. clypeatus* with molecular mass of 6.68 kDa and 116 kDa as determined by MALDI-TOF and SDS-PAGE, respectively, indicating that it is present in aggregate form [149].

6.2. Zymography

For the detection of β-glucosidase activity on gel matrix, number of substrates has been used by different researchers to visualize β-glucosidase on gel matrix [42,164,165]. For examples, Kwon *et al* used esculin to visualize β-glucosidase activity on Native-PAGE. The gel was removed and soaked in solution containing 0.1% esculin and 0.03% ferric ammonium citrate until a dark band appeared on the gel indicating the presence of β-glucosidase activity [139,166,167]. *P*-PNG has also been utilized to detect β-glucosidase activity on PAGE. The protein sample with β-glucosidase activity is mixed with SDS-PAGE buffer and loaded into native PAGE system and run for specific period of time. The gel is then incubated in p-NPG solution, and then immersed Na_2CO_3 solution till yellow bands appeared indicating the presence of β-glucosidase at that site [42,164]. MUG is another powerful substrate for visualization of β-glucosidase on gel matrix. Protein sample with β-glucosidase activity is loaded on native PAGE and run for specific period of time. After which the gel is incubated in buffered MUG solution and then visualized under UV light using UV transilluminator. Appearance of fluorescent bands indicates the liberation of methylumbelliferone due to enzyme activity [93,154,168,169].

6.3. Multiplicity of β-Glucosidase

Isoenzymes are the enzymes which catalyze the same biochemical reaction but differ in their composition e.g., amino acid sequences. β-Glucosidase multiplicity has been reported in number of filamentous fungi and yeast. For instances, multiplicity of β-glucosidase has been demonstrated in *T. reesei* [170,171]. *A. niger* NII-08121/MTCC 7956 was found to express four β-glucosidase isoforms when grown on lactose or cellulose, whilst 2 isoforms were expressed on wheat bran or rice straw as carbon source [172]. Sonia *et al* isolated thirteen thermophilic and thermotolerant fungi from composting soil and found that twenty-eight β-glucosidase isoforms were expressed when corn cob was used as carbon source. For instance, *A. caespitosus* produces four isoforms, *Chaetomium thermophilum* produced three isoforms, and *Absidia corymbifera* expressed 2 isoforms [173]. *A. tubingensis* CBS 643.92 expressed four β-glucosidases (I-IV) with MW of 131, 126, 54 and 54 kDa and an isoelectric points of 4.2, 3.9, 3.7 and 3.6, respectively [174]. *A. oryzae* secreted two isoforms when cultured on media containing various carbon source: major form with MW of 130 kDa and low glucose tolerance, and minor form with MW of 43 kDa and high glucose tolerance [122]. *C. subvermispora* expressed two distinct β-glucosidase isoforms with molecular weight of 110 and 53

kDA when grown under Solid State Fermentation (SSF) on *P. taeda* wood chips [115]. The thermotolerant *A. terreus* AN1 strain secreted four distinct β-glucosidase isoforms when corn cob was used as a carbon source. Three isoforms designated as βGI, βGII & βGIII had a MW of 29, 43, and 98 KDa, and isoelectric point of 2.8, 3.7, and 3.0, respectively [165]. Similarly, the filamentous fungus, *Penicillium funiculosum* NCL1, expressed multiple isoforms of β-glucosidase when cultivated on media containing different carbon source e.g., four isoforms on wheat bran, two isoforms on sugarcane bagasse, and one isoform on lactose containing media whereas no isoform was expressed on salicin containing media as sole carbon source [175]. *A. unguis* NII-08123 expressed five different β-glucosidase isoforms among which one novel isoform was highly glucose-tolerant with MW of 10 kDa [132]. In bacteria, *Bacillus subtilis* strain PS secreted three isoforms β-glucosidase with molecular mass of 193 kDa, 64 kDa and 42 kDa, when it was grown on glucose containing media [176].

