Minireview

Metabolic engineering of microorganisms for the production of multifunctional non-protein amino acids: γ-aminobutyric acid and δ-aminolevulinic acid

Anping Su,[1] Qijun Yu,[1] Ying Luo,[1] Jinshui Yang,[1] iD
Entao Wang[2] and Hongli Yuan[1,*]

[1]State Key Laboratory of Agrobiotechnology and Key Laboratory of Soil Microbiology, Ministry of Agriculture, College of Biological Sciences, China Agricultural University, No.2 Yuanmingyuan West Road,Haidian District, Beijing, 100193, China.
[2]Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, 11340, Mexico.

Summary

Gamma-aminobutyric acid (GABA) and delta-aminolevulinic acid (ALA), playing important roles in agriculture, medicine and other fields, are multifunctional non-protein amino acids with similar and comparable properties and biosynthesis pathways. Recently, microbial synthesis has become an inevitable trend to produce GABA and ALA due to its green and sustainable characteristics. In addition, the development of metabolic engineering and synthetic biology has continuously accelerated and increased the GABA and ALA yield in microorganisms. Here, focusing on the current trends in metabolic engineering strategies for microbial synthesis of GABA and ALA, we analysed and compared the efficiency of various metabolic strategies in detail. Moreover, we provide the insights to meet challenges of realizing industrially competitive strains and highlight the future perspectives of GABA and ALA production.

Introduction

Belonging to the non-protein amino acid, both gamma-aminobutyric acid (GABA) and delta-aminolevulinic acid (ALA) are not coded by DNA, but have great similar and important multifunction in overall metabolism of plants, animals and even humans (Sasaki, 2002; Kang *et al.*, 2012; Rashmi *et al.*, 2018). In plants, GABA is an endogenous signalling molecule involved in various physiological and biochemical processes that plays an important role in the promotion of plant growth and development, and relates to the plant metabolism in response to adverse environmental conditions (Fait *et al.*, 2008; Li *et al.*, 2016b). Similarly, ALA as the first product of tetrapyrrole biosynthesis can effectively control the biosynthesis of chlorophyll and plays vital functions in plant photosynthesis and cellular energy metabolism (Wu *et al.*, 2018). Based on these characters, GABA and ALA have been used as safe, environmentally compatible and biodegradable novel plant growth regulator (PGR) in agriculture (Meng *et al.*, 2016; Li *et al.*, 2019). In animals and humans, GABA as an important inhibitory neurotransmitter in mammalian nervous systems has great potential to be used as an anti-diabetic, anti-hypertensive, relaxation and immunity enhancing molecule (Yuan and Alper, 2019). Likewise, ALA as the second generation of photosensitizer with good curative effects and less side-effects has been widely applied as photodynamic medicine for cancer therapy and tumour localizing (Thunshelle *et al.*, 2016; Inoue, 2017). In addition, GABA and ALA are also used as widely available additives in food, feed, fertilizer and cosmetics (Kang *et al.*, 2017; Diez-Gutiérrez *et al.*, 2020).

Due to their wide function and significant application, GABA and ALA have become popular value-added products with increasing demand, and their anticipated global market size will be increased up to 50 million USD (https://www.qyresearch.com/index/detail/2135154/global-gaba-aminobutyric-acid-market) and 222.1 million USD (https://www.qyresearch.com/index/detail/1932502/global-5-aminolevulinic-acid-market) by 2026 respectively.

However, the current international market of GABA and ALA still relies heavily on their chemical synthesis, which is a complicated procedure with pollution, high price, low production and potential unsafety to animals and humans (Noh *et al.*, 2017; Kim *et al.*, 2018). To conquer those problems, microbial syntheses of GABA and ALA have become an inevitable trend based on their resource-conserving, environment-friendly and economically sustainable characteristics. Moreover, the rapid development of synthetic biology and metabolic engineering has accelerated the overproduction of GABA and ALA by microbes to constantly satisfy the demands of the growing global market.

Therefore, in view of the fact that GABA and ALA have some common characteristics, this paper reviewed the recent progress of metabolic engineering of microorganisms for their production, especially focusing on the advances of metabolic engineering strategies of microbes in GABA and ALA syntheses. Furthermore, the challenges and prospects of industrial production of GABA and ALA were also analysed with highlighting their potential application as novel PGR in agriculture.

## Biosynthesis pathways of GABA and ALA

GABA and ALA are 4-carbon and 5-carbon non-protein amino acids respectively. And the only difference in their chemical structures is that ALA has one more carbonyl group (C = O) than GABA. Both GABA and ALA have two alternative biosynthesis pathways, and their overall biosynthesis pathways are shown in Fig. 1.

GABA is naturally biosynthesized via glutamic acid decarboxylation (GAD) pathway (Choi *et al.*, 2015) or putrescine (Puu) pathway (Jorge *et al.*, 2016). In GAD pathway, GABA is synthesized by decarboxylation of glutamate via glutamate decarboxylase (GAD) (Fig. 1). GAD is a pyridoxal 5′-phosphate (PLP)-dependent enzyme composed of six identical subunits, which have strict substrate specificity towards glutamate and is the only key rate-limiting enzymes during the GABA biosynthesis (Yu *et al.*, 2019). In some bacteria, GAD has two isoforms named GadA and GadB (Wu *et al.*, 2017; Lyu *et al.*, 2018). Additionally, the *gadC* gene encodes the GABA antiporter (Soma *et al.*, 2017). GAD pathway is common and usually exists in *Lactobacillus* spp., *Escherichia coli* and *Listeria monocytogenes* (Diez-Gutiérrez *et al.*, 2020). In Puu pathway, the precursor putrescine can be converted into GABA in two sequential reactions catalysed by putrescine transaminase (PatA) and γ-aminobutyraldehyde dehydrogenase (PatD) (Jorge *et al.*, 2016) (Fig. 1). Puu pathway is not common and has been reported in *E. coli* (Cha *et al.*, 2014) and *Aspergillus oryzae* (Akasaka *et al.*, 2018). Lastly, GABA is decomposed to succinic semialdehyde (SSA) by GABA aminotransferase (GabT) and to succinate thereafter by SSA dehydrogenase (GabD). Additionally, the *gabP* and *gabC* genes encode the GABA-specific importer and antiporter respectively (Fig. 1) (Shi *et al.*, 2017).

Likewise, ALA is naturally biosynthesized via C4 or C5 pathway (Li *et al.*, 2016a). In the C4 pathway (the Shemin pathway), ALA is produced through one-step catalysis of ALA synthase (ALAS, encoded by *hemA* or *ALAS* gene) under the presence of essential cofactor PLP from the condensation of succinyl-CoA and glycine (Fig. 1). In this pathway, ALAS is the only rate-limiting key enzyme occurring in mammalian, fungi (like yeasts) (Hara *et al.*, 2019) and purple non-sulfur photosynthetic bacteria (like *Rhodobacter sphaeroides*) (Tangprasittipap *et al.*, 2007). In the C5 pathway, ALA is produced through catalysis by three enzymes, including glutamyl-tRNA synthetase (GluRS, encoded by *gltX*), a NADPH-dependent glutamyl-tRNA reductase (GluTR, encoded by *hemA*) and glutamate-1-semialdehyde aminotransferase (GSA-AT, encoded by *hemL*) (Fig. 1). In this pathway, glutamate-1-semialdehyde is an unstable intermediate that is quickly converted to ALA by the action of GSA-AT, and the GluTR and GSA-AT have a synergistic effect in this procedure. Moreover, GluTR is the rate-limiting enzyme in the C5 pathway and is strictly regulated by feedback inhibition of haem. C5 pathway exists in higher plants, algae and various bacteria (like *E. coli* and *Corynebacterium glutamicum*) (Yu *et al.*, 2015; Noh *et al.*, 2017), while, in very few microorganisms, like *Euglena gracilis* and *Arthrobacter globiformis* (Yang and Hoober, 1995), the C4 and C5 pathways coexist. In addition, intracellular membrane transport protein RhtA for threonine and

