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MINI-REVIEW

Microbial production of butyl butyrate, a flavor and fragrance compound

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Abstract

Butyl butyrate (BB) has been widely used as a flavor and fragrance compound in the beverage, food, perfume, and cosmetic industries. Currently, BB is produced through two-step processes; butanol and butyrate are first produced and are used as precursors for the esterification reactions to yield BB in the next step. Recently, an alternative process to the current process has been developed by using microorganisms for the one-pot BB production. In the one-pot BB process, alcohol acyl transferases (AATs) and lipases play roles in the esterification of butanol together with their co-substrates butyryl-CoA and butyrate, respectively. In this paper, we review the characteristics of two enzymes including AAT and lipase in the esterification reaction. Also, we review the one-pot processes for BB production by employing the wild-type and engineered *Clostridium* species and the engineered *Escherichia coli* strains, with the combination of AATs and lipases.

Keywords Butyl butyrate · Ester · Flavor · Alcohol acyltransferase · Lipase

Introduction

Butyl butyrate (BB) is one of the short-chain esters known as a flavor and fragrance compound (Matte et al. 2016; Varma and Madras 2008; Zabetakis and Holden 1997). In nature, BB has routinely been found in flowers, fruits, and fermented beverages. The sweet and sour flavor found in nature is often due to the presence of BB together with other short-chain esters. Thus, BB has been used as a flavoring agent in the beverages, foods, perfumes, and cosmetic industries (Jenkins et al. 2013; Santos et al. 2007).

For the industrial-scale production of BB, the catalytic and enzymatic processes have been developed and used. In the catalytic BB process, the esterification reaction has been performed under the high temperature and pressure conditions by supplying precursors butanol and butyrate and using hydrofluoric acid and sulfuric acid as catalysts (Han and Zhou 2011). By using such acid catalysts, the process has caused some problems in terms of corrosiveness, formation of environmentally hazardous byproducts, and difficulty in catalyst recovery (Han and Zhou 2011; Liu and Zhang 2018; Park et al. 2017a). To overcome such problems in BB production, an enzymatic process has been developed by employing immobilized lipases, which catalyze esterification reaction under atmospheric condition (Kirdi et al. 2017; Yeom and Go 2018). It has been demonstrated that the lipase-catalyzed esterification reaction allowed production of various esters including BB with high conversion yields (Chowdary and Prapulla 2002; Dhake et al. 2012; Gim and Kim 2018; Lozano et al. 2002; Park et al. 2005).

In both current catalytic and enzymatic processes for BB production, the precursors butanol and butyrate should be externally supplemented. Thus, the current BB processes typically comprise two independent steps including the precursor production step and the esterification reaction step. As an alternative to the current processes, one-pot processes have recently been developed for BB production by employing microorganisms (Horton and Bennett 2006; Rodriguez et al.

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2014). In these alternative processes, the precursors are produced as metabolic intermediates (or end-products) in microorganisms including *Clostridium* species and engineered *Escherichia coli* strains (Layton and Trinh 2014; Noh et al. 2018; Rodriguez et al. 2014).

In this paper, the characteristics of two key enzyme alcohol acyltransferase (AAT) and lipase involved in the esterification reaction for BB production in the one-pot processes are reviewed. The metabolic engineering strategies employed for BB production by microorganisms including *C. acetobutylicum* and *E. coli* are also reviewed. Furthermore, BB production by employing *Clostridium* species together with the extracellular lipases is reviewed. Finally, perspectives and future research directions are suggested.

AAT and lipase

AAT and lipase are used as key enzymes in the recent studies on the development of the one-pot processes for BB production (Horton et al. 2003; Langrand et al. 1990; Layton and Trinh 2016b). Thus, this section begins with a brief overview of their characteristics, which play important roles in the esterification reaction.

AAT

AAT is an enzyme catalyzing the condensation reaction of alcohols together with acyl-CoA to produce esters including BB (Fig. 1) (Günther et al. 2011; Nancolas et al. 2017; Olias et al. 1995; Salas 2004). Various AATs are found in yeasts as well as fruits including strawberry, banana, melon, and apple (Balbontin et al. 2010; Defilippi et al. 2005; Kruis et al. 2017). AATs are distinguished from wax synthase/diacylglycerol acyltransferases (WS/DGATs) by the substrate preference towards relatively shorter carbon length (Menendez-Bravo et al. 2017). For this reason, AATs have been employed for the production of short carbon chain esters like BB, while WS/DGATs have been used for the production of long carbon chain esters like biodiesel (d'Espaux et al. 2015; Kalscheuer et al. 2006; Kumar et al. 2018; Li et al. 2008; Park et al. 2017b; Sudheer et al. 2017).

