Front. Chem. Sci. Eng. 2023, 17(10): 1336–1353
https://doi.org/10.1007/s11705-022-2284-6

REVIEW ARTICLE

Strain and process engineering toward continuous industrial fermentation

Yufei Dong[1*], Ye Zhang[1*], Dehua Liu[1,2,3], Zhen Chen (✉)[1,2,3]

1 Key Laboratory of Industrial Biocatalysis (Ministry of Education), Department of Chemical Engineering, Tsinghua University, Beijing 100084, China
2 Tsinghua Innovation Center in Dongguan, Dongguan 523808, China
3 Center for Synthetic and Systems Biology, Tsinghua University, Beijing 100084, China

Abstract Most current biotechnology industries are based on batch or fed-batch fermentation processes, which often show low productivity and high production costs compared to chemical processes. To increase the economic competitiveness of biological processes, continuous fermentation technologies are being developed that offer significant advantages in comparison with batch/fed-batch fermentation processes, including: (1) removal of potential substrates and product inhibition, (2) prolonging the microbial exponential growth phase and enhancing productivity, and (3) avoiding repeated fermentation preparation and lowering operation and installation costs. However, several key challenges should be addressed for the industrial application of continuous fermentation processes, including (1) contamination of the fermentation system, (2) degeneration of strains, and (3) relatively low product titer. In this study, we reviewed and discussed metabolic engineering and synthetic biology strategies to address these issues.

Keywords continuous fermentation, productivity, contamination, strain degeneration, metabolic engineering

1 Introduction

The production of chemicals, fuels, and materials from abundant bioresources via biological fermentation is an important route for developing a sustainable bioeconomy [1–4]. Industrial production of several important bulk chemicals by fermentation (such as 1,3-propanediol, 1,4-butanediol, *n*-butanol, isobutanol) has been achieved in recent years [5–10]. Despite its enormous potential, large-scale production of low-value bulk chemicals and biofuels by fermentation remains economically challenging owing to its relatively high production cost. Increasing the titer, yield, and productivity of the biological processes is a major goal of many metabolic engineering efforts aimed at reducing production costs. Although increasing product yield and titer has been achieved in many examples, a substantial increase in process productivity remains a challenge owing to the inherent limitation of the employed fermentation processes.

Generally, biological fermentation can be classified into batch/fed-batch and continuous fermentation. In batch/fed-batch fermentation, microorganisms are inoculated into a bioreactor under appropriate conditions. With microbial growth, nutrients are gradually consumed, and the products are continuously accumulated. The products are harvested from the bioreactor after a short culture period. In continuous fermentation, the addition of substrates and harvest of final products are conducted continuously and simultaneously at an appropriate rate [2,4,8,22,23]. To date, continuous fermentation has been successfully implemented in several cases to produce bulk chemicals (Table 1). Compared to batch/fed-batch fermentation, continuous fermentation has several advantages, including (1) avoiding potential substrate and product inhibition by continuous substrate feeding and product removal; (2) prolonging the microbial exponential growth phase and increasing process productivity; (3) avoiding repeated fermentation operations, such as cleaning and sterilization between batches, which are laborious and time-consuming [5,8,10,22]. Thus, an ideal continuous process could reduce the installation and operation costs, making the process more economically competitive.

However, owing to long-term cultivation, continuous fermentation systems are exposed to a higher risk of

Received September 28, 2022; accepted November 17, 2022

E-mail: zhenchen2013@mail.tsinghua.edu.cn

* These authors contributed equally to this work.

contamination, including undesired microorganisms and phages [24–28]. Second, long-term cultivation may cause strain degeneration and process instability owing to plasmid loss or spontaneous mutations, which consequently reduces the strain's performance [29–31]. Finally, product titers in continuous processes are usually lower than those in batch/fed-batch fermentation because of the shorter retention time, which could result in higher separation costs (Table 2) [32,33].

In this study, we present an overview and discussion of potential approaches that could be employed to overcome the above-mentioned barriers to continuous fermentation (Fig. 1). In particular, we focused on strain engineering and process optimization strategies that can be implemented in continuous fermentation processes.

2 Strategies to prevent contamination

2.1 Microbial contamination

The most common bioprocess contamination is microbial contamination, which may significantly reduce process

Table 1 Examples of reported continuous fermentation processes

Product	Strain	Culture time	Ref.
PHA[a)]	*Halomonas campaniensis* LS21	65 days	[11]
Ethanol	*Saccharomyces cerevisiae*	141 days	[12]
Beer	Immobilized yeast cell	35 days	[13]
Ethanol and yeast	*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (SPSC 01)	40 days	[14]
Biohydrogen	Seed sludge from a local municipal wastewater treatment plant	200 days	[15]
Lactic acid	*Lactobacillus rhamnosus*	350 h	[16]
L-lysine	*Corynebacterium glutamicum* B-6	500 h	[17]
Succinic acid	*Actinobacillus succinogenes*	18 days	[18]
ABE[b)]	*Clostridium acetobutylicum*	300 h	[19]
1,3-Propanediol	*Clostridium butyricum*	16 days	[20]
Butanol	Immobilized *Clostridium acetobutylicum*	1000 h	[21]

a) Polyhydroxyalkanoates; b) acetone-butanol-ethanol.

