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ORIGINAL ARTICLE

Progression of Recombinant Cellulolytic and Ethanologenic *Escherichia coli* for Production of Bioethanol: A Review

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ABSTRACT - This review takes the readers to the current trend in the creating of recombinant cellulolytic and ethanologenic *Escherichia coli* for the production of bioethanol in recent years. The *E. coli* were employed in many bioprocesses due to their well-studied expression system and commercially available genetic modification tools resulting in them as the leading option in protein studies and production. This paper discusses the direction of the *E. coli* strains development and strains improvement as the implication to persisting challenges in the depolymerization of lignocellulosic biomass to fermentable sugars. The discussion includes the strategies applied for development of *E. coli* cellulolytic strains. Apart from that, the prospect of other modification strategies to achieve desirable recombinant protein expression learned from other *E. coli* applications were listed and considered. In the attempt to achieve a consolidated bioprocessing (CBP) of lignocellulose to bioethanol, more ethanologenic strains were discovered over the years and their refined characteristics were anticipated. Therefore, the approaches used in the development of ethanologenic strains in *E. coli* were discussed. The advantages and the limitations in the manipulation of *E. coli* expression system are to be understood in order to chart new pathways in developing better ethanologens and bioprocess that utilize them.

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INTRODUCTION

Lignocellulosic biomass from agricultural byproducts is potentially attractive raw material for value added products especially bioethanol production. Bioethanol is mainly important because it provides alternative to fossil fuel usage hence relaxes the world dependency on the non-renewable energy. However, bioethanol from starch is frown upon since starch is labelled as food. The conversion of food resources into bioethanol invites a never-ending debate whether to risk food security over energy security. The gap between the lignocellulosic biomass and bioethanol is bridged by the continuous effort in research and development to provide efficient hydrolysis and fermentable reactions.

Industrial hydrolysis reaction of biomass that occurs regularly in biofuel processing plant [1] or in paper and textile factories [2];[3] are partly enzymatic and catalyzed by commercial cellulases. Fungi species are the main producer of commercial cellulase. The scalable production is possible because fungi are the natural decomposers and recyclers in the ecosystem. Without the interference of genetic modification, fungal isolates can be obtained from decomposing cellulosic agro waste. The isolates were then screened for endoglucanase, exoglucanase and β-glucosidase activity. These activities are mode of action for cellulase. A single cellulase manifest a single activity or a combination of two activities [4]. Fungi have a more sophisticated natural delivery system of cellulases from the cell to the surrounding environment, which is absent in other microorganisms.

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The fungi adopt a collective structure called cellulosome, a bioconjugate protein that can be 'docked' with cellulases and lytic polysaccharide monooxygenases (LPMOs) which are all needed for efficient depolymerization of lignocellulosic material. To dock, the proteins are expressed to contain a domain called cohesin to enable the assembly between the enzymes and the scaffolding protein to form a functional cellulosome [4]. Fungi cellulases are available externally [5]. This is an extremely advantageous because cellulase production using other recombinant microorganisms are challenged with internal expression adding to processing cost.

While most fungal species are naturally existed to contain the genetic print to perform ethanol fermentation, the efficiency varies among species. The most common species to undertake hydrolysis process are *Trichorderma reesei, Trichoderma viride, Humicola insolens, Penicillium purpurogenum* and *Aspergillus niger*. *Saccharomyces cerevisiae*, a yeast, is an obvious choice for bioethanol fermentation. This species has an unrivalled efficiency in converting hexose sugar into ethanol. However, complex lignocellulosic material composition is known to contain both hexose and pentose sugar.

In 2009, a recombinant strain of *S. cerevisiae* had been reported to demonstrate the ability to utilize pentose sugars such as arabinose and xylose [6]. Several studies had shown the feasibility of simultaneous saccharification fermentation (SSF) through the (i) combination of heat-treated biomass with the action of thermo-tolerant yeast strain [7], (ii) medium optimization using two different fungal strains [8], (iii) using a microbial consortium [9] and (iv) optimizing the enzyme cocktail with fermenting fungus through statistical studies [10]. In SSF, fermentation occurs simultaneously when the biomass is subjected to saccharification process. Using genetic engineering, two fungal species such as *S. cerevisiae* and *Myceliophthora thermophile* [11];[12] were reported to be able to perform both hydrolysis and fermentation process. They demonstrated the desirable method in achieving CBP in bioethanol production.