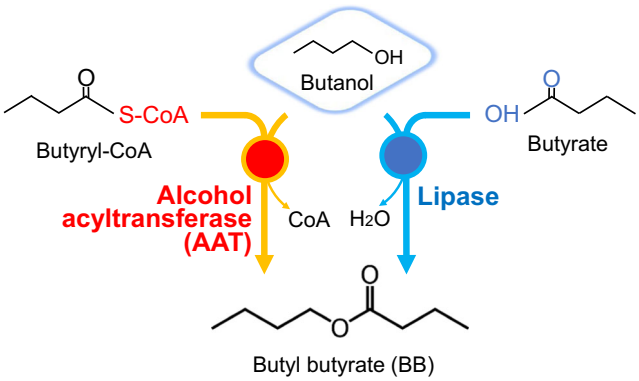


Fig. 1 Esterification reactions mediated by alcohol acyltransferases (AATs) and lipases to yield BB

In wild-type *Saccharomyces cerevisiae*, the AATs, ATF1 and ATF2, are encoded by the genes *ATF1* and *ATF2*, respectively (Saerens et al. 2010). ATF1 and ATF2 are mainly involved in the formation of acetate esters in *S. cerevisiae* (Kruis et al. 2017; Li et al. 2018; Nancolas et al. 2017; Zhang et al. 2013). While a recent report indicated that ATF1 had a substrate preference for C4 to C6 alcohols and acetyl-CoA in an engineered *E. coli* strain (Layton and Trinh 2016a), the substrate preference of ATF2 has not yet been defined in detail. The homologs of ATF1 and ATF2 have been identified in other yeast strains, including *Saccharomyces carlsbergensis*, *Candida glabrata*, and *Kluyveromyces lactis* (Fujii et al. 1996; Fujiwara et al. 1999; Kim et al. 2017; Schneiderbanger et al. 2016; Van Laere et al. 2008; Zhang et al. 2014).

AATs have also been isolated from fruits, such as strawberry (SAAT and FaAAT2 from *Fragaria* × *ananassa*, VAAT from *Fragaria vesca,* and FcAAT1 from *Fragaria chiloensis*), banana (BanAAT from *Musa sapientum*), melon (MAAT from *Cucumis melo*), and apple (AAAT from *Malus* sp.) (Beekwilder et al. 2004; Defilippi et al. 2005). The AATs from such fruits have wide substrate specificities that range from C1 alcohols to C10 or higher alcohols (Beekwilder et al. 2004). Beekwilder et al. (2004) reported that SAAT exhibited its highest activity in a reaction supplemented with geraniol and acetyl-CoA, but this activity was reduced to 5% and 11% of the highest activity when geraniol was replaced by methanol and butanol, respectively. When butyryl-CoA was supplied as a co-substrate in the SAAT-mediated esterification reaction, the highest activity was reported when octanol was used as the second substrate, and 81% of this activity level was retained when butanol was used as the second substrate for BB production (Beekwilder et al. 2004). However, in the reaction of butanol together with butyryl-CoA, the exact kinetics were limited by FaAAT2, which has a K_{cat}/K_M value of 0.04/s/μM (Cumplido-Laso et al. 2012).

Lipase

In contrast to AATs, which catalyze the esterification of alcohols with acyl-CoA, lipases catalyze the esterification of alcohols with acids to yield esters in the organic phase (Kim 2017). Thus, during the lipase-mediated production of BB, butanol and butyrate are used as precursors (Fig. 1). Lipase reacts with butyrate to yield a lipase-butyrate complex, followed by isomerization of the complex to form acyl-lipase intermediate. In the next step, acyl-lipase catches butanol to yield another complex, followed by isomerization of the complex to form butyl butyrate–lipase complex. In final, BB was released from the complex. Lipases can also catalyze the hydrolysis of fatty acid