Thus, the major factor influencing the expression of these isoforms is the carbon sources e.g., β-glucosidase are usually inducible enzymes synthesized by microorganisms in response to certain metabolites present the culture medium. These metabolites usually are low molecular weight carbohydrates incorporated in the culture medium or synthesized by constitutively expressed enzymes secreted to the medium [24]. Other factors which may influence the expression of these isoforms are their production method either Solid Sate Fermentation (SSF) or Submerged Fermentation (SmF), and fermentation conditions such as aeration, pH, temperature and nitrogen sources [177]. The mechanism through which these isoforms are generated is not well understood, although probably these isoform are produced by gene multiplicity, alternative splicing, and post-translational modifications [24]. Further studies are needed to pursue the exact mechanism through which the expression of different isoforms are being regulated is of crucial significance for industries e.g., in the designing of culture parameters for production of the desired isoforms. List of β-glucosidase produced from different fungi, yeast and bacteria along with their biochemical and molecular properties are given in Table 1.

Table 1. List of the Characterized Fungal, Yeast, and Bacterial β-Glucosidases

Source organism	GH Family	No. of subunits	MW(kDa)	pH Opt.	Temp. Opt	Substrate specificity	Reference
P. thermophile	NR	Homodimer	197	6.2	75	Broad specificity	[59]
A. niger Au0847	CH 3	Heterodimer	110 +120	4.6	65	Broad specificity	[69]
P. italicum	NR	Homotetramer	88.5/354	4.5	60	Broad specificity	[74]
P. simplicissimum	NR	Monomer	126	4.8	60	Broad specificity	[55]
A. awamori MIBA335	CH 3	monomer	120	4.5	55	Broad specificity	[227]
A. oryzae	NR	monomer	43	5	50	Broad specificity	[122]
Fomitopsis palustri	CH 3	Monomer	135	4.5	70	Cellobiose + p-NPG	[66]
T. aurantiacus	CH 3	Homotrimer	120/350	4.5	80	Broad specificity	[147]
T. lanuginosus–SSBP	CH 3	Homodimer	200	6.0	65	Broad specificity	[70]
Melanocarpus sp.	NR	Monomer	92	6.0	60	Broad specificity	[148]
A. fumigatus Z5	CH 3	Monomer	91.47	6.0	60	Broad specificity	[72]
Phoma sp.	CH 3	Tetramer	110/440	4.5	60	Broad specificity	[151]
D. eschscholzii	CH 3	Monomer	64.2	5.0	50	Broad specificity	[65]
P. purpurogenum	CH 3	Monomer	89.6	4	32	Broad specificity	[119]
F. velutipes	NR	Monomer	75	5.0-6.0	50	p-NPG	[113]
P. piceum	CH 3	Monomer	92	5.0	60	Broad specificity	[228]
P. decumbens	CH 3	Monomer	120	4.5-5.0	65-70	Broad specificity	[163]
Penicillium citrinum	CH 3	Monomer	72	5.0	70	Broad specificity	[229]
A. niger NII-08121	CH 3	Monomer	120	4.8	70	Broad specificity	[172]
Paecilomyces Bainier	CH 3	Monomer	115	3.5	60	Broad specificity	[230]
Termitomyces clypeatus	NR	Aggregate	6.68/116	5.0	45	Broad specificity	[149]
A. terreus EMOO 6-4	CH 3	Monomer	120	5.5	35	p-NPG	[231]
Agaricus bisporus	NR	Heterdimer	62+46	4.0	55	Broad specificity	[232]
T. citrinoviride	CH 3	Monomer	110	4.0	50	Broad specificity	[233]
Sporothrix schenckii,	CH 3	Dimer	97/204.9	5.5	45	Broad specificity	[160]
Rhizomucor miehei	CH 3	monomer	82.3	5.0	70	Broad specificity	[153]
Stachybotrys microbispora	CH 3	Monomer	78	6.0	50	Broad specificity	[234]
P. funiculosum	CH 3	Monomer	120	5.0	60	Broad specificity	[175]
A. niger KCCM 11239	CH 3	Monomer	123	4.0	70	Broad specificity	[117]
F. proliferatum ECU2042	CH 3	Monomer	78.7	5.0	50	Broad specificity	[235]
Humicola grisea	NR	Monomer	57	6.0	50	Broad specificity	[236]
C. peltata	NR	Monomer	43	5.0	50	Broad specificity	[131]
Penicillium janthinellum	NR	Monomer	120	4.5	65	Broad specificity	[237]

NR= not reported, MW= molecular weight, kDa= kilodalton, pH opt.= pH optima, Temp. opt.= temperature optima.

