Ethylene glycol and glycolic acid production from xylonic acid by *Enterobacter cloacae*

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Research

Keywords: Enterobacter cloacae, ethylene glycol, glycolic acid, xylonic acid, xylose

Posted Date: December 23rd, 2019

DOI: https://doi.org/10.21203/rs.2.19479/v1

Version of Record: A version of this preprint was published at Microbial Cell Factories on April 15th, 2020. See the published version at https://doi.org/10.1186/s12934-020-01347-8.

Abstract

Background

Biological routes of ethylene glycol production have been developed in recent years by constructing of the synthesis pathways in microorganisms. However, no microorganisms have been reported to produce ethylene glycol naturally.

Results

Xylonic acid utilizing microorganisms were screened from natural environments, and an Enterobacter cloacae strain was isolated. The major metabolites of this strain were ethylene glycol and glycolic acid. However, the metabolites were switched to 2,3-butanediol, acetoin or acetic acid when this strain was cultured with other carbon sources. The metabolic pathway of ethylene glycol and glycolic acid synthesis from xylonic acid in this bacterium was identified. Xylonic acid was converted to 2-dehydro-3-deoxy-D-pentonate with the catalysis of D-xylonic acid dehydratase. 2-Dehydro-3-deoxy-D-pentonate was converted to form pyruvate and glycolaldehyde, and this reaction was catalyzed by an aldolase. D-xylonic acid dehydratase and 2-dehydro-3-deoxy-D-pentonate aldolase were encoded by yjhG and yjhH , respectively. The two genes are part of the same operon and are located adjacent on the chromosome. Besides yjhG and yjhH , this operon contains four other genes. However, individually inactivation of these four genes had no effect on either ethylene glycol or glycolic acid production; both formed from glycolaldehyde. YqhD exhibits ethylene glycol dehydrogenase activity in vitro . However, a low level of ethylene glycol was still synthesized by *E. cloacae* Δ yqhD . Parameters for ethylene glycol and glycolic acid production by the *E. cloacae* strain were optimized, and aerobic cultivation at neutral pH were found to be optimal. In fed batch culture, 34 g/L of ethylene glycol and 13 g/L of glycolic acid were produced in 46 hours, with a total conversion ratio of 0.99 mol/mol xylonic acid.

Conclusions

A novel route of xylose biorefinery via xylonic acid as an intermediate has been established.

Background

Ethylene glycol is an important bulk chemical that is used primarily as a precursor for polyethylene terephthalate, polyurethane, and polyethylene succinate synthesis. Ethylene glycol is also used as feed stock for the synthesis of glyoxal, glycolic acid, methyl glycolate and other chemicals [1]. Industrially, ethylene glycol is produced chemically from ethylene. However, with the development of synthetic biology, ethylene glycol production by biological routes has become a research hotspot in recent years. Liu et. al. constructed an ethylene glycol synthesis pathway in Escherichia coli. This pathway consists of four steps: xylose→xylonate→2-dehydro-3-deoxy-D-pentonate→glycolaldehyde→ethylene glycol (Fig. 1). The first step converting xylose to xylonic acid was catalyzed by D-xylose dehydrogenase, which was originally obtained from Caulobacter crescentus. The residual three steps were catalyzed by host native

enzymes of D-xylonic acid dehydratase, 2-Dehydro-3-deoxy-D-pentonate aldolase, and aldehyde reductase, respectively. This strain produced 11.7 g/L ethylene glycol from 40 g/L xylose and glycolic acid as a by-product of this process [2]. Beside this pathway, a synthetic pathway of xylose→xylulose→xylulose-1P→glycolaldehyde→ethylene glycol was constructed in E. coli to produce ethylene glycol from xylose [3]. Following these strategies, other pentoses were used as substrates for ethylene glycol synthesis in E. coli [4]. Beside pentose, glucose was also used for ethylene glycol production. This synthesis pathway was constructed in Corynebacterium glutamicum and E. coli by using serine as an intermediate [5, 6].

Xylose is the second most abundant sugar in nature after glucose. Xylose can be used as a carbon source for culture of microorganisms. However, the catabolism of xylose by microorganisms is not as easy as that of glucose. In our previously research, xylonic acid production by Klebsiella pneumoniae was developed, and this process has a high conversion ratio and productivity [7]. Thus we proposed to use xylonic acid as an intermediate for xylose biorefinery. The enzymes that catalyze the conversion of xylose to xylonic acid belong to three classes based on the cofactor used. Glucose dehydrogenase was identified to catalyze the reaction in K. pneumoniae, and this enzyme is located in the inner membrane of the periplasmic space and uses pyrroloquinoline quinine (PQQ) as the cofactor. D-xylose dehydrogenase from Trichoderma reesei uses NADPH as the cofactor [8] whereas D-xylose dehydrogenase from C. crescentus uses NADH as the cofactor [9]. These two D-xylose dehydrogenases are located in the cytoplasm. Different cofactors are used and the different location of the enzymes lead to the different efficiency of xylonic acid production. 103 g/L xylonic acid was produced in 79 hours by K. pneumoniae using glucose dehydrogenase [7]. While only 19 g/L xylonic acid was produced in 150 hours of culture by Trichoderma reesei, using a NADPH dependent D-xylose dehydrogenase [8]. 39 g/L xylonic acid was produced after 36 hours of culture by E. coli, using a NADH dependent D-xylose dehydrogenase [9].

Unlike gluconic acid, which is an intermediate of the glucose oxidization pathway [10], xylonic acid cannot be further catabolized by K. pneumoniae. Therefore, in this work xylonic acid utilizing microorganisms were screened from nature, and an Enterobacter cloacae strain was selected. This bacterium was a native ethylene glycol producer, and the metabolic pathway of ethylene glycol and glycolic acid synthesis from xylonic acid was identified. Furthermore, the process conditions for ethylene glycol and glycolic acid production were optimized.

Results

Screening of xylonic acid utilizing microorganisms and strain identification

Xylonic acid utilizing microorganisms were enriched from soil samples and 4 colonies with different morphologies were isolated from LB agar plates and cultured in flasks. E. coli W3110 was also cultured at the same time as a control. Fermentation results of these strains are shown in Table 1.

Table 1
Metabolites produced by xylonic acid utilizing microorganisms tested.

Strains	Residual xylonic acid (g/L)	Metabolites (g/L)		
		Ethylene glycol	Glycolic acid	Acetic acid
1	0	11.1	3.1	0
2	27.5	0	0	0
3	28.0	0	0	0
4	37.9	0	0	0
W3110	10.1	3.3	1.9	2.5

Xylonic acid was consumed by isolated strains (1–3) and E. coli W3110, but not by strain 4. Of the xylonic acid utilizing strains, no known metabolites were detected in the broth of strains 2 and 3. For strain 1 and E. coli W3110, ethylene glycol (assumed) and glycolic acid (assumed) were the major metabolites. The identification of ethylene glycol and glycolic acid are shown in the following section. Acetic acid was found as a metabolite of E. coli W3110, but not for any of the other strains.

Strain 1 has a higher ethylene glycol and glycolic acid productivity and yields than E. coli W3110. This strain was identified by the 16S rRNA gene. The sequence has been submitted to GenBank with the accession number of MG779638. This gene sequence was blasted in the NCBI, and a dendrogram was then composed to elucidate evolutionary relationships between strain 1 and some related strains (see the Supplementary Fig. 1). Based on 16S rRNA gene sequence and the dendrogram, strain 1 was tentatively identified as E. cloacae, and named E. cloacae S1. The genome of this strain was subsequently sequenced and the raw sequence has been submitted to GenBank with the accession numbers of VSZU00000000. This strain was used for further investigation.

Ethylene glycol and glycolic acid were the major metabolites of E. cloacae S1 using xylonic acid as the carbon source. The strain was also grown using xylose, glucose, gluconic acid, 2-ketogluconic acid and glycerol as the sole carbon source and the metabolites detected are listed in Supplementary Table 2.

Ethylene glycol and glycolic acid were the main metabolites of E. cloacae S1 using xylonic acid as the sole carbon source. However, the two chemicals were not synthesized by this strain using any of the other carbon sources tested. 2,3-Butanediol and acetic acid were the major metabolites using xylose and 2-ketogluconic acid as the sole carbon sources, respectively. Acetoin and 2,3-butanediol were the major metabolites using glycerol as the sole carbon. When using glucose or gluconic acid as the sole carbon source, acetic acid, acetoin, and 2,3-butanediol were all synthesized by this strain.

Ethylene glycol and glycolic acid identification

^{1}H and ^{13}C NMR spectral data of glycolic acid sample compared to the spectra of a standard glycolic acid (Sodium salt commercial product) are given in Supplementary Fig. 2.

^{1}H NMR chemical shift for CH_2 of glycolic acid was 3.83 and 4.00 ppm for sample and standard, respectively. ^{13}C NMR chemical shifts of glycolic acid were 179.52 (C1), 60.95 (C2) ppm for the sample, and 176.14 (C1), 59.12 (C2) ppm for the standard. The NMR data of the sample correlated well with the standard glycolic acid data. From this comparison, it was concluded that the compound was glycolic acid.

Retention times of standard ethylene glycol and sample were all 12.2 min for HPLC and all 8.2 min for GC analysis. These results confirmed that ethylene glycol was the presumed metabolite.

Gene recombination method development

Gene recombination using linear DNA with 39 and 40 nt homologous extensions that was directly amplified from plasmid pIJ778 was tried first. However, no colonies were obtained on selection plates. So linear DNA with 500 bp of homologous regions was used for gene recombination in E. cloacae. Commonly, 100 colonies were obtained in a single recombination experiment using this method.

Identification of genes responsible for glycolaldehyde synthesis from xylonic acid

Glycolaldehyde is an intermediate of the Dahms pathway. Ethylene glycol, and glycolic acid are all synthesized directly from glycolaldehyde. There are two D-xylonic acid dehydratases (YjhG, YagF) catalyzing the conversion of xylonic acid to 2-dehydro-3-deoxy-D-pentonate, and two 2-dehydro-3-deoxy-D-pentonate aldolases (YjhH, YagE) that catalyze the conversion of 2-dehydro-3-deoxy-D-pentonate to glycolaldehyde in E. coli [2]. yjhG, yagF, yjhH and yagE gene sequences of E. coli were blasted against the NCBI database and the genome of E. cloacae S1 to find the homologous genes of E. cloacae. However, only homologues of yjhG and yjhH were found. The two genes were located nearby in the yjh operon (Fig. 1, A). Beside, this operon contains genes of yhcH, yagG, xyl and iclR, which encoding a beta subunit of beta-galactosidase, a sugar transporter, a beta-D-xylosidase, and a regulatory gene, respectively. yjhG and yjhH were knocked out individually to generate mutant strains of E. cloacae ΔyjhG and E. cloacae ΔyjhH, respectively.