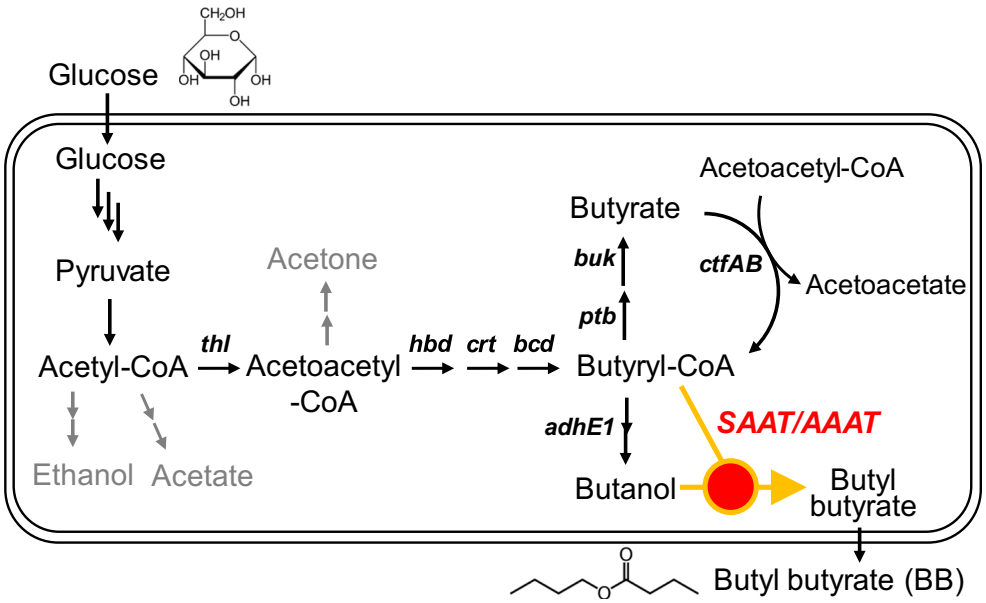


Fig. 2 Metabolic pathway of the engineered *C. acetobutylicum* strain harboring the *AAT* genes for BB production (Noh et al. 2018). The *SAAT* and *AAAT* genes were introduced from *Fragaria* × *ananassa* and *Malus* sp., respectively, into *C. acetobutylicum*. Gene abbreviations encoding enzymes: *ctfAB*, CoA transferase; *adhE1*, aldehyde/alcohol dehydrogenase; *buk*, butyrate kinase; *ptb*, phosphotransbutyrylase; *thl*, thiolase; *hbd*, 3-hydroxybutyrate dehydrogenase; *crt*, crotonase; *bcd*, butyryl-CoA dehydrogenase

esters, including triacylglycerol, via their α/β-hydrolase activity (Haque et al. 2018; Jung et al. 2010; Langrand et al. 1990; Mancheno et al. 2003; Yu et al. 2012).

Lipases have been identified from various microorganisms, including *Achromobacter* sp., *Bacillus* sp., *Burkholderia* sp., *Thermomyces lanuginosus*, *Candida antarctica*, and *Candida rugosa* (Gupta et al. 2004; Martins et al. 2013; Selvam et al. 2013). In particular, *Candida antarctica* lipase B (CALB) is a well-known enzyme that robustly catalyzes diverse reactions, including the syntheses of flavor and fragrance esters (short-chain esters), biodiesels (long-chain esters), and modified glycerides. For commercial applications, CALB is typically used in an immobilized form under biphasic conditions because the free enzyme is unstable in the aqueous condition (Dhake et al. 2012; Hasan et al. 2006; Kim and Suh 2016). In a study for BB synthesis, kinetic parameters of a

