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*Full Length Research Paper*

# Isolation and characterization of a new mucoid-free *Klebsiella pneumoniae* strain for 2,3-butanediol production

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**The secretion of mucoid substances by *Klebsiella pneumoniae*, a natural 2,3-butanediol (2,3-BD) hyper-producer, hinders its application in large-scale fermentation because of pathogenicity, fermentation instability, and downstream difficulty. In this study, 14 *K. pneumoniae* strains were isolated from a waste water treatment plant and their 2,3-BD production efficiencies were assessed with the strain *K. pneumoniae* DSM2026. Among various strains isolated, *K. pneumoniae* GSC010 and GSC112 produced relatively large amounts of 2,3-BD compared to other isolates; and their 2,3-BD production was consistent with DSM2026. Meanwhile, mucoidic characteristics of GSC010 were more or less similar to DSM2026, which was observed by scanning electron microscope (SEM) as a characteristic intercalated thread anchored on the surface of the cells. However, no polysaccharide materials were found in a non-mucoid cell, GSC112. Fed-batch culture of GSC112 with continuous glucose feeding resulted in the production of 2,3-BD at 52.4 g/l with 2,3-BD yield and overall productivity of 0.27 g/g glucose and 0.52 g/l/h, respectively. These results strongly suggest that the newly isolated mucoid-free *K. pneumoniae* GSC112 has potential for industrial production of 2,3-BD.**

**Key words:** 2,3-Butanediol, *Klebsiella pneumoniae*, isolation, capsular polysaccharides, scanning electron microscopy.

## INTRODUCTION

Production of bio-based fuels and chemicals from renewable resources is becoming more important as fossil resources diminish and global climate changes threaten environmental safety and human life. One of such chemicals, 2,3-butanediol (2,3-BD) possesses a wide range of industrial applications, and its production by microbial fermentation has been extensively studied for more than 100 years (Zeng and Sabra, 2011; Harden and Walpole, 1906). For example, 2,3-BD with a high heating value of 27,200 kJ/kg has been proposed as a liquid fuel additive or gasoline blend stock that either alone or with other alcohols enhances octane rating (Alam et al., 1990; Magee and Kosaric, 1987; Flickinger, 1980). More recently, the shortages of petroleum-based C4 compounds such as methyl ethyl ketone (MEK), used as solvent and 1,4-butadiene, used as a substrate of synthetic rubber, have encountered dramatic increases in price and strongly revived interest in the microbial production of 2,3-BD from renewable feedstocks. 2,3-BD can be easily converted into both compounds by chemical processes (Cho et al., 2008; Zeng and Sabra, 2011; Celińska and Grajek, 2009).

In order to avoid intracellular acidification, a variety of microorganisms, including Aerobacter, Bacillus, Klebsiella, Pseudomonas, and Serratia, have been reported to produce 2,3-BD via a mixed acid fermentation pathway under oxygen-limited conditions (Figure 1) (Ji et al., 2011;

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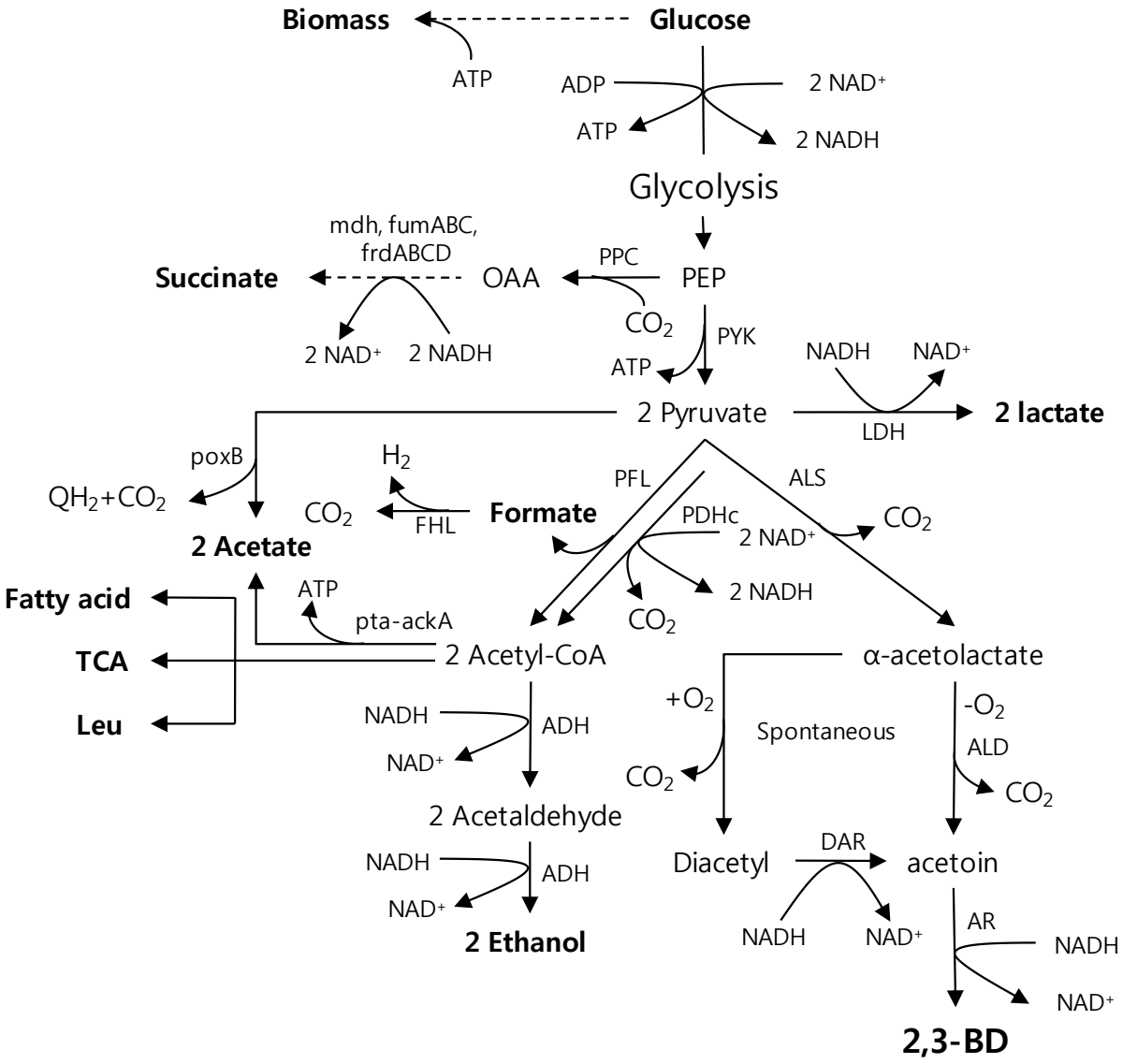