Physiological characteristics of these strains were determined by culturing them in M9 medium with xylonic acid or xylose as the sole carbon source, and results are presented in Fig. 2.

Growing with xylose as the sole carbon source 2.2, 1.2 and 1.5 g/L of 2,3-butanediol and 1.1, 2.3 and 1.8 g/L acetic acid were synthesized after 24 h culture by E. cloacae S1, E. cloacae ΔyjhG and E. cloacae

ΔyjhH, respectively. There was not any distinct differences between these strains for xylose utilization. Thus it appears that YjhG and YjhH are not directly involved in xylose metabolism.

Using xylonic acid as the sole carbon source, 2.1 g/L ethylene glycol and 0.7 g/L glycolic acid were synthesized by E. cloacae S1. However, E. cloacae ΔyjhG and E. cloacae ΔyjhH were unable to grow in this medium, and no metabolites were synthesized. These results indicated that yjhG and yjhH encoding D-xylonic acid dehydratase and 2-dehydro-3-deoxy-D-pentonate aldolase respectively were responsible for glycolaldehyde synthesis from xylonic acid, and these two enzymes seem to have no isoenzymes in this strain.

The roles of other genes in the yjh operon on xylose and xylonic acid catabolism

As yjhG and yjhH are responsible for xylonic acid catabolism it was suspected that other genes in the same operon might also be related to xylose or xylonic acid catabolism. iclR, yhcH, yagG, and xyL were disrupted individually to obtain strains E. cloacae ΔiclR, E. cloacae ΔyhcH, E. cloacae ΔyagG and E. cloacae ΔxyL, respectively. Physiological characteristics of these 4 strains were determined, and the results are presented in Fig. 3.

Xylose was used by E. cloacae ΔiclR, E. cloacae ΔyhcH, E. cloacae ΔyagG, and E. cloacae ΔxyL, and 2.2–2.3 g/L of 2,3-butanediol were produced by these strains. The cell growth and 2,3-butanediol synthesis were comparable to that of E. cloacae S1 (shown in Fig. 3). Xylonic acid was used by all these strains, and 0.3–0.5 g/L of glycolic acid and 1.9–2.4 g/L of ethylene glycol were synthesized by these strains. Also, these titers were similar to that of E. cloacae S1 (shown in Fig. 3). On the whole, the fermentation results showed that there were no distinct differences between the wild strain and these mutants regarding xylose and xylonic acid utilization.

Identification of genes responsible for ethylene glycol synthesis from glycolaldehyde

YqhD, a NADPH-dependent aldehyde reductase, was shown to catalyze the conversion of glycolaldehyde to ethylene glycol in E. coli [2]. E. cloacae contains a homologous gene called yqhD, and this gene was amplified from E. cloacae S1. yqhD was cloned into a pet 28a plasmid and over-expressed in E. coli. YqhD was obtained from the cell lysate. The ethylene glycol dehydrogenase activity of YqhD and the cell lysate of E. cloacae S1 were assayed using either NADH or NADPH as cofactor.

Ethylene glycol dehydrogenase activities of cell lysate of E. cloacae S1 using NADH or NADPH as cofactor were 0.006 and 0.13 U/mgP, respectively. Whereas the activity of purified YqhD was 0.004 and 0.175 U/mgP that using NADH or NADPH as the cofactor respectively. These results indicated that the

ethylene glycol dehydrogenase in E. cloacae S1 uses NADPH as the cofactor, and YqhD of E. cloacae S1 is an ethylene glycol dehydrogenase.

To further investigate the in vivo function of yqhD in ethylene glycol formation, yqhD was knocked out and also a YqhD over-expressing strain was constructed. E. cloacae S1, E. cloacae ΔyqhD and E. cloacae + yqhD were cultured in flasks for ethylene glycol production. Fermentation medium was used, and the results are presented in Fig. 4.

Xylonic acid was exhausted by E. cloacae S1 after 18 h of culture, and 8.3 g/L ethylene glycol and 2.1 g/L of glycolic acid were produced. Xylonic acid utilization by E. cloacae ΔyqhD was much slower, however, ethylene glycol synthesis ability was not totally lost; the strain still produced 1.6 g/L of ethylene glycol. Similar to ethylene glycol, glycolic acid synthesized by E. cloacae ΔyqhD was decreased to 0.1 g/L. The final levels of ethylene glycol and glycolic acid produced by E. cloacae + yqhD were only slightly lower compared to that of the wild-type strain. These results indicate YqhD is responsible for the conversion of glycolaldehyde to ethylene glycol in vivo. However, other ethylene glycol dehydrogenase isoenzymes exist in the cell that could explain the small quantities of ethylene glycol synthesized by the deletion mutant.

Identification of genes responsible for glycolic acid synthesis from glycolaldehyde

aldA was identified as coding for an aldehyde dehydrogenase for glycolic acid synthesis from glycolaldehyde in E. coli [2]. However, no homologous genes of aldA were found in the genome of E. cloacae S1. aldB, betB, ad1, and ad2 that are presumed to be aldehyde dehydrogenases or putative aldehyde dehydrogenases in the genome of E. cloacae were cloned and over-expressed in E. coli to obtain E. coli BL21/aldB, E. coli BL21/betB, E. coli BL21/ad1, and E. coli BL21/ad2. Purified enzymes of these genes were obtained from the lysate of these strains and analyzed for their glycolaldehyde dehydrogenase activities. The results are shown in Supplementary Table 3. The cell lysate of E. cloacae S1 was used as a control for the glycolaldehyde dehydrogenase activity assay.

Glycolaldehyde dehydrogenase activity of cell lysate of E. cloacae S1 using NAD as cofactor was 0.0021 U/mgP. While no activity was measured using NADP as the cofactor. For the purified enzymes, only BetB showed a distinct glycolaldehyde dehydrogenase activity of 0.21 U/mgP when using NAD as the cofactor. All other enzymes exhibited a very low level of glycolaldehyde dehydrogenase activity using NAD as the cofactor. When using NADP as the cofactor, all these selected enzymes showed a very low level of activity. These results indicate that BetB might be responsible for glycolic acid formation from glycolaldehyde.

To further investigate the role of BetB in the glycolic acid formation from glycolaldehyde, a gene knock-out strain E. cloacae ΔbetB and an over-expression strain E. cloacae + betB were constructed. These

strains were cultured in flasks for ethylene glycol production, and fermentation results are shown in Fig. 5.

The cell growth of these three strains was similar. Glycolic acid and ethylene glycol synthesis by E. cloacae ΔbetB were slightly decreased compared with that of the wild-type strain. However, glycolic acid and ethylene glycol synthesized by E. cloacae + betB was a bit decreased compared with wild type strain and E. cloacae ΔbetB.

Culture parameters optimization

E. cloacae S1 was batch cultured in 5L stirred tank bioreactors for ethylene glycol and glycolic acid production. The culture pH was kept stable at 6.0, 6.5 7.0 and 7.5, respectively. Agitation rate was maintained at 500 rpm, and cell growth and metabolites produced are presented in Fig. 6.

After 6 hours of lag phase, cells started to grow and reached the exponential phase after about 10–12 hours. Xylonic acid was not used by cells until cell density reached about OD 7. Cells could grow in the whole experimental culture pH range with cells at pH 6.5 had the fastest growth rate, whereas cells grown at pH 7.5 had the lowest growth rate. The effect of culture pH on cell growth, xylonic acid consumption, ethylene glycol, and glycolic acid production showed a similar trend with the pH 6.5 culture showing fastest utilization of xylonic acid in parallel with the fastest production of ethylene glycol and glycolic acid. Thus pH 6.5 was selected as the optimal culture pH.

Oxygen supplementation is a key parameter for cell growth and product synthesis. The agitation rate was set at 200, 400, 600 and 800 rpm to give micro-aerobic condition at the lowest rate to fully aerobic conditions at the highest rate. Fermentation results of E. cloacae S1 at different agitation rates are presented in Fig. 7, culture pH was kept constant at pH 6.5.

Cells growth showed a positive correlation with agitation rate with cells grown at 600 rpm and 800 rpm gave the highest cell densities, and those at 200 rpm had the lowest cell density (OD 8.0). The trend of xylonic acid consumption was similar to that of cell growth, with cells grown at 600 rpm gave the fastest xylonic acid consumption rate, and those grown 200 rpm had the lowest xylonic acid consumption rate (0.9 g/lh). Ethylene glycol and glycolic acid production were positively correlated to agitation rate from 200 to 600 rpm. However, the product synthesis was strictly inhibited at the condition of 800 rpm agitation. Thus, medium level of oxygen supply appears to favor both ethylene glycol and glycolic acid synthesis, and therefore 600 rpm was selected as the optimal agitation condition.

3.9 Ethylene glycol production in fed-batch fermentation

E. cloacae S1 was cultured in a 5L stirred tank bioreactor, and xylonic acid was fed in the process using bolus additions. Fermentation results are presented in Fig. 8.

Similar to the batch fermentations, xylonic acid was quickly consumed after cells reached the exponential phase. After 15 h of batch culture, xylonic acid was fed for the first time, and 8 bolus additions of xylonic acid were made in total as shown in Fig. 8B. The highest cell density of 16.4 (OD) was reached at 21 h; after that cell density started to decrease. Ethylene glycol had a high production rate from about 10 h to 30 h, and then the productivity decreased. The trend of glycolic acid synthesis was similar to that of ethylene glycol production. In total, 34.1 g/L ethylene glycol and 13.2 g/L glycolic acid were produced after 46 h of culture. The molecular conversion ratio calculated was 0.217 mol/mol for glycolic acid and 0.772 mol/mol for ethylene glycol, and the total conversion ratio reached 0.989 mol/mol xylonic acid.