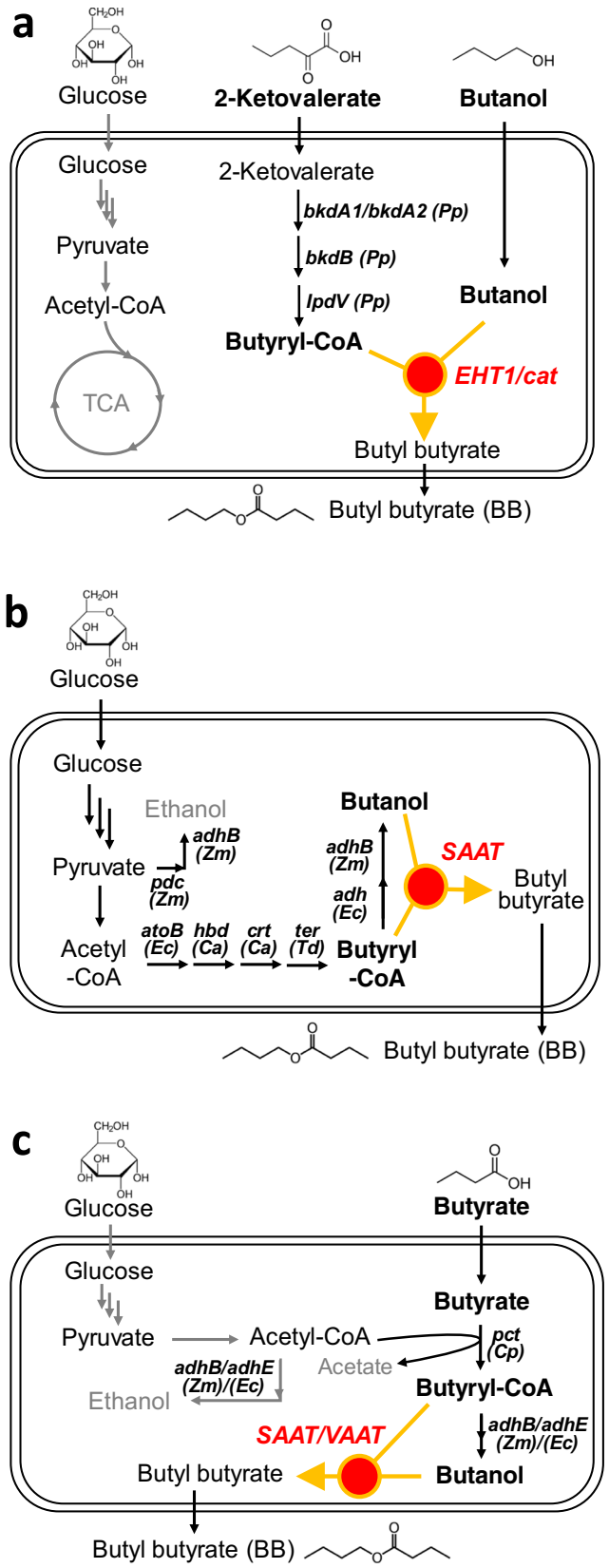
Table 1 Butyl butyrate production using the engineered microorganisms containing the heterologous *AAT* genes

Microorganisms	Constructed pathway for forming precursors	*AATs*[a]	Externally added precursors	Titer (mg/L)	Selectivity[b] (%)	References
C. acetobutylicum (wild-type)	Native pathway	*SAAT* (*Fr*)	None	50.07	84.8	(Noh et al. 2018)
C. acetobutylicum (wild-type)	Native pathway	*AAAT* (*Mal*)	None	40.60	87.4	(Noh et al. 2018)
E. coli (Δ*adhE*, Δ*frd*, Δ*ldhA*, Δ*pta*, Δ*pflB*, Δ*fnr*, Δ*yqhD*, Δ*adhP*, Δ*eutG*, Δ*yiaY*, Δ*yjgB*, Δ*fucO*)	KDHC operon (*bkdA1-bkdA2-bkdB-lpdV*)	*EHT1* (*Sc*)	3 g/L 2-Ketovalerate and 3 g/L butanol	14.9	100	(Rodriguez et al. 2014)
E. coli (Δ*adhE*, Δ*frd*, Δ*ldhA*, Δ*pta*, Δ*pflB*, Δ*fnr*, Δ*yqhD*, Δ*adhP*, Δ*eutG*, Δ*yiaY*, Δ*yjgB*, Δ*fucO*)	KDHC operon (*bkdA1-bkdA2-bkdB-lpdV*)	*cat*	3 g/L 2-Ketovalerate and 3 g/L butanol	10.6	100	(Rodriguez et al. 2014)
E. coli (Δ*zwf*, Δ*ndh*, Δ*sfcA*, Δ*maeB*, Δ*ldhA*, Δ*frdA*, Δ*poxB*, Δ*pta*, Δ*fadE*)	Acyl-CoA and ethanol pathways (*atoB*, *hbd*, *crt*, *ter*, *pdc*, *adhB*)	*SAAT* (*Fr*)	None	36.83[c]	1.98	(Layton and Trinh 2014)
E. coli (Δ*zwf*, Δ*ndh*, Δ*sfcA*, Δ*maeB*, Δ*ldhA*, Δ*frdA*, Δ*poxB*, Δ*pta*, Δ*fadE*)	Acid-to-alcohol pathways (*pct*, *pdc*, *adhB*)	*SAAT* (*Fr*)	2 g/L Butyrate	47.63	26	(Layton and Trinh 2016a)
E. coli (Δ*zwf*, Δ*ndh*, Δ*sfcA*, Δ*maeB*, Δ*ldhA*, Δ*frdA*, Δ*poxB*, Δ*pta*, Δ*fadE*)	Acid-to-alcohol and isobutanol pathway (*alsS*, *ilvC*, *ilvD*, *kivd*, *adhE*, *pct*)	*SAAT* (*Fr*)	2 g/L Butyrate	21.34	32	(Layton and Trinh 2016b)

