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REVIEW

Polysaccharide hydrolysis with engineered *Escherichia coli* for the production of biocommodities

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Abstract *Escherichia coli* can ferment a broad range of sugars, including pentoses, hexoses, uronic acids, and polyols. These features make *E. coli* a suitable microorganism for the development of biocatalysts to be used in the production of biocommodities and biofuels by metabolic engineering. *E. coli* cannot directly ferment polysaccharides because it does not produce and secrete the necessary saccharolytic enzymes; however, there are many genetic tools that can be used to confer this ability on this prokaryote. The construction of saccharolytic *E. coli* strains will reduce costs and simplify the production process because the saccharification and fermentation can be conducted in a single reactor with a reduced concentration or absence of additional external saccharolytic enzymes. Recent advances in metabolic engineering, surface display, and excretion of hydrolytic enzymes provide a framework for developing *E. coli* strains for the so-called consolidated bioprocessing. This review presents the different strategies toward the development of *E. coli* strains that have the ability to display and secrete saccharolytic enzymes to hydrolyze different sugar-polymeric substrates and reduce the loading of saccharolytic enzymes.

Keywords *Escherichia coli* · Metabolic engineering · Biocommodities · Polysaccharides · Saccharolytic enzymes secretion

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Introduction

Currently, the world is facing the end of the availability of abundant and cheap oil [9, 62, 63, 72]. Several countries are hungry for energy and global oil production has reached its peak [9, 62, 63, 72]. Additionally, the pollution produced by burning fossil fuels has caused a massive accumulation of greenhouse gases in the atmosphere [38]. For these reasons, it is imperative to produce chemicals from renewable and environmentally friendly resources. One such option is the transformation of sugars by fermentation processes into metabolites that can replace petrochemicals [19].

Lignocellulose, the most abundant biopolymer on earth, is a material rich in fermentable sugars [94]. The transformation and use of this biopolymer does not compete with the chain production of foods. Among others, lignocellulose can be obtained from agricultural and forest residues, waste paper, and dedicated lignocellulosic crops [26, 31, 55]. The lignocellulose structure is principally composed of three fractions: cellulose, hemicellulose, and lignin [52, 78]. However, some lignocellulosic materials, such as sugar beet pulp, citrus peel, and apple pomace, can also contain an important fraction of pectin [20]. Another good source of fermentable sugars is seaweed, which has the advantage that its production does not require arable land and fresh water; furthermore, lignin is present only in small amounts or is absent in this material [36, 40]. Although some seaweeds are farmed extensively in China, Japan, and Korea for human consumption, there are many species that are not used for feed or food production and can potentially be used for the production of biocommodities [47, 89]. The carbohydrates that are found in seaweed are alginate, agar, ulvan, laminarim, mannitol, starch, and cellulose [53, 74].

To release the fermentable sugars in lignocellulose, the biomass is first subjected to a pretreatment process with the

aim of making the carbohydrate polymers more accessible to the enzymes during the saccharification process, which is performed in some cases to hydrolyze a portion of the sugar polymers [15, 60, 61]. The pretreatment can be physical, chemical, or a combination of both, and will determine the optimal enzyme mixture required in the next step [15, 60, 61]. After pretreatment, the solids from the biomass are squeezed, washed, and conditioned for the saccharification process, whereupon the polymers are hydrolyzed by the synergistic action of cellulases and β-glucosidases [10, 52]. If necessary, the enzymatic cocktail can be enriched with accessory enzymes, such as xylanases, β-xylosidases, esterases, arabinosidases, pectinases, etc. [1, 6, 15, 27]. Additionally, the accessibility of cellulose to cellulases can be enhanced by the addition of non-hydrolyzing proteins that promote amorphogenesis [2] such as swollenins [13, 44]. In the case of seaweeds, the low content or absence of lignin allows for the release of some sugars with water after milling or crushing the biomass [36, 90]. The polymeric sugars can then be hydrolyzed with alginate lyases, laminarases, amylases, cellulases, etc. [67, 90]. The hydrolysis of lignocellulose and seaweed produces a wide variety of fermentable sugars including hexoses, such as glucose, mannose and galactose; and pentoses, such as xylose and arabinose [52, 53, 74]. The hydrolysis of pectin-rich biomass and seaweeds also produces rhamnose, mannitol, and uronic acids, such as galacturonic, mannuronic, and guluronic acids [20, 53, 74].