Table 2. List of Fungal, Bacterial and Metagenomic Recombinant β-Glucosidases

Origin of BGL gene Fungal species	Host for expression	GH Family	No. of a.a	MW kDa	pH Optima	Temp optima	Potential applications	Reference
A. niger	*P. pastoris.*	CH 3	860	116	4.0	60	Biofuel production	[92]
A. fumigatus Z5	*P. pastoris*	CH 3	844	130	6.0	60	Biofuel production	[238]
Periconia sp.	*T. reesei*	CH 3	867	130	5.0	70	Biofuel production	[184]
Volvariella volvacea	*E. Coli*	CH3	NR	97	6.4	50	Biofuel production	[239]
Neosartorya fischeri	*E. coli*	CH 1	488	56/ 480	6.0	40	Food Technology	[86]
Neosartorya fischeri P1	*P. pastoris*	CH 3	739	80	5.0	80	Isoflavone hydrolysis	[155]
T. emersonii	*T. reesei*	CH 3	857	130	5.0	71.5	Biofuel production	[187]
T. reesei	*T. reesei*	CH 3	744	75	4.6	70	Biofuel production	[190]
M. thermophila,	*P. pastoris.*	CH 3	733	90	5.0	70	Biofuel production	[79]
A. saccharolyticus	*T. reesei*	CH 3	860	130	4.2	50-60	Biofuel production	[240]
T. reesei	*P. pastoris*	CH 3	744	76	5.0	70	Biofuel production	[180]
Aspergillus aculeatus	*T. reesei*	CH 3	841	130	5.5	65	Biofuel production	[191]
T. aurantiacus	*P. pastoris*	CH 3	861	130	5.0	70	Biofuel production	[43]
P. brasilianum	*A.oryzae*	CH 3	842	115	4.8	70	Biofuel production	[183]
P. funiculosum NCL1	*P. pastoris*	CH 3	857	130	5.0	60	Bioethanol production	[134]
N. patriciarum W5	*P. pastoris*	CH 3	776	85.1	5.0-6.0	40	Biofuel production	[118]
Periconia sp.	*P. pastoris*	CH 3	866	130	5.0	70	Biofuel production	[241]
N. fischeri NRRL181	*E. coli.*	CH 1	529	60	6.0	40	Food technology	[242]
Bacterial species								
C. saccharolyticus	*E. coli*	CH1	453	54	5.5	70	Biofuel production	[98]
Exiguobacterium sp.	*E. coli*	CH 1	450	52	7	45	Oligosaccharide synthesis	[199]
Exiguobacterium oxidotolerans A011	*E. coli*	CH 1	448	51.6	7	35	Food technology	[89]
Halothermothrix orenii,	*E. coli*	CH 1	451	53	6.0	65-75	Galactooligosaccharide synthesis	[116]
Weissella cibaria 37	*E. coli*	CH 1	415	50	5.5	45	Biofuel production	[243]
Sphingomonas sp. 2F2	*E. coli*	CH 1	447	49.3	5.0	37	Ginsenoside transformation	[244]
Pseudonocardia sp.	*E. coli*	CH 3	743	79	7.0	37	Ginsenoside transformation	[44]
Bacteroides uniformis	*E. coli*	CH 3	740	81.5	6.0	40	Secoisolariciresinol diglucoside hydrolysis	[245]