[a] *Fr, Fragaria* × *ananassa*; *Mal*, *Malus* sp.; *Sc*, *Saccharomyces cerevisiae*; and *cat*, chloramphenicol resistance gene

[b] BB selectivity to total esters produced

[c] The value was obtained from the fermentation with *in situ* recovery system



◀ **Fig. 3** Metabolic pathway of the engineered *E. coli* strains harboring the *AAT* genes for BB production. **a** Butyryl-CoA was formed from the externally added 2-ketovalerate through the branched-chain keto acid dehydrogenase complex (KDHC) encoded from the *P. putida bkdA1, bkdA2, bkdB, and lpdV* genes (Rodriguez et al. 2014). The other precursor butanol was also externally supplied for BB production. The *S. cerevisiae EHT1* and the chloramphenicol resistant *cat* genes were used for the esterification reaction. **b** Butyryl-CoA was formed from glucose through the chimeric butanol pathway involving the enzymes encoded from the *atoB, hbd, crt,* and *ter* genes (Layton and Trinh 2014). Butanol could form through enzymes encoded from the *adh* and *adhB* genes. The *Fragaria* × *ananassa SAAT* gene was used for the esterification reaction. **c** Butyryl-CoA was formed from the externally added butyrate through the acyl-CoA transferase encoded from the *C. propionicum pct* gene (Layton and Trinh 2016a). The other precursor butanol was formed from butyryl-CoA via the enzymes encoded from the *adhB* and endogenous *adhE* genes. The *Fragaria* × *ananassa SAAT* and *Fragaria vesca VAAT* genes were used for the esterification reaction. Abbreviations for microorganisms: Pp, *P. putida*; Zm, *Z. mobilis*; Ca, *C. acetobutylicum*; Td, *Treponema denticola*; Ec, *E. coli*; Cp, *C. propionicum*. Gene abbreviations encoding enzymes: *bkdA1*, 2-oxoisovalerate dehydrogenase α subunit; *bkdA2*, 2-oxoisovalerate dehydrogenase β subunit; *bkdB*, dihydrolipoyl transacylase; *lpdV*, dihydrolipoamide dehydrogenase; *atoB*, acetyl-CoA acetyltransferase; *hbd*, 3-hydroxybutyrate dehydrogenase; *crt*, crotonase; *ter*, trans-2-enoyl-CoA reductase; *adhE*, alcohol dehydrogenase; *adhB*, alcohol dehydrogenase II; *pdc*, pyruvate decarboxylase, *pct*, propionyl-CoA transferase

CALB lipase immobilized on acrylic resin, Novozym 435, were determined in the absence of the product: V_{max} of 2.22 mol/g/h, K_M with butanol of 530 mM, and K_M with butyrate of 350 mM (Varma and Madras 2008). The immobilized CALB used in such work is routinely produced from recombinant yeast and fungi (Emond et al. 2010; Han et al. 2009; Tamalampudi et al. 2007).