**Figure 1.** Pathway for the production of 2,3-BD from glucose.

Zhang et al., 2010; Alam et al., 1990; Sablayrolles and Goma, 1984). Among them, *K. pneumoniae* has been considered as a promising industrial host because of its ability to produce large amounts of 2,3-BD using a wide range of renewable feed-stocks. Fed-batch fermentation demonstrated that *K. pneumoniae* produced a very high titer of 2,3-BD with a productivity of 4.21 g/l/h using glucose (Ma et al., 2009). Many researchers have also reported that *K. pneumoniae* can use various alternative carbon sources such as whey permeate, *Jerusalem artichokes*, and corncob in place of glucose for the production of 2,3-BD, which can be very economical (Wang et al., 2010; Sun et al., 2009; Frazer and McCaskey, 1991; Lee and Maddox, 1984). In addition, *K. pneumoniae* utilizes glycerol, a promising raw material available in surplus due to biodiesel production, as a sole carbon and energy source for 2,3-BD production (da Silva et al., 2009; Biebl et al., 1998).

However, *K. pneumoniae* inherently produces copious amounts of capsular polysaccharides (CPS) that often cause serious problems in industrial applications (Lin et al., 2009; Guo et al., 2010). Immunologically, CPS acts as a significant virulence determinant of *K. pneumoniae* that establishes infection in humans (Brisse et al., 2009; Yu et al., 2007). Fermentatively, high-cell density culturing is limited due to the presence of flocculating CPS, and the separation of mucoidic cells is difficult during downstream processing (Guo et al., 2010). For these reasons, obtaining CPS-free *K. pneumoniae* would be very useful. To limit CPS content of *K. pneumoniae*, several findings reported mutagenesis and genetic modifications. For instance, random UV and chemical mutagenesis (Guo et al., 2010), deletion of *rmpA/rmpB* gene (Wacharotayankun et al., 1993; Nassif et al., 1989), or

**Table 1.** Production of 2,3-BD by various *K. pneumoniae* isolates including the reference strain DSM 2026 using glucose at 50 mM.

| Strain | Metabolite (g/l) | | | | | | Residual glucose (g/l) |
|---|---|---|---|---|---|---|---|
| | 2,3-BD | Lactic acid | Succinic acid | Formic acid | Acetic acid | Total | |
| DSM 2026 | 2.64 | 0.20 | 0.35 | 0.00 | 0.32 | 3.51 | 0.00 |
| GSC010 | 1.93 | 1.16 | 0.39 | 0.00 | 0.41 | 3.89 | 1.07 |
| GSC013 | 2.15 | 0.94 | 0.39 | 0.11 | 0.17 | 3.76 | 1.81 |
| GSC022 | 2.47 | 0.53 | 0.47 | 0.00 | 0.37 | 3.84 | 0.01 |
| GSC028 | 2.40 | 0.69 | 0.27 | 0.00 | 0.42 | 3.78 | 0.04 |
| GSC038 | 2.42 | 0.80 | 0.46 | 0.00 | 0.39 | 4.08 | 0.00 |
| GSC041 | 2.33 | 0.46 | 0.41 | 0.00 | 0.40 | 3.59 | 0.01 |
| GSC042 | 2.21 | 0.31 | 0.46 | 0.00 | 0.32 | 3.30 | 0.00 |
| GSC049 | 2.77 | 0.95 | 0.26 | 0.13 | 0.10 | 4.22 | 0.41 |
| GSC067 | 0.87 | 1.54 | 0.31 | 0.00 | 0.64 | 3.36 | 3.01 |
| GSC070 | 2.27 | 0.63 | 0.27 | 0.00 | 0.23 | 3.41 | 0.09 |
| GSC072 | 2.14 | 0.65 | 0.41 | 0.00 | 0.31 | 3.51 | 0.01 |
| GSC073 | 2.33 | 0.41 | 0.42 | 0.00 | 0.20 | 3.36 | 0.02 |
| GSC086 | 2.48 | 1.13 | 0.33 | 0.00 | 0.22 | 4.15 | 0.04 |
| GSC112 | 3.00 | 0.37 | 0.28 | 0.00 | 0.35 | 4.00 | 0.00 |

curing of mega plasmids (Nassif et al., 1989) in *K. pneumoniae* reduces CPS content to some degree. On the other hand, Thakker et al. (2006) isolated *K. pneumoniae* from environmental samples showing very low $LD_{50}$ of $3.3 \times 10^8$ CFU/ml which is normally considered to be non-virulent. Brisse et al. (2009) also suggested that *K. pneumoniae* is a heteromorphic strain.

Speculating that the environment may have various phenotypes of *K. pneumoniae*, the present study aimed at the isolation of *K. pneumoniae* strains from the environment devoid of mucoid secretion. The obtained isolates were further subjected for the production of 2,3-BD in batch and fed-batch fermentations.