Fig. 1. Overall metabolic pathways for ALA and GABA biosynthesis and transformation/degradation (drawn based on references). Dark green is Puu pathway, and light green is GAD pathway for GABA synthesis. Red is C5 pathway, and orange is C4 pathway for ALA synthesis. Genes abbreviated are as follows: *zwf*, glucose-6-phosphate carboxylase; *pgi*, glucose-6-phosphate isomerase; *pfkA*, 6-phosphofructokinase; *gapA*, glyceraldehyde 3-phosphate dehydrogenase; *pgk*, phosphoglycerate kinase; *ldhA*, L-lactate dehydrogenase; PDH, pyruvate dehydrogenase complex (E1, E2 and E3 components encoded by *aceE*, *aceF* and *lpd* genes respectively); *pta*, phosphate acetyltransferase; *acsA*, acetyl-CoA synthetase; *ackA*, acetate kinase; *ppc*, phosphoenolpyruvate carboxylase; *pyc*, pyruvate carboxylase; *pck*, phosphoenolpyruvate carboxykinase; *gltA*, citrate synthase; *sucAB*, α-ketoglutarate dehydrogenase; *sucCD*, succinyl-CoA synthetase; *sdh*, succinate dehydrogenase; *mdh*, malate dehydrogenase; *gdh*, glutamate dehydrogenase; *hemA*, glutamyl-tRNA reductase (C5 pathway); *hemA*, ALA synthase (C4 pathway); *hemL*, glutamate-1-semialdehyde aminotransferase; *hemB*, δ-aminolevulinic acid dehydratase; *rhtA*, inner membrane transporter for L-threonine; *GAD* (*gadA*, *gadB*), glutamate decarboxylase; *gabT*, γ-aminobutyric acid transaminase; *gabD*, succinate semialdehyde dehydrogenase; *gabC*, Glu/GABA antiporter; *speC*, L-ornithine decarboxylase; *patA*, putrescine transaminase; *patD*, γ-aminobutyraldehyde dehydrogenase; *gabP*, GABA-specific importer.

(A)

(B)
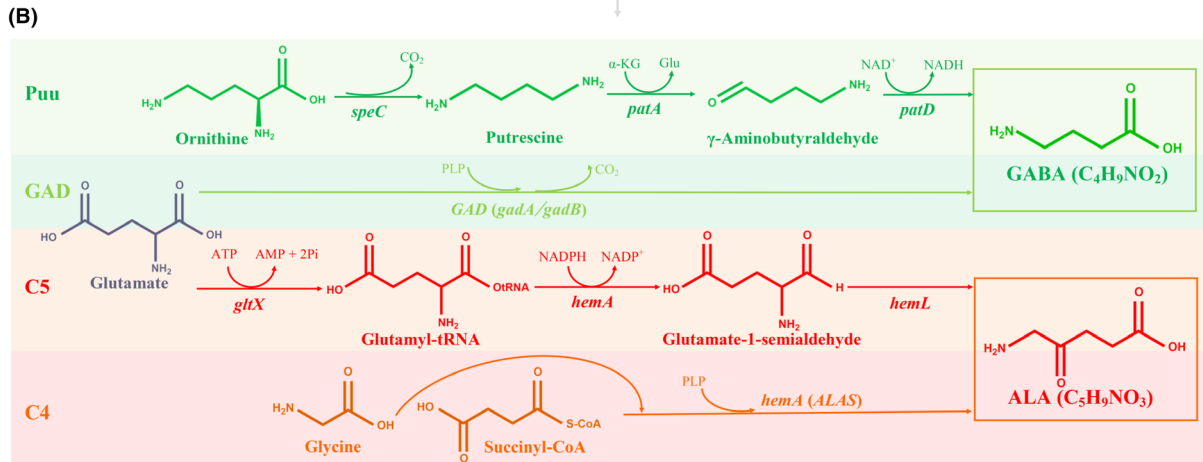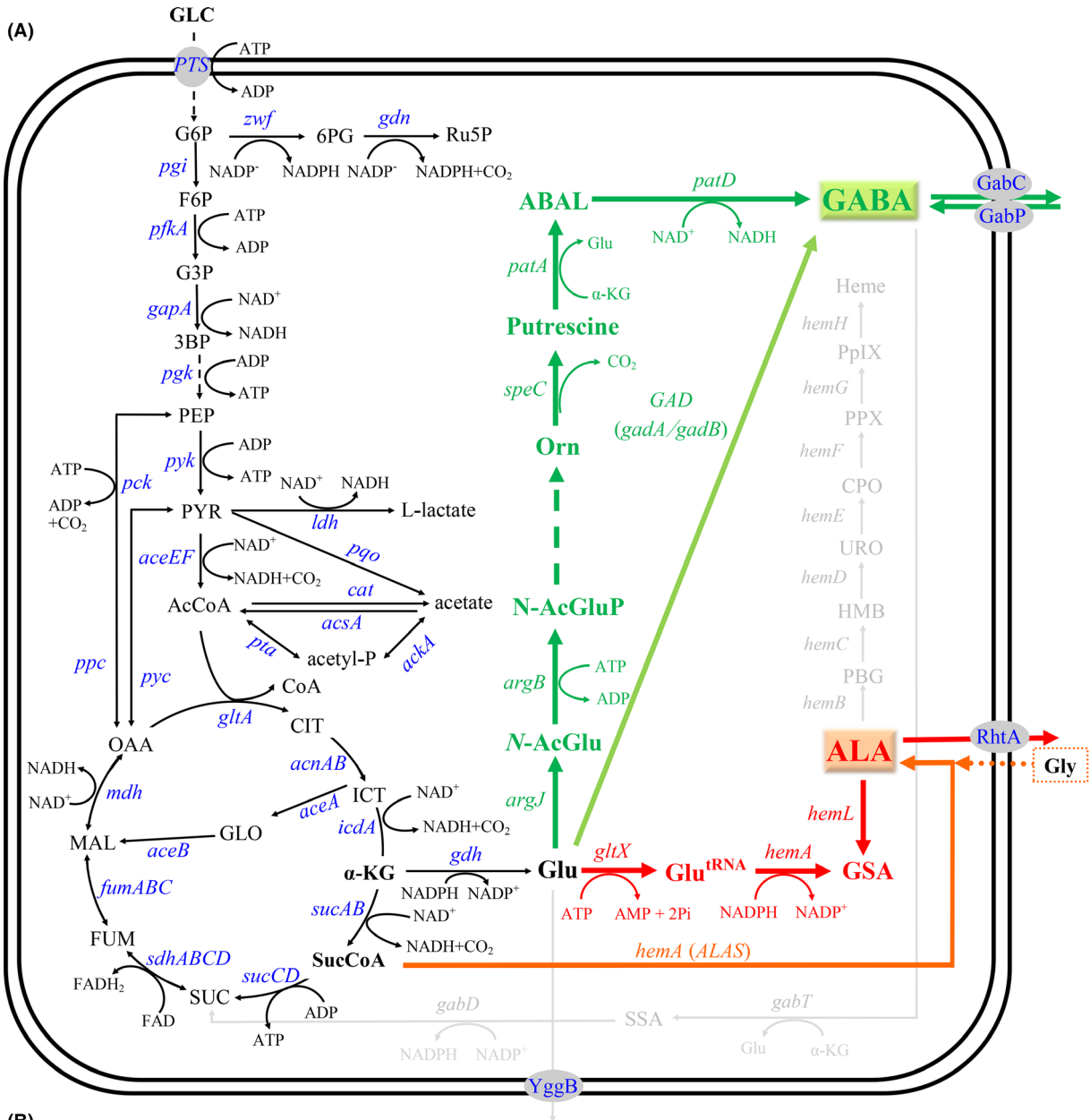
homoserine exporting also approved the ALA efflux (Kang *et al.*, 2011), and two molecules of ALA are condensed into one molecule of porphobilinogen (PBG) by ALA dehydratase (ALAD, encoded by *hemB*) that was further converted into other haem compounds (Fig. 1) (Su *et al.*, 2019).