Escherichia coli is a Gram-negative bacterium that has the ability to ferment a broad range of substrates, including pentoses, hexoses, uronic acids, and polyols [16, 39, 69]. Non-pathogenic, industrial, and laboratory *E. coli* strains can grow in mineral salt media without complex supplements [22, 58, 70, 85]. Although it is known that many of the toxic compounds that are formed during lignocellulose pretreatment can inhibit the fermentation performance of *E. coli* [30, 59], at present, there are reports of some engineered strains that are able to directly ferment the sugars obtained from the pretreated biomass even in the presence of toxic compounds such as hydroxymethylfurfural, furfural and acetate [22, 29, 30]. From the microbiological, physiological, and genetic points of view, *E. coli* is a well-known microorganism. A wide array of genetic tools is available to facilitate its genetic modification and this microorganism is widely used on an industrial scale to produce several biotechnological products [16]. For these reasons, *E. coli* has been the target of metabolic engineering for the production of commodity fuels and chemicals that can replace petrochemicals such as ethanol [22, 37, 70], butanol [8, 18, 28], lactate [84, 85], succinate [95], fatty alcohols [18, 82], fatty acids [18], fatty esters (biodiesel) [8, 82], pinene [8], and methyl ketones [73], etc. To reduce costs and simplify the production process, a microorganism with the capacity to secrete saccharolytic enzymes and to ferment whole sugars into wanted biocommodities is required [68]. This idea was first presented in 1936 by Veldhuis et al. [88]. It was first designated as Direct Microbial Conversion (DMC) [35] and later renamed Consolidated Bioprocessing (CBP) by Lynd [57]. By taking advantage of the aforementioned characteristics of *E. coli*, this microorganism is a good candidate for CBP.

Although *E. coli* can ferment a broad range of sugars, it cannot hydrolyze polymeric sugars or oligosaccharides, such as cellodextrins or xylodextrins. This limitation has motivated the development of strategies for the secretion of saccharolytic enzymes to produce biocommodities directly from structural and storage sugars (i.e., without the addition of an exogenous saccharifying catalyst). The secretion of heterologous and homologous hydrolytic enzymes has many advantages. These enzymes have free access to insoluble substrates because cellulose and soluble oligosaccharides can be hydrolyzed without the need to cross the cell envelope. If the enzymes are attached to the cell surface, the biocatalyst becomes more stable. It can then be recovered with the cells for subsequent fermentations or saccharification processes. In the present review, we discuss the strategies that various research groups are developing for conducting CBP with *E. coli*. Specifically, we describe the state-of-the-art strategies for the secretion of saccharolytic enzymes.

Cell surface display of saccharolytic enzymes

The cell surface display of heterologous proteins has many biotechnological applications. These applications include the development of live vaccines [12, 54, 66], bioadsorbents for the removal of harmful chemicals and heavy metals [5, 92], high-throughput screening of enzyme libraries [48, 56] and whole-cell biocatalysts [64, 79, 81, 83], among other applications. Currently, the attaching of saccharolytic enzymes to *E. coli* for the hydrolysis of non-natural substrates is becoming more relevant for the production of biocommodities, especially for the secretion of enzymes involved in the depolymerization of low-cost polysaccharides [64, 79, 81, 83]. The cell surface is associated with the use of outer membrane proteins (OMP) as carriers. The passenger protein (secreted enzyme) is fused by its N- or C- terminus to the OMP, and when the chimeric protein arrives in the periplasmic space, the passenger protein is translocated across the outer membrane and anchored with the OMP (Figs. 1, 2). Table 1 summarizes the reports and applications of the cell surface display of saccharolytic enzymes.