## MATERIALS AND METHODS

### Bacterial strains and maintenance

The *K. pneumoniae* strains used in this study are listed in Table 1. *K. pneumoniae* DSM 2026 obtained from the German Collection of Microorganisms was used as the reference strain, and several additional *K. pneumoniae* strains were isolated for this work. GSC112 was identified as *K. pneumoniae* by its genotypic characteristics confirmed by 16S rDNA gene sequencing (accession no. JN897382). The strain was deposited in the Korean Collection for Type Cultures (http://www.brc.re.kr) under registration no. KCTC 11888BP. The strains were routinely maintained in Luria-Bertani (LB) agar (Difco Laboratories, Detroit, MI) plates for isolation, including dilution plating, Voges-Proskauer (VP) test, and DNA preparation. Glycerol stocks (15%, v/v) were stored at -80°C as master cell banks for further fermentation studies. Unless otherwise noted, all chemicals used in this study were of reagent grade and purchased either from Sigma-Aldrich (St. Louis, MO) or Junsei Chemical (Tokyo, Japan).

### Isolation of *K. pneumoniae* strains

Water and sludge samples were collected from a municipal wastewater treatment plant (Daejeon, Korea). In order to enrich *K. pneumoniae* strains, 1% inoculum was added to 9 ml M9 medium supplemented with 100 mM glycerol and potassium phosphate buffer (pH 7.0) in serum bottles. The bottles were incubated in a rotary shaker at 150 rpm and 30°C for 24 h. Followed by three consecutive transfers, dilution plating of the cultures grown under anaerobic condition was done on LB agar plates. About thousand colonies were randomly picked and were subjected to Voges Proskauer (VP) test in 96-well deep well plates. The VP positive strains were named as GSC001, GSC002, and so on. Arbitrarily, 14 VP positive strains were further investigated by polymerase chain reaction (PCR) using eubacterial 16S ribosomal RNA primers 27F and 1492R (Frank et al., 2008). The sequencing results of the PCR products were analyzed by BLAST (Basic Local Alignment Search Tool) sequence search using the NCBI (National Center for Biotechnology Information) database. Phylogenetic trees were constructed for the 16S rDNA sequences of the isolates by ClustalW2 Multiple Sequence Alignment online tool for the diversity analysis. The partial 16S rDNA sequence of GSC112 was deposited in the NCBI data base (Genebank: JN897382.1)

### Scanning electron microscopy

For SEM, cells were fixed in a mixture of 2.5% glutaraldehyde and 2.0% paraformaldehyde solution buffered in 0.1 M sodium cacodylate (pH 7.2) at 4°C for 2 h. They were then immobilized on a glass slide coated with poly-L-lysine. Secondary fixation was conducted in a mixture of 1.5% potassium ferricyanide and 1.0% osmium tetroxide. Prior to complete dehydration with hexamethyl disilizane, samples were pre-dehydrated using 50, 60, 70, 80, 90, and 95% ethanol in series. All chemicals used in SEM preparation were of reagent-grade from Sigma-Aldrich (USA). The dehydrated samples were sputter coated with gold at 30 mA plasma current under argon gas for 150 s. Microscopic examinations were carried

out using a SEM (Quanta™ 250FEG, FEI Company, Hillsboro, OR) at an acceleration voltage of 10 kV.

**Cultivation and analytical procedures**

Test tube cultures were conducted at 150 rpm and 37°C in a rotary shaker in a medium containing 10 g/l peptone, 3 g/l beef extract, and 5 g/l NaCl with 50 mM glucose. The medium used in fermentation experiments contained (g/l): bacto yeast extract 5.0 (Becton Dickinson, Le Pont de Claix, France), $FeSO_4 \cdot 7H_2O$ 0.05, $ZnSO_4 \cdot 7H_2O$ 0.001, $MnSO_4 \cdot H_2O$ 0.001, $CaCl_2 \cdot 2H_2O$ 0.001, $MgSO_4 \cdot 7H_2O$ 0.25, $(NH_4)_2SO_4$ 6.6, $K_2HPO_4$ 8.7, and $KH_2PO_4$ 6.8. The initial pH was adjusted to 6.8 by adding 5 N NaOH or HCl. Batch fermentations were carried out in a 6.6 L BIOFLO® and CELLIGEN® 310 fermentor (New Brunswick. Scientific Co., Edison, NJ) containing 2.7 L of the medium plus 500 mM glucose with 10% (v/v) of an inoculum cultured in a 500 ml Erlenmeyer flask. The temperature and agitation speed were kept at 37°C and 150 rpm, respectively. The pH was controlled at 6.5±0.1 by the automatic addition of 5 N NaOH. The fermentor was continuously flushed with air through a 0.2 $\mu$m membrane filter at a flow rate of 3.0 l/min. Fed-batch fermentations were carried out under the same condition as the batch fermentations. A concentrated solution containing 700 g/l glucose and 20 g/l $MgSO_4 \cdot 7H_2O$ was continuously fed into the fermentor at a predetermined rate via a peristaltic pump (Cole-Parmer, Vernon Hills, IL).

Cell growth was monitored by measuring the $OD_{600}$ using a UV-Vis spectrophotometer (DR5000, Hach Company, CO). 2,3-BD and metabolites including formic acid, acetic acid, ethanol, lactic acid, acetoin, and succinic acid were measured using high-performance liquid chromatography (Agilent 1200, Agilent Technologies, Waldbronn, Germany) equipped with a refractive index detector (Shodex RI-71, Tokyo, Japan) and Aminiex HPX-87H column (300 mm × 7.8 mm, Bio-Rad, Hercules, CA). The column was isocratically eluted using 0.01N $H_2SO_4$ at a flow rate of 0.6 ml/min with the column oven temperature of 80°C. Glucose concentration was determined using the YSI 2700 SELECT™ Biochemistry Analyzer (YSI Inc., Yellow Springs, OH) and confirmed by the HPLC system.

