Bioresource Technology 288 (2019) 121523

Contents lists available at ScienceDirect

Bioresource Technology

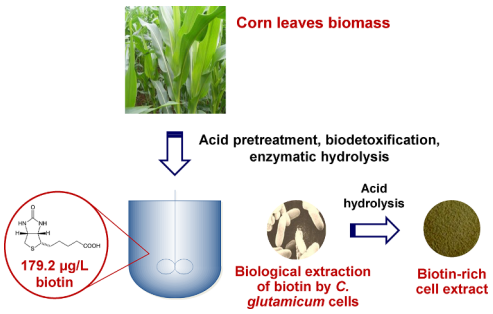
journal homepage: www.elsevier.com/locate/biortech

Microbial extraction of biotin from lignocellulose biomass and its application on glutamic acid production

Xushen Han[1], Li Li[1], Jie Bao*

State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Biotin
Corynebacterium glutamicum
BioYMN transporter
Lignocellulose
Glutamic acid production

ABSTRACT

Biotin (vitamin B_7) is an important nutrient for various fermentations. It is abundant in agricultural lignocellulose biomass and maintains stable in biorefinery processing chain including acid pretreatment, biodetoxification and saccharification. Here we show a microbial extraction of biotin from biotin-rich corn leaves hydrolysate. *Corynebacterium glutamicum* was found to have the highest biotin uptake capacity among different biotin auxotrophic microorganisms, and it was further significantly increased by overexpressing the *bioYMN* gene cluster encoding biotin transporter. Finally 250 folds greater biotin was extracted by recombinant *C. glutamicum* (303.8 mg/kg dry cell) from virgin corn leaves (1.2 mg/kg), which was far higher than that in commonly used fermentation additives including yeast extract (~2 mg/kg), molasses (~1 mg/kg) and corn steep liquor (~0.75 mg/kg). The biotin extracted from corn leaves was successfully applied to glutamic acid fermentation. This is the first report on microbial extraction of biotin from lignocellulose biomass and fermentation promotion application.

1. Introduction

Biotin (vitamin B_7) is an essential coenzyme in carboxylation reactions. Proper biotin addition significantly promotes the production performance of various microbial fermentations for glutamic acid (Cao et al., 2014; Eggeling et al., 2001; Wen et al., 2018), lysine (Coello et al., 2002; Chen et al., 2019), arginine (Zhan et al., 2019), succinic acid (Chen et al., 2000), ethanol (Alfenore et al., 2002; Nikolic et al., 2009), and lactic acid (Kwon et al., 2000). Chemical production of biotin generally takes multiple steps with toxic reagents use and generates chiral isomers of d-biotin (Seki et al., 2004; Seki, 2006; Zhong et al., 2012). Microbial production of biotin by fermentation (Saito et al., 2000; Seki, 2006; Survase et al., 2006) or direct extraction from eggs, yeast, liver etc. (Alban et al., 2000) is very costly. When biotin is used for fermentation enhancement, the biotin-rich additives

* Corresponding author.
E-mail address: jbao@ecust.edu.cn (J. Bao).
[1] These authors are equally contributed to this work.

https://doi.org/10.1016/j.biortech.2019.121523
Received 22 April 2019; Received in revised form 17 May 2019; Accepted 18 May 2019
Available online 21 May 2019
0960-8524/

are usually used instead of pure biotin such as molasses, corn steep liquor and yeast extract (Chen et al., 2019; Wen et al., 2018).

Recently we identified that agricultural lignocellulose biomass such as corn stover contains surprisingly high biotin and the biotin maintains stable in biorefinery processing chain including acid pretreatment, biodetoxification and enzymatic hydrolysis (Wen et al., 2018). Biotin in plant cells is either bound to organelles as cofactors of carboxylases for fatty acid synthesis, amino acid catabolism, and carbohydrate gluconeogenesis (Alban et al., 2000; Baldet et al., 1992), or present in cytosol as free biotin reserve (Baldet et al., 1993). Biotin content of plant leaves is generally higher than that of stems because of the active de novo fatty acid synthesis in plastids of leaves (Rawsthorne, 2002; Wen et al., 2018).

This study showed a microbial extraction of biotin from corn leaves after dry acid pretreatment, biodetoxification, and enzymatic hydrolysis and its application for glutamic acid production enhancement. The biotin uptake capacity of several biotin auxotrophic microbes was assayed including *Saccharomyces cerevisiae*, *Corynebacterium glutamicum*, and *Trichosporon cutaneum*. *C. glutamicum* was found to behave a strong ability to accumulate intracellular biotin, and its biotin uptake capacity was further enhanced by genetic modification. The biotin-rich cell extract of *C. glutamicum* was used as biotin additive in glutamic acid production and the production performance was significantly promoted. This is the first report on microbial extraction of biotin from lignocellulose biomass and fermentation promotion application.

2. Materials and methods

2.1. Materials and reagents

Corn stover was harvested in September of 2016 from Weifang, Shandong Province of China. After collection, it was air-dried, anatomical fractionated into stems and leaves, and then milled by a hammer crusher (10 mm diameter apertures) respectively. The corn leaves contained 26.9% cellulose, 20.0% xylan, 17.9% lignin, 12.4% ash on a dry weight basis assayed using NREL protocols (Sluiter et al., 2005, 2012). The details of cellulase (Han et al., 2018), pure biotin, corn steep liquor, biotin assay medium, *Lactobacilli* broth, and other reagents were shown in Supplemental Materials.