Accordingly, GAD and C4 pathways are related to one key enzyme with common cofactor PLP, while Puu and C5 pathways are related to multiple key enzymes. On the other hand, GAD and C5 pathways share the common metabolic routes at the early stage (from glycolysis to TCA) and common precursor glutamate. Since GABA and ALA have common metabolic pathway, their metabolic engineering strategies are comparable and can inspire each other.

Comparative analysis of metabolic engineering strategies for GABA and ALA biosyntheses

Microbial fermentation has obvious advantages of low cost, no chemical residue and high yield, and is an ideal way to produce value-added compounds (Yuan and Alper, 2019). GABA and ALA are emerging value-added non-protein amino acids with great significance to realize their high yield by microbial fermentation. Initially, efforts for increasing the yields focused on natural producers and usually performed through random mutagenesis and optimizing fermentation conditions. Recently, remarkable efforts have been made to improve the yield of biosynthetic GABA and ALA, through natural or engineered strains (Choi *et al.*, 2015; Cui *et al.*, 2019; Zhang *et al.*, 2019; Zhang *et al.*, 2020). We screened the development in recent five years from the representative and authoritative journals, and summarized their metabolic engineering strategies in detail, which include every metabolic engineering step and its effectiveness, as well as the synthetic pathway, substrate, final titre, biomass, fermentation time, form and scale, as well as the calculated yields based on the substrate (Tables 1 and 2).

To further analyse the metabolic engineering strategies and its effectiveness of GABA and ALA biosyntheses, the proportion and distribution of host strains were counted (Fig. 2A), and the metabolic engineering strategy-related genes were summarized and analysed (Fig. 2B). Moreover, the improved titre times of each metabolic strategy in different host strains were calculated and compared (Fig. 2C).

Diversity of GABA- and ALA-producing microbes

For GABA biosynthesis, *Lactobacillus* spp. are the most common and efficient natural producers (Table 1 and Fig. 2A up), which are usually isolated from traditional fermented products and present food safety character;

hence, their GABA product has better application prospect and market value (Tajabadi *et al.*, 2015; Cui *et al.*, 2020). Among the *Lactobacillus* spp., *L. brevis* is the most frequently reported producer with naturally high GABA productivity (Lyu *et al.*, 2017; Lyu *et al.*, 2018; Gong *et al.*, 2019). However, due to the lack of effective genetic manipulation in *Lactobacillus* spp., *E. coli* and *C. glutamicum* are still accounted for a large proportion in the current studies on metabolic engineering (Table 1 and Fig. 2A up). As for ALA biosynthesis, relatively few kinds of host strains have been researched (Table 2 and Fig. 2A), which may be due to the fact that natural ALA high-yielding microbes are rarely discovered. Similar to the GABA production, *E. coli* and *C. glutamicum* still are the most studied chassis in ALA production (Fig. 2A) due to their well-characterized genetics and advanced metabolic engineering tools.

Metabolic engineering strategies for improving the GABA and ALA production

Figure 2B, C obviously showed that the biggest increase in gene manipulation is amplifying the key pathway genes. For GABA synthesis, GAD pathway is common in the producing strains, in which *GAD* or *gadB* gene plays a key role and this gene usually from *Lactobacillus* spp. has been used for metabolic engineering. On the other hand, Jorge *et al.* (2016) first reported that the heterologous expression of *patA* and *patD* from *E. coli* in a putrescine producer *C. glutamicum* enabled it to produce GABA via the Puu pathway. Then, they further developed the Puu route to synthesis GABA in *C. glutamicum* and obtained up to the titre of 63.2 g l^{-1}, reached the highest volumetric productivity for fermentative GABA production (1.34 g l^{-1} h^{-1}) by the time of the report (Jorge *et al.*, 2017). And this glucose-based GABA production via Puu route presented a higher volumetric productivity than that via the GAD pathway. For ALA synthesis, *ALAS* (*hemA*) usually from *R. sphaeroides* and *Rhodopseudomonas palustris* in C4 pathway, and *hemA* usually from the mutated *Sazlmonella arizonae* and *hemL* from *E. coli* in C5 pathway have been employed for metabolic engineering. In addition, the optimization of the metabolic flux was also achieved by releasing the feedback regulation of key enzymes (*gadT* and *gadD* for GABA; *hemB*, *hemD*, *hemF* for ALA); increasing export of target amino acids out of the cells (*gadC* for GABA; *rhtA* for ALA); and common reducing metabolic fluxes of TCA cycle (*sucA*, *sucCD*) to downregulate competitive pathway. Besides, the upstream common key genes (*pyc* and *gapA*) also related their overproduction. In this case, promoter and RBS engineering strategies are most applied to balance and regulate gene expression levels. Moreover, from Tables 1

Table 1. Metabolic engineering of microorganisms for the production of GABA.

Host strain	Metabolic engineering strategies	Titre (g l⁻¹)	Pathway	Fermentation results	Yield (g g substrate⁻¹)	Substrate	References
C. glutamicum	*E. coli* wild-type *gadB*↑ with strong synthetic P$_{H36}$ promoter	0.34	GAD	38.6 g l⁻¹; 0.54 g l⁻¹ h⁻¹; OD:76.6; 72 h; 5 l	0.40	Glucose	Choi *et al.* (2015)
	E. coli gadB mutant (Glu89Gln/Δ452-466)↑ with strong synthetic P$_{H36}$ promoter	5.89					
	Add optimal biotin concentration: 50 μg l⁻¹	6.32					
	Adjusting pH 7 to 5	8.34					
	Fed-batch cultivations (pH 6)	38.6					
L. plantarum	*GAD*↑	0.18	GAD	1.14 g l⁻¹; 60 h SF	0.02	MSG	Tajabadi *et al.* (2015)
	Optimization of GABA production conditions (WT)	0.74					
	Optimization of GABA production conditions (*GAD*↑)	1.14					
C. glutamicum	Δ*argF*, Δ*argR*, *speC*↑, *argF$_{21}$*↑, *patA*↑(*E. coli*), *patD*↑(*E. coli*)	5.3	Puu	8.0 g l⁻¹; DCW:15 g l⁻¹; 26 h; SF	0.20	Glucose	Jorge *et al.* (2016)
	Δ*cgmA*	5.1					
	Δ*snaA*	5.7					
	Modified CGXII medium	6.6					
	Δ*gabTDP* operon	8.0					
B. methanolicus	*gadB*↑(*E. coli*); *gad*st↑ (*S. thermosulfidooxidans*)	0.03; 0.03	GAD	9.0 g l⁻¹; DCW: 47.5 g l⁻¹; 31 h; 3 l	-	Methanol	Irla *et al.* (2017)
	gadB↑, 50 for 10–12 h and 37 to 24 h	0.41					
	gadst↑, pH shift from 6.5 to 4.6 after 27 h	9.0					
C. glutamicum	Δ*argF*, Δ*argR*, Δ*snaA*, Δ*gabTDP*, *speC*↑, *argF$_{21}$*↑, *patDA*↑	9.2	Puu	63.2 g l⁻¹; 69 h; 1 l	0.24	Glucose	Jorge *et al.* (2017)
	The *odha* and *odhI* genes were replaced by the alleles (*odhA*^{TTG}, *odhI*^{T15A})	9.8					
	Δ*yggb*, Δ*cgma*	9.8					
	gapA↑; *gapA*↑, *pyc*↑; *gapA*↑, *arg*$^{ba49v/M54V}$↑	8.6; 7.8; 8.9					
	Δ*pyc*	10					
	Cg3170↑	8.1					
	Fed-batch	63.2					
E. coli	TA3000 (*gdhA*↑, *gadB*↑), TA4024 (*gdhA*↑, *gadB*↑, *gadC*↑)	1.8	GAD	4.8 g l⁻¹; 32 h; SF	0.28	Glucose	Soma *et al.* (2017)
	TA4024, TA4076 (*gdhA*↑, *gadB*↑, *gadC*↑, *sucA* control unit) TA4077 (*gdhA*↑, *gadB*↑, *gadC*↑, *sucA* control unit, *pyc*↑)	1.54; 1.54; 3.86					
	TA4053 combined a *sucA aceE* control unit in TA4077	4.66					
	IPTG was added at 6 h	4.8					
L. brevis	Wild type	35.81	GAD	43.65 g l⁻¹; OD:6.5; 48 h; 5 l	0.69	MSG	Lyu *et al.* (2017)
	gadA↑	41.49					
	F$_o$F$_1$-ATPase-defective mutants	43.65					
E. coli	Opt *gadB*^{mut}↑(*L. lactis*)	306.65	GAD	308.26 g l⁻¹; 7 h; 5 l	0.70	Glutamate	Yang *et al.* (2018)
	Removed the C-plug of *gadC*	307.12					
	Molecular chaperones *Gro7*↑	307.4					
	Δ*gadA*, Δ*gadB*	308.26					
L. brevis	Native strain *L. brevis* CK	82.47	GAD	104.38 g l⁻¹; OD:6.5; 72 h; 5 l	0.56	MSG	Lyu *et al.* (2018)
	gadB↑, *gadC*↑, Fed-batch fermentation (0-24 h: pH 5.2, 35 ; 24-102 h: pH 4.4, 40)	104.38					
L. brevis	*L. brevis* ATCC 367	9.65	GAD	177.74 g l⁻¹; OD:13; 36 h; 3 l	1.19	MSG	Gong *et al.* (2019)
	Mutant strain with much higher expression level of *gadR*	11.62					
	pH-controlled, mixed-feed fermentation	177.74					