## RESULTS

### Isolation and characterization of *K. pneumoniae*

Samples collected from waste water treatment plants were enriched under anaerobic condition using glycerol as a sole carbon and energy source in M9 medium. *K. pneumoniae* ferments glycerol and produces 1,3-propanediol as the main metabolite under anaerobic condition, a redox neutral pathway which permits them to grow under this condition (Bouvet et al., 1995). By this approach, over a thousand strains were picked for VP analysis and 14 strains were confirmed as *K. pneumoniae* by 16S rDNA ribotyping (data not shown). To qualitatively check the mucoidity of the isolates, a string test of the colonies on LB agar plate was first conducted. Centrifugation studies of overnight grown broth cultures of isolates were also carried out (Lin et al., 2009). Figure 2 shows the precipitation profile of mucoidic DSM2026 and non-mucoidic GSC112 which represents how polysaccharides interfere in the cell separation in the broth culture.

The colonies could be divided into four groups based on their mucoid characteristics analyzed by tooth pick in LB agar plate; small mucoidic (GSC010), small partial mucoidic (GSC013, GSC042, and GSC067), small non-mucoidic (GSC022, GSC041, GSC086, GSC112 and etc), and large non-mucoidic (GSC077) phenotypes (data not shown). Figure 3 shows the phylogram of 16S rDNA sequences of the new isolates generated by ClustalW, an online-tool used for the analysis of bacterial diversity. Most of the isolates showed 16S rDNA sequence identity of more than 98% to *K. pneumoniae* MGH78578, except GSC010 (96% identity). The mucoidic (GSC010 and DSM 2026) and non-mucoidic (GSC112) phenotypes of the strains observed on plate and broth cultures were further subjected to SEM analysis for the confirmation of their microscopic cell morphology. At the magnification of 150,000×, there were dense polysaccharide materials tangled between cells of GSC010 and DSM 2026. No such materials were evident from GSC112 (Figure 4). Moreover, the cells GSC010 and DSM 2026 did not settle down by centrifugation at 12,000 x *g* for 1 min, even with a longer spin time, resulting in a loose pellet that was easily dislodged. On the other hand, GSC112 settled down by centrifugation at the same speed and duration as a tight pellet (Figure 2).

### Fermentation

The 14 isolates, which were confirmed as *K. pneumoniae* by 16S rDNA ribotyping, produced 2,3-BD under semi-aerobic condition in a 50 ml test tube (Table 1) using glucose. Maximum titer of 2,3-BD was 3.0 g/l by the strain GSC112. Likewise, most of the other strains could also produce 2,3-BD at about 2.5 g/l. After 2,3-BD, lactic acid was the second dominant metabolite produced by most of the strains. GSC067 produced a maximum concentration of lactic acid (1.54 g/l) among the isolates, and thereby resulted in a very low production of 2,3-BD (Table 1). Conversely, the strains which produced lactic acid at minor amounts resulted in the production of 2,3-BD at higher concentrations. This is mainly due to the requirement of cells to maintain a redox balance by which it can produce both 2,3-BD and lactic acid at a certain balance. Notably, unlike other metabolites, lactic acid was produced at a very broad range of concentrations among different strains. Acetic acid levels by all isolates ranged from 0.10 to 0.64 g/l. The concentration of succinic acid was at a narrow range among isolates. Formic acid was the least among other metabolites by all the strains being semi-aerobic condition adopted for the production.

For further evaluation of the isolates, batch fermentations were carried out for few strains selected based on fermentation efficiency in terms of titer and selectivity of 2,3-BD production from the test tube studies. They were GSC010, GSC022, GSC041, GSC086, and GSC112. The