2.2. Strains, media and seed culture

All the strains used were shown in Table 1. *Escherichia coli* DH5α was used as the host for plasmid construction. *Amorphotheca resinae* ZN1 was used for biodetoxification of the acid pretreated corn leaves (Zhang et al., 2010). *Corynebacterium glutamicum* S9114 (Wen et al., 2018), *Saccharomyces cerevisiae* DQ1 (Zhang et al., 2011), *Saccharomyces cerevisiae* XH7 (Li et al., 2016; Liu et al., 2018), and *Trichosporon cutaneum* ACCC 20271 (Wang et al., 2016) were biotin auxotrophic microorganisms and used for biotin extraction from corn leaves hydrolysate. The origin, media and seed culture of the strains were shown in Supplemental Materials.

2.3. Overexpression of bioYMN gene cluster in C. Glutamicum

Plasmids, primers and strains used were listed in Table 1. The gene cluster *bioYMN* encoding biotin biosynthesis protein (*bioY*), cobalt ABC transporter ATP-binding cassette protein (*bioM*) and ABC transporter permease (*bioN*) were amplified from the genome of *C. glutamicum* S9114 by PCR. The plasmid pH36mob was constructed using the synthesized strong promoter *H36* (Yim et al., 2013) into pTRCmob by Generay Biotech Co., Shanghai, China. The expression plasmid pH36mob-*bioYMN* was constructed by inserting the *bioYMN* gene cluster into the plasmid pH36mob at EcoRI and XbaI, and it was consequently transformed into *C. glutamicum* S9114 according to Ruan et al. (2015) and Schaefer et al. (1994) by electroporation (Gene Pulser Xcell, Biorad, Hercules, CA, USA) to obtain the recombinant strain *C. glutamicum* pH36mob-*bioYMN*.

The cassette *H36-bioYMN* was integrated into the genome by deleting the *CGS9114_RS02700* gene. A 1,000-bp fragment at the upstream and the other 1,000-bp fragment at the downstream of *CGS9114_RS02700* were amplified. The two fragments were overlapped with the *H36-bioYMN* fragment and inserted into the suicide plasmid pK18mobsacB to construct the integration plasmid pK18mobsacB-Δ*CGS9114_RS02700*::(*H36-bioYMN*) using the HB-infusion Seamless Cloning Kit (HanBio, Shanghai, China). The plasmid was introduced into *C. glutamicum* S9114 by electroporation and the substitution of *CGS9114_RS02700* with *H36-bioYMN* was conducted by homologous recombination (Wang et al., 2018). The final recombinant *C. glutamicum* Δ*CGS9114_RS02700*::(*H36-bioYMN*) was obtained and verified by sequencing. The recombinant *C. glutamicum* Δ*CGS9114_RS02700*::(*H36-bioYMN*) was cultured by adaptive evolution in corn leaves hydrolysate every 12 h at 10% (v/v) inoculum size, 30 °C, pH 7.0, 200 rpm until the cell growth and glucose consumption were stable.

2.4. Pretreatment, biodetoxification and hydrolysate preparation

Corn leaves was dry acid pretreated (Han and Bao, 2018; Zhang et al., 2011), biodetoxified (Zhang et al., 2010; He et al., 2016) and enzymatically hydrolyzed at 15% (w/w) solids loading, cellulase dosage of 10 mg/g cellulose, pH 5.5, 50 °C for 48 h. The hydrolyzed slurries were centrifuged at 10,000 × g for 15 min, and the supernatant was autoclaved at 115 °C for 20 min and sterile filtrated. The prepared corn leaves hydrolysate contained 38.1 g/L glucose with no furfural and HMF. No free liquid was generated in dry acid pretreatment and biodetoxification processes, and therefore biotin was effectively preserved in the prepared corn leaves hydrolysate.

2.5. Biotin extraction and biotin-rich cell extract preparation

The seeds of *S. cerevisiae* DQ1, *S. cerevisiae* XH7 and *T. cutaneum* ACCC 20271, *C. glutamicum* S9114 and recombinants *C. glutamicum* were respectively inoculated at 10% (v/v) inoculum size into 250 mL flasks containing 30 mL corn leaves hydrolysate with supplement of 5 g/L $(NH_4)_2SO_4$ at 30 °C, 200 rpm for biotin extraction. For *C. glutamicum* S9114 and its recombinants, the pH was adjusted to 7.0 using 5 M NaOH solution before inoculation and maintained by supplement of 20% (w/w) urea solution. Samples were taken and centrifuged at 15,000 × g for 5 min, in which the supernatant was used for extracellular biotin and glucose assay, and the cells were used for intracellular biotin assay. Biotin uptake capacity was calculated by multiplying the intracellular biotin content (μg/g dry cell weight, DCW) and dry cell concentration (g/L). Cell growth was assayed according to optical density at 600 nm (OD_{600}), and the dry cell concentration (g/L) was calculated by multiplying OD_{600} and 0.45 g/L. The yield of biotin extraction was obtained using biotin uptake capacity divided by the initial biotin content in the corn leaves hydrolysate.

The biotin extraction using *C. glutamicum* was scaled up in a 3 L fermentor (Biotech-3BG-4, Baoxing Co., China) at 30 °C, 600 rpm, and aeration rate of 1.4 vvm. One liter of corn leaves hydrolysate was used with supplement of 5 g/L $(NH_4)_2SO_4$ at the inoculum size of 10% (v/v) and pH of 7.0 by automatic feeding of 20% (w/v) ammonium hydroxide solution. Biotin uptake capacity and biotin extraction yield were obtained as above mentioned. The harvested cells were hydrolyzed using 3% (w/w) H_2SO_4 at 10% (w/w) cells content, 121 °C for 30 min. The slurries after acid hydrolysis were centrifuged at 15,000 × g for 5 min and the supernatant was used as biotin-rich extract.