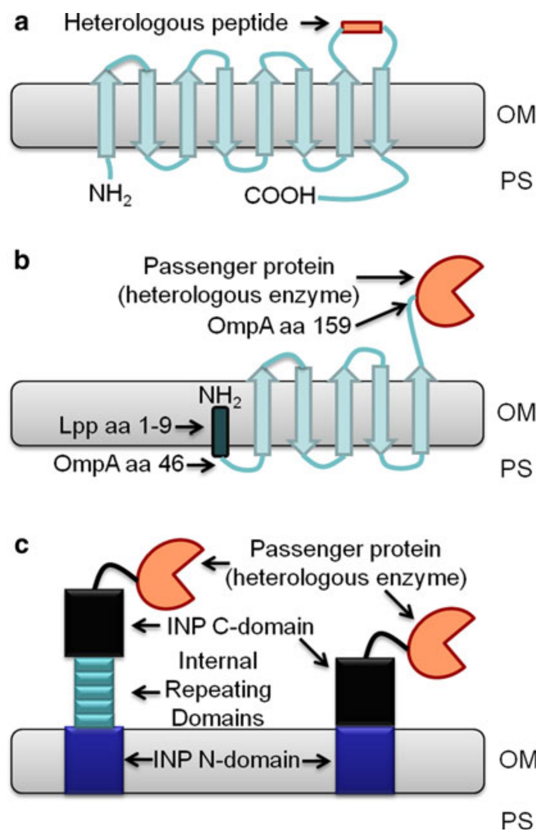

Fig. 1 Examples of cell surface displays. **a** Insertion of a heterologous peptide within a loop of OmpA [25]. The peptide is attached by its N- and C-termini in a sandwich conformation. **b** Attachment of a heterologous enzyme by its N-terminus to the hybrid protein Lpp-OmpA [23]. **c** Attachment of a heterologous enzyme by its N-terminus to the INP [43]. The internal repeating domains can be modulated in length or removed. *OM* outer membrane, *PS* periplasmic space, *aa* amino acid, *OmpA* outer-membrane protein A, *Lpp* lipoprotein, *INP* ice nucleation protein