F. islandicum	*E. coli*	CH1	459	53	6.0-7.0	90	Biofuel production	[137]
Thermobifida fusca,	*E. coli*	CH 1	485	53.4	7.0	50	Biofuel production	[123]
T. thermosaccharolyticum	*E. coli.*	CH 1	443	52	6.4	70	Biofuel production	[246]
Bacillus licheniformis	*E. coli.*	CH1	471	53	6.0	50	Biofuel production	[200]
Thermoanaerobacter brockii	*E. coli*	CH 1	450	52	5.5	75	Biofuel production	[247]
Bacillus halodurans	*E. coli*	CH 1	447	51.6	8.0	45	Biofuel production	[99]
Bacillus licheniformis	*E. coli*	CH 1	469	53.4	6.0	47	Biofuel production	[248]
Anoxybacillus sp. DT3-1	*E. coli*	CH 1	453	53	8.5	70	Biofuel production	[83]
E. antarcticum B7	*E. coli*	CH 1	448	52	7.0	30	Biofuel production & Food Technology	[84]
T. aotearoense	*E. coli*	CH 1	446	52.6	6.0	60	Bioethanol production	[136]
Thermotoga thermarum	*E. coli*	CH 1	490	55	4.8	90	Ginsenoside hydrolysis	[138]
Bacillus halodurans	*E. coli*	GH 1	453	52.5	7.0	50	Biofuel production	[249]
Streptomycete sp.	*E. coli*	CH 1	456	51	6.0	45	Biofuel production	[152]
Metagenomics BGL								
Marine metagenomic	*E. coli*	CH 1	442	50	7.5	45	Food Technology	[64]
Soil metagenome	*E. coli*	CH 1	469	51.6	6.0	50	Bioethanol production	[106]
Kusaya gravy metagenome	*E. coli*	CH 1	452	54	5.0-6.5	45	Biofuel production Food technology	[250]
Hot spring metagenome	*E. coli*	CH 1	495	57.2	6.5	90	Biofuel production	[107]
Marine metagenomic	*E. coli*	CH 1	442	51	6.5	40	Biofuel production	[139]
Agricultural soil metagenome	*E. coli*	CH 3 CH 1	762 475	80.9 54.8	8 8	40 60	Waste paper recycling Detergent industries	[111]
Compost metagenome	*E. coli*	CH 1	445	52	5.5	75	Biofuel production Oligosaccharide synthesis	[108]
Termite gut	*E. coli*	CH 1	455	51	6.0	90	Biofuel production	[105]
Environmental DNA	*E. coli*	CH 1	485	55	6.5	55	Ginsenoside conversion Biofuel production	[49]
Cow rumen metagenome	*E. coli*	CH 3	NR	NR	5.0	50	Biofuel production	[110]
Cattle rumen metagenome	*E. coli*	CH 3	779	85	5.0	38	Biofuel production	[109]
Bioreactor contents	*E. coli*		620	66	7.0	37	Food Technology	[104]
Mangrove metagenome	*E. coli*	CH 3	663	71	7.0	40	Conversion of polydatin to resveratrol	[251]
Mangrove metagenome	*E. coli*	CH 3	442	48	6.0	40	Isoflavone hydrolysis	[140]