2.6. Glutamic acid production

Glutamic acid production was performed in a 3 L fermentor with a working volume of 1 L. The seed of *C. glutamicum* S9114 was prepared and inoculated at 10% (v/v) inoculum size, 30 °C, and the aeration rate of 1.4 vvm. The pH was maintained at 7.0 by automatic feeding of 20%

Table 1
Strains, plasmids and primers used.

Strains	Characteristics	Sources
Escherichia coli DH5α	Host for plasmid construction	Lab stock
Amorphotheca resinae ZN1	Biodetoxification fungus isolated in our lab	Zhang et al. (2010)
Saccharomyces cerevisiae DQ1	Biotin-deficient	Zhang et al. (2011)
Saccharomyces cerevisiae XH7	Biotin-deficient	Liu et al. (2018)
Trichosporon cutaneum ACCC 20,271	Biotin-deficient	Wang et al. (2016)
Corynebacterium glutamicum S9114	Biotin-deficient	Purchased from SIIM
C. glutamicum pH36mob-*bioYMN*	*C. glutamicum* S9114 harboring *bioYMN* expression plasmid pH36mob-*bioYMN*	This study
C. glutamicum Δ*CGS9114_RS02700*::(*H36-bioYMN*)	Integration of *H36-bioYMN* into *CGS9114_RS02700* locus of *C. glutamicum* S9114 genome	This study
Plasmids	**Characteristics**	**Sources**
pTRCmob	Shuttle expression vector, P*trc* promoter, Km^R	Yim et al. (2013)
pH36mob	Expression vector, P_{h36} promoter	This study
pH36mob-*bioYMN*	Expression vector containing *bioYMN* gene cluster	This study
pK18mobsacB	Mobilizable vector for integration, Km^R, sacB	Wang et al. (2018)
pK18mobsacB-Δ*CGS9114_RS02700*::(*H36-bioYMN*)	Plasmid for integration of the expression cassette *H36-bioYMN*	This study
Primers	**Sequences (5′–3′)**	
bioYMN-F	GAATTCTTGTTGAACACTGTTCAGGTGTAT	
bioYMN-R	TCTAGATTAATCGCCGGCACCA	
H36-bioYMN-F	ATTTTTTCTCCCCGTCAACCACCATCAAACAGGA	
H36-bioYMN-R	CAACAACTGAAAGGGCTACCGCTTCTGCGTTCTGATTTAATCTGTAT	
CGS9114_RS02700-up-F	ACAGCTATGACATGATTACGCGCCGAAAACCTGCTGTCTAT	
CGS9114_RS02700-up-R	GGTGGTTGACGGGGAGAAAAAATG	
CGS9114_RS02700-down-F	GCAGAAGCGGTAGCCCTTTCAGTTGT	
CGS9114_RS02700-down-R	TTGCATGCCTGCAGGTGGCGAAGCCGAGGAAGACA	

Note: The underline indicates the digestion site, and the double underline indicates the homologous sequence of over-lap PCR. Km^R, kanamycin resistance.

(w/v) ammonium hydroxide and the dissolved oxygen (DO) was maintained in the range of 10%-40% by changing the agitation rate. The fermentation medium contained 120 g/L of glucose, 2.5 g/L of urea, 0.6 g/L of $MgSO_4$, 1.5 g/L of KH_2PO_4, 2.0 mg/L of $MnSO_4$, 2.0 mg/L of $FeSO_4$. Proper biotin-rich extract was added into the fermentation medium. Corn steep liquor and pure biotin were also added into the medium respectively as the control of the biotin-rich extract.

2.7. Analytical methods

The biotin content was determined by a microbiological method using *Lactobacillus plantarum* ATCC 8014 (Wen et al., 2018). Glutamic acid and glucose were measured using the SBA-40D biosensor (Shandong Academy of Sciences, China). Acetic acid, furfural, HMF and xylose were analyzed using a HPLC system (Shimadzu, Kyoto, Japan) equipped with a LC-20AD pump, a RID-10A refractive index detector, and a Aminex HPX-87H column (Bio-Rad, CA, USA) at 65 °C and 0.6 mL/min 5 mM H_2SO_4.

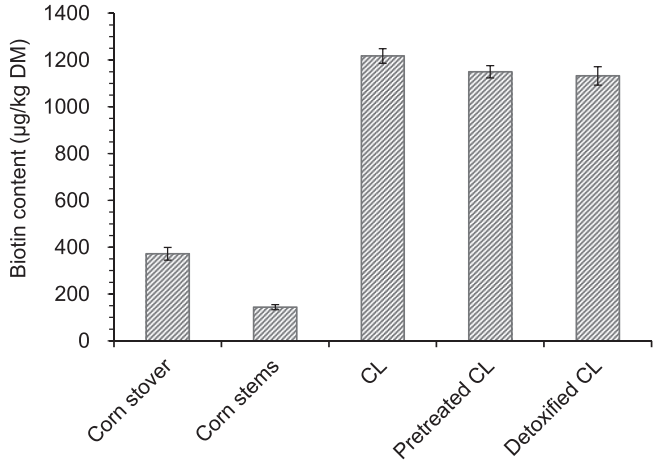


Fig. 1. Biotin distribution in different parts of corn stover and its stability in biorefinery processing chain. CL, corn leaves. The biotin content was assayed in duplicate. The data shown here was taken from the average of the two experiments, and the error bars represented the standard deviation of the two experiments.