Table 1. (Continued)

Host strain	Metabolic engineering strategies	Titre (g l^{-1})	Pathway	Fermentation results	Yield (g g substrate^{-1})	Substrate	References
E. coli	Native *E. coli* K12	0.11	GAD	19.79 g l^{-1};	0.57	MSG	Yu *et al.*
	ΔgabT	0.32		DCW:0.85 g l^{-1};			(2019)
	ΔgabT, ΔgabP	0.55		33 h;			
	ΔgabT, ΔgabP, ΔpuuE	0.73		1 l SF			
	gadA↑, gadB↑, gadC↑	6.4					
	1 l SF fermentation, adjust the pH to 4.2	19.79					

;: and; ;: or; ↑: gene overexpression; ↓: gene knockdown; Δ: gene knockout; opt: codon-optimized; MSG: l-monosodium glutamate; OD: OD$_{600}$; DCW: dry cell weight; SF: shake flask.

and 2, we can also see that the fermentation time of *E. coli* is shorter than that of *C. glutamicum*, but Fig. 2C showed the gene operation of *C. glutamicum* is more obviously effective than that of *E. coli* for improving the yield, whatever in GABA and ALA synthesis.

Optimization of GABA and ALA fermentation conditions

Up to now, the highest titre of GABA production by metabolic engineering is 308.26 g l^{-1} in *E. coli* through heterologously expressed *gadB* gene from *L. lactis* via GAD pathway (Yang *et al.*, 2018), while the highest titre of ALA bioproduction is 18.5 g l^{-1} in *C. glutamicum* through heterologously expressed *ALAS* (*hemA*) gene from *R. palustris* via C4 pathway (Chen *et al.*, 2020). However, these significant achievements realized by combining whole-cell biocatalysts using 3 M glutamate as substrate and 4 g l^{-1} glycine supply respectively. In fact, most of the GABA and ALA production contributed to the use of the complex medium in complicated cultivation process and continual feeding of the precursors (Feng *et al.*, 2016; Yang *et al.*, 2016; Li *et al.*, 2016a; Hara *et al.*, 2019; Zhu *et al.*, 2019; Chen *et al.*, 2020). On the one hand, whole-cell biocatalysts indeed remarkably enhance production, but depending on the amount of substrate and the number of cells uses. On the other hand, comparing with the C4 pathway, an obvious advantage of the C5 pathway is that it can achieve de novo synthesis of ALA just from glucose, and the C5 pathway is usually more efficient than the C4 pathway with the exogenous glycine or succinic acid supplement. Furthermore, we conclude that the two-stage pH and temperature control with substrate-feeding strategy is mostly applied in current fermentation optimization. However, even a good fermentation optimization in fermenter can only improve the yield by 3–4 times (Tables 1 and 2, Fig. 2C), and these strategies are undesirable for economical and sustainable industrial GABA and ALA production. Hence, higher titre still depends on the initial strain engineering transformation and it is still rather attractive to directly generate GABA and ALA from glucose. Indeed, the application of metabolic engineering strategies is the inexhaustible driving force for GABA and ALA sustained high yield. Meanwhile, the metabolic engineering can save production more costs than the complex fermentation processes do.

Future prospects: challenges and insights

Recently, GABA and ALA as novel PGRs attracted great concern. By regulating photosynthesis, epigenetic modifications, nutrient distribution, and growth and development, GABA and ALA can break seed dormancy, improve drought tolerance and water use efficiency, enhance temperature tolerance and nitrogen use efficiency, promote shoot elongation and generation, increase shoot and root mass, and ameliorate the plants adapting to adverse environmental stress (Small and Degenhardt, 2018). They can also be widely used in field crops (corn, soya bean, wheat), fruits, vegetables, ornamental plants and lawns, and these huge planting areas are the future application market of GABA and ALA (Wu *et al.*, 2018; Li *et al.*, 2019). In addition, GABA and ALA application could avoid the drug resistance in the traditional insecticides, fungicides and herbicides, could reduce over-reliance on synthetic fertilizer and pesticides, and could improve the utilization rate of fertilizers. Moreover, the application dosage of GABA and ALA is low, safe and environmentally friendly. Therefore, the future increase in agricultural economy will benefit greatly from the application of GABA and ALA. However, the PGRs only occupy a proportion of 4–5% in the current international pesticide market. Furthermore, the application of GABA and ALA in medicine, food and feed requires the biosynthesis products. So, it is very important to develop a safe, high-activity, green and sustainable production strategy, and it is the time to develop and realize the industrial production of GABA and ALA by microorganisms.

Theoretically, the thermodynamic maximum yields of GABA and ALA produced from glucose were calculated according to Dugar and Stephanopoulos (2011) and are

Table 2. Metabolic engineering of microorganisms for the production of ALA.