However, *E. coli* remains an interesting option for bioethanol production mainly because the relatively accessible genetic material to be manipulated, expressed and characterized. The diversity of genes required in the biochemistry of microbial species' fermentation and hydrolysis pathways provided an infinite repertoire of genetic materials for heterologous gene expression in recombinant *E. coli*.

HETEROLOGOUS EXPRESSION OF RECOMBINANT CELLULASES

Recombinant cellulases are proteins from modified cellulase genes that are heterologously expressed. The host can be from a closely related species or a completely different species altogether. Recombinant cellulase expression is carried out to characterize the resulting enzyme activity and to participate in pilot experiments in bioreactor or process designs. In protein characterization, the enzymes were extracted from the growth media by harvesting the cells pellet. Generally, recombinant proteins produce from *E. coli* expression system is internally expressed [13]. In an optimized buffer solution, the enzymes were suspended and exposed with pure form of known carbon substrates. The carboxymethyl cellulose (CMC), Avicel, and p-nitrophenyl-D-glucopyranoside (pNPG) are examples of carbon source that are used to detect the activity of endoglucanase, exoglucanase and β-glucosidase respectively [14]. Enzyme activities are examined using assays that measure the amount of reducing sugars produced by the processes. Subsequently, further manipulation of substrate concentrations and the enzyme kinetics of the reactions can be studied. Enzyme activity measures the functionality of the cellulases whereas constants in enzyme kinetics reveal the relationship between substrate and cellulase affinity for each other, maximum velocity of cellulase reaction rate at substrate saturation phase and the turnover number of the enzymes [4];[15];[16];[17];[18];[19].

The *E. coli* expressing cellulase are then introduced to a suitable lignocellulosic biomass for the production of commercial cellulase. The expanding range of choice to include Oil Palm Empty Fruit Bunch (OPEFB) [4];[15] as carbon source for cellulase production has promised a new gateway through which the accumulation of palm oil industrial wastes in Malaysia could be utilized fully. Elsewhere, where grapefruit processing waste (GPW) is common, the study by Ibrahim *et al.* [14];[17] demonstrated the role of cellulases harvested from *E. coli* in converting the mentioned waste into biofuel precursors. Their study had detected a considerable amount of reducing sugars (i.e. xylose, arabinose, galactose, mannose and rhamnose) originating from pectin- or hemicellulose-rich fraction of the GPW biomass composition.

Table 1 summarizes the recently reported enzyme activities of cellulase genes sourced from various microbial species and expressed using the *E. coli* as their host expression system.

CELLULASE MODIFICATION WITH NON-CATALYTIC DOMAINS

Desirable characteristics of cellulase modification are either improved enzymatic activity, better adsorption on cellulosic materials or extracellular protein expression. These characteristics are achieved by joining the cellulase gene with non-catalytic domains.

Table 2 shows the fusion of cellulase genes and non-catalytic domains retrieve from natural genetic pool across the species. Peptide signals were added at the C-terminal of the expressed proteins (cellulase) to ensure the catalytic sites are available on the surface of the cells. In this way, cellulase purification become unnecessary. In addition, the cell-surface display of cellulolytic *E. coli* will increase the surface area to facilitate hydrolytic reactions [20];[21].

Site-directed mutagenesis is used to change the sequence of relevant domains in order to map the structure and function of genetic material to their phenotypic manifestations, with the help of genetic sequence homology analysis made feasible by the developing bioinformatics database. This approach is especially true in the creation of knock-out mutants. In a more relevant studies, conserved regions are studied to alter gene structure in the attempt to create cellulase with a better enzymatic efficiency [22];[23]. Other investigations used a protein fusion technique to develop cellulase variants that are compatible with the conditions seen in a conventional bioreactor.

Recent trends appear to prefer the addition of carbohydrate binding modules (CBM) to the cellulase protein to enhance structure stability in the presence of high pH and temperature of growth medium, which are common in a bioreactor for hydrolytic reaction. The affinity for cellulose is promoted with the addition of CBM because the cellulase-cellulose interface is made available. However, the role of linkers in fusion cellulase architecture should not be overlooked, as the structure could become dysfunctional without them. Linker sequences are thought to provide flexibility within the structure of the two domains terminally situated [24].