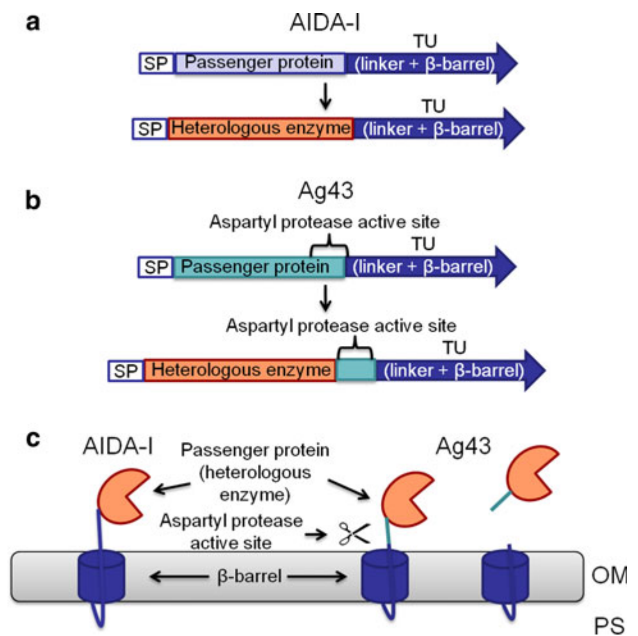

Fig. 2 Secretion of heterologous enzymes using the autotransporter pathway. **a** The structure of the AIDA-I pre-protein autotransporter multidomain. The complete native passenger protein is substituted by the heterologous enzyme [64]. **b** The structure of the multidomain Ag43 pre-protein autotransporter. The aspartyl protease active site remains after replacing the passenger protein with the heterologous enzyme to allow for the secretion of the enzyme into the milieu [90]. **c** When the protein arrives in the periplasmic space, the SP is cleaved and the β-barrel is embedded in the OM. Subsequently, the passenger protein is translocated across the OM and attached to the cell surface by its C-terminus via a linker (AIDA-I). Some autotransporters, such as Ag43, process the linker after the passenger protein is translocated, this feature had been used to release heterologous proteins into the milieu [90]. *SP* signal peptide, *TU* translocation unit, *OM* outer membrane, *PS* periplasmic space

Surface display using outer membrane proteins (OMPs) from *E. coli*

An important characteristic of OMPs is their β-barrel structure, which is formed by anti-parallel transmembrane β-strands joined by loops [50]. In addition to their function as structural components, virulence factors, and porins, OMPs are receptors of many phages [50]. Early studies with phage OMP-resistant mutants showed that mutations occurred in very specific areas, which suggests that these zones are cell surface-exposed loops that can be targets for the cell surface display of peptides or proteins [12, 25]. With this in mind, Freudl et al. [25] and Charbit et al. [11] fused a peptide by its N- and C-termini to an external loop of the OMPs OmpA and LamB, respectively. This sandwich conformation resulted in the presentation of the peptide at the cell surface (Fig. 1a). The use of surface-exposed loops is restricted to small peptides because the insertion of large peptides or proteins can affect the assembly of the carrier protein into the outer membrane [86]. However, this strategy opened the door for the development of live vaccines by displaying epitopes [12, 54, 66]. These pioneering works served as the starting point of bacterial surface display.

To display a complete protein, Francisco et al. [23] developed a secretion system consisting of the signal peptide and the first nine amino acids of the lipoprotein Lpp that was linked to five of the eight membrane-spanning loops of OmpA (Fig. 1b). In this case, the passenger protein was fused by its N-terminus to the C-terminus of Lpp-OmpA. This construction allowed for the display of the first saccharolytic enzyme in *E. coli*, the *Cellulomonas fimi* exoglucanase Cex [24]. Recently, the OMP Blc was used to display the *Thermobifida fusca* β-glucosidase BglC [81, 83]. This enzyme was fused by its N- or C-terminus and secreted in an active form [83]. An engineered *E. coli* strain, which expressed the same system, hydrolyzed and fermented 139 mM (47.5 g/l) cellobiose into 69 mM (4.1 g/l) isopropanol [81].

Table 1 Cell surface display of saccharolytic enzymes in *E. coli* and its applications

Anchor	Passenger protein(s)	Application	Source
E. coli Lpp-OmpA	*C. fimi* exoglucanase	Hydrolysis of cellulose	[24]
E. coli Blc	*T. fusca* β-glucosidase	Hydrolysis of cellobiose	[83]
E. coli Blc	*T. fusca* β-glucosidase	Isopropanol production from cellobiose	[81]
E. coli AIDA-I	*T. fusca* β-glucosidase	Ethanol production from cellobiose	[64]
P. syringae INP	*B. subtillis* carboxymethylcellulase	Hydrolysis of carboxymethylcellulose	[43]
P. syringae INP	*B. subtillis* carboxymethylcellulase	High throughput screening of enzyme libraries	[48]
P. syringae INP	*Clostridium phytofermentans* endoglucanase	High-throughput screening of enzyme libraries	[56]
B. subtilis PgsA	*Streptococcus bovis* α-amylase	Cornstarch hydrolysis	[65]
B. subtilis PgsA	*Clostridium cellulolyticum* endocellulase, exocellulase, and β-glucosidase	Ethanol production from corn stover cellulose	[79]
B. subtilis PgsA	*C. fimi* xylanase	Enhancing the stability of the enzyme	[14]