a.a= amino acids, NR= not reported.

7. Cloning and Expression of Microbial β-Glucosidases

Generally, cloning and expression of microbial genes encoding for industrially important enzymes aims to: i) produce enzymes in industrially compatible microorganisms such as *Aspergillus*, and *Trichoderma* species, if it is difficult to grow or handle the original microbes, ii) enhance enzyme production by expressing it in highly efficient host, using either multiple gene copies, and/or strong promoter, iii) produce enzymes in safe host if the origin of the genes is pathogenic or toxin producing microorganism, and iv) improve enzymes specificity, and stability by genetic engineering e.g., mutagenesis and direct evolution [178,179]. Number of β-glucosidase genes from bacteria, yeast and fungi have been cloned and expressed in *E. coli* and eukaryotic systems such as *Saccharomyces cerevisiae*, *Pichia pastoris*, and *T. reesei* [168,180,181,182]. Cloning and expression has started with either 1) genomic DNA digestion, construction of genomic DNA library and then functional screening for β-glucosidase [183], or 2) construction of cDNA library and function-based screening of β-glucosidase [169,184].

Filamentous fungi are known to synthesize and secrete high amounts of extracellular enzymes including β-glucosidase and reports on cloning and expression of β-glucosidase genes from filamentous fungi are quiet less [185]. In addition, fungal genes complexity e.g., presence of introns, and post-transcriptional and post-translational modifications such as glycosylation, acts as hurdle for cloning of these genes [186]. Nevertheless, β-glucosidase genes from *T. emersonii*, *P. brasilianum* and *T. aurantiacus* have been cloned and expressed in filamentous fungi such as *T. reesei* [187], *A. oryzae* [183], and *P. pastoris* [43], respectively. *P. pastoris* is known to secrete high amount of extracellular protein therefore it is preferred for heterologous expression of recombinant proteins [188,189]. Similarly, *T. reesei* produces cellulase component (endoglucanase and exoglucanase) in high amount with excellent properties such as thermostability and catalytic efficiency but it lacks sufficient amount of β-glucosidase. Researchers therefore have heterologously expressed exogenous β-glucosidase genes in *T. reesei* under strong promoter in order to enhance its β-glucosidase activity so that cellulose hydrolysis rate was improved considerably [112,187,190,191,192]. Majority of recombinant fungal β-glucosidase belong to glycoside hydrolase family 3. Fungal species are usually transformed with linearized plasmid which integrates to the chromosomal DNA enhancing its stability and expression efficiency [187,193].

Bacterial β-glucosidase genes has been cloned from number of species and expressed in *E. coli* because of its high growth rate, easy handling, genetic simplicity, easy transformation and plasmid uptake, and it can grow to high cell density 200 g/1 [194,195]. However, expression in *E. coli* has several drawbacks such as formation of inclusion bodies, low secretion efficiency, absence of splicing machinery and inability to perform post-translational modifications such as glycosylation explaining why it is not successfully being used for expression of fungal β-glucosidase enzymes [196,197,198]. *E.coli* is usually transformed with self-replicated plasmid that does not integrate to chromosomal DNA and keeps on replicating itself independently from cell divisions [199,200].

Finally, metagenome represents an excellent culture-independent approach for obtaining industrially important enzymes from unculturable microorganisms. A number of β-glucosidase has been cloned, expressed and characterized from different environment such as cattle rumen, marine, compost, soil metagenomes etc. In this approach, environmental DNA is first extracted and digested. The digested DNA is ligated into a particular vector for construction of metagenomic library which is then screened using function-based approach. List of recombinant β-glucosidase from fungi, bacteria and metagenome sources are shown in Table 2.

8. Applications of β-Glucosidase

β-Glucosidases are a group of enzymes that catalyze the hydrolysis of nonreducing β-glucosyl terminal from wide variety of aryl, alkyl glycosides, disaccharides and cello-oligosaccharides. It is a component of cellulase system involving in hydrolysis of cellobiose and short oligosaccharides to glucose thus eliminating the inhibitory effect of cellobiose on both endo- and exo-glucanases [201]. In addition to hydrolytic activity, β-glucosidase, under certain circumstances, have synthetic activity via transglycosylation [24,202]. As a result, β-glucosidase has wide range of potential biotechnological applications, some of which are based on the hydrolytic activity and some are based on the synthetic activity.

8.1. Applications Based on Hydrolytic Activity

Biofuel production from lignocellulose material is very attractive area for research in the current era especially with arising of energy crisis, depletion of fossil fuel, energy high prices and global warming. β-Glucosidase is utilized in hydrolysis of cellobiose and short oligosaccharides to glucose during cellulose hydrolysis so that glucose can be fermented to ethanol or other biofuel [13,28,128]. Currently, *T. reesei* is the major source of the commercial cellulase enzyme used for cellulose hydrolysis and biofuel production but it lacks sufficient β-glucosidase activity therefore supplementation of exogenous β-glucosidase from other filamentous fungi such as *Aspergillus niger* is mandatory [28].