Discussion

Xylonic acid utilization by microorganisms

Xylose is the second most abundant sugar in nature after glucose, and many microorganisms can catabolize xylose through the pentose phosphate pathway. However, catabolism of xylonic acid is not common by microorganisms. There are two pathways of xylonic acid catabolism have been reported in Pseudomonas fragi. One way consists of: D-xylose → D-xylonate → 3-deoxy-D-pentulosonic acid → α-ketoglutarate semi-aldehyde → α-ketoglutarate. α-Ketoglutarate is then fed into the TCA cycle for further metabolism. This pathway was named the Weimberg pathway, in recognition of the scientist Ralph Weimberg [11]. Another way contains the following steps: D-xylose → D-xylonate → 3-deoxy-D-pentulosonic acid → pyruvate + glycolaldehyde. This metabolic pathway was named the Dahms pathway after the scientist A. Stephen Dahms [12]. Glycolaldehyde produced can be converted to ethylene glycol by a reduction reaction or converted to glycolic acid by an oxidization reaction. Microorganisms that contain any of the two pathways can grow on xylonic acid as the sole carbon source. Our results showed that strains 2 and 3 could use xylonic acid as the sole carbon source, but no known metabolites were detected. Thus these two strains might contain the Weimberg pathway. Xylonic acid was used by strain 1 and E. coli W25113 and both ethylene glycol and glycolic acid were produced by these strains suggesting that these two strains might use the Dahms pathway. By contrast strain 4 doesn't seem to possess any of the two pathways.

Physiological characterization of E. cloacae S1

E. cloacae is a facultative anaerobic Gram-negative bacterium belonging to the family of Enterobacteriaceae. Like most Enterobacter, E. cloacae occurs as a commensal microorganism in water, sewage, soil, meat, hospital environments, the skin, and in the intestinal tracts of humans and animals [13]. In biotechnology, this bacterium was used as a producer of hydrogen and 2,3-butanediol, and the two chemicals were produced under anaerobic and aerobic conditions, respectively [14, 15]. Acetoin is an intermediate of 2,3-butanediol, and commonly produced together with 2,3-butanediol [16]. In this study, acetoin and 2,3-butanediol were the main metabolites of E. cloacae S1 using glucose, xylose, gluconic

acid, and glycerol as carbon sources. Whereas ethylene glycol and glycolic acid were produced by E. cloacae S1 using xylonic acid as the sole carbon source.

Xylonic acid is not a common chemical, and we have not found any reports about using xylonic acid as the sole carbon source for cultivation of microorganisms. Xylonic acid used throughout this study was synthesized by K. pneumoniae, in which a PQQ-dependent glucose dehydrogenase catalyzed the reaction [7]. We have checked the genome of E. cloacae S1 and found that this bacterium has the gene coding for a PQQ-dependent glucose dehydrogenase, however, this bacterium does not hold the PQQ synthesis genes. Therefore, xylose cannot be converted to xylonic acid by E. cloacae.

Red recombinase associated gene recombination method is effective for E. cloacae

Gene recombination is a commonly used tool in molecular biology. Traditionally, suicide plasmid homologous recombination was used for gene recombination in bacteria, and it was used in E. cloacae until recently [15]. Red recombinase associated gene recombination was first developed in E. coli [17] and improved in Streptomyces. This method has the advantage of high efficiency and is easy to operate. Linear DNA with 36-nt homologous extensions was sufficient to obtain successful recombination [18]. The Red recombinase system has been modified as recombination tool suitable for many microorganisms, such as Burkholderia cepacia [19] Pseudomonas aeruginosa [20], Pantoea ananatis [21], Salmonella enterica [22], and Vibrio cholerae [23]. However, the minimal sizes of homologous extension are different ranging from 50 to 1000-nt. Initially no colony was obtained on selection plates using liner DNA with 39 and 40 nt homologous extensions in this study. Linear DNA with long homologous extensions was constructed following the method we have developed for gene recombinase in K. pneumonia, of which high recombination ratio was obtained with linear DNA containing 500 nt homologous extensions [24]. Similarly, high recombination ratio was obtained in E. cloacae in this study with 500 nt homologous extensions, and successful recombinants was obtained after a single experiment.

The function of genes in the yjh operon

yjhG and yjhH were the sole enzymes responsible for glycolaldehyde formation from xylonic acid in E. cloacae. This finding is different to E. coli, where the two enzymes both have one isoenzyme [2]. Other genes in this operon were suspected be important for xylose or xylonic acid metabolism, since yagG has been noted as a putative D-xylonate transporter for xylonic acid catabolism in E. coli [25]. However, the xylose metabolism was not affected by disrupting any of these genes. Our results indicate that xylonic acid utilisation was catalysed only by E. cloacae yjhG and E. cloacae yjhH. The activities of other genes in this operon have no effect on xylonic acid catabolism. Further work is needed to determine the native physiological function of this operon.

Identification of genes responsible for ethylene glycol and glycolic acid synthesis from glycolaldehyde

The E. cloacae YqhD has ethylene glycol dehydrogenase activity, similar to the YqhD in E. coli [2]. However, this enzyme was not solely responsible for this reaction. Generally, many short-chain alcohol dehydrogenases have a broad substrate range. Other short-chain alcohol dehydrogenases in the cell might be responsible for ethylene glycol synthesis in E. cloacae ΔyqhD.

AldA has been noted to catalyze the reaction of glycolic acid oxidation from glycolaldehyde in E. coli [2]. Some strains of E. cloacae have homologous genes of aldA, however, some strains including E. cloacae S1 do not have this gene in their genome. betB encodes a betaine aldehyde dehydrogenase. The substrate specificity of this enzyme from E. coli was strictly limited to betaine aldehyde [26]. By contrast our results showed that this enzyme catalyzes the reaction of glycolaldehyde oxidation to glycolic acid in vitro. However, the in vivo experimental results show that this enzyme was not the enzyme responsible for glycolic acid formation from glycolaldehyde. Further research is needed to identify the enzyme that responsible for this reaction.

Ethylene glycol and glycolic acid synthesis have an inherent relationship

YqhD was responsible for ethylene glycol synthesis from glycolaldehyde, and ethylene glycol synthesis was reduced in E. cloacae ΔyqhD. As glycolaldehyde synthesis was not being affected in E. cloacae ΔyqhD, we hypothized that glycolic acid synthesis would not be affected. However, glycolic acid synthesis was also decreased (Fig. 4). Furthermore, ethylene and glycolic acid synthesis did not change in the yqhD over-expression strain E. cloacae + yqhD. Similarly, glycolic acid synthesis was decreased in E. cloacae ΔbetB and E. cloacae + betB, and ethylene glycol synthesis was also decreased (Fig. 5). This finding is different from the metabolite production of engineered E. coli, in which over-expression of yqhD resulted in an increase of ethylene glycol but a decrease of glycolic acid synthesis [2]. In the culture parameter optimization experiments, ethylene glycol production varied in different conditions. Glycolic acid produced in these experiments showed a similar trend to that of ethylene glycol (Fig. 6, 7). Thus, the formation of ethylene glycol and glycolic acid are closely linked in E. cloacae. This is in contrast to production of these two metabolites in engineered E. coli in which fully aerobic condition favor ethylene glycol formation and microaerobic condition favor glycolic acid formation [3]. The mechanism of this relationship needs further investigation.

Ethylene glycol production by E. cloacae

Different ethylene glycol synthesis pathways have been developed, and several bacteria have been used as host cells. Utilising the Dahms pathway, 11.7 g/L ethylene glycol was produced from 40 g/L xylose by

an engineered E. coli strain, with the productivity of 0.24 g/L h [2]. Furthermore, 20 g/L of ethylene glycol was produced with a molar yield of 0.38 g/g xylose and productivity of 0.37 g/L h by an modified strain of E. coli using xylulose as an intermediate [3]. In another study 40 g/L ethylene glycol was produced with a yield of 0.63 g/g xylose and productivity of 0.55 g/L h after some optimization of the conditions [4]. Using glucose as substrate, 3.5 g/L ethylene glycol was produced by engineering C. glutamicum, with a yield of 0.08 g/g glucose and productivity of 0.05 g/L h [5]. Using E. coli as the host cell, 4.1 g/L ethylene glycol was produced with a yield of 0.14 g/g glucose and productivity of 0.03 g/L h were obtained [6]. In this report, 34.1 g/L ethylene glycol was produced, with the yield 0.288 g/g xylonic acid and productivity of 0.74 g/L h. The productivity obtained here is higher than all previous published reports that using xylose or glucose as the substrate. Based on the amount of xylonic acid supplied, the total molecular conversion ratio reached nearly 1 mol/mol xylonic acid. The high conversion ratio indicates that all the xylonic acid added was metabolized in the cell through one pathway, and the glycolaldehyde formed was completely converted to ethylene glycol and glycolic acid. However, pyruvate produced in the process was utilized by cells. In a research that using engineered E. coli of ethylene glycol and glycolic acid production, pyruvate was partly recovered for glycolic acid synthesis and the total yield of the process was improved [4].

Ethylene glycol and glycolic acid synthesis by E. cloacae was started at 8–10 hours culture, and was into a high rate at 12 hours culture or later. However, cell growth of these processes was into high growth rate at 3–6 hours culture (Figure. 4–8). Cell growth and the synthesis of ethylene glycol and glycolic acid were not coincided. This is different to all reports that using E. coli as the producer, in which the cell growth and ethylene glycol synthesis are coincided [3, 4]. Ethylene glycol and glycolic acid synthesis was inhibited at an agitation rate of 800, but xylonic acid consumption was at a high rate, (Figure. 7). This experiment was repeated several times, and the results were the same. It indicated some undetectable chemicals were produced in the process, and it is interesting for further investigation.

Conclusions

Ethylene glycol is a highly important commodity chemical. However, there are no known natural pathways to directly synthesize ethylene glycol from carbohydrates [27]. In this study, it was shown that ethylene glycol can be produced by E. cloacae S1 using xylonic acid as the sole carbon source. This synthesis pathway presents an alternative route for ethylene glycol production from sugars. Ethylene glycol production by E. cloacae S1 yielded the highest productivity and the second highest product titer compared with reported data. This was achieved with little process optimisation and it is anticipated that the fed-batch process can be further improved in terms of product titer and yield. This work forms the basis to develop a new industrial process for ethylene glycol and glycolic acid production by a biological route.