Host strain	Metabolic engineering strategies	Titre (g l⁻¹)	Pathway	Fermentation results	Yield (g g substrate⁻¹)	Substrate	References
C. glutamicum	Native *hemA*↑, *hemL*↑	0.07984	C5	1.79 g l⁻¹; OD:20; 144 h; SF	0.04	Glucose	Yu *et al.* (2015)
	hemA^M↑(mutated *Salmonella arizona*), *hemL*↑(*E. coli*)	0.42511					
	Reduced dissolved oxygen and Fe²⁺ concentrations	0.83					
	Add 0.3 mm maleic acid; add 0.1 mm of phthalic acid	1.289; 1.507					
	Add degradation ASV tag at the C-terminus of ALAD	1.79					
E. coli	Opt *hem1*↑(*S. cerevisiae*)	0.94	C4, C5	3.58 g l⁻¹ 48 h; SF	-	Glycine, succinic acid, Glucose	Li *et al.* (2016)
	Opt *hem1*↑ in various recombinant *E. coli*	1.609					
	Constructing T7 RNA polymerase gene on the plasmid	2.013					
	Δ*ispH* Δ*folK* and re-locating them to the plasmid	1.725					
	Auto-induction (IPTG-free) system	3.584					
C. glutamicum	Native C5 pathway	0.0251	C4	14.7 g l⁻¹; 16 h; SF	0.40 (glucose); 1.56 (glycine)	Glucose, glycine	Yang *et al.* (2016)
	SucCD↓	0.09287					
	Opt *hemA*↑(*R. capsulatus* SB1003)	7.6					
	Two-stage fermentation	12.46					
	rhtA↑(*E. coli*)	14.7					
C. glutamicum	Opt *hemA*↑(*R. sphaeroides*) with add 7.5 g l⁻¹ glycine	1.44	C4	7.53 g l⁻¹; OD:140; 33 h; 5 l	1.60 (glycine); 0.37 (glucose)	Glycine, glucose	Feng *et al.* (2016)
	Δ*pqo*, Δ*pta*, Δ*ackA*, Δ*cat* (acetate) and Δ*idhA* (lactate)	1.92					
	ppc↑	2.06					
	Δ*pbp1a*; Δ*pbp1b*; Δ*pbp2b* (HMW-pbps)	2.35; 2.61; 2.53					
	rhtA↑(*E. coli*)	3.14					
	Fed-batch culture	7.53					
E. coli	Native	0.01	C5	3.4 g l⁻¹; OD:18.5; 18 h; SF	0.28	Glucose	Noh *et al.* (2017)
	hemA^mut↑(*S. typhimurium*), *hemL*↑	0.74					
	Δ*sucA*	0.56					
	gltA↑	0.37					
	Varying the transcriptional strength of *aceA*	1.09					
	Induction timing was delayed from 0.8 to 5.0 of OD₆₀₀	3.4					
E. coli	*hemA* and *hemL* were integrated into chromosome with 98 copy number	2.72	C5	4.55 g l⁻¹; OD:24; 72 h; SF	~0.23	Glucose	Cui *et al.* (2019)
	Optimization of fermentation conditions	3.1					
	Add a degradation tag ssra to the C-terminus of ALAD	1.2					
	yaaA↑; *katG*↑	1.7; 2.8					
	Δ*recA* in MG136a (adaptive evolution of MG136)	4.55					
S. cerevisiae	Native	0.000058	C4	0.00136 g l⁻¹; 48 h; SF	Too low	Glucose, glycine	Hara *et al.* (2019)
	hem1↑	0.00022					
	hem1↑*Aco2*↑, add 5 mM glycine	0.00136					
E. coli	Assembled higher RBS of *hemA* and medium of *hemL*	2.41	C5	5.25 g l⁻¹; 0.16 g l⁻¹ h⁻¹; OD:16; 33 h; 3 l	0.15	Glucose	Zhang *et al.* (2019)
	ALA dehydratase was rationally regulated	2.68					
	Cofactor PLP↑	2.86					
	Δ*recA*, Δ*endA*	2.86					
	rhtA↑, *hemD*↑, *hemF*↑	3.77					
	Carrying a pH two-stage strategy	5.25					
E. coli	*E. coli* bw24	5.3	C4	11.5 g l⁻¹; OD:55.3; 22 h; 5 l	-	Glucose, glycine	Zhu *et al.* (2019)
	Kate↑	9.6					
	SodB↑	8.7					
	KatE↑, *sodB*↑	11.5					
E. coli	Chromosomal integration with 7 copies of *hemA^M* and *hemL*	0.1696	C5	1.997 g l⁻¹; OD:22; 42 h; 5 l	0.03	Glucose	Su *et al.* (2019)
	hemB↓	0.862					
	Fed-batch fermentation with engineered strain	1.997					
C. glutamicum	*hemA*↑ (*R. palustris* ATCC 17001)	3.8	C4	18.5 g l⁻¹; OD:177.2; 39 h; 5 l	-	Cassava bagasse, glycine	Chen *et al.* (2020)
	Replace the original RBS with relatively high translational activities RBS	4.4					
	Native *ppc*↑	3.243					
	ppc expression was optimized using RBSs	5.5					
	Fed-batch fermentation from glucose	16.3					
	Fed-batch fermentation from cassava bagasse	18.5					

Table 2. (Continued)

Host strain	Metabolic engineering strategies	Titre (g l^{-1})	Pathway	Fermentation results	Yield (g g substrate^{-1})	Substrate	References
C. glutamicum	Native	0.0053	C5	3.16 g l^{-1};	0.07	Glucose	Zhang *et al.* (2020)
	Endogenous *hemA*↑, *hemL*↑	0.0299		Biomass: 9 g l^{-1};			
	hemA^M↑(mutated *S. arizona*), *hemL*↑(*E. coli*)	0.51		64 h;			
	Constitutive overexpression of *hemA*^{M} and *hemL*	0.62		SF			
	pyc↑ and *ppc*↑ under P$_{tuf}$	0.68; 0.7					
	gltA was overexpressed under P$_{tuf}$	0.79					
	pcka$_{ms}$ driven by p$_{dapA}$ (weak promoter)	0.89					
	Native *icd* driven by P$_{tuf}$, P$_{sod}$ and p$_{dapA}$	1.01; 0.96; 0.92					
	gapA^M (*C. glutamcium*) was integrated into the genome driven p$_{dapA}$	1.28					
	Add 5 mg l^{-1} PLP	1.61					
	Cgl0788-Cgl0789 operon↑ (PLP synthase) under p$_{dapA}$	1.48					
	ODHC activity was decreased by 68.5%	1.78					
	Two-stage fermentation	1.93					
	odhA↓driven by P$_{CP\_2836}$	2.38					
	rhtA ↑ driven by P$_{tuf}$, P$_{sod}$; p$_{dapA}$	1.6; 1.8; 2.3					
	rhtA↑ in an IPTG-inducible manner	2.95					
	rhtA was inserted downstream of the *hmuO* promoter	3.16					

,: and; ;: or; ↑: gene overexpression; ↓: gene knockdown; Δ: gene knockout; opt: codon-optimized; OD: OD$_{600}$; DCW: dry cell weight; SF: shake flask.
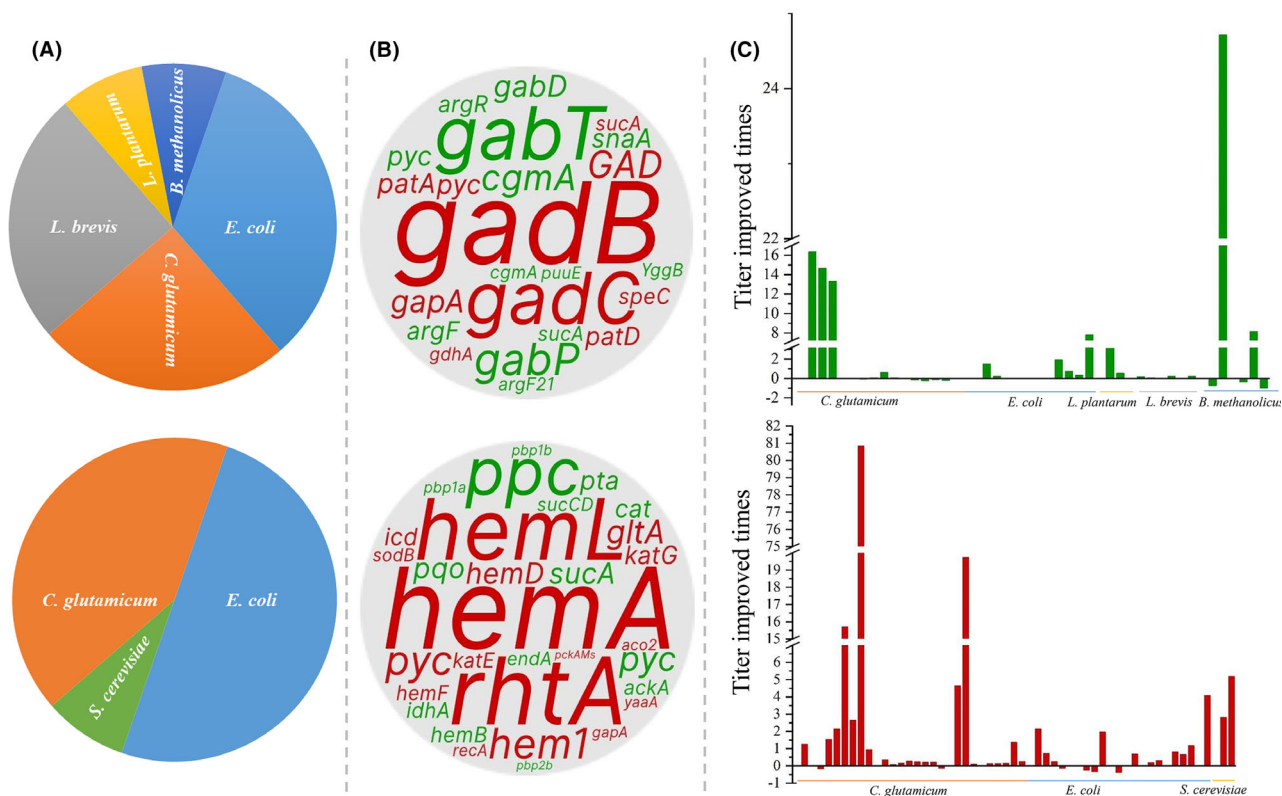
Fig. 2. Host strains, genes and efficiency of each metabolic strategy in different host strains for GABA (up) and ALA (down) production.
A. The proportion of host strain used for GABA and ALA production.
B. Word clouds of gene manipulation increased production, font size correlates with the frequency of occurrence, red means upregulation, and green means downregulation.
C. The improved titre times of each metabolic strategy in different host strains. Green means GABA production, and red means ALA production.