β-Glucosidase is also utilized in aroma enhancement of wine and fruit juices by liberation of aromatic compounds from their glycosidic precursors present in fruit juices, musts and wines [203]. Since endogenous plant β-glucosidase is less efficient, supplementation with exogenous enzyme from microbial sources enhances the release of aromatic compounds from their glyosidic precursors [204,205].

β-Glucosidase is also used in the hydrolysis of phenolic compounds such as flavonoids and isoflavone glycosides present in soybean, fruit, vegetables, and other plant-derived foods [156,206,207,208]. These compounds are known to have phytoestrogenic [209], antioxidant [210,211], anticancer [212], and antinflammatory activities [213]. However, they are present mainly in glycosides

form thus decreasing their absorption from small intestine. As a result, hydrolysis of these phenolic glycosides by β-glucosidases increases their absorption and bioavailability [211,214]. These compounds may include diadzin, genistin, sesaminol glycosides, hesperidin, neohesperidin, naringin, narirutin, rutin, neoponcirin [61,211,215].

β-Glucosidases can also be used for debittering of fruit juices by hydrolysis of bitter compounds such naringenin and oleuropein [215,216]. Another important application of β-glucosidase is cassava detoxification, a carbohydrate rich food and a staple food in tropical countries. Because cassava is known to contain toxic cyanogenic glycosides such as linamarin and lotaustralin, prolonged consumption of cassava has been associated with CNS syndrome "Konzo" [217,218]. These cyanogenic glycosides can be eliminated with addition of exogenous linamarase and β-glucosidase from microbial sources during cassava processing [22,29,219]. Finally, β-glucosidase, along with other cellulases and hemicellulases enzymes, is utilized in waste paper recycling and ink removal for production of biofuel [1,30,220,221].

8.2. Applications Based on Synthetic Activity

The synthetic activity of β-glucosidase can be invested for synthesis of different alkyl- and aryl-β-glycosides and oligosaccharides. Alkyl- and aryl-β-glycosides have a wide range of applications in pharmaceutical and medical sciences increasing the demand of these valuable compounds. The enzymatic methods for synthesis of such compounds provides the advantage of high regio- and stereo-selectivity and utilization of mild conditions over the chemical methods which are nonspecific and require very harsh conditions [143]. Alkyl polyglucosides (APGs) are used for improving lubricating properties of water since they are biodegradable and have surface activity. Alkyl- and aryl-β-glycosides are nonionic surfactants with chemical stability, safety, biogradability, and antimicrobial activity. They can be used in detergent, pharmaceutical, food, and cosmetics industries [222]. On the other hand, synthetic oligosaccharides have broad spectrum of applications particularly in biomedical science as therapeutic agents; growth promoting agents for probiotics bacteria, vaccines, and diagnostic tools [35]. Synthetic Galacto-oligosaccharides (GOS) produced from lactose have a fiber properties relieving the symptoms of constipation in adult populations and modulate bowel function and stool characters [223,224]. GOS supplemented in infant formula stimulate intestinal *Bifidobacteria* and *Lactobacilli* to the same extent the breast-fed infants could have [202,225,226].

9. Conclusion

β-Glucosidases are heterogeneous group of enzymes found in bacteria, fungi, plants and animals. β-Glucosidases are diverse enzymes exhibiting different pH and temperature optima, substrate specificity, localization, and biological roles. β-Glucosidases have broad spectrum of potential biotechnological applications particularly in biofuel production, isoflavone hydrolysis, bitterness removal, cassava detoxification, etc. These potential applications call for effective large scale production of this valuable enzyme from fungal, bacterial and metagenome sources. Moreover, application of β-glucosidases in biofuel production require a novel enzymes exhibiting high thermostability, acidophilicity, and glucose tolerance therefore the search for enzymes exhibiting high tolerance to temperature, acidic pH and high glucose concentration is highly encouraged. Synthetic activity of β-glucosidases through transglycosylation is another attractive activity in biomedical and pharmaceutical applications and the search for new β-glucosidases with an excellent transglycosylation activity is another avenue of research in this field.

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