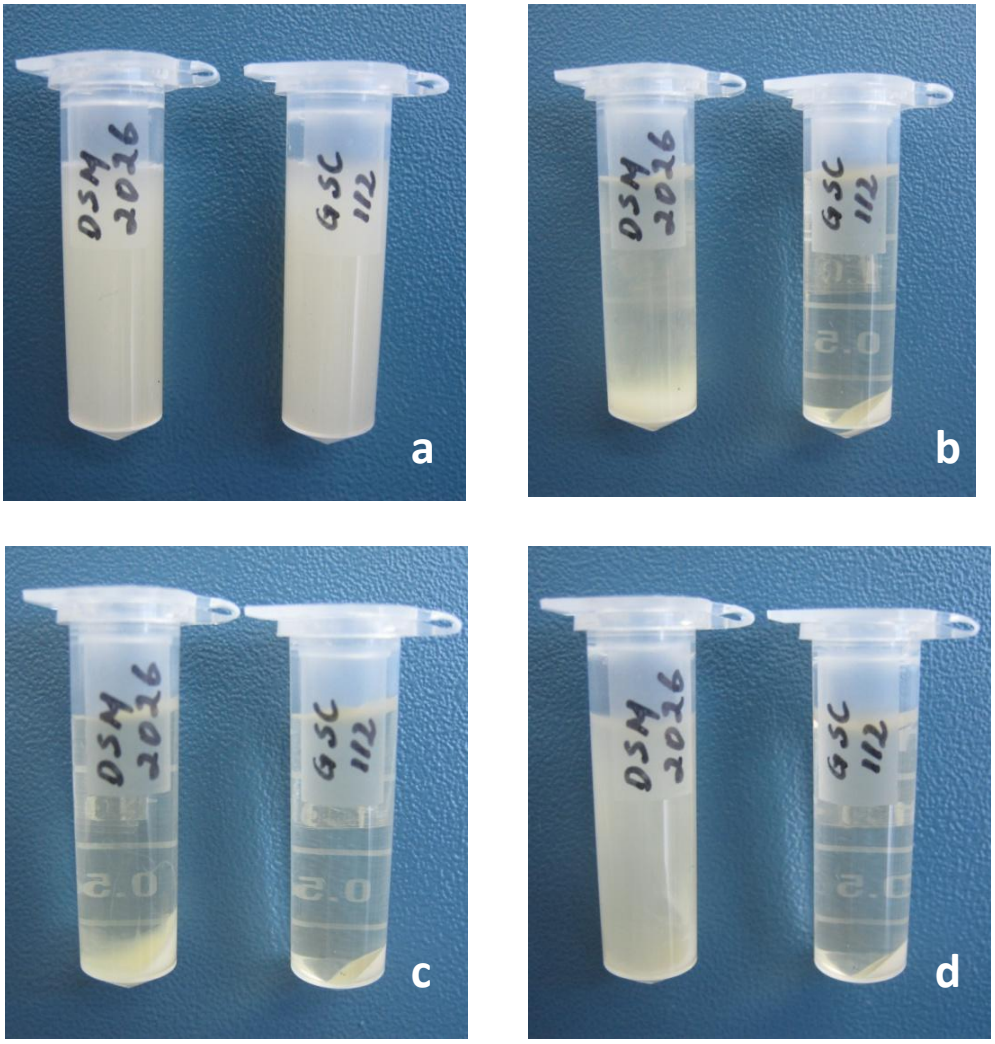


**Figure 2.** Centrifugation profile of DSM2026 and GSC112 at 13,000 rpm. (a), Overnight grown culture before centrifugation; (b), centrifuged for 1 min; (c), 10 min; and (d) gentle invert mixing of 10 min spun cultures.