Methods

Strains, plasmids, and primers

Bacterial strains and plasmids used in this study are listed in Table 2. Primers used for PCR are listed in Supplementary Table 1.

Table 2
Strains and plasmids

Strain or plasmid	Relevant genotype and description	Reference or source
K. pneumoniae Δgad	Δgad	(Wang, et al., 2016)
E. coli W3110	Wild type	Lab stock
E. coli BL21/yqhD	Over-expression of yqhD	This work
E. coli BL21/aldB	Over-expression of aldB	This work
E. coli BL21/betB	Over-expression of betB	This work
E. coli BL21/ad1	Over-expression of ad1	This work
E. coli BL21/ad2	Over-expression of ad2	This work
Enterobacter cloacae S1	Wild type,	This work
E. cloacae ΔyjhG	ΔyjhG, Str^r	This work
E. cloacae ΔyjhH	ΔyjhH, Apr^r	This work
E. cloacae ΔyhcH	ΔyhcH, Apr^r	This work
E. cloacae ΔyagG	ΔyagG, Apr^r	This work
E. cloacae ΔiclR	ΔiclR, Apr^r	This work
E. cloacae ΔxyL	Δxyl, Apr^r	This work
E. cloacae ΔyqhD	ΔyqhD, Str^r	This work
E. cloacae ΔbetB	ΔbetB, Str^r	This work
E. cloacae + yqhD	pSARI-yqhD, Kan^r	This work
E. cloacae + betB	pSARI-betB, Kan^r	This work
pMD18-T-simple	Amp^r, TA cloning vector, 2,692 bp	Takara®
pMD18T-yhcH	Amp^r, carries yhcH, 4,237 bp	This work
pMD18T-ΔyhcH	Amp^r, carries part of yhcH, Apr^r, 5,077 bp	This work
pMD18T-yjhH	Amp^r, carries yjhH, 4,844 bp	This work
pMD18T-ΔyjhH	Amp^r, carries part of yjhH, Apr^r, 5,285 bp	This work

Strain or plasmid	Relevant genotype and description	Reference or source
pMD18T-yjhG	Amp^r, carries yjhG, 6,034 bp	This work
pMD18T-ΔyjhG	Amp^r, carries part of yjhG, Str^r, 5,416 bp	This work
pMD18T-yagG	Amp^r, carries yagG gene, 5,237 bp	This work
pMD18T-ΔyagG	Amp^r, carries part of yagG gene, Apr^r, 5,897 bp	This work
pMD18T-xyL	Amp^r, carries β-xylosidase gene, 5,642 bp	This work
pMD18T-ΔxyL	Amp^r, carries part of β-xylosidase gene, Apr^r, 6,187 bp	This work
pMD18T-iclR	Amp^r, carries iclR, 4,637 bp	This work
pMD18T-ΔiclR	Amp^r, carries part of iclR, Apr^r, 5,228 bp	This work
pMD18T-yqhD	Amp^r, carries yqhD, 3,856 bp	This work
pMD18T-ΔyqhD	Amp^r, carries part of yqhD, Str^r, 4,268 bp	This work
pMD18T-betB	Amp^r, carries betB, 5308 bp	This work
pMD18T-ΔbetB	Amp^r, carries part of betB, Str^r, 5261 bp	This work
pIJ773	Apr^r, aac(3)IV with FRT sites, 4,334 bp	(Gust, et al., 2003)
pIJ778	Str^r, aadA with FRT sites, 4,337 bp	(Gust et al., 2003)
pIJ790	Cm^r, encodes λ-Red genes, 6,084 bp	(Gust et al., 2003)
pSARI	Kan^r, PR, 4,914 bp (Genbank MH037013)	Lab stock
pSARI-red	Kan^r, carries λ-Red genes, 6,799 bp	This work
pSARI-yqhD	Kan^r, carries the yqhD, 6,078 bp	This work
pSARI-betB	Kan^r, carries the betB, 6630 bp	This work
Pet 28a	Vector carries N-terminal His Tag, Kanr, 5369 bp	Novagen®
Pet 28a-yqhD	Amp^r, carries the yqhD, 6,520 bp	This work
Pet 28a-aldB	Kan^r, carries the aldB, 6,895 bp	This work
Pet 28a-betB	Kan^r, carries the betB, 6,772 bp	This work

Strain or plasmid	Relevant genotype and description	Reference or source
Pet 28a-ad1	Kan^r, carries the ad1, 6,727 bp	This work
Pet 28a-ad2	Kan^r, carries the ad2, 6,826 bp	This work

Xylonic acid preparation

Xylonic acid (Ammonium salt) was produced from xylose by K. pneumoniae, as described previously [7]. The fermentation broth was centrifuged to eliminate cells and other insoluble impurities. 1% of activated carbon was added to the supernatant and filtrated with paper. The discolored liquid was concentrated to 700 g/L with a rotary evaporator at 70 °C. The xylonic acid crystals were formed after keeping the liquid at room temperature for 1 week. This xylonic acid obtained was used in the following experiments.

Screening of xylonic acid utilizing microorganisms

Soil samples were collected from the campus of Shanghai Advanced Research Institute. 1 g of soil sample was inoculated to a 250 ml flask with 50 ml enrichment medium and then incubated aerobically at 37 °C on a rotary shaker (120 rpm). After one day of incubation, 0.1 ml of the culture broth was transferred to another flask with the same enrichment medium and incubated for 1 day. After 3 rounds of such enrichment operation, 1 ml of 10^8-fold diluted culture broth was plated on Luria–Bertani (LB) agar plate and cultured at 37 °C overnight. Colonies grown on the plates were inoculated to a 250 ml flask with 50 ml confirmation medium and then incubated on a rotary shaker at 37 °C and 120 rpm for 1 day. Chemical compounds in the broth were quantified by high performance liquid chromatography (HPLC) as described previously [7].

The enrichment medium was M9 medium and xylonic acid 40 g/l was used. The confirmation medium contained: xylonic acid 40 g/L, Yeast extract 5 g/L, Tryptone 10 g/L, NaCl 10 g/L.

Strains identification and carbon source utilization

16S rRNA gene sequence analysis was used for strains identification. The isolated strain E. cloacae S1 was cultured in M9 medium with either glucose, gluconic acid, 2-ketogluconic acid, xylose, xylonic acid or glycerol as the sole carbon source (20 g/L), respectively. Gluconic acid and 2-ketogluconic acid used were in the form of sodium salt, and 2-ketogluconic acid was prepared as reported previously [28].

Ethylene glycol and glycolic acid structure confirmation

Glycolic acid produced by E. cloacae S1 was purified from the fermentation broth by ion-exchange chromatography and the structure confirmed by nuclear magnetic resonance (NMR) spectroscopic analysis. A Bruker spectrometer was used and chemical shift values were reported in ppm (δ).

Ethylene glycol was confirmed by comparison with the standard chemical by HPLC and gas chromatography (GC). A gas chromatograph system (Shimadzu GC 2010) equipped with a flame ionization detector and a DB-WAX column (30 m × 0.25 mm), with nitrogen as the carrier gas was used.

Construction of mutants of E. cloacae

Red recombinase system was used for E. cloacae mutant construction, and following the method that we developed in K. pneumoniae [24]. pIJ790 is a plasmid that contains the red recombinase genes and used in E. coli for gene recombination. However, this plasmid could not be used directly for gene recombination in E. cloacae. pSARI is a low copy number plasmid containing a temperature induced promoter and kanamycin resistance gene. pSARI can be transferred into E. cloacae and was used for red recombinase meditated gene manipulations. Red recombinase encoding genes were amplified from PIJ790 and ligated into the pSARI to generate plasmid pSARI-red.

E. cloacae ΔyjhG construction is described in detail as an example. Other mutants were constructed in the same way and using corresponding primers and resistance genes.

The yjhG gene in the genome of E. cloacae and flanking sequences was amplified by PCR using the primer pair yjhG-s and yjhG-a. The PCR product was ligated into the pMD18-T-simple vector to generate pMD18-T-yjhG. A linear DNA with 39 and 40 nt homologous extensions flanking streptomycin resistance gene aadA was amplified with plasmid pIJ778 as the template using the primer pair yjhG-FRT-s/yjhG-FRT-a. pMD18-T-ΔyjhG was constructed by replacing yjhG in plasmid pMD18-T-yjhG with the aadA cassette using the Red recombination system in E. coli.

The plasmid pMD18-T-ΔyjhG was further used as the template for PCR preparation of a linear DNA containing the streptomycin resistance gene aadA with 500 bp of homologous regions on both sides. Finally, the linear DNA was transformed into E. cloacae/red, which already hosted the plasmid pSARI-red. Homologous recombination between the linear DNA and the chromosome was facilitated by Red recombinase and led to yjhG deletion in E. cloacae.

Construction of strains for protein over-expression

The ORF of yqhD in E. cloacae S1 was amplified using the primer pair yqhD-s2 and yqhD-a2. The PCR product was ligated into the pMD18-T-simple vector to generate pMD18-T-yqhD. pMD18-T-yqhD was digested with BamH I and Nco I to obtain the yqhD fragment, and this fragment was ligated into pET28a to generate pET28a-yqhD. pET28a-yqhD was transformed into E. coli BL21 for protein expression.

E. coli BL21/aldB, E. coli BL21/betB, E. coli BL21/ad1, and E. coli BL21/ad2 were constructed in the same way as E. coli BL21/yqhD.

pMD18T-yqhD was digested and ligated into pSARI to generate SARI-yqhD. SARI-yqhD was transformed into E. cloacae S1 to obtain E. cloacae + yqhD. E. cloacae + betB was constructed following the same method.

Physiological characterisation of strains

Wild-type and these constructed strains were inoculated in 250 ml flasks containing 50 ml fermentation medium or M9 medium (either xylose and xylonic acid used as carbon sources) and incubated on a rotary shaker at 37 °C and 120 rpm for 1 day. All experiments were done in triplicate, and data are expressed as the mean ± standard error.

Enzyme preparations and assay

YqhD and other protein were purified from the culture broth of E. coli BL21/yqhD and other E. coli strains by affinity chromatography using a His-Trap column. The enzyme assay follows the method for butanediol dehydrogenase activity assay [16]. Ethylene glycol or glycolaldehyde was used as substrates.