Table 3. Thermodynamic maximum yield of GABA and ALA.

Compounds	Chemical formula	MW g mol^{-1}	Degree of reduction	Thermodynamic maximum yield	
				g g glucose^{-1}	g g glutamate^{-1}
Glucose	$C_6H_{12}O_6$	180	24	–	–
Glutamate	$C_5H_9NO_4$	147	18	1.09	–
GABA	$C_5H_9NO_3$	103	18	0.76	1.4
ALA	$C_4H_9NO_2$	131	20	0.87	–
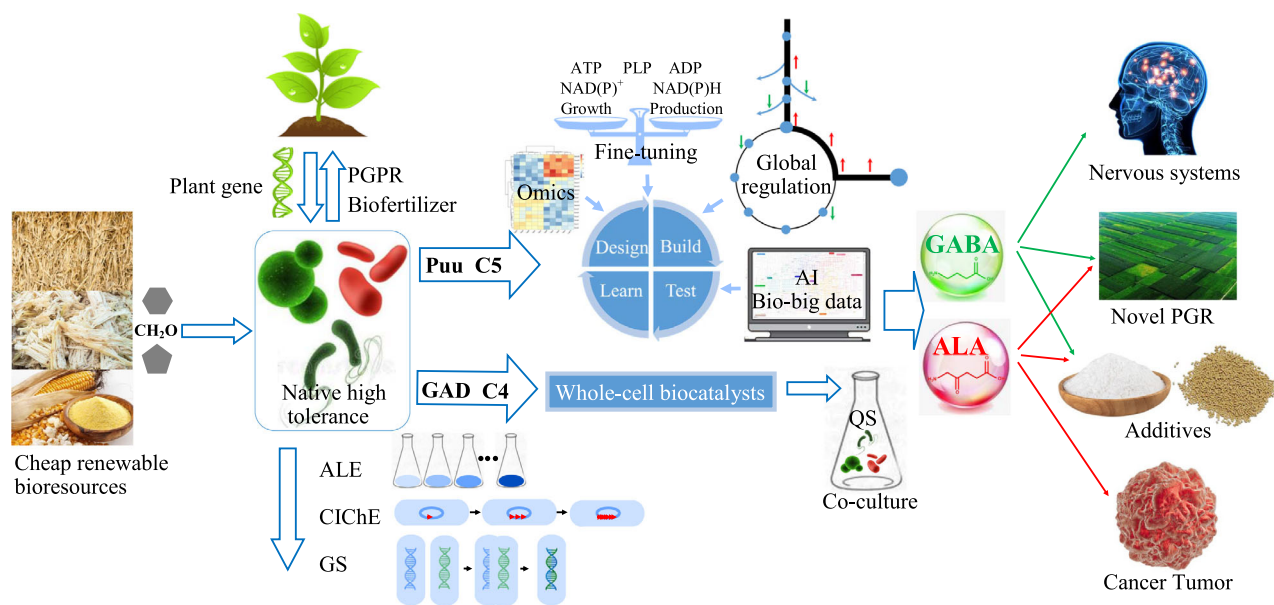
Fig. 3. Future insights for developing the production of GABA and ALA.

shown in Table 3. The maximum yields of GABA and ALA and their precursor glutamate in mol/mol glucose and g g glucose^{-1} are nearby. However, from the data in Tables 1 and 2, the titre of GABA is generally higher about 10 times than that of ALA. What's more, up to now, the highest yields of GABA and ALA are only 0.40 g g glucose^{-1}. In this case, the microbial production of ALA and GABA still has more potential to be tapped.

Novel host strains with native high tolerance

First, the low yields of GABA and ALA may be caused by the low intrinsic tolerance of the microbes to these products. For GABA, it was reported that 113g l^{-1} GABA caused a 50% decrease of the growth rate in *C. glutamicum* (Jorge *et al.*, 2016). In *Bacillus methanolicus*, 7.2 g l^{-1} GABA caused a 50% decrease of the growth rate, and growth arrested completely at 16.5 g l^{-1} (Irla *et al.*, 2017). For ALA, 15 g l^{-1} ALA caused a 36% decrease in initial specific growth rate of *E. coil*, and its

growth was completely stopped when the ALA concentration reached 18 g l^{-1} (Zhu *et al.*, 2019). Although Zhu *et al.* (2019) explained the cytotoxicity of ALA via generate reactive oxygen species (ROS). Attention also should be paid to that the ALA accumulation causes pH decrease, while GABA accumulation causes pH increase. Hence, high tolerance of acid–base stress microorganisms is needed for improving GABA and ALA production (Fig. 3), which is usually ignored and may be another breakthrough point to further increase the yield of GABA and ALA.

Hence, screening and selecting novel host strains with high tolerance and natural over productive features are a preferred way to improve GABA and ALA productions. While in fact, compared with traditional conventional microorganisms, extremophilic bacteria are better adapted to the rough conditions in industrial production, as well as greatly reducing the production cost (Chen and Liu, 2021). On the other hand, the non-conventional strains might have the capability to consume cheap

renewable bioresources (Fig. 3) including lignocellulosics, wastes, agro-industrial residues and C1 (methane, methanol, formic acid and carbon dioxide) compounds (Haldar and Purkait, 2020). However, it should also be noted that the genetic manipulation of wild-type strains is limited to stubborn bacterial issues, such as restricted modification systems, cell wall thickness and endophytic plasmids. Fortunately, the advances in high-throughput screening techniques and genome sequencing technologies have accelerated the isolation and engineering of non-conventional hosts (Li *et al.*, 2020). Moreover, evolutionary engineering like adaptive laboratory evolution (ALE), chemically induced chromosomal evolution (CIChE) and genome shuffling (GS) are especially suitable for non-conventional strains which lack genetic tools (Fig. 3). Hence, now it is the adequate time to develop novel cell factories.

Develop whole-cell biocatalysts to co-culture for GAD and C4 pathway

Ignoring the cost, up to now, the highest titre GABA was realized by whole-cell biocatalysis through GAD pathway. Similarly, the C4 pathway is also catalysed by a key enzyme ALAS. Hence, the GAD and C4 pathways are more suitable for the whole-cell biocatalysts (Fig. 3). However, the yield heavily depends on the amount of precursor: glutamate, glycine and succinic acid. Among those precursors, glutamate is cheaper. Nonetheless, whole-cell transformations are not only retarded cell growth, but also unsustainable with high cost. Co-culture can diminish the metabolic burden on each microbial strain, so that the optimized metabolic pathway can be parallel constructed in a modular way to take the advantage of the favourable traits from each co-cultured organism (Zhang and Wang, 2016). Thus, co-culture strategy might be a resolve for the problem of high cost (Fig. 3). For example, co-culture of the succinic acid-producing engineering strain, glycine-producing engineering strain and expressing ALAS engineering strain could be used as a consortium for ALA production. Furthermore, use quorum sensing (QS) in co-culture regulation is a promising way.