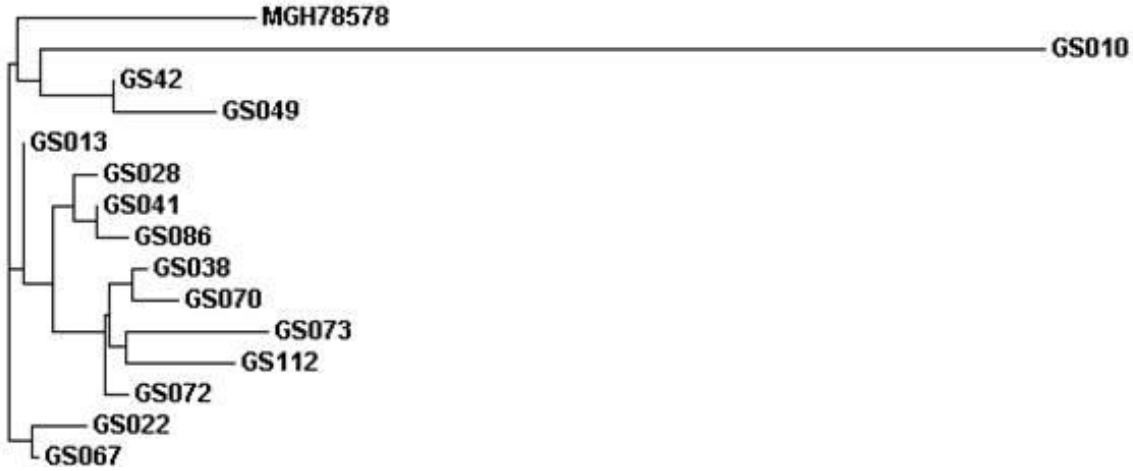


**Figure 3.** Phylogenetic tree for the 16S rDNA sequence of different *K. pneumoniae* isolates.

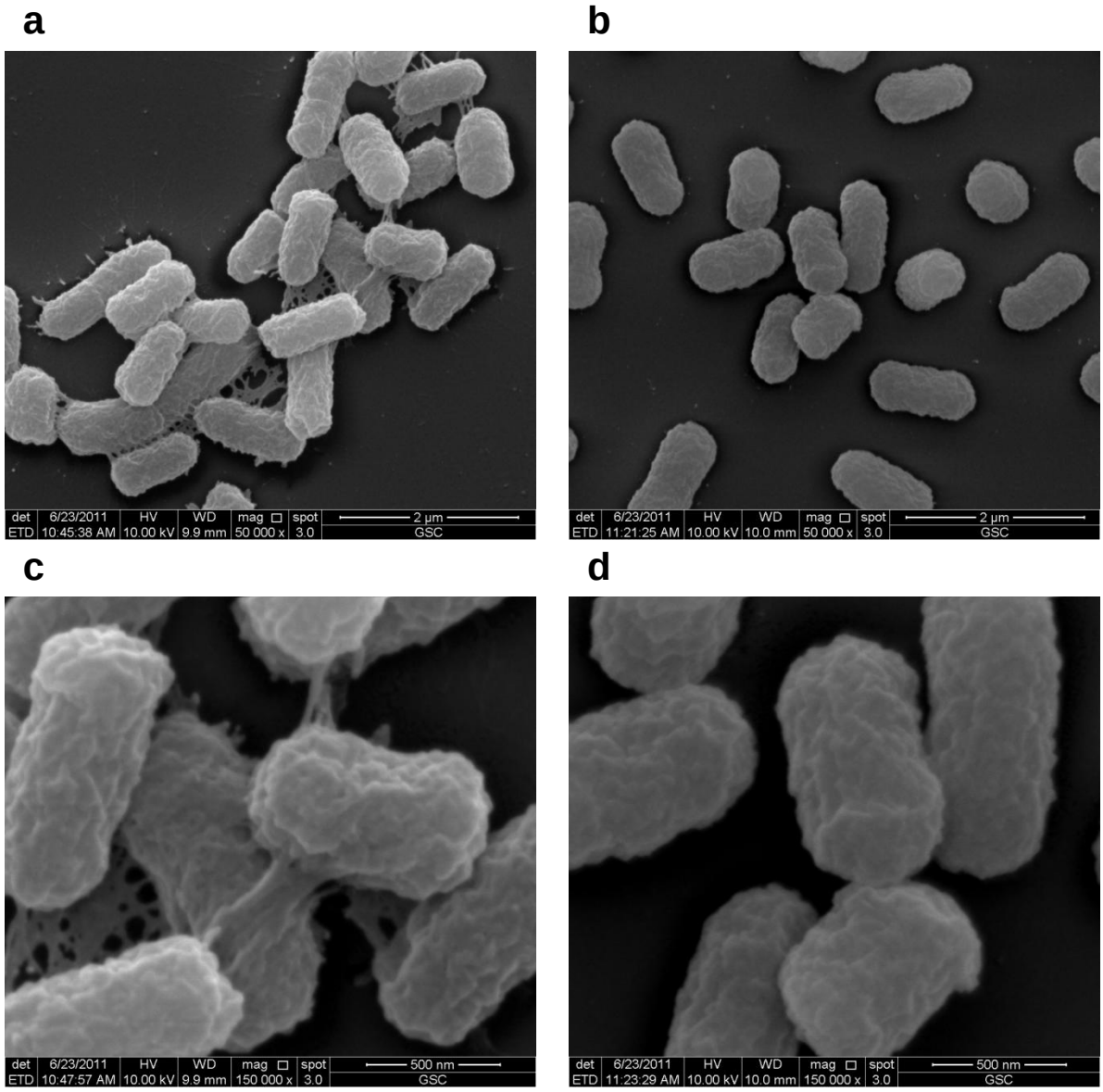


**Figure 4.** Scanning electron microscopic images of *K. pneumoniae* GSC010 (a, c) and GSC122 (b, d) at different magnitudes: (a) and (b), 50,000×; (c) and (d), 150,000×.

strain GSC112 produced a maximum of 24 g/l of 2,3-BD in 15 h by consuming 500 mM of glucose. The batch fermentations continued until the time point when the strains completely consumed glucose. For instance, fermentation lasted for a maximum period of 21 h by GSC086, 18 h by GSC041, and so on (Table 2). Generally, strains that took a short time for fermentation showed high rates of 2,3-BD production and glucose consumption. The 2,3-BD production and glucose consumption rates by GSC112 were found to be substantially higher than those of the other strains studied. At the same time, selectivity of 2,3-BD production over all metabolites ($S_{e,BD}$), including 2,3-BD, formic acid, acetic acid, ethanol, lactic acid, acetoin, and succinic acid, by GSC112 as well was highly comparable with other strains. Time course profiles of cell growth, 2,3-BD production, and substrate consumption are shown in Figure 5. Conversely, specific cell growth rates (µ/h) of GSC112, and GSC041 were found to be slightly lower when compared to other strains, but did not reflect the outcome to a large extent that is, titer or rate of 2,3-BD production. As far as the final titer of 2,3-BD is concerned, GSC022 was the highest compared to other strains, but the rate of 2,3-BD production was relatively low.

Production of 2,3-BD by the fed-batch fermentation of GSC112 was observed at the maximum of 52 g/l (Figure 6). The 2,3-BD production rate was 1.65 g/l/h during the period between 10 and 15 h fermentation which later

**Table 2.** Carbon distribution of glucose metabolism by 2,3-BD producing *K. pneumoniae* strains at the end of the fermentation.

| Strain | *K. pneumonia* | | | | | |
|---|---|---|---|---|---|---|
| | DSM 2026[a] | 010[b] | 22[c] | GSC 41[c] | 86[d] | 112[a] |
| **Substrate(g/l)** | | | | | | |
| Glucose | 90.9 | 93.6 | 94.2 | 93.5 | 93.3 | 95.0 |
| Biomass | 8.23 | 8.56 | 6.97 | 6.34 | 5.62 | 6.55 |
| **Metabolites (g/l)** | | | | | | |
| 2,3-BD | 24.68 | 20.35 | 25.52 | 23.79 | 23.45 | 23.90 |
| Lactic acid | 6.26 | 17.72 | 6.07 | 10.55 | 18.86 | 10.37 |
| Succinic acid | 2.52 | 2.59 | 4.12 | 2.93 | 2.59 | 2.85 |
| Formic acid | 8.52 | 7.38 | 6.12 | 6.89 | 7.57 | 5.53 |
| Acetic acid | 0.85 | 1.65 | 1.59 | 1.25 | 0.78 | 1.88 |
| Acetoin | 0.46 | 0.62 | 0.56 | 0.66 | 0.47 | 0.58 |
| Ethanol | 9.61 | 7.30 | 8.00 | 7.66 | 4.94 | 8.03 |
| μ | 0.89 | 0.88 | 0.82 | 0.59 | 0.83 | 0.59 |
| $Y_{m,BDO}$ (g/g) | 0.27 | 0.22 | 0.27 | 0.25 | 0.25 | 0.25 |
| $P_{v,BDO}$ (g/l/h) | 1.65 | 1.27 | 1.42 | 1.32 | 1.11 | 1.59 |
| $S_{e,BDO}$ (g/g) | 0.47 | 0.35 | 0.49 | 0.44 | 0.40 | 0.45 |
| $C_{v,Glu.}$ (g/l/h) | 6.06 | 5.85 | 5.23 | 5.19 | 4.44 | 6.33 |

Termination time point of different fermentations; [a]15 h, [b]16 h, [c]18 h, and [d]21 h; μ, specific cell growth rate/h; $Y_{m,BD}$, 2,3-BD yield on glucose; $P_{v,BD}$, 2,3-BD production rate; $S_{e,BD}$, 2,3-BD selectivity over all metabolites; $C_{v,Glu}$, glucose consumption rate.

decreased considerably. 2,3-BD production continued for the whole period when glucose consumption took place (Figure 6), indicating that the enzymes in a pathway leading to the production of 2,3-BD remained active. A total of 200 g/l of glucose was consumed in 97 h of fermentation with an average 2,3-BD yield of 0.26 g/g. Lactic acid was produced at 43 g/l which is 83% of the quantity of 2,3-BD. This high amount of lactic acid is one of the causes of low 2,3-BD yield on glucose. Even though the titer of 2,3-BD and lactic acid seems comparable, the production rates between the two metabolites were quite different. About 60% of 2,3-BD was produced by 20 h of fermentation, by then lactic acid produced less than 40%. Ethanol and succinic acid were the second major metabolites produced in the fermentation broth. Formic and acetic acids were the least of all the other metabolites.