3. Results and discussion

3.1. Microbial uptake capacity of biotin from corn leaves hydrolysate

We manually separated corn stover into stems and leaves, then measured the biotin content of each part and also the corn leaves at different biorefining processing stages (Fig. 1). Corn leaves contained

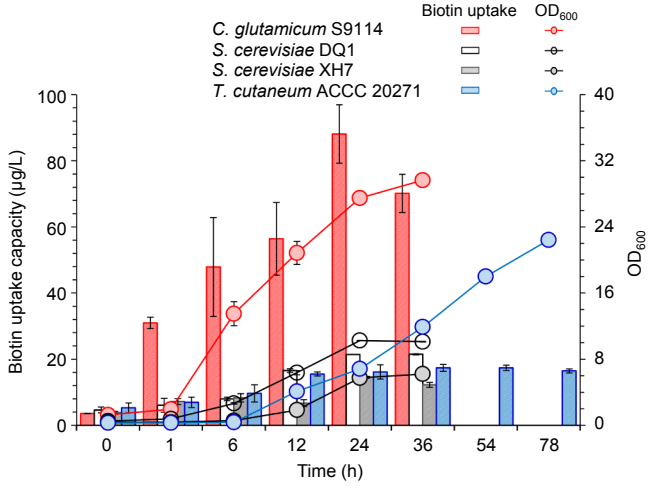


Fig. 2. Microbial extraction of biotin from corn leaves hydrolysate by biotin auxotrophic strains. Biotin uptake capacity was calculated by multiplying the intracellular biotin content (μg/g dry cell weight) and dry cell concentration (g/L). The experiments were carried out in flasks in duplicate. The data shown here was taken from the average of the two experiments, and the error bars represented the standard deviation of the two experiments.

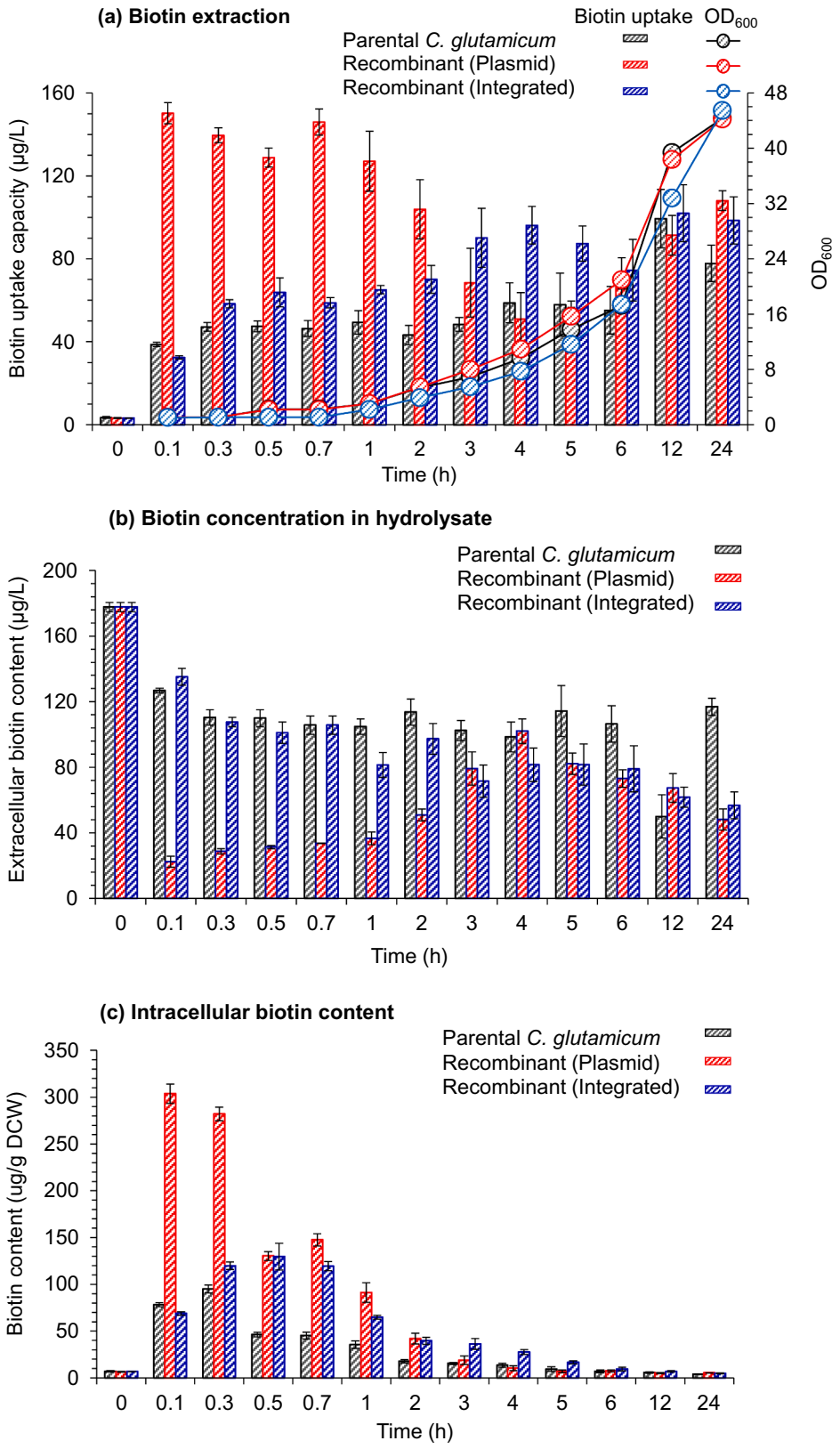


Fig. 3. Biotin extraction from corn leaves hydrolysate by the parental *C. glutamicum* S9114 and two recombinants. (a) Biotin extraction; (b) Biotin concentration in hydrolysate; (c) Intracellular biotin content. Recombinant (Plasmid), *C. glutamicum* pH36mob-*bioYMN*; Recombinant (Integrated), *C. glutamicum* Δ*CGS9114_RS02700*::(*H36-bioYMN*). The experiments were carried out in 3L fermentors in duplicate. The data shown here was taken from the average of the two experiments, and the error bars represented the standard deviation of the two experiments.