Develop DBTL for Puu and C5 pathway

The Puu and C5 pathways are more suitable for metabolic engineering, based on the classical Design–Build–Test–Learn (DBTL) system to realize the sustainable production from glucose or cheap raw materials (Fig. 3). However, we have to admit that simply using metabolic engineering is a time-consuming and labour-intensive process. Luckily, with the development of artificial intelligence (AI) and the bio-big data, the metabolic simulation and prediction on genome-scale could guide experiments more accurately, reasonably and quickly (Fig. 3) (Ryu *et al.*, 2019; Wytock and Motter, 2019). In addition, omics such as genomics, transcriptomics, proteomics, metabolomics and fluxomics have provided faster and comprehensive progress in metabolic engineering research (Fig. 3). What's more, fine-tuning and balance could use the minor force winning energetically. On the one hand, it can be realized by RBS and promoter library, CRISPRi and point mutation strategy. On the other hand, regenerate cofactor ($NADP^+$/NADPH) and energy (ATP/ADP) are necessary. In detail, $NADP^+$/NADPH supply via the glycolytic pathway and the pentose phosphate pathway (Fig. 1), in which the related genes were *gapA*, *aceEF*, *ldh*, *zwf* and *gdn*; ATP regenerate-related genes were *pgk*, *pyk* and *sucCD*, need to be considered. Moreover, global regulation realizes growth-coupled production is also necessary for improving the production.

Maximum utilization of fermentation

Since GABA and ALA are extracellular secretion products, to maximize the use of microbial cell factories, complement each other or no compete intracellular products with extracellular simultaneous production might be considered. Besides, the most common aerobic problem in large-scale fermentation can be solved by overexpressing haemoglobin to increase aeration.

Other strategies relate to plants and agriculture

Since plants also have GABA and ALA synthesis pathways, introducing plants synthesis pathways or functional genes under rational design may bring surprising discovery. On the other hand, in agriculture, direct engineering the GABA- and ALA-producing microorganisms themselves can also be used as live therapeutics, that is to say as a novel plant growth-promoting rhizobacteria (PGPR) (Fig. 3). GABA- and ALA-producing strain can also be used as a novel species to add and enrich biofertilizer (Fig. 3). Furthermore, combinate GABA-producing strain with ALA-producing strain also may enhancement their effectiveness in agriculture. Thus, modification of GABA- and ALA-producing strains or construction of stable recombinant strains that can breed in the soil should be a novel direction for future studies.

Concluding remarks

In summary, GABA and ALA have many common properties: (i) both belong to non-protein amino acids; (ii) both have multiple functions and are widely used in agriculture, medicine and other fields; (iii) both as novel

PGRs can increase plant resilience to abiotic stress and promote plants growth; (iv) they share a common synthesis system, with common precursor glutamate and common cofactor PLP; and (v) they have similar system for metabolic engineering strategies. Hence, putting GABA and ALA together to analyse and compare their metabolic engineering strategies will benefit to each other. Likewise, these strategies provide some ideas for microbial production research of other non-protein amino acids (such as the precursor of Puu pathway ornithine) or PGRs or value-added compounds. We believe that the increasing adoption of the strategies described here will allow development of strains capable of efficiently producing GABA and ALA on an industrial scale with reduced effort, time and cost. And their application and development can better promote the sustainable development of related industries.

Funding Information

Conflict of interest

The authors declare no competing financial interest.

References

Akasaka, N., Kato, S., Kato, S., Hidese, R., Wagu, Y., Sakoda, H., and Fujiwara, S. (2018) Agmatine production by *Aspergillus oryzae* is elevated by low pH during solid-state cultivation. *Appl Environ Microbiol* **84:** e00722-18

Cha, H.J., Jeong, J.H., Rojviriya, C., and Kim, Y.G. (2014) Structure of putrescine aminotransferase from *Escherichia coli* provides insights into the substrate specificity among class III aminotransferases. *PLoS One* **9:** e113212.

Chen, G.Q., and Liu, X. (2021) On the future fermentation. *Microb Biotechnol* **14:** 18–21.

Chen, J., Wang, Y., Guo, X., Rao, D., Zhou, W., Zheng, P., *et al.* (2020) Efficient bioproduction of 5-aminolevulinic acid, a promising biostimulant and nutrient, from renewable bioresources by engineered *Corynebacterium glutamicum*. *Biotechnol Biofuels* **13:** 41.

Choi, J.W., Yim, S.S., Lee, S.H., Kang, T.J., Park, S.J., and Jeong, K.J. (2015) Enhanced production of gamma-aminobutyrate (GABA) in recombinant *Corynebacterium glutamicum* by expressing glutamate decarboxylase active in expanded pH range. *Microb Cell Fact* **14:** 21.

Cui, Y., Miao, K., Niyaphorn, S., and Qu, X. (2020) Production of gamma-aminobutyric acid from Lactic acid bacteria: A systematic review. *Int J Mol Sci* **21:** 995.

Cui, Z., Jiang, Z., Zhang, J., Zheng, H., Jiang, X., Gong, K., *et al.* (2019) Stable and efficient biosynthesis of 5-aminolevulinic acid using plasmid-free *Escherichia coli*. *J Agric Food Chem* **67:** 1478–1483.

Diez-Gutiérrez, L., San Vicente, L.R., Barrón, L.J., Villarán, M.D.C., and Chávarri, M. (2020) Gamma-aminobutyric acid and probiotics: Multiple health benefits and their future in the global functional food and nutraceuticals market. *J Funct Foods* **64:** 103669.

Dugar, D., and Stephanopoulos, G. (2011) Relative potential of biosynthetic pathways for biofuels and bio-based products. *Nat Biotechnol* **29:** 1074–1078.

Fait, A., Fromm, H., Walter, D., Galili, G., and Fernie, A.R. (2008) Highway or byway: the metabolic role of the GABA shunt in plants. *Trends Plant Sci* **13:** 14–19.

Feng, L., Zhang, Y., Fu, J., Mao, Y., Chen, T., Zhao, X., and Wang, Z. (2016) Metabolic engineering of *Corynebacterium glutamicum* for efficient production of 5-aminolevulinic acid. *Biotechnol Bioeng* **113:** 1284–1293.

Gong, L., Ren, C., and Xu, Y. (2019) Deciphering the crucial roles of transcriptional regulator GadR on gamma-aminobutyric acid production and acid resistance in *Lactobacillus brevis*. *Microb Cell Fact* **18:** 108.

Haldar, D., and Purkait, M.K. (2020) Lignocellulosic conversion into value-added products: a review. *Process Biochem* **89:** 110–133.

Hara, K.Y., Saito, M., Kato, H., Morikawa, K., Kikukawa, H., Nomura, H., *et al.* (2019) 5-Aminolevulinic acid fermentation using engineered *Saccharomyces cerevisiae*. *Microb Cell Fact* **18:** 194.

Inoue, K. (2017) 5-Aminolevulinic acid-mediated photodynamic therapy for bladder cancer. *Int J Urol* **24:** 97–101.

Irla, M., Nærdal, I., Brautaset, T., and Wendisch, V.F. (2017) Methanol-based γ-aminobutyric acid (GABA) production by genetically engineered *Bacillus methanolicus* strains. *Ind Crop Prod* **106:** 12–20.

Jorge, J.M., Leggewie, C., and Wendisch, V.F. (2016) A new metabolic route for the production of gamma-aminobutyric acid by *Corynebacterium glutamicum* from glucose. *Amino Acids* **48:** 2519–2531.

Jorge, J.M., Nguyen, A.Q., Perez-Garcia, F., Kind, S., and Wendisch, V.F. (2017) Improved fermentative production of gamma-aminobutyric acid via the putrescine route: Systems metabolic engineering for production from glucose, amino sugars, and xylose. *Biotechnol Bioeng* **114:** 862–873.

Kang, Z., Ding, W., Gong, X., Liu, Q., Du, G., and Chen, J. (2017) Recent advances in production of 5-aminolevulinic acid using biological strategies. *World J Microbiol Biotechnol* **33:** 200.

Kang, Z., Wang, Y., Gu, P., Wang, Q., and Qi, Q. (2011) Engineering *Escherichia coli* for efficient production of 5-aminolevulinic acid from glucose. *Metab Eng* **13:** 492–498.

Kang, Z., Zhang, J., Zhou, J., Qi, Q., Du, G., and Chen, J. (2012) Recent advances in microbial production of delta-aminolevulinic acid and vitamin B12. *Biotechnol Adv* **30:** 1533–1542.