## DISCUSSION

*K. pneumoniae* can produce 2,3-BD at a high titer and yield (Celińska and Grajek, 2009). However, mucoid secretions, mainly CPS, do not make it suitable for the industrial production of bulk chemicals at large scale (Guo et al., 2010). Therefore, obtaining a user-friendly host for industrial fermentation is essential. In the present study, several *K. pneumoniae* strains were isolated from environmental samples which were confirmed by 16s rDNA genotyping. According to string formation of the colonies on agar plates, the isolates were grouped as mucoidic, partial mucoidic, and non-mucoidic types (Lin et al., 2009). Broth cultures of mucoidic cells (DSM 2026 and GSC010) did not easily get pelleted down upon centrifugation due to the presence of more CPS (Lin et al., 2009). SEM images also clearly differentiated mucoidic over non-mucoidic cells (Figure 4). Possible reasons for variations in CPS content observed among isolates could be due to mutations, gene displacement, or curing of plasmids that occurred spontaneously or were induced by environmental factors. Thakker et al. (2006) isolated non-virulent *K. pneumoniae* strains from environmental samples. Frequently, virulence factors exchange genes though horizontal gene transfer and weakly associate with the genome, consequently losing virulent genes. In turn, *K. pneumoniae* is diverse and heteromorphic in nature (Brisse et al., 2009). Hence, it can be speculated here that the strains isolated must have undergone genotypic changes and in turn there were phenotypic modifications.

Production of 2,3-BD by all the 14 isolates checked by test tube cultivations showed a broad range of titers, irrespective of mucoidity of the strains (Table 1). Considering the 2,3-BD production rate, titer, and glucose consumption rate, five strains were selected from mucoidic and non-mucoidic background and subjected for batch fermentations under controlled conditions (Figure 5). Except the specific cell growth rate, 2,3-BD

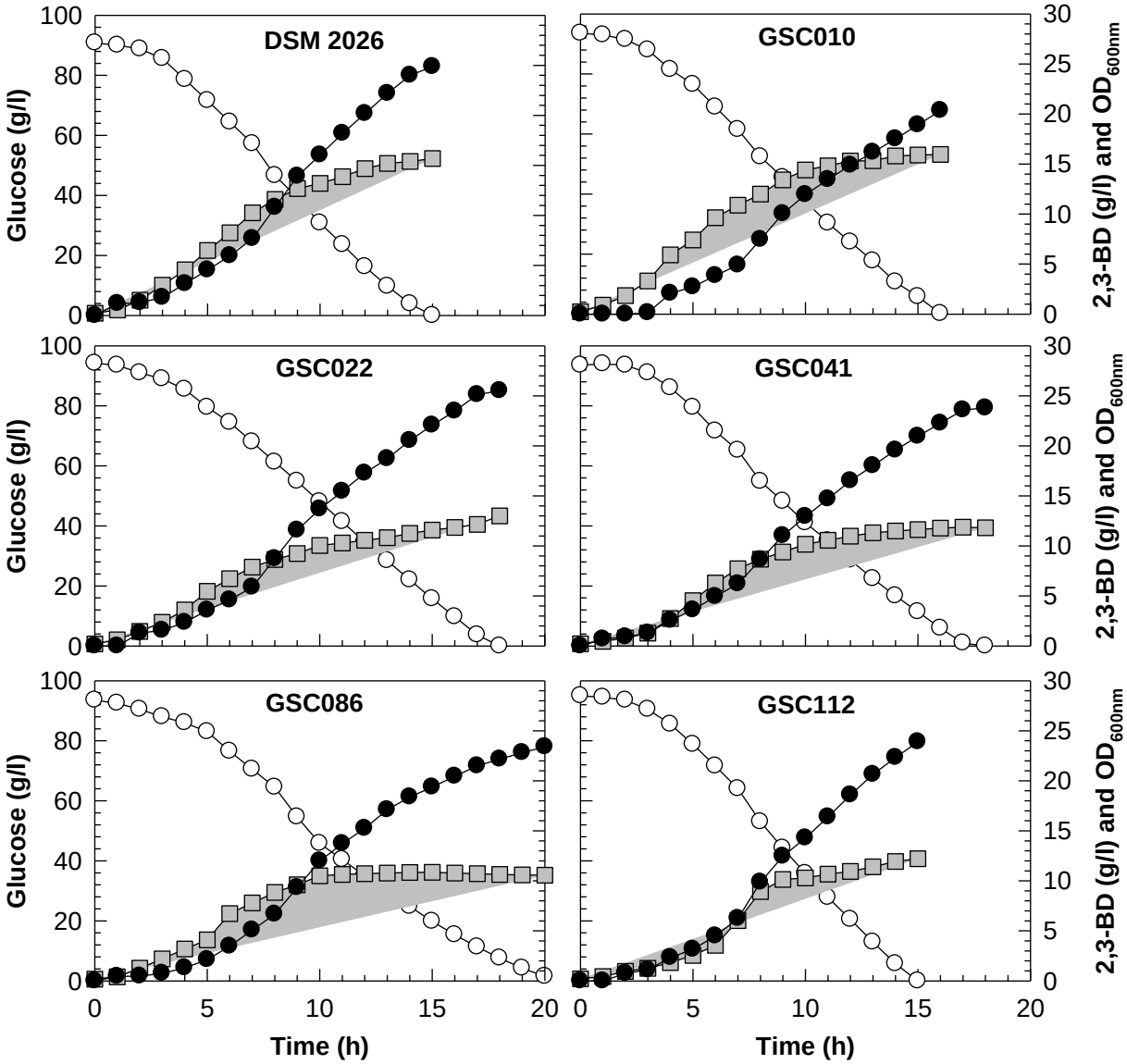


**Figure 5.** Time course profiles of different *K. pneumoniae* strains under batch fermentation. Closed circle, 2,3-BD; open circle, glucose; grey square, DCW.