Culture parameters optimization and fed batch culture condition

Stirred tank bioreactors were used for culture parameters optimization. For the seed culture, 250-mL flasks containing 50 mL of LB medium were incubated on a rotary shaker at 37 °C and 200 rpm overnight. The seed culture was inoculated into a 5-L bioreactor (BIOSTAT-A plus Sartorius) with a working volume of 3 L and air supplement of 2 L/min. Culture pH and stirring rate were optimized individually. The fermentation medium contained: xylonic acid 30 g/L, corn steep liquor 4 g/L, $(NH_4)_2SO4$ 5 g/L, KCl 0.4 g/L, and MgSO4 0.1 g/L.

Fed batch cultures were performed at optimized conditions, with culture pH 6.5, culture temperature 37 °C and agitation rate of 600 rpm. When xylonic acid in the broth was consumed to 5 g/L, 100 ml 500 g/L of xylonic acid solution was added. All experiments were done in triplicate, and data were expressed as the mean ± standard error.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The 16S rRNA gene sequence has been submitted to GenBank with the accession number of MG779638. The genome sequence data was submitted to submitted to GenBank with the accession numbers of VSZU00000000

Competing interests

The authors declare that they have no competing interests

Funding

This work was supported by National Key R&D Program of China (Grant No. 2017YFE0112700), Royal Society-Newton Advanced Fellowship (Grant No. NAF\R2\180721), Natural Science Foundation of Shanghai (Grant No. 19ZR1463600) and National Natural Science Foundation of China (Grant No. 21576279).

Authors' contributions

FB and JH designed this study. ZZ, YY, YW, JG and XLu conducted the research. ZZ, YY, XLiao, JS, CK, GL, FB and JH analysed the data. ZZ, YY, FB and JH wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgments FB would like to thank the Chinese Academy of Sciences for the award of a President's International Fellowship Initiative (Grant No. 2019VCB0007).

References

1. Yue H, Zhao Y, Ma X, Gong J: Ethylene glycol: properties, synthesis, and applications. Chem Soc Rev. 2012, 41:4218-4244.
2. Liu, Ramos, Kristine RM, Valdehuesa, Kris NG, Nisola, Grace M, Chung, Wook-Jin: Biosynthesis of ethylene glycol in *Escherichia coli*. Appl Microbiol Biotechnol 2013, 97:3409-3417.
3. Alkim C, Cam Y, Trichez D, Auriol C, Spina L, Vax A, Bartolo F, Besse P, François JM, Walther T: Optimization of ethylene glycol production from (D)-xylose via a synthetic pathway implemented in *Escherichia coli*. Microb Cell Fact, 2015, 14:127.
4. Pereira B, Li Z-J, De Mey M, Lim CG, Zhang H, Hoeltgen C, Stephanopoulos G: Efficient utilization of pentoses for bioproduction of the renewable two-carbon compounds ethylene glycol and glycolate. Metab Eng 2016, 34:80-87.

5. Chen Z, Huang J, Wu Y, Liu D: Metabolic engineering of *Corynebacterium glutamicum* for the de novo production of ethylene glycol from glucose. Metab Eng 2016, 33:12-18.
6. Pereira B, Zhang H, De MM, Lim CG, Li ZJ, Stephanopoulos G: Engineering a novel biosynthetic pathway in *Escherichia coli* for production of renewable ethylene glycol. Biotechnol Bioeng 2016, 113:376-383.
7. Wang C, Dong W, Zhang Z, Wang D, Shi J, Kim CH, Jiang B, Han Z, Jian H: Production of xylonic acid by *Klebsiella pneumoniae*. Appl Microbiol Biotechnol 2016, 100:10055-10063.
8. Nygård Y, Toivari MH, Penttilä M, Ruohonen L, Wiebe MG: Bioconversion of D-xylose to D-xylonate with *Kluyveromyces lactis*. Metab Eng 2011, 13:383-391.
9. Liu H, Valdehuesa KNG, Nisola GM, Ramos KRM, Chung W-J: High yield production of D-xylonic acid from D-xylose using engineered *Escherichia coli*. Bioresour Technol 2012, 115:244-248.
10. Wang D, Wang C, Wei D, Shi J, Kim CH, Jiang B, Han Z, Hao J: Gluconic acid production by gad mutant of *Klebsiella pneumoniae*. World J Microbiol Biotechnol 2016.
11. Weimberg R: Pentose oxidation by *Pseudomonas fragi*. Westview Press; 1961.
12. Dahms AS: 3-Deoxy-D-pentulosonic acid aldolase and its role in a new pathway of D-xylose degradation. Biochem Biophys Res Comm 1974, 60:1433.
13. Grimont F, Grimont PAD: The Genus Enterobacter. Springer New York; 2006.
14. Khanna N, Kotay SM, Gilbert JJ, Das D: Improvement of biohydrogen production by Enterobacter cloacae IIT-BT 08 under regulated pH. J Biotechnol 2011, 152:9-15.
15. Li L, Li K, Wang Y, Chen C, Xu Y, Zhang L, Han B, Gao C, Tao F, Ma C: Metabolic engineering of Enterobacter cloacae for high-yield production of enantiopure (2R, 3R)-2, 3-butanediol from lignocellulose-derived sugars. Metab Eng 2015, 28:19-27.
16. Chen C, Wei D, Shi J, Wang M, Hao J: Mechanism of 2, 3-butanediol stereoisomer formation in *Klebsiella pneumoniae*. Appl Microbiol Biotechnol 2014, 98:4603-4613.
17. Murphy KC, Campellone KG, Poteete AR: PCR-mediated gene replacement in *Escherichia coli*. Gene 2000, 246:321-330.
18. Gust B, Challis GL, Fowler K, Kieser T, Chater KF: PCR-targeted *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. Proc Natl Acad Sci USA 2003, 100:1541-1546.
19. Jia B, Yang JK, Liu WS, Li X, Yan YJ: Homologous overexpression of a lipase from *Burkholderia cepacia* using the lambda Red recombinase system. Biotechnology Lett 2010, 32:521-526.
20. Lesic B, Rahme L: Use of the lambda Red recombinase system to rapidly generate mutants in Pseudomonas aeruginosa. BMC Mol Biol 2008, 9:20.
21. Katashkina J, Hara Y, Golubeva L, Andreeva I, Kuvaeva T, Mashko S: Use of the λ Red-recombineering method for genetic engineering of *Pantoea ananatis*. BMC Molecular Biology 2009, 10:34.
22. Doublet B, Douard G, Targant H, Meunier D, Madec JY, Cloeckaert A: Antibiotic marker modifications of λ Red and FLP helper plasmids, pKD46 and pCP20, for inactivation of chromosomal genes using

PCR products in multidrug-resistant strains. J microbiol methods 2008, 75:359-361.

23. Yamamoto S, Izumiya H, Morita M, Arakawa E, Watanabe H: Application of λ Red recombination system to *Vibrio cholerae* genetics: Simple methods for inactivation and modification of chromosomal genes. Gene 2009, 438:57-64.
24. Wei D, Wang M, Shi J, Hao J: Red recombinase assisted gene replacement in *Klebsiella pneumoniae*. J Ind Microbiol Biotechnol 2012, 39:1219-1226.
25. Shimada T, Yamamoto K, Yamanaka Y, Ishihama A, Momiyama E, Watanabe H: Regulatory role of XynR (YagI) in catabolism of xylonate in *Escherichia coli* K-12. FEMS Microbiol Lett 2017, 364.
26. Boyd LA, Adam L, Pelcher LE, McHughen A, Hirji R, Selvaraj G: Characterization of an *Escherichia coli* gene encoding betaine aldehyde dehydrogenase (BADH) : structural similarity to mammalian ALDHs and a plant BADH. Gene 1991, 103:45-52.
27. Zhang Y, Liu D, Chen Z: Production of C2–C4 diols from renewable bioresources: new metabolic pathways and metabolic engineering strategies. Biotechnol biofuels 2017, 10:299.
28. Wei D, Xu J, Sun J, Shi J, Hao J: 2-Ketogluconic acid production by *Klebsiella pneumoniae* CGMCC 1.6366. J Ind Microbiol Biotechnol 2013, 40:561-570.