1217.4 μg biotin per kilogram of dry matter (DM), approximately 8.5 folds greater than corn stems (143.9 μg/kg DM), three folds greater than the unclassified corn stover (371.6 μg/kg DM), and 42 folds greater than corn grain (29 μg/kg DM). The biotin content of corn leaves maintained stable during biorefinery processing: 1149.5 μg/kg DM after pretreatment, 1132.0 μg/kg DM after biodetoxification vs.

1217.4 μg/kg DM of the virgin corn leaves (Fig. 1). The pretreated and biodetoxified corn leaves were enzymatically hydrolyzed at 15% (w/w) solids loading and the obtained corn leaves hydrolysate contained 179.2 μg/L biotin, equivalent to 1015.5 μg/kg biotin from the pretreated and biodetoxified corn leaves (dry base) with approximately 10% loss. The result indicates that most of the free and bound biotin in corn leaves was liberated into the hydrolysate after biorefinery processing.

We assayed the biotin uptake capacity of several biotin auxotrophic strains (Fig. 2). *C. glutamicum* S9114 extracted more biotin even at the low cell density within one hour (biotin uptake capacity of 31.0 μg/L), compared with the limited uptake of *S. cerevisiae* DQ1 (6.1 μg/L), *S. cerevisiae* XH7 (7.2 μg/L), and *T. cutaneum* ACCC 20271 (6.9 μg/L). The biotin extraction by *C. glutamicum* S9114 reached 88.1 μg/L with the extraction yield of 49.2% after 24 h' culture, significantly higher than *S. cerevisiae* DQ1 (21.4 μg/L, 11.9%), *S. cerevisiae* XH7 (14.6 μg/L, 8.1%) and *T. cutaneum* ACCC 20,271 (16.1 μg/L, 9.0%). The result indicates that *C. glutamicum* was the proper microorganism for biotin uptake from corn leaves hydrolysate.

3.2. Increasing biotin uptake by overexpressing biotin transporter gene in C. Glutamicum

We overexpressed the *bioYMN* gene cluster encoding biotin transporter proteins in *C. glutamicum* S9114 to strengthen the biotin transport and uptake capacity (Brune et al., 2012; Schneider et al., 2012). The plasmid harboring the expression cassette *H36-bioYMN* was introduced into *C. glutamicum* S9114 to construct the recombinant *C. glutamicum* pH36mob-*bioYMN* with overexpression in plasmid, then the cassette was integrated into the genome of *C. glutamicum* S9114 to construct the integrated recombinant *C. glutamicum* Δ*CGS9114_RS02700*::(*H36-bioYMN*) by homologous recombination.

Biotin uptake capacity of the two recombinants was then assayed in the fermenter (Fig. 3). The biotin uptake capacity of the recombinant *C. glutamicum* pH36mob-*bioYMN* (in plasmid form) reached 150.2 μg/L with the extraction yield of 83.8% within just six minutes (0.1 h), and maintained stable for 2 h (Fig. 3a). Afterwards the intracellular biotin released from cytosol and led to the increased biotin content in the hydrolysate (Fig. 3b). The recombinant *C. glutamicum* Δ*CGS9114_RS02700*::(*H36-bioYMN*) (in integrated form) also showed the improved biotin uptake ability compared to the parental strain, but not as high as the recombinant in plasmid form due to the less copy number of the *bioYMN* gene in the integrated form. In a conclusion, the highest biotin extraction yield (83.8%) and intracellular biotin content (303.8 mg biotin per kg of dry cell weight) were obtained at a short time (0.1 h) by using *C. glutamicum* pH36mob-*bioYMN* (in plasmid form) (Fig. 3c), which concentrated 250 folds of biotin from virgin corn leaves (1.2 mg biotin per kg of dry corn leaves). According to the previous reports about the biotin distribution in the biotin auxotrophic microbes