Metabolic engineering of microorganisms for AAT-mediated BB production

In recent studies, AATs have been used to construct synthetic pathways for BB production in *C. acetobutylicum* and *E. coli* (Layton and Trinh 2014, 2016a, b; Noh et al. 2018). In the engineered microorganisms, BB was formed via butanol and butyryl-CoA, which were obtained from glucose catabolism or external supplementation. To generate these two precursors for BB production, the native pathway was used in *C. acetobutylicum*, whereas a synthetic pathway was constructed in *E. coli* (Layton and Trinh 2014; Noh et al. 2018; Rodriguez et al. 2014).

Wild-type *C. acetobutylicum* forms butanol via butyryl-CoA from glucose (Fig. 2), making the strain a promising host for BB production (Desai et al. 1999; Horton et al. 2003; Noh et al. 2018; Woo et al. 2018). In this organism, butyryl-CoA and butanol are formed from the sequential transformation of two acetyl-CoA molecules by four enzymes: thiolase, 3-hydroxybutyryl-CoA dehydrogenase, crotonase, and butyryl-CoA dehydrogenase (Jang et al. 2012; Wiesenborn et al. 1988;

Yeon et al. 2016). Butyryl-CoA can also be formed by the reassimilation of butyric acid by CoA transferase (Desai et al. 1999; Lee et al. 2012; Wiesenborn et al. 1988). In a recent study, SAAT and AAAT from *F. ananassa* and *Malus sp.*, respectively, were introduced into *C. acetobutylicum* (Fig. 2). The codon-optimized *SAAT* and *AAAT* genes were expressed under the control of the *thl* promoter (Noh et al. 2018). In anaerobic cultures of the engineered *C. acetobutylicum* strains, BB productions of 50.07 mg/L (SAAT) and 40.60 mg/L (AAAT) were achieved without precursor feeding (Table 1). The BB selectivity of this ester production was 84.8% and 87.4% for *C. acetobutylicum* strains harboring the *SAAT* and *AAAT* genes, respectively (Noh et al. 2018).

Unlike *C. acetobutylicum*, wild-type *E. coli* does not possess a native pathway for forming butanol and butyryl-CoA. Thus, genetic modules must be heterologously introduced to enable the generation of precursors if the goal is to produce BB in *E. coli*. In one study, to supply butyryl-CoA for BB production, Rodriguez et al. (2014) engineered an *E. coli* mutant, that had low levels of aldehyde and alcohol dehydrogenase activity, by introducing the branched-chain keto acid dehydrogenase complex (KDHC) operon from *Pseudomonas putida*. 2-Ketovalerate was externally supplied to yield butyryl-CoA via KDHC, as was butanol, which was not produced by the mutant *E. coli* (Fig. 3a). For BB production, the engineered *E. coli* strain was further transformed by constructs encoding one of two AATs: *EHT1* from *S. cerevisiae* or the chloramphenicol acetyltransferase-encoding *cat* gene (Rodriguez et al. 2014). When the final strains were cultured in medium containing 3 g/L of 2-ketovalerate and 3 g/L butanol, the BB productions were 14.9 mg/L (*ETH1* strain) and 10.6 mg/L (*cat* strain) (Table 1).

In the same year, another group used two different modules to generate butyryl-CoA and alcohol in an engineered *E. coli* (Layton and Trinh 2014). The butyryl-CoA-producing module was constructed on the basis of the butanol pathway known in *C. acetobutylicum* and previous work demonstrating butanol production in *E. coli* (Inui et al. 2008). An alcohol production module was constructed by cloning the *Zymomonas mobilis adhB* and *pdc* genes, which encode alcohol dehydrogenase II and pyruvate decarboxylase, respectively (Fig. 3b). To enable the esterification reaction, the *SAAT* gene was incorporated downstream of the T7 promoter in the butyryl-CoA production module. Culture of the mutant *E. coli* strain harboring these modules without feeding of any precursor yielded production of 0.75 mg/L for BB and 37.16 mg/L for ethyl butyrate (Layton and Trinh 2014). In a fermentation with in situ recovery using the same strain, Layton and Trinh (2014) obtained production of 36.83 mg/L for BB and 134.00 mg/L for ethyl butyrate (Table 1).