Surface display using OMPs from other microorganisms

One of the most popular carriers for cell surface display in *E. coli* is the ice-nucleation protein (INP) from *Pseudomonas syringae*. This OMP is produced from ice-nucleation-active bacteria such as *P. syringae*, *Xanthomonas campestris* and *Erwinia herbicola.* It catalyzes the formation of ice on supercooled water [43, 46]. The first studies performed with INP expressed in *E. coli* demonstrated that the protein retains its function [71], which introduces the possibility of using INP as a carrier for the cell surface display of heterologous proteins. The structure of INP consists of three domains: the N-terminal hydrophobic domain, which interacts with the OM to attach the INP to the cell surface; the central hydrophilic repeated domain, which catalyzes the ice nucleation; and the C-terminal hydrophilic domain [43, 46]. To display a heterologous protein using this system, the N-terminal domain of the protein is fused with the C-terminal domain of the INP (Fig. 1c). Additionally, the internal repeated domain can be modulated in length, allowing the passenger protein to move further from or closer to the outer membrane (Fig. 1c) [43]. *P. syringae* INP has been used for the secretion of *Bacillus subtilis* carboxymethylcellulase [43, 48] and *Clostridium phytofermentans* endoglucanase [56]. However, its principal application is for the high-throughput screening of glucanase libraries [48, 56].

PgsA is a protein that has been implicated in the synthesis of poly-γ-glutamate (PGA), a polymer produced from *B. subtilis* [4]. When PgsA was heterologously expressed in *E. coli*, the protein was detected in a cell membrane preparation [3]. With the use of PgsA as a carrier for cell surface display, Narita et al. [65] developed a secretion system that allows for the N-terminus fusion of the *Streptococcus bovis* α-amylase. The attachment of *C. fimi* xylanase Cex to PgsA results in increased enzyme stability [14]. The cell surface display of Cex increased the thermal stability of this enzyme and its activity over a broader pH range relative to the soluble enzyme [14]. Finally, PgsA was used for attaching a *Clostridium cellulolyticum* endocellulase, exocellulase, and β-glucosidase to the cell surface of an ethanologenic *E. coli* strain, which allowed for the direct fermentation of 10 g/l phosphoric acid-swollen cellulose into 3.59 g/l ethanol, and 1 g/l pretreated corn stover cellulose into 0.3 g/l ethanol [79].

Surface display via the type V (autotransporter) system

The autotransporter system that belongs to the type V secretion system is considered to be the simplest secretion system among Gram-negative bacteria [17, 34]. The complete information for protein secretion is contained within a single gene that produces a multidomain pre-protein that consists of an N-terminal signal peptide (SP), a passenger protein, and a C-terminal translocation unit (TU) (Fig. 2a) [17, 42]. The SP directs the pre-protein into the periplasmic space where it is cleaved by a signal peptidase. Subsequently, the passenger protein is translocated across the outer membrane by the TU, which is formed by a linker region and a β-barrel (at the C-terminus). The linker region anchors the passenger protein to the cell surface; in some cases, autotransporters process the passenger protein after it has been translocated, thereby releasing the protein into the milieu (Fig. 2c) [17]. To display a heterologous protein, the native sequence of the passenger protein is replaced with the heterologous protein sequence. The first autotransporter described was the immunoglobulin A1 (IgA1) protease of *Neisseria gonorrhoeae* [76] and this transporter was also the first autotransporter used for the cell surface display of a heterologous protein in *E. coli* [49]. However, the most commonly used autotransporter for displaying heterologous proteins is the adhesin involved in diffuse

adherence (AIDA-I) of diarrheagenic *E. coli* [41, 42]. Recently, Muñoz-Gutiérrez et al. [64] used AIDA-I to attach the *T. fusca* β-glucosidase BglC in an ethanologenic *E. coli* strain for the hydrolysis and fermentation of 40 g/l cellobiose to 17 g/l ethanol.