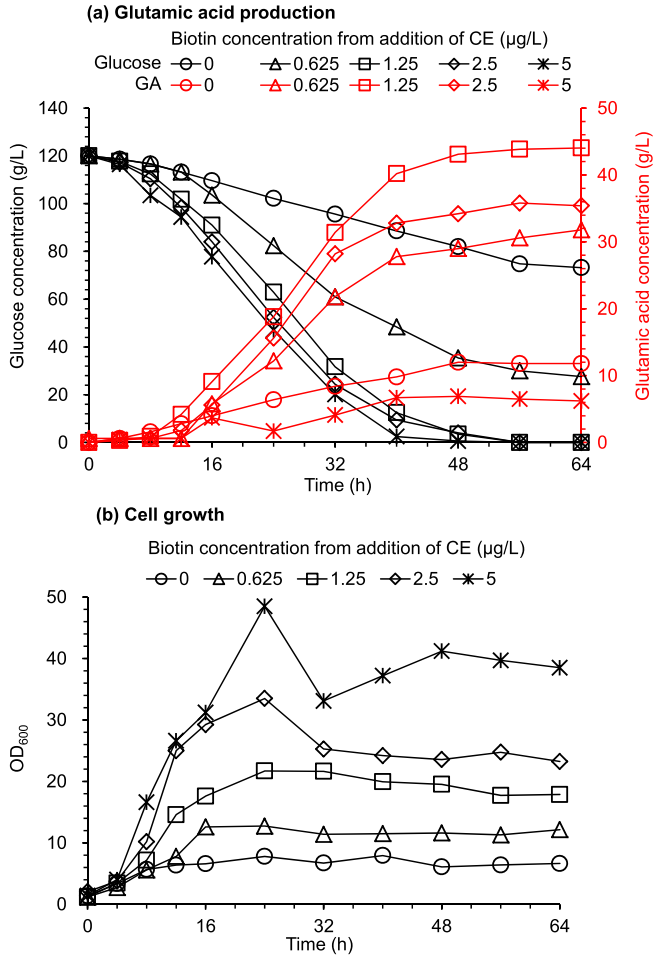


Fig. 4. Application of biotin-rich cell extract (CE) in glutamic acid (GA) production. (a) Glutamic acid production; (b) Cell growth. Biotin-rich cells were acid hydrolyzed as the cell extract and added at the biotin concentration of 0, 0.625, 1.25, 2.5 and 5 μg/L as the sole biotin source (except 0.6 μg/L biotin from the seed culture).

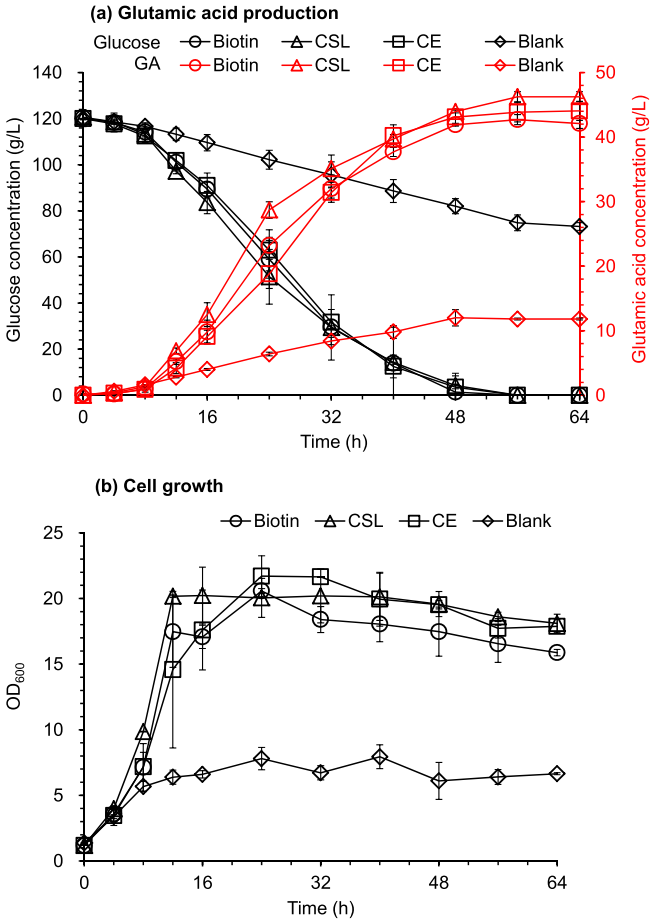


Fig. 5. Comparison of biotin-rich cell extract (CE), corn steep liquor (CSL) and pure biotin in glutamic acid (GA) production. (a) Glutamic acid production; (b) Cell growth. CE, CSL and pure biotin were supplemented into glutamic acid production respectively at the biotin concentration of 1.25 μg/L as the sole biotin source (except 0.6 μg/L biotin from the seed culture). Blank, no biotin was added except 0.6 μg/L biotin from the seed culture. The experiments were carried out in duplicate. The data shown here was taken from the average of the two experiments, and the error bars represented the standard deviation of the two experiments.

Saccharomyces cerevisiae (Rogers and Lichstein, 1969) and *Lactobacillus plantarum* (Waller and Lichstein, 1965), we predicted that only a little part of the biotin in *C. glutamicum* was bound to the enzyme, and most of the biotin was present in free form.

Overall, the biotin content in the *C. glutamicum* cells was two orders of magnitude greater than that of the commonly used fermentation additives including yeast extract (~2 mg/kg), molasses (~1 mg/kg), and corn steep liquor (CSL) (~0.75 mg/kg). Furthermore, the glucose (36.0 g/L) in corn leaves hydrolysate was well preserved and could be used for various fermentations after the cells were recovered for biotin extraction.