Figures

Figure 1

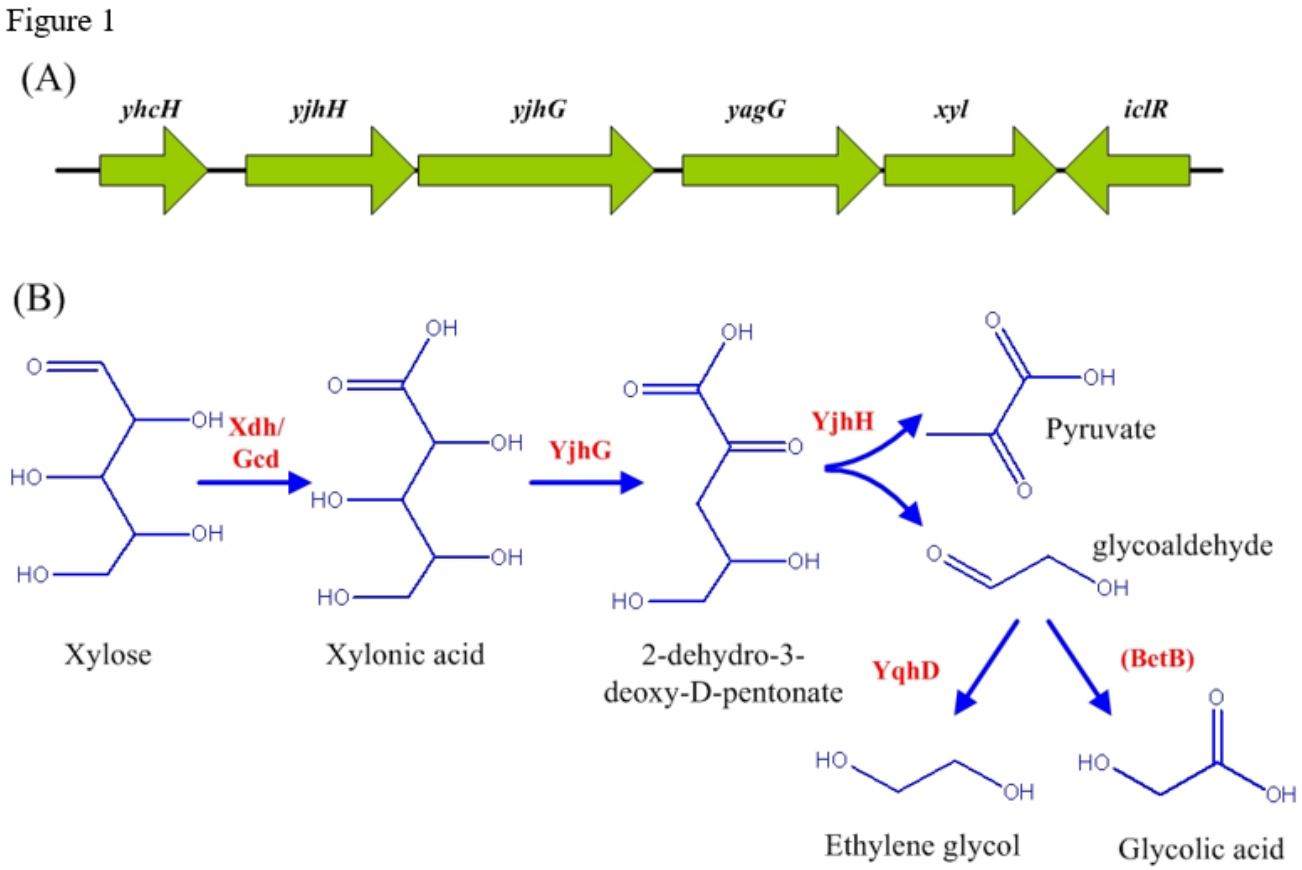

Figure 1

Ethylene glycol and glycolic acid synthesis pathway and yjh operon of E. cloacae. A: yjh operon of E. cloacae containing 6 genes. B: Metabolic pathway of ethylene glycol and glycolic acid synthesis from xylose. Xdh: D-xylose dehydrogenase of C. crescentus; GcD Glucose dehydrogenase of K. pneumoniae; YjhG 2-Dehydro-3-deoxy-D-pentonate aldolase of E. cloacae; YjhH: aldehyde reductase of E. cloacae; YqhD: alcohol dehydrogenase of E. cloacae; BetB: aldehyde dehydrogenase of E. cloacae.

Figure 2

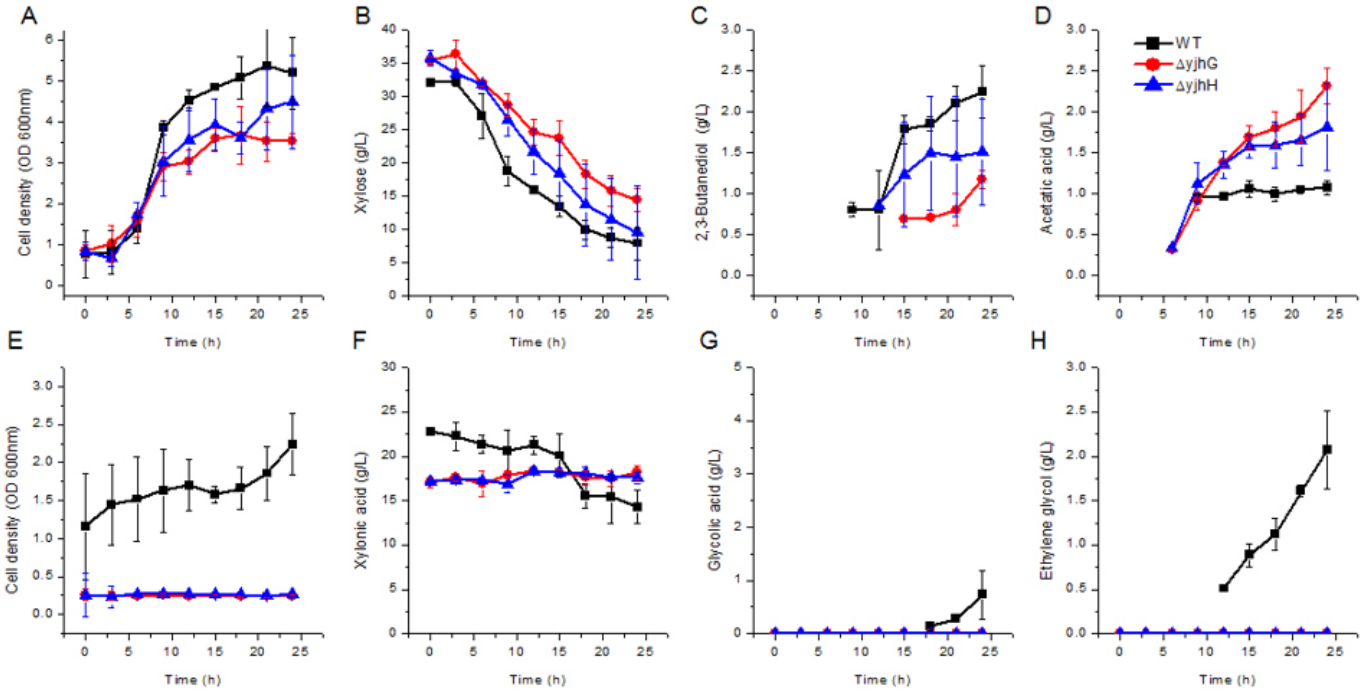

Figure 2

Growth and metabolite production of E. cloacae ΔyjhG and E. cloacae ΔyjhH grown on xylose (A-D) and xylonic acid (E-H). A, B, C, D: Cell density, xylose utilization, 2,3-butanediol, and acetic acid production; E, F, G, H: Cell density, xylonic acid utilization, glycolic acid, and ethylene glycol production. WT: E. cloacae S1 (filled square), ΔyjhG: E. cloacae ΔyjhG (filled circle); ΔyjhH: E. cloacae ΔyjhH (filled triangle). Data points are the average of n = 3; error bars represent standard error.

Figure 3

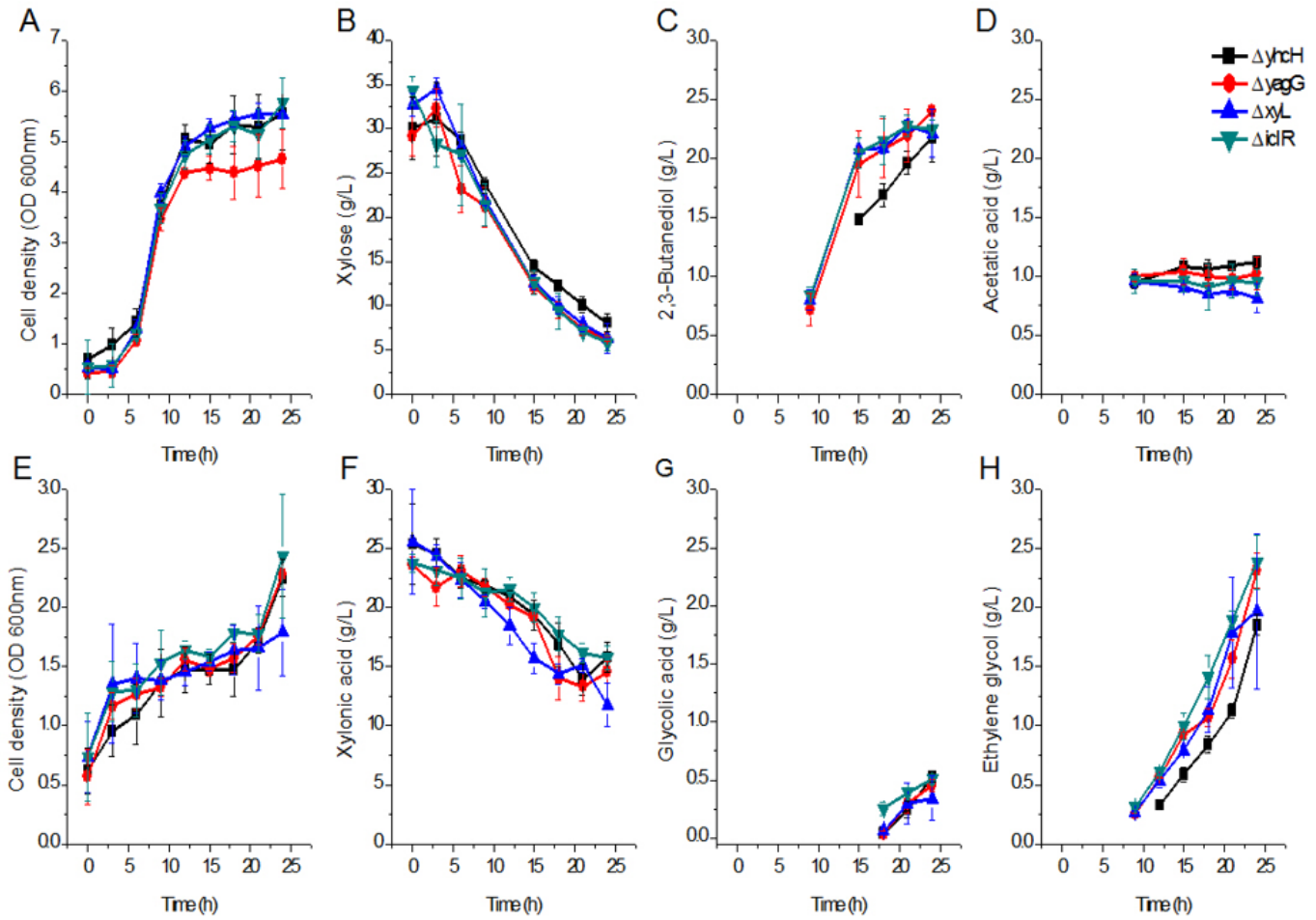

Figure 3

Growth and metabolite production of E. cloacae ΔiclR, E. cloacae ΔyhcH, E. cloacae ΔyagG and E. cloacae ΔxyL grown on xylose (A-D) and xylonic acid (E-H). A, B, C, D: Cells growth, xylose utilization, 2,3-butanediol, and acetic acid production; E, F, G, H: Cells growth, xylonic acid utilization, glycolic acid, and ethylene glycol production. ΔyhcH : E. cloacae ΔychH (filled square), ΔyagG: E. cloacae ΔyagG (filled circle); ΔxyL: E. cloacae ΔxyL (filled up triangle) ΔiclR: E. cloacae ΔiclR (filled down triangle). Data points are the average of n=3; error bars represent standard error.