titer, yield on glucose, and production rate of GSC112 were quite high and comparable to DSM 2026. The glucose consumption rate was recorded as the highest (6.33 g/l/h) among the other isolates tested (Table 2). Ability of the mucoid-free GSC112 did not compromise its 2,3-BD production and was selected undoubtedly as a suitable candidate to be evaluated further. It is also worth noting that GSC022 could also be highly competitive to GSC112 and the type strain (DSM 2026) except the prolonged fermentation time and 2,3-BD production rate. Constant glucose feeding under fed-batch mode GSC112 produced 2,3-BD at the maximum of 52 g/l with an overall 2,3-BD production rate of 0.54 g/l/h (Figure 6). Lactic acid was the competing byproduct during the fermentation of 2,3-BD. Mutating *ldhA*, which encodes lactate dehydrogenase, proved to show substantial reduction of lactic acid and thereby improved the titer of 2,3-BD in *Klebsiella* strains (unpublished data). This is the first report describing *K. pneumoniae* isolates that secrete less or no CPS and can serve as organisms of choice for the large-scale production of 2,3-BD. Pathogenicity studies of GSC112 for the presence of virulence factors would be required.

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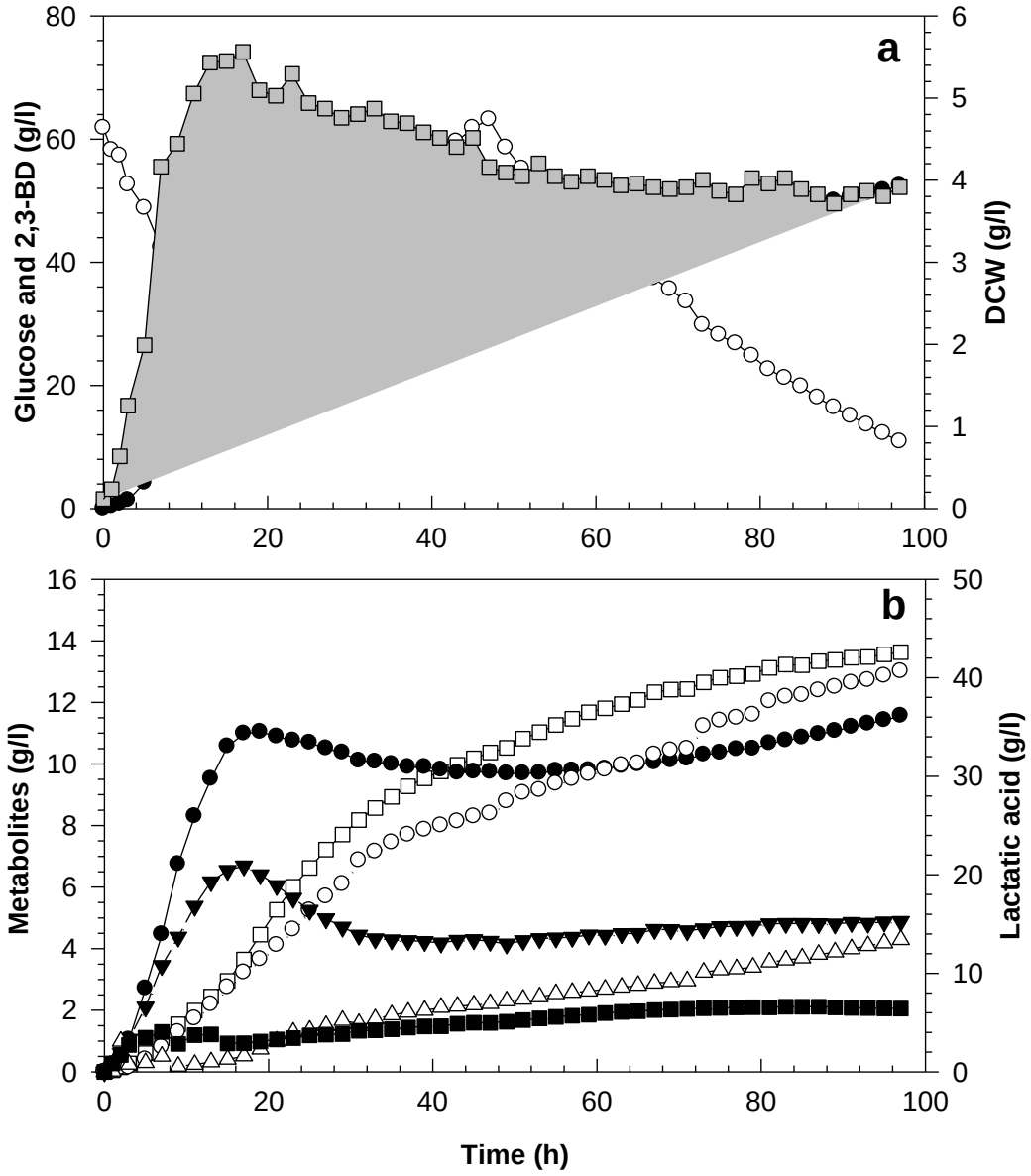


**Figure 6.** Fed-batch fermentation profiles of *K. pneumoniae* GSC112 on the production of 2,3-BD (a); Closed circle, 2,3-BD; open circle, glucose; grey square, DCW and metabolites production (b); open square, lactic acid; closed circle, ethanol; open circle, succinic acid; closed triangle down, formic acid; open triangle up, acetone; closed square, acetic acid.

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