POTENTIAL ADAPTATIONS FROM SECRETORY PROTEINS USING *E. COLI* EXPRESSION SYSTEM

Table 3 summarizes of the externally expressed protein within the scope of *E. coli* expression system under various applications.

The strategy to express heterologous gene using *E. coli* expression system include the co-expression of the desired gene with Sec pathway signal peptides [25];[26], secretion signal protein [27] co-expression with another secretory enzyme [28], the host's periplasmic protein and anchored protein of *E. coli* membranes [29];[30];[31];[32].

In commercial scale protein manufacturing, such as commercial enzymes, protein-based medicine production, and protein substitutes of medically significant compounds, the desire to enable protein secretion to the outer environment is a pressing concern. By expressing the desired protein externally, processing cost can be reduced significantly. This is because the additional step of protein extraction from the cell lysate can be omitted when the large fraction of synthesized protein is available in the growth medium. It is also appealing to the process design because a continuous production can easily be achieved with the addition of fresh medium load and the productivity of the cell factory is restored. On the other hand, when expressed internally, the cells are harvested, and protein is extracted from the cell lysate or the cellular membranes.

Proteins which are expressed on the surface of the recombinant *E. coli,* are commonly found in the development of biosensors and bioremediation applications [29];[32]. Surface area of the cells are optimized with the spread of the reaction sites across the membrane [29]. For cells delineating the carbon sensors, the cellular membrane is enriched with lead binding domain (PnBD) should the proteins containing the domain is cell surface-displayed on the cells. The accumulation of lead ions from the polluted environment to the binding sites, creates a potential gradient between two interfacing surface of the electrode hence generating electrical signal for the biosensor [29].

For bioremediation applications, laccase had been reported to be cell surface-displayed [31];[32] on *E. coli* to catalyze the oxidation of phenolic compounds to a less environmentally damaging compounds. Taking advantage of the oxidizing nature of laccase, the presence of laccase as electron acceptor for the conversion of benzene (1,2-benzoquinone) into catechol (pyrocatechol) open ways to detect benzene pollution using an electrode. The electrode forms a sensor that detects benzene compounds by donating electrons to drive laccase catalytic reaction. The intensity of catalytic reaction by laccase is correlated with electrical signal generated by the use of the electrode. When the laccase is mounted on the surface of *E. coli*, this strategy provides structural stability for both bioremediation and pollutant detection process [32].

GENETIC MODIFICATIONS ENABLING FERMENTABLE SUGAR ASSIMILATION BY ETHANOLOGENIC STRAIN

Table 4 summarizes the resulting outcomes of ethanologenic *E. coli* modification strategies. In the development of ethanologenic *E. coli* strain, it is advantageous to have the β-glucosidase surface displayed [33]. This is because cellobiose (glucose dimer) are rich in hydrolyzed lignocellulosic biomass. Complete utilization of glucose is hampered by the underutilized cellobiose in fermentation step as this is limited to the enzyme load available in the reaction [33].

To facilitate the conversion of dissolve cellobiose into glucose, ethanologenic strain exhibiting β-glucosidase functionality would ensure complete substrate assimilation in bioethanol production [33]. Indirect implication to the reusable enzyme on the cell surface is the reduction of dependency on enzyme load to supply the β-glucosidase [33].

Apart from that, recent study reported ethanol production using cellobiose as substrate by co-expressing β-glucosidase from *Paenibacillus polymyxa* with an inducible periplasmic protein (OsmY) tag under the regulation of a strong constitutive *gapA* promoter. In its native host, the *gapA* promoter regulates the transcription of glyceraldehyde-3-phosphate dehydrogenase, GAPDH protein [33]. Another direction in ethanologenic strain development is to create transport channels for cellobiose assimilation. However, this approach has only been reported across recombinant strain of *S. cerevisea* that produce β-glucosidase internally [34].