Secretion of saccharolytic enzymes into the milieu

Non-complexed saccharolytic enzymes are secreted by their native organism to permit the hydrolysis of insoluble substrates. Additionally, cellulases and xylanases have carbohydrate-binding domains (CBDs) that allow these enzymes to be adsorbed onto insoluble substrates, thus bringing the catalytic domain into close proximity with the substrate. These characteristics might allow similar secretion systems in *E. coli* to be developed to improve the hydrolysis process of polymeric sugars for the production of biocommodities.

Secretion using the type II and type V (autotransporter) system

Ag43 is an *E. coli* autotransporter encoded by the *flu* gene that confers the autoaggregation phenotype, and it is produced by many strains, including non-pathogenic species [87]. Although the passenger protein of Ag43 is cleaved after it has been translocated across the outer membrane (OM), this protein remains bound to the TU via noncovalent interactions [33, 87]. An aspartyl protease active site within the passenger protein has been postulated to be the site responsible for the autocleavage of the passenger protein (Fig. 2b) [33, 90]. To secrete *Pseudoalteromonas* sp. SM0524 alginate lyase (Aly) into the milieu, Wargacki et al. [90] replaced the passenger protein sequence with the *aly* gene while maintaining the aspartyl protease active site sequence (Fig. 2b, c). This construction was used for the fermentation of alginate from a brown macroalga into ethanol with an engineered *E. coli* strain [90]. The engineered *E. coli* strain developed by Wargacki et al. directly fermented 150 g/l of the seaweed *Saccharina japonica* to 37.8 g/l ethanol [90]. Recently, Bio Architecture Lab opened an experimental pilot facility in Chile to produce ethanol from the native seaweed *Macrocystis pyrifera* [7].

Unlike the autotransporter system, the type II secretion system (T2SS) is a complex structure that contains 12–15 different proteins and spans both the inner and outer membranes [51] (Fig. 3). The passenger proteins have an N-terminal signal peptide that directs the protein to the periplasmic space where the protein is folded and subsequently translocated across the outer membrane via the T2SS apparatus [51] (Fig. 3). *Erwinia chrysanthemi* is a plant pathogen and Gram-negative bacterium that secretes a battery of cell wall-degrading enzymes using the Out system, which belongs to the T2SS [32]. When the *E. chrysanthemi out* genes are cloned in *E. coli*, it is possible to drive the secretion of four pectate lyase isozymes [32], an exo-poly-α-D-galacturonosidase [32], a pectin methylesterase [32], and an endoglucanase [96] into the milieu, all of which are from the same microorganism. Edwards et al. [21] engineered an ethanologenic *E. coli* strain with the *E. chrysanthemi out* genes for the secretion of a pectate lyase and an oligogalacturonide lyase from the same bacterium. This allowed for the fermentation of 100 g/l sugar beet pulp into 14.6 g/l ethanol, with the help of commercial cellulases and a pectin methylesterase [21]. A limitation of the T2SS is the high specificity of its target proteins, even for closely related bacteria. The *E. chrysanthemi* T2SS cannot secrete proteins from the *Erwinia carotovora* ssp. *carotovora* T2SS [32].

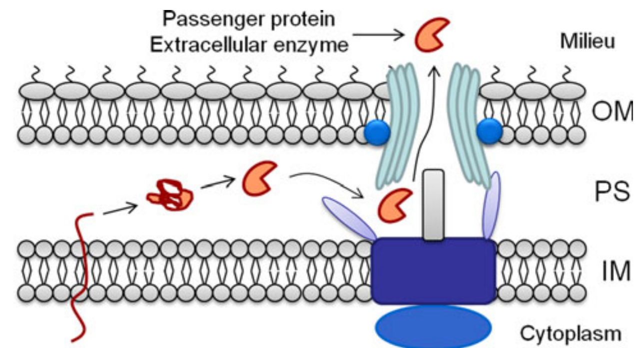

Fig. 3 Schematic representation of a type II secretion system. The passenger protein is folded in the PS and is subsequently secreted through the T2SS (type II secretion system) apparatus that consists of approximately 15 different proteins [51]. *OM* outer membrane, *PS* periplasmic space, *IM* inner membrane