In subsequent studies, the same research group replaced the butyryl-CoA production module with the acyl-CoA transferase encoded by the *Clostridium propionicum pct* gene (Layton and Trinh 2016a, b). Butyrate was externally fed to form butyryl-CoA through acyl-CoA transferase in the engineered *E. coli* (Fig. 3c). Layton and Trinh (2016a) used the alcohol-forming enzymes encoded from the *adhB* and *pdc* genes and tested five different AATs for the esterification reaction in the mutant *E. coli*. Among the engineered *E. coli* strains, those harboring the *SAAT* and *VAAT* genes yielded BB productions of 47.63 mg/L and 2.76 mg/L, respectively, and ethyl butyrate productions of 134.43 mg/L and 141.60 mg/L, respectively (Layton and Trinh 2016a). Conversely, strains expressing ATF1, ATF2, and AeAT9 (*Actinidia eriantha*) exhibited negligible BB productions of 0.17–0.28 mg/L (Layton and Trinh 2016a).

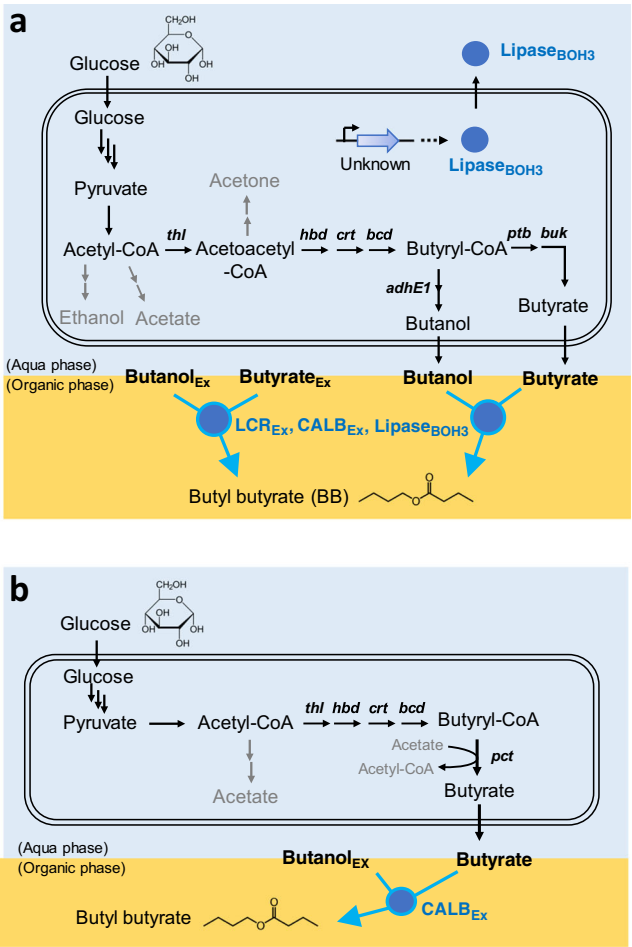


Fig. 4 Production of BB using *Clostridium* species together with lipases. **a** BB production using the solventogenic *C. acetobutylicum*, *Clostridium* sp. BOH3, and *C. beijerinckii spo0A* mutant (Seo et al. 2017; van den Berg et al. 2013; Xin et al. 2016). **b** BB production using the acidogenic *C. tyrobutyricum* (Zhang et al. 2017). Abbreviations: LCR$_{Ex}$, externally added *Candida rugosa* lipase; CALB$_{Ex}$, externally added *C. antarctica* lipase B; Lipase$_{BOH3}$, native lipase secreted from *Clostridium* sp. BOH3; butanol$_{Ex}$, externally added butanol; and butyrate$_{Ex}$, externally added butyrate. Gene abbreviations encoding enzymes: *thl*, thiolase; *hbd*, 3-hydroxybutyrate dehydrogenase; *crt*, crotonase; *bcd*, butyryl-CoA dehydrogenase; *adhE1*, aldehyde/alcohol dehydrogenase; *ptb*, phosphotransbutyrylase; *buk*, butyrate kinase; and *pct*, propionate:acetate CoA transferase