In other recent study, an improved benchmark in ethanol tolerance among ethanologenic *E. coli* had been reported [35]. Two strains originating from the KO11 strain were developed. The first strain contains a defective *hscA* gene. From UniPROT database, the mentioned gene translates into a chaperon protein, Hsca. The second strain is structurally similar to the first with a compensating plasmid bearing a complete *hscA* gene and constitutive overexpression of Iron-sulfur Cluster protein (ICS). This study demonstrated an example of gene knockout strategy to prove the importance of ICS in the breaking down of Reactive Oxygen Species (ROS) molecules that accumulated in ethanol-producing cells. The increased level of ROS molecules was associated with the increased level of ethanol. The concentration level of ROS molecules was regulated by the production of modified fatty acids as an adaptive mechanism to withstand the high level of ethanol. This was validated in the study when overexpression of the ICS protein resulted in the synthesis of various types of fatty acids [35]. An enrichment approach was used in a previous study to promote high tolerance for ethanol in an existing strain. The strategy improved the growth medium of cultivated strains to promote the desired ethanol tolerance [19]. Repeated strain adaptation to stress is an approach that had also been reported to cultivate strains with improved tolerance for acidic environment [36].

Deletion of certain genes proved beneficial in ethanol-producing strains [37];[38]. A study had reported an increased level of ethanol production with the absence of genes whose expression lead to byproducts formation. Byproducts from ethanol that were synthesized using biochemical pathways existing in ethanologenic *E. coli* include lactate, succinate, acetate and formate. By turning off the pathways for byproducts formation, carbon substrate was contained within the pathway for ethanol synthesis [38]. Alternatively, the high production of ethanol can be promoted by optimizing the growth conditions statistically [39].

A mutant exhibiting high tolerance for acetate, an inhibitor to ethanol fermentation reaction, was reported [37]. In the study, an acquired ability to convert the acetate molecule into adenosine

triphosphate (ATP) molecules had been presented. The ATP molecules are energy currency at molecular level and mainly synthesized via cellular aerobic respiration pathway. This strategy offers another clever way to ensure complete utilization of carbon substrate in the fermenting process.

Table 1. Heterologous cellulases and their reported activities on various carbon sources.

Year	Cellulase and Microbial Strains	Activity	Carbon source / substrate	Ref.
2021	Cel48S exoglucanase from *Clostridium thermocellum*	153.5 ± 2.4 U/mL	N/A	[38]
2021	CelA from *Caldicellulosiruptor bescii*	1.86 ± 0.06 U/mL	CMC	[39]
2021	Endoglucanase from *Clostridium thermocellum*	1185 U/mg	CMC	[40]
2019	A glucose tolerant β-glucosidase isolated from archaeon *Thermococcus sp*	N/A	*p*-nitrophenyl-D-glucopyranoside (pNPGl)	[28]
2019	β-glucosidase from *Clostridium cellulovorans* (CcBglA); bifunctional cellulase/xylanase thermophilic *Clostridium thermocellum* (CtCel5E)	higher β-glucosidase activity of CtCel5E-CcBglA than that of CcBglA in using cellobiose at different pH and temperatures and the optimal temperature of the fusion enzyme became higher at 50 °C	CMC; regenerated amorphous cellulose (RAC); Beechwood (BW); Salicin; pNPG; Cellobiose	[42]
2019	CMCase from *Thermotoga naphthophila*	12464 U/mg	CMC	[18]
2019	UV-mutated cellulase gene from *Aspergillus niger*	i) Wild type *A. niger*; 96 umol /min /mg ii) *A. niger* UVMT-I; 330 umol /min /mg iii) recombinant *E. coli* ; 441 umol /min /mg	CMC	[42]
2018	Glucose tolerant , β-glucosidase mutant B9L147 (V169C) from *Thermomicrobium roseum*	279.9 ± 5.2 U/mg (B9L147) 317.3 ± 16.9 U/mg (V169C)	*p*-nitrophenyl-D-glucopyranoside (pNPGc)	[24]
2018	β-glucosidase and endoglucanase from *Bacillus methylotrophicus*	β-glucosidase; 1670.15 ± 18.94 U/mL endoglucanase; 0.130 ± 0.002 U/mL	p-NPG; CMC	[43]
2018	Cellulase gene of *Bacillus licheniformis*	985.2mU/mL	CMC	[20]
2018	Endo- β-1,4-glucanase from *Bacillus sp.* RK2 isolate	2.403 U/ml	CMC	[16]
2018	Endo- β-1,4-glucanase from *Bacillus sp.* RK2 isolate	Vmax; 1.581 K_M; 0.0709	Oil Palm Empty Fruit Bunch (OPEFB)	[4]
2018, 2017	*Cel12B, Cel8C* and *peh* genes from *Pectobacterium carotovarum* subsp. *carotovarum*	cel12B; 1.53 U/mL (exoglucanase activity), 1.21 U/mL (endoglucanase) cel8C; 14.7 U/mL (endoglucanase)	Grapefruit processing waste CMC, Avicel	[14], [17]
2017	Endo-β-1.4-glucanase from *Bacillus sp. RK2*	2.665 U/mL	Oil Palm Empty Fruit Bunch (OPEFB)	[15]
2017	Endoglucanase (Cel5A) from *Fusarium graminearum*	i) extracellular YebF-Cel5A product reached 0.30 IU/mL ii) Cel5A; 0.18 IU/mL	CMC	[44]
2017	Endoglucanase gene of *Bacillus subtilis* M015	18.56 U/mg	CMC, Microcrystalline cellulose, Corn cob	[45]
2015	Secretory β-glucosidase gene from *Paenibacillus polymyxa*;	<3.2 μmol/min/mL	wheat straw hydrolysate	[33]