Kim, D.-H., Dasagrandhi, C., Park, S.-K., Eom, S.-H., Huh, M.-K., Mok, J.-S., and Kim, Y.-M. (2018) Optimization of gamma-aminobutyric acid production using sea tangle extract by lactic acid bacterial fermentation. *Lwt* **90:** 636–642.

Li, J., Rong, L., Zhao, Y., Li, S., Zhang, C., Xiao, D., *et al.* (2020) Next-generation metabolic engineering of

non-conventional microbial cell factories for carboxylic acid platform chemicals. *Biotechnol Adv* **43:** 107605.

Li, T., Guo, Y.Y., Qiao, G.Q., and Chen, G.Q. (2016a) Microbial synthesis of 5-aminolevulinic acid and its coproduction with polyhydroxybutyrate. *ACS Synth Biol* **5:** 1264–1274.

Li, W., Liu, J., Ashraf, U., Li, G., Li, Y., Lu, W., *et al.* (2016b) Exogenous γ-aminobutyric acid (GABA) application improved early growth, net photosynthesis, and associated physio-biochemical events in Maize. *Front Plant Sci* **7:** 919.

Li, Z., Huang, T., Tang, M., Cheng, B., Peng, Y., and Zhang, X. (2019) iTRAQ-based proteomics reveals key role of gamma-aminobutyric acid (GABA) in regulating drought tolerance in perennial creeping bentgrass (*Agrostis stolonifera*). *Plant Physiol Biochem* **145:** 216–226.

Lyu, C.J., Zhao, W.R., Hu, S., Huang, J., Lu, T., Jin, Z.H., *et al.* (2017) Physiology-oriented engineering strategy to improve gamma-aminobutyrate production in *Lactobacillus brevis*. *J Agric Food Chem* **65:** 858–866.

Lyu, C., Zhao, W., Peng, C., Hu, S., Fang, H., Hua, Y., *et al.* (2018) Exploring the contributions of two glutamate decarboxylase isozymes in *Lactobacillus brevis* to acid resistance and gamma-aminobutyric acid production. *Microb Cell Fact* **17:** 180.

Meng, Q., Zhang, Y., Ju, X., Ma, C., Ma, H., Chen, J., *et al.* (2016) Production of 5-aminolevulinic acid by cell free multi-enzyme catalysis. *J Biotechnol* **226:** 8–13.

Noh, M.H., Lim, H.G., Park, S., Seo, S.W., and Jung, G.Y. (2017) Precise flux redistribution to glyoxylate cycle for 5-aminolevulinic acid production in *Escherichia coli. Metab Eng* **43:** 1–8.

Rashmi, D., Zanan, R., John, S., Khandagale, K., and Nadaf, A. (2018) γ-Aminobutyric acid (GABA): biosynthesis, role, commercial production, and applications. *Stud Nat Products Chem* **57:** 413–452.

Ryu, J.Y., Kim, H.U., and Lee, S.Y. (2019) Deep learning enables high-quality and high-throughput prediction of enzyme commission numbers. *Proc Natl Acad Sci USA* **116:** 13996–14001.

Sasaki, M.W.T.T.T.K. (2002) Biosynthesis, biotechnological production and applications of 5-aminolevulinic acid. *Appl Microbiol Biot* **58:** 23–29.

Shi, F., Si, H., Ni, Y., Zhang, L., and Li, Y. (2017) Transaminase encoded by *NCgl2515* gene of *Corynebacterium glutamicum* ATCC13032 is involved in γ-aminobutyric acid decomposition. *Process Biochem* **55:** 55–60.

Small, C.C., and Degenhardt, D. (2018) Plant growth regulators for enhancing revegetation success in reclamation: a review. *Ecol Eng* **118:** 43–51.

Soma, Y., Fujiwara, Y., Nakagawa, T., Tsuruno, K., and Hanai, T. (2017) Reconstruction of a metabolic regulatory network in *Escherichia coli* for purposeful switching from cell growth mode to production mode in direct GABA fermentation from glucose. *Metab Eng* **43:** 54–63.

Su, T., Guo, Q., Zheng, Y., Liang, Q., Wang, Q., and Qi, Q. (2019) Fine-tuning of *hemB* using CRISPRi for increasing 5-aminolevulinic acid production in *Escherichia coli. Front Microbiol* **10:** 1731.

Tajabadi, N., Baradaran, A., Ebrahimpour, A., Rahim, R.A., Bakar, F.A., Manap, M.Y., *et al.* (2015) Overexpression and optimization of glutamate decarboxylase in *Lactobacillus plantarum* Taj-Apis362 for high gamma-aminobutyric acid production. *Microb Biotechnol* **8:** 623–632.

Tangprasittipap, A., Prasertsan, P., Choorit, W., and Sasaki, K. (2007) Biosynthesis of intracellular 5-aminolevulinic acid by a newly identified halotolerant *Rhodobacter sphaeroides. Biotechnol Lett* **29:** 773–778.

Thunshelle, C., Yin, R., Chen, Q., and Hamblin, M.R. (2016) Current advances in 5-aminolevulinic acid mediated photodynamic therapy. *Curr Dermatol Rep* **5:** 179–190.

Wu, Q., Tun, H.M., Law, Y.S., Khafipour, E., and Shah, N.P. (2017) Common distribution of *gad* operon in *Lactobacillus brevis* and its GadA contributes to efficient GABA synthesis toward cytosolic near-neutral pH. *Front Microbiol* **8:** 206.

Wu, Y., Liao, W., Dawuda, M.M., Hu, L., and Yu, J. (2018) 5-Aminolevulinic acid (ALA) biosynthetic and metabolic pathways and its role in higher plants: a review. *Plant Growth Regul* **87:** 357–374.

Wytock, T.P., and Motter, A.E. (2019) Predicting growth rate from gene expression. *Proc Natl Acad Sci USA* **116:** 367–372.

Yang, H.-S., and Hoober, J.K. (1995) Divergent pathways for δ-aminolevulinic acid synthesis in two species of *Arthrobacter. FEMS Microbiol Lett* **134:** 259–263.

Yang, P., Liu, W., Cheng, X., Wang, J., Wang, Q., Qi, Q., and Liu, S.J. (2016) A new strategy for production of 5-aminolevulinic acid in recombinant *Corynebacterium glutamicum* with high yield. *Appl Environ Microb* **82:** 2709–2717.

Yang, X., Ke, C., Zhu, J., Wang, Y., Zeng, W., and Huang, J. (2018) Enhanced productivity of gamma-amino butyric acid by cascade modifications of a whole-cell biocatalyst. *Appl Microbiol Biotechnol* **102:** 3623–3633.

Yu, P., Ren, Q., Wang, X., and Huang, X. (2019) Enhanced biosynthesis of γ-aminobutyric acid (GABA) in *Escherichia coli* by pathway engineering. *Biochem Eng J* **141:** 252–258.

Yu, X., Jin, H., Liu, W., Wang, Q., and Qi, Q. (2015) Engineering *Corynebacterium glutamicum* to produce 5-aminolevulinic acid from glucose. *Microb Cell Fact* **14:** 183.

Yuan, S.F., and Alper, H.S. (2019) Metabolic engineering of microbial cell factories for production of nutraceuticals. *Microb Cell Fact* **18:** 46.

Zhang, C., Li, Y., Zhu, F., Li, Z., Lu, N., Li, Y., *et al.* (2020) Metabolic engineering of an auto-regulated *Corynebacterium glutamicum* chassis for biosynthesis of 5-aminolevulinic acid. *Bioresource Technol* **318:** 124064.

Zhang, H., and Wang, X. (2016) Modular co-culture engineering, a new approach for metabolic engineering. *Metab Eng* **37:** 114–121.

Zhang, J., Weng, H., Zhou, Z., Du, G., and Kang, Z. (2019) Engineering of multiple modular pathways for high-yield production of 5-aminolevulinic acid in *Escherichia coli. Bioresource Technol* **274:** 353–360.

Zhu, C., Chen, J., Wang, Y., Wang, L., Guo, X., Chen, N., *et al.* (2019) Enhancing 5-aminolevulinic acid tolerance and production by engineering the antioxidant defense system of *Escherichia coli. Biotechnol Bioeng* **116:** 2018–2028.