3.3. Promotion of biotin-rich cell extract on glutamic acid production

The harvested *C. glutamicum* cells with high biotin content (303.8 mg/kg) were acid hydrolyzed to release intracellular biotin as biotin-rich additive for fermentation applications. Glutamic acid production by the industrial strain *C. glutamicum* S9114 was used to test the function of biotin-rich extract. Fig. 4 shows that the biotin level of the medium reached 5.6 μg/L when the cell extract (CE) was added at biotin concentration of 5 μg/L (0.6 μg/L biotin from the seed culture), leading to the fast glucose consumption and cell growth but the suppressed glutamic acid generation (only 6.9 g/L). When the biotin-rich extract was added at 2.5 μg/L of biotin, the glucose consumption and the cell growth were slowed, and the glutamic acid generation reached 35.8 g/L. When the biotin from cell extract was adjusted to 1.25 μg/L, the maximum glutamic acid titer of 44.1 g/L was obtained. When the biotin from cell extract was further reduced to 0.0625 μg/L, glutamic acid generation declined to 31.8 g/L with 27.6 g/L of the residual glucose. If no biotin-rich extract was added with the only biotin from the seed culture (0.6 μg/L), a reduced glutamic acid titer of 11.8 g/L was obtained. The phenomenon was consistent with the general glutamic acid production in which the absence of biotin restricted the cell growth and the excessive biotin inhibited glutamic acid secretion, while only the “suboptimal” biotin level (1–5 μg/L) was appropriate for glutamic acid production (Eggeling et al., 2001).

We further compared the addition of biotin rich extract with the commercial additives corn steep liquor (CSL) and pure biotin at the same biotin level of 1.25 μg/L (equivalent to 4.11 mg/L of biotin-rich cell extract, 1.67 g/L of CSL, and 1.25 μg/L pure biotin, respectively) in glutamic acid production. Fig. 5 shows that the three scenarios with the same biotin level were essentially the same in cell growth and glutamic acid production: 44.1 g/L, 46.3 g/L, and 42.1 g/L, respectively. The results clearly suggest that the biotin extracted from corn leaves could be used as effective biotin nutrient additives in glutamic acid production process. The biotin-rich extract also has industrial potentials for promotion of other biotin-required fermentations.

4. Conclusion

Dry acid pretreatment and biodetoxification well preserved the biotin of corn leaves. *C. glutamicum* cells efficiently extracted biotin from corn leaves hydrolysate, and overexpression of the biotin transporter gene *bioYMN* significantly increased biotin uptake rate and yield. The biotin extracted from corn leaves by *C. glutamicum* was successfully applied to glutamic acid production. This is the first report on microbial extraction of biotin from lignocellulose biomass and fermentation promotion application.

Acknowledgement

This research was supported by the Natural Science Foundation of China (31961133006).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2019.121523.

References

Alban, C., Job, D., Douce, R., 2000. Biotin metabolism in plants. Annu. Rev. Plant Biol. 51, 17–47.
Alfenore, S., Molina-Jouve, C., Guillouet, S.E., Uribelarrea, J.L., Goma, G., Benbadis, L., 2002. Improving ethanol production and viability of *Saccharomyces cerevisiae* by a vitamin feeding strategy during fed-batch process. Appl. Microbiol. Biotechnol. 60, 67–72.
Baldet, P., Alban, C., Axiotis, S., Douce, R., 1992. Characterization of biotin and 3-methylcrotonyl-coenzyme a carboxylase in higher plant mitochondria. Plant Physiol. 99, 450–455.
Baldet, P., Alban, C., Axiotis, S., Douce, R., 1993. Localization of free and bound biotin in cells from green pea leaves. Arch. Biochem. Biophys. 303, 67–73.
Brune, I., Gotker, S., Schneider, J., Rodionov, D.A., Tauch, A., 2012. Negative transcriptional control of biotin metabolism genes by the TetR-type regulator BioQ in biotin-auxotrophic *Corynebacterium glutamicum* ATCC 13032. J. Biotechnol. 159, 225–234.
Cao, Y., Duan, Z., Shi, Z., 2014. Effect of biotin on transcription levels of key enzymes and glutamate efflux in glutamate fermentation by *Corynebacterium glutamicum*. World J. Microbiol. Biotechnol. 30, 461–468.
Chen, K., Li, J., Ma, J., Jiang, M., Wei, P., Liu, Z., Ying, H., 2000. Succinic acid production by *Actinobacillus succinogenes* using hydrolysates of spent yeast cells and corn fiber. Bioresour. Technol. 102, 1704–1708.
Chen, Z., Liu, G., Zhang, J., Bao, J., 2019. A preliminary study on L-lysine fermentation from lignocellulose feedstock and techno-economic evaluation. Bioresour. Technol. 271, 196–201.
Coello, N., Montiel, E., Concepcion, M., Christen, P., 2002. Optimisation of a culture medium containing fish silage for L-lysine production by *Corynebacterium glutamicum*. Bioresour. Technol. 85, 207–211.
Eggeling, L., Krumbach, K., Sahm, H., 2001. L-glutamate efflux with *Corynebacterium glutamicum*: why is penicillin treatment or tween addition doing the same? J. Mol. Microbiol. Biotechnol. 3, 67–68.
Han, X., Bao, J., 2018. General method to correct the fluctuation of acid based pretreatment efficiency of lignocellulose for highly efficient bioconversion. ACS Sustain. Chem. Eng. 6, 4212–4219.
Han, X., Hong, F., Liu, G., Bao, J., 2018. An approach of utilizing water-soluble carbohydrates in lignocellulose feedstock for promotion of cellulosic L-lactic acid production. J. Agric. Food Chem. 66, 10225–10232.
He, Y., Zhang, J., Bao, J., 2016. Acceleration of biodetoxification on dilute acid pretreated lignocellulose feedstock by aeration and the consequent ethanol fermentation evaluation. Biotechnol. Biofuels 9, 19.
Kwon, S., Lee, P.C., Lee, E.G., Chang, Y.K., Chang, N., 2000. Production of lactic acid by *Lactobacillus rhamnosus* with vitamin-supplemented soybean hydrolysate. Enzyme Microb. Technol. 26, 209–215.
Li, H., Shen, Y., Wu, M., Hou, J., Jiao, C., Li, Z., Liu, X., Bao, X., 2016. Engineering a wild-type diploid *Saccharomyces cerevisiae* strain for second-generation bioethanol production. Bioresour. Bioproc. 3, 51.
Liu, G., Zhang, Q., Li, H., Qureshi, A.S., Zhang, J., Bao, X., Bao, J., 2018. Dry biorefining maximizes the potentials of simultaneous saccharification and co-fermentation for cellulosic ethanol production. Biotechnol. Bioeng. 115, 60–69.
Nikolic, S., Mojovic, L., Rakin, M., Pejin, D., 2009. Bioethanol production from corn meal by simultaneous enzymatic saccharification and fermentation with immobilized cells of *Saccharomyces cerevisiae* var. *ellipsoideus*. Fuel 88, 1602–1607.
Rawsthorne, S., 2002. Carbon flux and fatty acid synthesis in plants. Prog. Lipid Res. 41, 182–196.
Rogers, T.O., Lichstein, H.C., 1969. Characterization of the biotin transport system in *Saccharomyces cerevisiae*. J. Bacteriol. 100, 557–564.
Ruan, Y., Zhu, L., Li, Q., 2015. Improving the electrotransformation efficiency of *Corynebacterium glutamicum* by weakening its cell wall and increasing the cytoplasmic membrane fluidity. Biotechnol. Lett. 37, 2445–2452.
Saito, I., Honda, H., Kawabe, T., Mukumoto, F., Shimizu, M., Kobayashi, T., 2000. Comparison of biotin production by recombinant *Sphingomonas sp.* under various agitation conditions. Biochem. Eng. J. 5, 129–136.
Schaefer, A., Tauch, A., Jaeger, W., Kalinowski, J., Thierbach, G., Puehler, A., 1994. Small mobilizable multi-purpose cloning vectors derived from the *Escherichia coli* plasmids pK18 and pK19: Selection of defined deletions in the chromosome of *Corynebacterium glutamicum*. Gene 145, 69–73.
Schneider, J., Peters-Wendisch, P., Stansen, K.C., Gotker, S., Maximow, S., Kramer, R., Wendisch, V.F., 2012. Characterization of the biotin uptake system encoded by the biotin-inducible *bioYMN* operon of *Corynebacterium glutamicum*. BMC Microbiol. 12, 6.
Seki, M., Hatsuda, M., Mori, Y., Yoshida, S., Yamada, S., Shimizu, T., 2004. A practical synthesis of (+)-biotin from L-cysteine. Chem. Eur. J. 10, 6102–6110.
Seki, M., 2006. Biological significance and development of practical synthesis of biotin. Med. Res. Rev. 26, 434–482.
Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., 2005. Determination of ash in biomass. NREL/TP-510-42622. National Renewable Energy Laboratory, Golden, CO.
Sluiter, A., Hames, B., Ruiz, R., Scarlat, C., Sluiter, J., Templeton, D., Crocker, D., 2012.