Table 2. The fusion of cellulase genes with non-catalytic domains and their resulting applications.

Year	Cellulase gene	Non-catalytic domain	Application	Ref.
2018	Cellulase gene of *Bacillus licheniformis*	Ice-nucleation protein (INP); INP, an outer membrane-bound protein from *Pseudomonas syringae*	Surface displayed cellulase	[20]
2018	Glycoside hydrolase 5, GH5 (bifunctional cellulase) from *Bacillus sp*.	Carbohydrate binding module; CBM3 from *Bacillus sp*. CBM9 from *Thermotoga maritima* Xyn10A	Addition of CBM increased the thermostability of the enzyme; enhanced the GH5 affinity (Km)	[41]
2018	Endoglucanase cel12B genes from *Thermotoga maritima*	The cellulose binding domain (CBD) of the N-terminal of xylanases from *Thermotoga maritima* and *Thermotoga thermarum,* CBD1, CBD2, CBD3, and CBD4 fusion proteins	CBD4 shows the best adsorption on microcrystalline cellulose; Reduced cellulase activity; lack of linker in protein fusion was suspected	[24]
2018	β-Glucosidase Bgl1A(A24S/F297Y)	Carbohydrate-binding modules (CBMs)	The fusion proteins were expressed in *E. coli* and adsorbed onto cellulose; The Bgl-CBM24 was found to have the highest immobilization efficiency at room temperature within 1 h adsorption; Improved ethanol tolerance ability (<35% v/v); Bgl-CBM24 effectively hydrolyze soybean isoflavone glycosides	[46]
2018	CelA gene from *D. turgidum*	48 aa linker peptide; CBM11 (part of CelH gene) from *Clostridium thermocellum*; pET20b-CelA-linker-CBM11	Soluble form proteins are expressed; The addition of a CBM resulted in a cellulase with enhanced stability at extreme pHs, higher affinity and activity on insoluble cellulose.	[47]
2019	β-glucosidase (Bgl1), exoglucanase (Exo5), from *Caldicellulosiruptor saccharolyticus*	carbohydrate-binding modules (CBMs) from *Caldicellulosiruptor saccharolyticus*	The fused enzyme show both β-glucosidase and exoglucanase activities; to improve the degradation efficiency of lignocellulosic biomass	[48]
2019	β-1,4-glucosidase, CtGH1 from *Clostridium thermocellum*	Site-directed mutagenesis of β-1,4-endoglucanase from family 5 glycoside hydrolase (CtGH5) from *Clostridium thermocellum* (CtGH5-F194A)	Improved structural integrity, thermostability and enhanced bi-functional enzyme activities	[22]
2020	Cel48Ft, from *R. cellulolyticum* Cel5Af, from *R. cellulolyticum* Cel9Gc, from *R. cellulolyticum*	*R. thermocellum* dockerins; *Ruminococcus flavefaciens* dockerins; *R. cellulolyticum* dockerins; hybrid scaffoldin, Scaf6; cohesins; curli protein CsgA; Carbohydrate binding module	*E. coli* MG1655 which massively displays at the cell surface a hybrid scaffolding whose three cohesins can robustly and specifically anchor three proteins/enzymes simply engineered to bear the cognate dockerin modules	[21]
2021	Single mutation cellulase gene (BglC-M) from *Bacillus amyloliquefaciens*	Single mutation of carbohydrate-binding module (CBM) of the (BglC-M) from *Bacillus amyloliquefaciens*	Compact and stable structure; increased catalytic efficiency; The BglC-M enzyme showed a more significant amount of reducing sugar released from all lignocellulosic wastes than the control; The first evidence that altering the base composition of the cellulose binding module enhanced the catalytic activity.	[23]

Table 3. Strategies adopted to express recombinant proteins externally by *E. coli*.