Figure 4

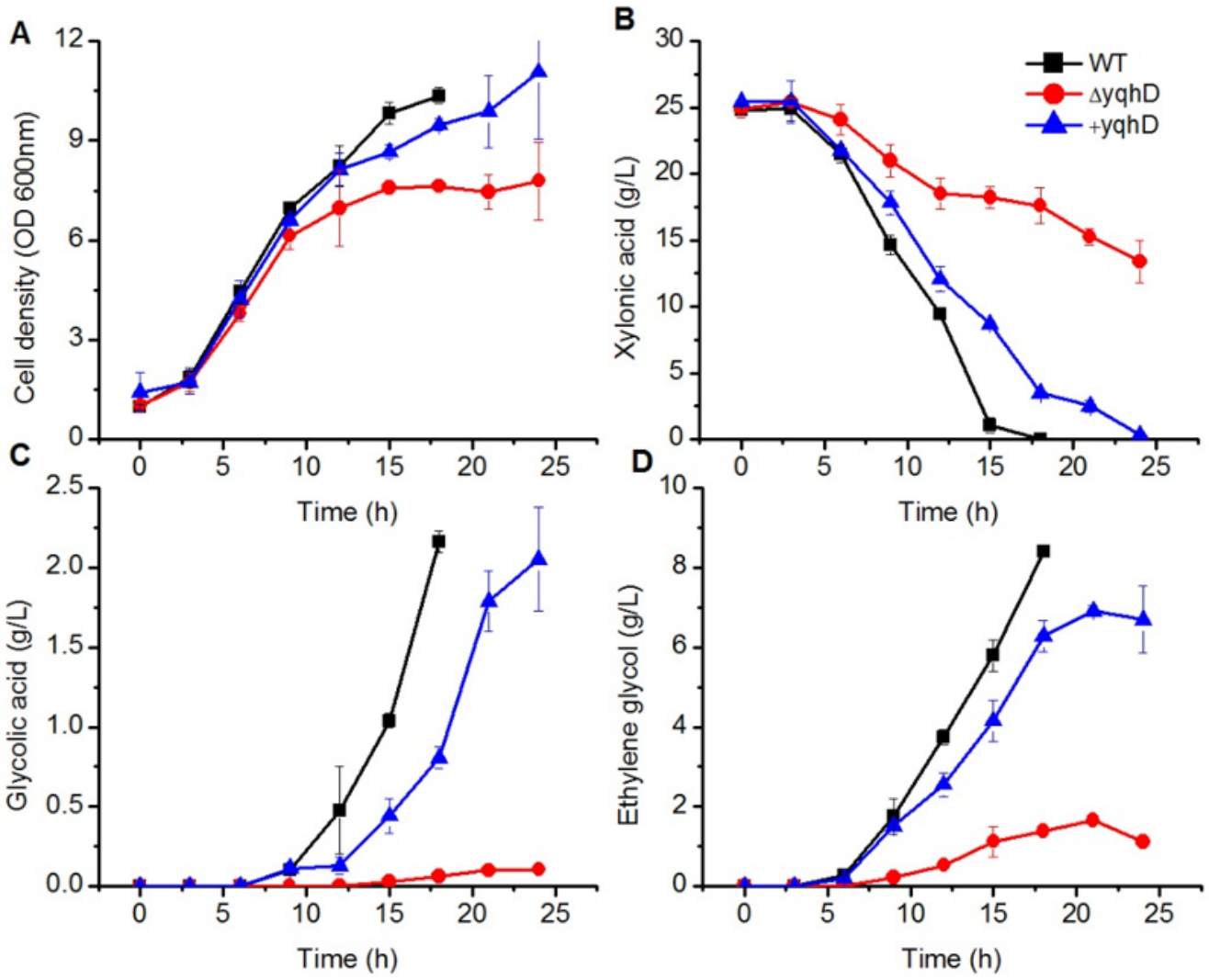

Figure 4

Ethylene glycol synthesis by yqhD knock-out and over-expression strains. E. cloacae S1 (filled square), E. cloacae ΔyqhD (filled circle) and E. cloacae+yqhD (filled triangle). Data points are the average of n=3; error bars represent standard error.

Figure 5

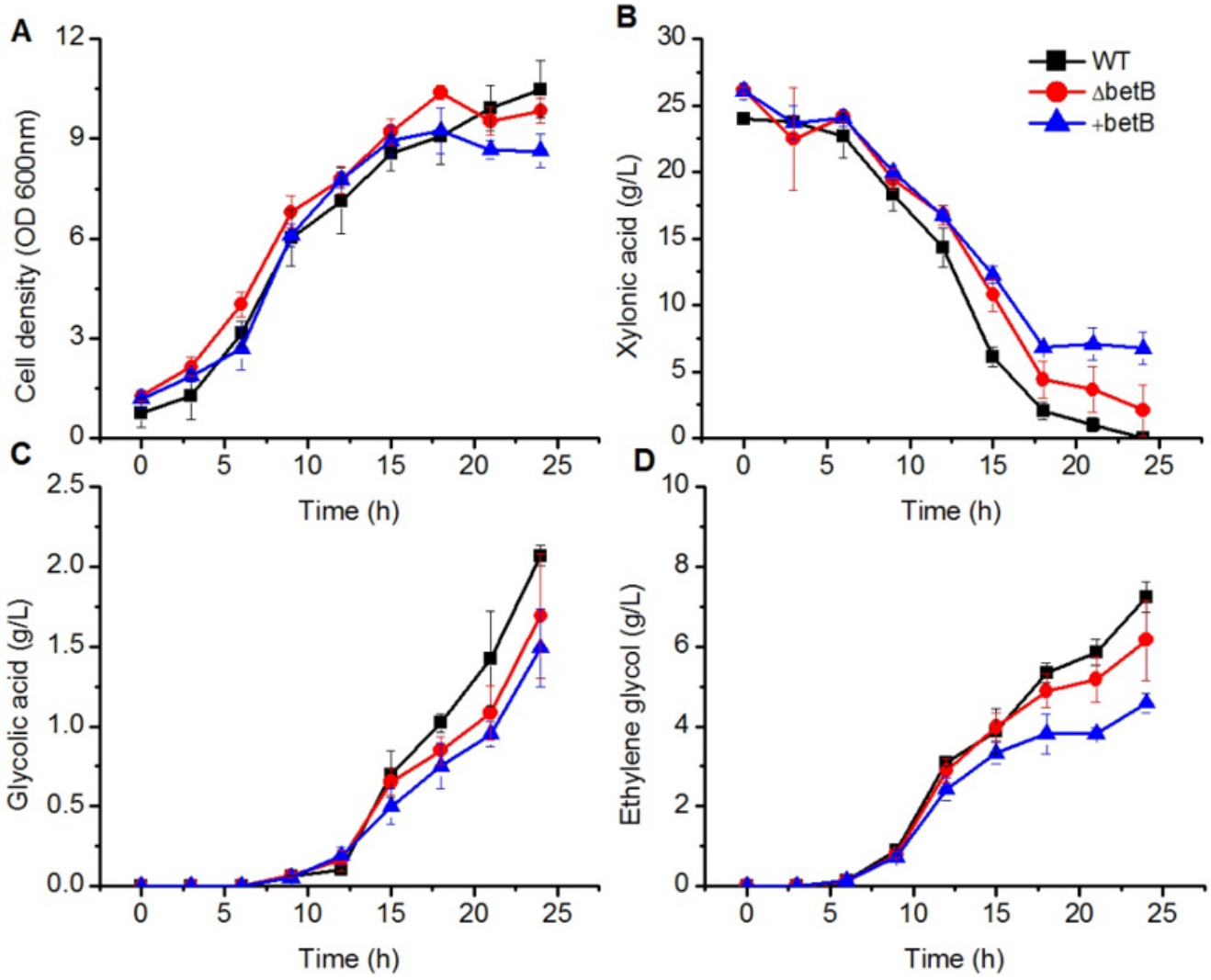

Figure 5

Ethylene glycol synthesis by betB knock- out and over-expression strains. E. cloacae S1 (filled square), E. cloacae ΔbetB (filled circle) and E. cloacae+betB (filled triangle). Data points are the average of n = 3; error bars represent standard error.