Determination of structural carbohydrates and lignin in biomass. NREL/TP-510-42618. National Renewable Energy Laboratory, Golden, CO.
Survase, S.A., Bajaj, I.B., Singhal, R.S., 2006. Biotechnological production of vitamins. Food Technol. Biotechnol. 44, 381–396.
Waller, J.R., Lichstein, H.C., 1965. Biotin transport and accumulation by cells of *Lactobacillus plantarum*. I. General properties of the system. J. Bacteriol. 90, 843–852.
Wang, J., Gao, Q., Zhang, H., Bao, J., 2016. Inhibitor degradation and lipid accumulation potentials of oleaginous yeast Trichosporon cutaneum using lignocellulose feedstock. 218, 892–901.
Wang, Y., Cao, G., Xu, D., Fan, L., Wu, X., Ni, X., Zhao, S., Zheng, P., Sun, J., Ma, Y., 2018. A novel *Corynebacterium glutamicum* L-glutamate exporter. Appl. Environ. Microbiol. 84, e02691–17.
Wen, J., Xiao, Y., Liu, T., Gao, Q., Bao, J., 2018. Rich biotin content in lignocellulose biomass plays the key role in determining cellulosic glutamic acid accumulation by *Corynebacterium glutamicum*. Biotechnol. Biofuels 11, 132.
Yim, S., An, S.J., Kang, M., Lee, J., Jeong, K.J., 2013. Isolation of fully synthetic promoters for high-level gene expression in *Corynebacterium glutamicum*. Biotechnol. Bioeng. 110, 2959–2969.
Zhan, M., Kan, B., Dong, J., Xu, G., Han, R., Ni, Y., 2019. Metabolic engineering of *Corynebacterium glutamicum* for improved l-arginine synthesis by enhancing NADPH supply. J. Ind. Microbiol. Biotechnol. 46, 45–54.
Zhang, J., Zhu, Z., Wang, X., Wang, N., Wang, W., Bao, J., 2010. Biodetoxification of toxins generated from lignocellulose pretreatment using a newly isolated fungus, *Amorphotheca resinae* ZN1, and the consequent ethanol fermentation. Biotechnol. Biofuels 3, 26.
Zhang, J., Wang, X., Chu, D., He, Y., Bao, J., 2011. Dry pretreatment of lignocellulose with extremely low steam and water usage for bioethanol production. Bioresour. Technol. 102, 4480–4488.
Zhong, Z., Wu, X., Chen, F., 2012. Recent progresses in total synthesis of (+)-biotin. Chin. J. Org. Chem. 32, 1792–1802.