Year	Heterologous genes	Secretory / Cell surface display	Strategy	Ref.
2013	Xylanase, α-amylase, xylose isomerase and trehalose synthase	Secretory	Co-expression with *Thermobifida fusca* cutinase (increases membrane permeability via its phospholipid hydrolysis activity)	[49]
2018	Haem	Secretory	Using the C5 pathway, the downstream pathway for haem biosynthesis is optimized by knocking out ldhA, pta and also yfeX—encoding a putative haem-degrading enzyme	[50]
2019	Feruloyl esterase derived from *Lactobacillus crispatus*	Secretory	Exhibit secretory effect without genetic intervention	[51]
2019	PETase from *Ideonella sakaiensis*	Secretory	Fusion with sec-dependent signal peptides	[26]
2019	Three protein antigens from enterohemorrhagic *E. coli* (EHEC)	Secretory	Fusion to the C-terminal secretion signal of HlyA (The hemolysin (Hly) secretion system of *E. coli* allows the one-step translocation of hemolysin A (HlyA) from the bacterial cytoplasm to the extracellular medium, without a periplasmic intermediate)	[27]
2018	Laccase CotA from *Bacillus subtilis*	Cell surface display	Poly-γ-glutamate synthetase A protein (PgsA) from *B. subtilis* as an anchoring matrix.	[31]
2018	Dibenzothiophene monooxygenase DszC (*dszC* gene) from *Gordonia sp.*	Cell surface display	*bclB* gene (provides anchoring motif) from *B. anthracis sterne* at the upstream of *dscZ* gene	[30]
2018	Binding domain (PbBD) from *Cupriavidus metallidurans* CH34 PbrR ($Pb2^{+}$-sensing transcriptional factor PbrR)	Cell surface display	Fusion with surface anchor Lpp-OmpA (LOA)	[29]
2018	Laccase (a multi-copper oxidase that catalyzes the oxidation of one electron of a wide range of phenolic compounds)	Cell surface display (biosensor application to measure catechol)	Increased tandem-aligned anchors with three repeats of the N-terminal domain of an ice nucleation protein (INP) were used to construct a highly active *E. coli* whole cell laccase-based catalytic system	[32]
2018	Monomeric and dimeric repeats of the BPA-binding peptide (KSLENSY)	Cell surface display	Fused to the C-terminus of omp C genes	[25]

Table 4. Ethanologenic *E. coli* strain and their modification strategies

Ethanologenic strain	Strategy	Characteristics	Carbon source / substrate	Pretreatment	Cellulolytic agent	Ethanol (g/L or mM); Ethanol Productivity (g/L.hr)	Year / Ref.
E. coli TC4	The use of expression vector pLOI308-10 to express pyruvate decarboxylase and alcohol dehydrogenase II from *Zymomonas mobilis*	Ethanol was produced during both anaerobic and aerobic growth	Glucose	N/A	N/A	Anaerobic; 482 mM Aerobic; 337 mM	1988 / [52]
Mutant of *Escherichia coli* KO11 (LY01 mutant)	Enrichment method which selects alternatively for ethanol tolerance during growth in broth and for ethanol production on solid medium	Higher ethanol tolerance of yeasts in the LY01 strain	Xylose	N/A	N/A	60g/L; 0.83 g/ L.h	1998 / [19]
FBR5	The use of expression vector pLO1297 to express genes converting pyruvate into ethanol from *Zymomonas mobilis*	Ethanol was produced using the given substrate	Corn fiber hydrolysate containing arabinose, glucose, and xylose	N/A	N/A	0.46 g of ethanol/g of available sugar	2000 / [53]
FBR5 improved variant (FBHW)	Repeated strain adaptation	FBHW was resistant to the toxicity of hydrolysate	Hot-water wood extract hydrolysate	Acid treatment	-	36.8 g/L ethanol	2010/ [36]
KO11	The use of corn steep liquor (CSL) media	Current benchmark ethanologenic strain	i) CSL/glucose; ii) CSL/ glucose/ xylose; iii) CSL/xylose	N/A	Enzymatic hydrolysis	i) 44.3 ± 0.5 g/ L; 0.79 g/L.h ii) 41.9 ± 0.8 g/L; 0.72 ± 0.01 g/L.h iii)43.1 ± 0.1 g/L; 0.72 ± 0.01 g/L.h	2010/ [54]
KO11	Simultaneous saccharification and ethanol fermentation of *L. japonica*	-	N/A	Acid treatment	Commercially available hydrolytic enzymes	0.4 g/g carbohydrate	2011/ [55]