Secretion using extracellular proteins as carriers

As OMPs can be used as carriers for cell surface display, extracellular proteins can be used as carriers for secreting enzymes into the milieu. YebF is an *E. coli* protein of unknown function that is secreted into the medium [93]. To study the secretion of YebF, Zhang et al. [93] fused the *B. subtilis* X-23 α-amylase to the C-terminus of YebF and discovered that most of the activity observed was detected in the medium. However, a more popular carrier is the osmotically inducible protein Y (OsmY), which was identified in an extracellular proteome analysis of *E. coli* by Qian et al. [77]. The potential of this carrier was demonstrated using the secretion of an *E. coli* alkaline phosphatase, a *B. subtilis* α-amylase, and a human leptin; all of these proteins were fused by their N-termini to OsmY [77]. Subsequently, the application of OsmY in the production of

Table 2 Secretion of saccharolytic enzymes in *E. coli* and its applications

Secretion system	Passenger protein(s)	Application	Source
E. coli Ag43	*Pseudoalteromonas* sp. SM0524 alginate lyase	Ethanol production from brawn macroalgae	[90]
E. chrysanthemi out genes	*Erwinia chrysanthemi* pectate lyases isozymes, exo-poly-α-D-galacturonosidase and pectin methylesterase	Pectin hydrolysis	[32]
E. chrysanthemi out genes	*E. chrysanthemi* endoglucanase	Cellulose hydrolysis	[96]
E. chrysanthemi out genes	*E. chrysanthemi* pectate lyase PelE and oligogalacturonide lyase Ogl	Ethanol production from sugar beet pulp	[21]
E. coli OsmY	*B. subtilis* α-amylase	Starch hydrolysis	[77]
E. coli OsmY	*Clostridium stercorarium* endoxylanase catalytic domain and *Bacteroides ovatus* xylanase	Fatty-acid production from hemicellulose	[82]
E. coli OsmY	*Bacillus* sp. D04 endocellulase, *C. stercorarium* endoxylanase catalytic domain	Fatty-acid ethyl esters, butanol, and pinene production from switchgrass	[8]
E. coli OsmY	*Fusarium graminearum* endoxylanase, *Clostridium stercorarium* endoxylanase, *Fibrobacter succinogenes* endoxylanase; *Bacteroides ovatus* xylosidase, *Bacillus pumilus* IPO xylosidase, *Selenomonas ruminantium* xylosidase, *F. graminearum* xylosidase, and *B. subtilis* α-arabinofuranosidase	Succinate production from xylan	[95]
E. coli YebF	*B. subtilis* X-23 α-amylase	Starch hydrolysis	[93]

biocommodities was demonstrated by the secretion of the *Clostridium stercorarium* endoxylanase catalytic domain and the *Bacteroides ovatus* xylanase in an engineered *E. coli* strain designed for fatty acid ethyl esters (FAEEs) production [82]. This engineered *E. coli* strain produced 11.6 mg/l FAEEs from a medium containing 2 % xylan and 0.2 % glucose, a threefold increase in FAEEs production compared to glucose alone [82]. The hydrolysis of switchgrass for the production of butanol, pinene, and FAEEs was achieved using OsmY as the protein carrier to secrete the *Bacillus* sp. D04 endocellulase and the *C. stercorarium* endoxylanase catalytic domain in a metabolically engineered *E. coli* strain that also expressed the *Cellvibrio japonicus* β-glucosidase and xylobiosidase [8]. The production of 71 mg/l FAEEs, 28 mg/l butanol, and 1.7 mg/l pinene was achieved, without using externally supplied hydrolases, from three different batches of switchgrass pretreated with ionic liquids. Furthermore, Zheng et al. [95] studied the hydrolysis of xylan for succinate production by combining endoxylanases and xylosidases from many bacteria (Table 2); the best combination was the *Fibrobacter succinogenes* endoxylanase with the *Fusarium graminearum* xylosidase. To improve xylan hydrolysis, the *B. subtilis* α-arabinofuranosidase was also secreted using OsmY as the protein carrier [95]. These researchers engineered an *E. coli* strain that secreted the three selected hemicellulases and produced 14.44 g/l of succinate from a mixture containing 3 % beechwood xylan and 1 % xylose, more than three times the titer reached with 1 % xylose alone [95].