Table 2 Butyl butyrate production using *Clostridium* species and lipases

Microorganisms	Lipases[a]	Externally added precursors	Extractants (or inducers)	Fermentation conditions	Titer (g/L)	References
Clostridium acetobutylicum	*Candida antarctica* lipase B (CALB)	Butyric acid[b]	Hexadecane	Fed-batch fermentation with 40 g/L initial glucose	4.9	(van den Berg et al. 2013)
Clostridium sp. strain BOH3	Native lipase	None	Olive oil	Batch fermentation with 70 g/L xylose	1.7	(Xin et al. 2016)
Clostridium sp. strain BOH3	*Candida rugosa* lipase (LCR)	7.9 g/L Sodium butyrate	Kerosene	Fed-batch fermentation with 70 g/L initial xylose	22.4	(Xin et al. 2016)
Clostridium beijerinckii spo0A mutant	*Candida antarctica* lipase B (CALB)	5 g/L Butanol	Hexadecane	Batch fermentation with 60 g/L glucose	3.32	(Seo et al. 2017)
Clostridium tyrobutyricum	*Candida antarctica* lipase B (CALB)	10 g/L Butanol	Hexadecane	Batch fermentation with 80 g/L glucose	34.7	(Zhang et al. 2017)

[a] CALB and LCR were externally added in the culture

[b] The exact butyrate feeding was not reported. Feeding solution contained 80 g/L glucose and 160 g/L butyrate

BB production by employing *Clostridium* species and lipase supplementation

As *Clostridium* species can generate precursors for BB production, some studies have employed *C. acetobutylicum*, *Clostridium beijerinckii*, and *Clostridium tyrobutyricum*, together with lipases, to produce BB (Fig. 4 and Table 2). For example, hexadecane-extractive fed-batch fermentation of *C. acetobutylicum* in the presence of beads harboring immobilized *Candida antarctica* lipase B (CALB) yielded a BB production of 4.9 g/L from glucose (van den Berg et al. 2013).

Clostridium sp. strain BOH3, which harbors native lipase activity, was recently used for BB production (Xin et al. 2016). This strain yielded 1.7 g/L BB from xylose under olive oil–based lipase induction and 6.3 g/L BB from xylose using an oil sludge remover for lipase induction and extraction (Xin et al. 2016). In the same study, BB production of 22.4 g/L was achieved from 70 g/L xylose and 7.9 g/L exogenous butyrate in kerosene-extractive fed-batch fermentation with externally supplemented *Candida rugosa* lipase (Table 2).

The *C. beijerinckii spo0A* mutant has also been tested for BB production, as it has a high capability for producing butanol and butyrate (Seo et al. 2017). In hexadecane-extractive batch-fermentation using the *spo0A* mutant, 3.32 g/L BB was produced from 60 g/L glucose and 5 g/L exogenous butanol (Table 2).

In a more recent study, the hyper butyrate producer, *C. tyrobutyricum,* was tested for BB production in medium containing exogenous butanol and CALB (Zhang et al. 2017). In hexadecane-extractive fermentation using *C. tyrobutyricum*, 34.7 g/L BB production was achieved by CALB from 80 g/L glucose and 10 g/L butanol (Table 2).

Conclusions

One-pot processes for producing BB have been developed by employing wild-type and engineered *Clostridium* species as well as engineered *E. coli* strains. In these processes, AATs and lipases contribute to esterifying butanol together with butyryl-CoA and butyrate, respectively, to yield BB. Butanol, butyryl-CoA, and butyrate are formed as metabolites in wild-type *Clostridium* species, and a number of studies have shown that these strains are promising hosts for BB production (Noh et al. 2018; Seo et al. 2017; van den Berg et al. 2013; Xin et al. 2016; Zhang et al. 2017). On the other hand, as wild-type *E. coli* does not produce butanol and butyryl-CoA, synthetic modules were constructed to form precursors for BB production in the engineered strains (Layton and Trinh 2014, 2016a, b; Rodriguez et al. 2014). Although the engineered and/or externally supplemented AATs and lipases function properly in these one-pot processes, the BB yields obtained to date are not sufficient to allow these strategies to replace the current catalytic and enzymatic processes. To overcome this hurdle, it will be necessary to improve the affinity (K_M) of AATs for butanol and butyryl-CoA by evolutionary enzyme engineering. Moreover, for processes involving lipases, system metabolic engineering could be used to optimize the metabolic pathways of *Clostridium* to produce butanol and butyrate at a proper precursor ratio for BB production.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals by any of the authors.

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