Figure 6

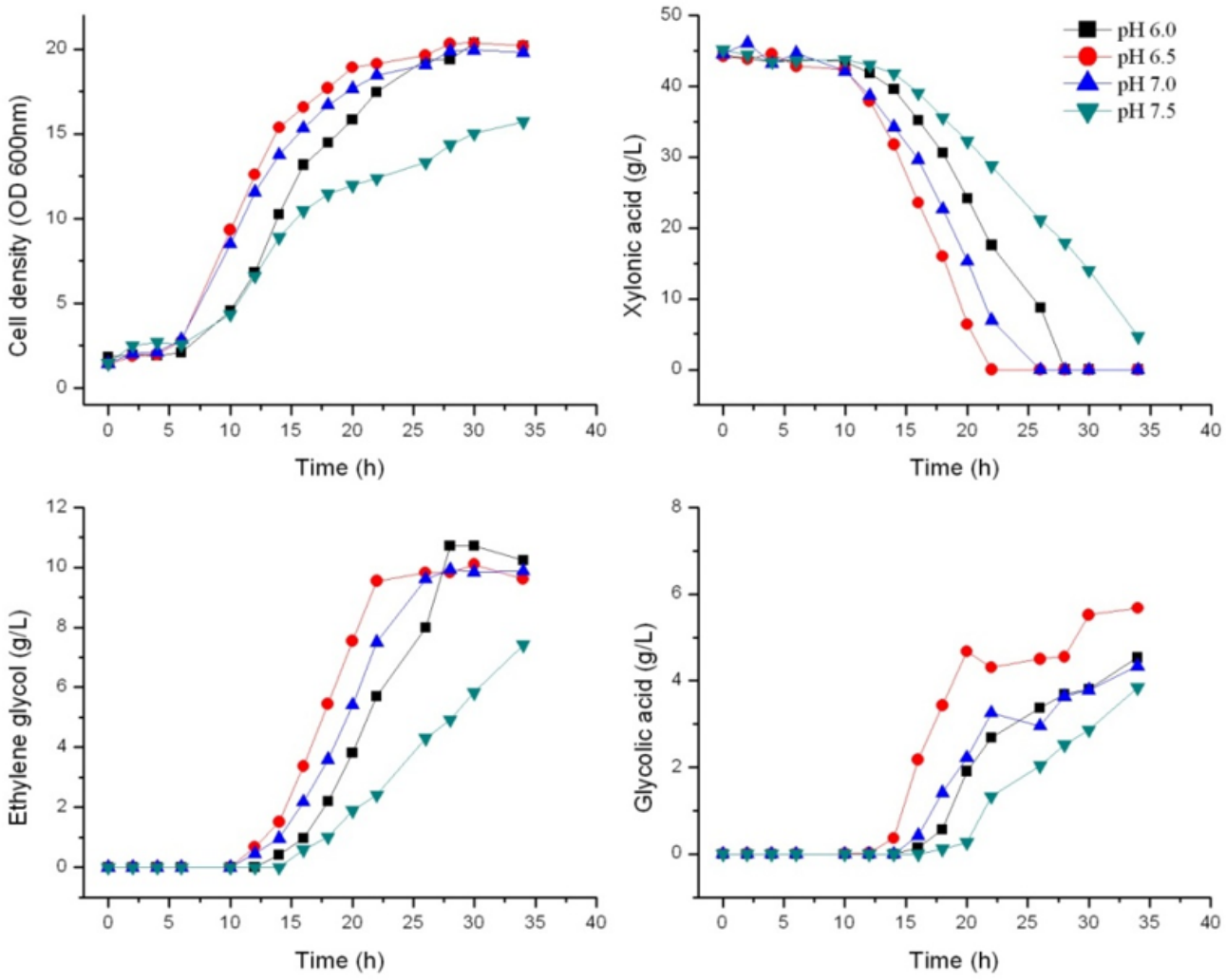

Figure 6

Cell growth and metabolite production of E. cloacae S1 grown on xylonic acid in batch culture at different pH values in 5L bioreactors operated at 500 rpm.

Figure 7

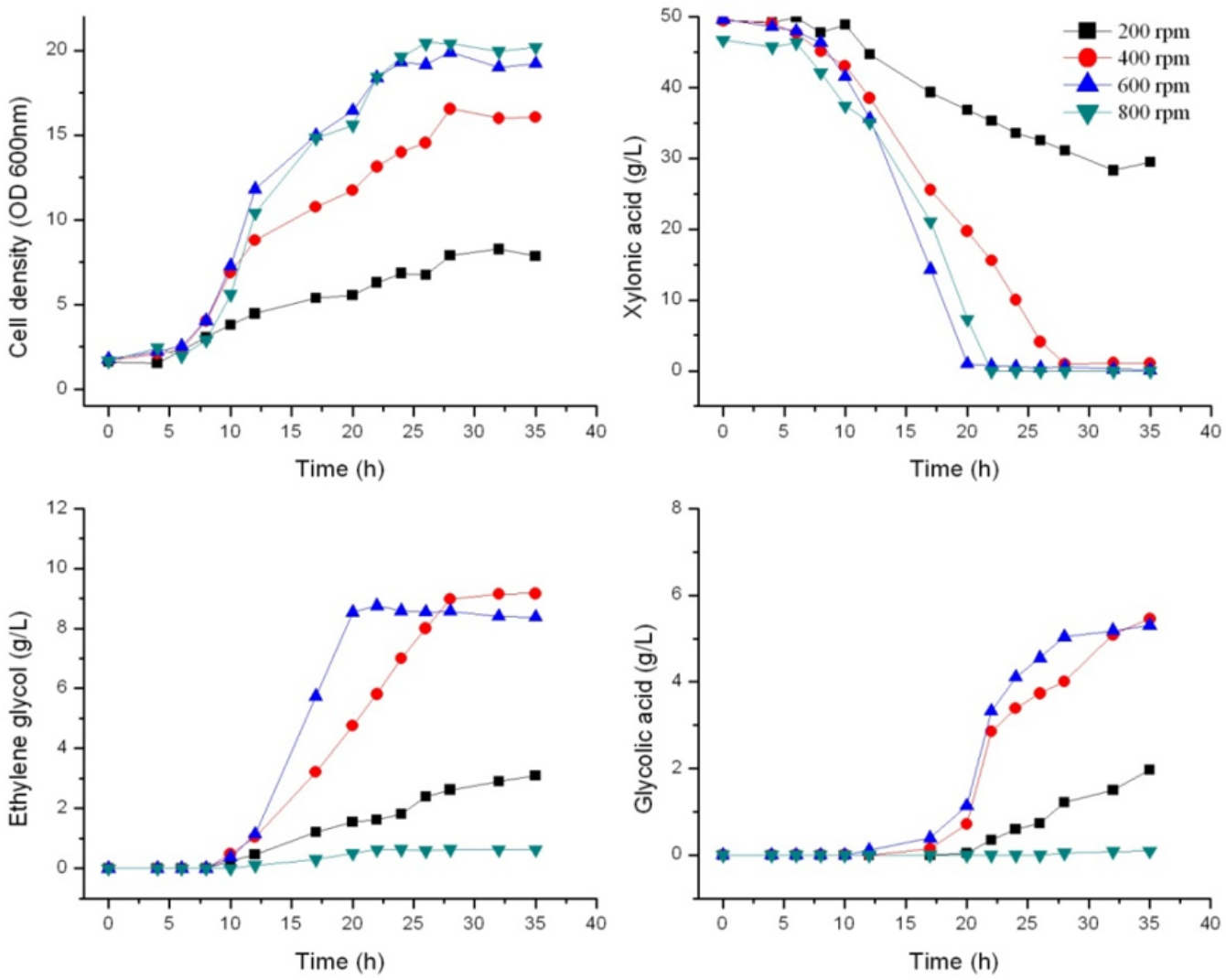

Figure 7

Cell growth and metabolite production of E. cloacae S1 grown on xylonic acid in batch culture at different different agitation rate in 5L bioreactors operated at pH 6.5.

Figure 8

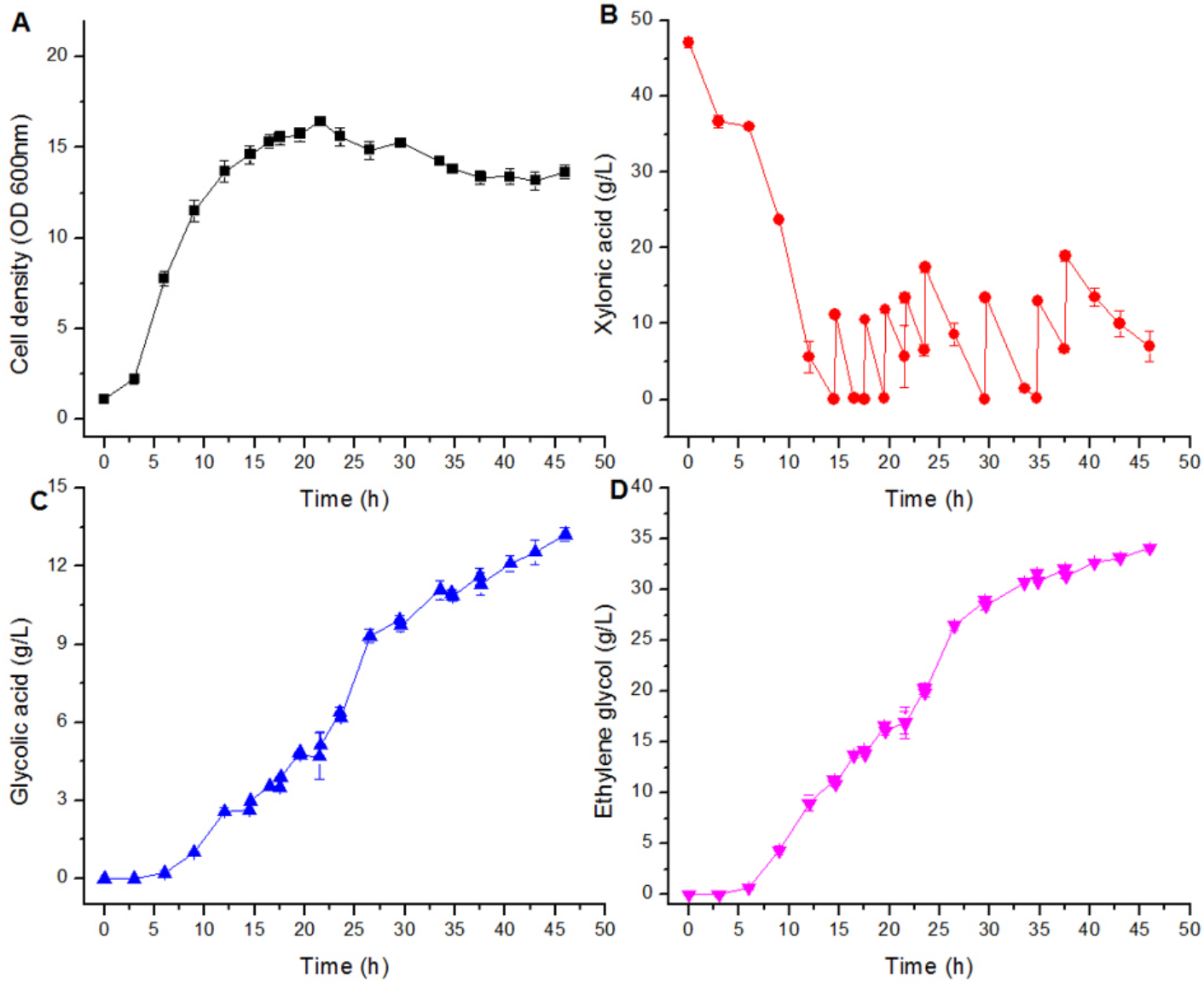

Figure 8

Cell growth and metabolite production of E. cloacae S1 grown on xylonic acid in fed-batch culture at pH 6.5 values in a 5L bioreactor operated at 600 rpm. A: cell density; B: xylonic aid; C: Glycolic acid; D: Ethylene glycol.Data points are the average of n = 3; error bars represent standard error.

Supplementary Files

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