	with high mannitol content						
The *E. coli* strain SSY09 (pZSack)	Modulating the expression of pyruvate dehydrogenase and acetate kinase and deleting pathways for competing co-products. Lactate, succinate, acetate and formate are deleted.	SSY09 (pZSack) primarily producing ethanol	i) Xylose ii) Glucose iii) Glucose and xylose	N/A	N/A	i) 6.84 mmol/L/h, ii) 12 mmol/L/h, iii) 15 mmol/L/h	2012/ [38]
E. coli MG1655, MS04	i) Gene deletion ii) Isolating acetate tolerant mutants (generate ATP from acetate)	Acetate tolerant strain (MSO4); produce < 40 g/L of sodium acetate	Glucose	N/A	N/A	<30 g/L; 0.67±0.05 g/L.h	2012/ [37]
Derivative of *Escherichia coli* KO11 (SL100)	Box-Behnken experimental design to maximize sugars released and minimize inhibitors.	Inhibitor-resistant strain	Chips of i)*Eucalyptus benthamii* ii) *Eucalyptus grandis*	Acid and heat treatment	Enzyme hydrolysis	i) 0.217g /g DW biomass; ii) 0.243 g /g DW biomass	2014/ [56]
The *E. coli* strain SSY09 (pZSack)	The use of *gapA* promoter for β-glucosidase from *Paenibacillus polymyxa* ; Co-expression with an inducible periplasmic protein (OsmY) tag	A strain that constitutively secretes a cellulolytic enzyme	Wheat straw hydrolysate containing glucose, xylose and cellobiose	Ammonium-treated wheat straw;	Enzyme hydrolysis	204 mM of ethanol	2015/ [33]
KO11	Simultaneous saccharification and co-fermentation) using *T. reesei* cellulase and *E. coli* KO11	-	Poplar wood	Heat treatment	*Trichoderma reesei* RUT C-30	3.6 g/L	2017/ [57]
Strain SL100 (a derivative of *E. coli* KO11)	-	Inhibitor-resistant strain	i) Sweet sorghum juice; ii) Residual	Acid and heat treatment	Enzyme hydrolysis	i) 140 to 170 g/L ii) 27.5 g /L	2017/ [39]

			bagasse				
KO11 (mutants)	i) KO11 origin ii) Kmp (hscA mutant decreased ethanol tolerance) iii) Kmp with complementary plasmid for iron-sulfur cluster (ISC)	Improved ethanol tolerance Low organic acids production	Glucose	Acid and heat treatment	N/A	i) 9.216 g/L; 0.064 g/L.h ii) 7.488 g/L; 0.052g/L.h iii) 10.656 g/L; 0.074 g/L.h	2019/ [35]
Strain MS04	i) Single-stage continuous cultures micro-aerated ii) Batch process iii) Micro-aerated two-stage continuous culture	-	Xylose and glucose	N/A	N/A	i) 18 g/L; 0.9 g/L.h ii) 22 g/L; 1.3 g/L.h iii) 21g/ L; 1.6 g/L.h	2019/ [58]
Strain SL100	Fermentation using i) Over-limed hydrolysate ii) Activated charcoal detoxifying hydrolysate	-	Exhausted olive pomace (EOP)	Acid treatment	N/A	i)13.6 g/L; 0.11 g/L.h ii)14.48 g/L; 0.73 g/L.h	2022/ [59]

CONCLUSION

The development of recombinant *E. coli* strains for bioethanol production applications is the focus of this review, which focuses on strains for the saccharification and fermentation steps. Modifications are achieved mainly via genetic alterations. While the productivity of recombinant *E. coli* is behind other interspecies counterpart as ethanol-producers, research and development activities have been greatly facilitated by the use of *E. coli* expression system. The system provides a common testing ground for the expression of genes available in nature for saccharification and fermentation of cellulosic biomass applications.

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