Secretion by increasing outer membrane permeability

Outer membrane leakage has been employed as a strategy of protein secretion instead of using secretion pathways or protein carriers [80]. An *E. coli* mutant that does not produce the lipoprotein Lpp was used for the secretion of *Bacillus halodurans* C-125 xylanase and *Clostridium thermocellum* cellulase [80]. The enzymes were directed to the periplasmic space where they were subsequently secreted into the milieu as a result of the increased outer membrane leakage caused by the absence of Lpp [80]. However, the main disadvantage of this strategy is the damage to the cell envelope, which makes the cells more susceptible to any toxic compound present in the medium and/or to the produced biocommodity.

Concluding remarks

The secretion of heterologous proteins by *E. coli* has been a challenge since the beginning of biotechnology. However, this topic has reappeared for the development of CBP with *E. coli*. Many of the renewable sources of sugars are in polymeric forms and cannot cross the cell envelope. Therefore, these polysaccharides must be enzymatically depolymerized into fermentable sugars and the saccharolytic enzymes must be secreted. Non-pathogenic laboratory strains of *E. coli* are not good protein secretors. Whereas the principal producers of saccharolytic enzymes at industrial level, the cellulolytic fungus *Trichoderma reesei*

and *Aspergillus niger*, can secrete up to 100 g/l cellulases [45, 75], the highest extracellular protein titers reported with the engineered *E. coli* strains presented in this review are less than 1 g/l employing the protein carrier OsmY [77, 95]. However, engineered *E. coli* strains, even showing a relative low secreted saccharolytic activity, can hydrolyze oligosaccharides or polysaccharides and simultaneously grow and ferment monomeric sugars; hence opening the possibility of producing biocommodities from a wide variety of polymeric sugars.

There are many challenges that have to be surpassed in order to reach a CBP with *E. coli*, among others: it is important to develop strains with improved secretion capabilities without affecting cell viability and performance and without the need of complex medium supplements [64, 95]; use selected promoters to optimize the secretion of saccharolytic enzymes [8]; also eliminating the use of expensive commercial inductors, such as isopropyl β-D-1-thiogalactopyranoside; and finding saccharolytic enzymes that well match with *E. coli* growth conditions, i.e., pH 7 and 37 °C [64, 79, 81, 83, 95]. It is feasible to design *E. coli* strains to decompose specific lignocellulosic fractions or specific types of biomass, as described in the present review with the hydrolysis and fermentation of xylan, pectin, cellulose, or alginate. These strains can be co-cultured to ferment all the lignocellulose fractions as already shown by Bokinsky et al. [8].

Although the titers shown in the present review for metabolites such as FAEEs, butanol, and pinene are in the range of mg/l [8, 82], these works are proof-of-concept showing that the production of advanced biofuels directly from polymeric sugar using *E. coli* is promising. However, Muñoz-Gutierrez et al. [64] and Zheng et al. [95] engineered *E. coli* to hydrolyze and ferment cellobiose and xylan, respectively, in mineral medium without complex supplements, with very low β-glucosidase and xylanase activities as compare with *Aspergillus niger* and *T. reesei* [45, 91], but reaching 17 g/l of ethanol and 14.44 g/l of succinate, respectively. Furthermore, Wargacki et al., from Bio Architecture Lab [90], showed that depolymerization and fermentation process with the alginate-fermenting *E. coli* strain can reach 37.8 g/l ethanol from seaweed as a source of fermentable carbohydrates. These titers, in the range of g/l, are promising for future industrial applications.

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