Table 2 Comparison of typical chemical production via batch/fed-batch and continuous fermentation

Product	Strain	Fermentation type	Titer	Yield	Productivity	Ref.
Ethanol	*Saccharomyces cerevisiae*	Batch fermentation	~114.0 $g \cdot L^{-1}$	97% of the theoretical	16.2 $g \cdot L^{-1} \cdot h^{-1}$	[8]
	Saccharomyces cerevisiae	Continuous fermentation	60.5 $g \cdot L^{-1}$	95% of the theoretical	32.0 $g \cdot L^{-1} \cdot h^{-1}$	[8]
Lactic acid	*Lactobacillus casei*	Fed-batch	210 $g \cdot L^{-1}$	90.3%	2.14 $g \cdot L^{-1} \cdot h^{-1}$	[9]
	Lactobacillus rhamnosus	Continuous fermentation	Not reported	68.8%	8.18 $g \cdot L^{-1} \cdot h^{-1}$	[16]
L-lysine	*Corynebacterium glutamicum*	Fed-batch fermentation	100 $g \cdot L^{-1}$	Not reported	~2.1 $g \cdot L^{-1} \cdot h^{-1}$	[17]
	Corynebacterium glutamicum	Continuous fermentation	105 $g \cdot L^{-1}$	0.385 $g \cdot g^{-1}$	5.6 $g \cdot L^{-1} \cdot h^{-1}$	[17]
1,3-Propanediol	*Clostridium butyricum*	Fed-batch fermentation	67.9 $g \cdot L^{-1}$	0.55 $g \cdot g^{-1}$	0.78 $g \cdot L^{-1} \cdot h^{-1}$	[20]
	Clostridium butyricum	Continuous fermentation	30.1 $g \cdot L^{-1}$	0.52 $g \cdot g^{-1}$	1.87 $g \cdot L^{-1} \cdot h^{-1}$	[20]
Butanol (ABE)	*Clostridium acetobutylicum*	Batch fermentation	<11 $g \cdot L^{-1}$	0.175 $g \cdot g^{-1}$	0.17 $g \cdot L^{-1} \cdot h^{-1}$	[19]
	Clostridium acetobutylicum	Continuous fermentation	5.1 $g \cdot L^{-1}$	0.42 $g \cdot g^{-1}$	4.6 $g \cdot L^{-1} \cdot h^{-1}$	[21]

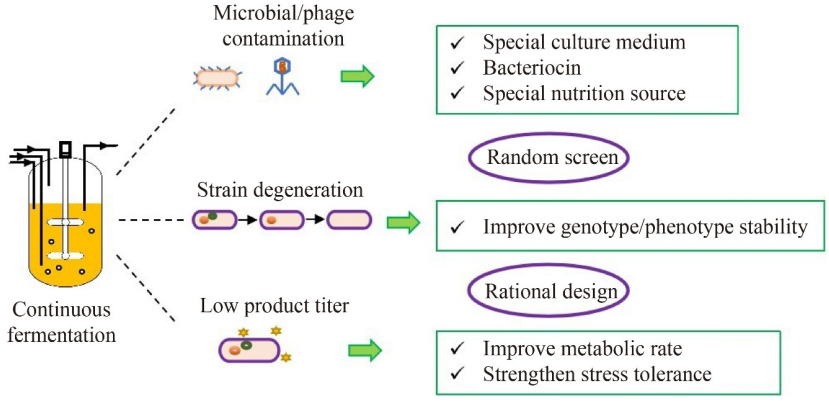

Fig. 1 Barriers to continuous fermentation and potential solutions.

yield and productivity [34–36]. Antibiotics can be used to prevent contamination during specific processes. However, the use of antibiotics increases overall production costs. Furthermore, antibiotics remaining in waste potentially lead to the emergence and spread of antibiotic-tolerant mutants and consequently have a huge negative impact on public health and food safety [27,28]. Therefore, there is an urgent need to develop effective approaches to prevent microbial contamination.

The development of fermentation processes based on specific microorganisms that can grow under harsh conditions is a potential approach to prevent microbial contamination (Fig. 2). Harsh conditions may be as follows: (1) extreme culture conditions, such as very low/high temperature, very low/high pH, and high osmotic pressure; (2) medium containing toxic substances; (3) medium containing unusual nutrients. Fermentation processes that contain one or more of the above-mentioned features are expected to efficiently resist microbial contamination, as most environmental microorganisms cannot grow under such harsh conditions. Some extremophilic bacteria, such as acidophiles, alkaliphiles, psychrophiles, thermophiles, and halophiles, which show significant growth advantages under extreme culture conditions, are promising platform strains for developing continuous fermentation processes.

Successful continuous production processes based on the tolerance of industrial strains to osmotic pressure and acidic environment have been achieved [37,38]. Using a hyperosmotic medium with approximately 6% (w/v) NaCl, a 65-day continuous fermentation was maintained to produce PHAs by the halophile *Halomonas campaniensis* LS21 [4,11,38]. In addition, *Lactobacillus amylovorus* was used to maintain lactic acid production at pH 5.5 for 10 days [39,40]. Some extremophilic bacteria, including alkaliphiles, psychrophiles, and thermophiles, are considered the next-generation industrial strains that allow bioprocesses to be conducted under non-sterile conditions [11,41].

Based on the sensitivity of bacteria to certain compounds, special substances, including organic acids and alcohols, were added to the initial medium for effective contamination control [26,34,35]. Katakura et al. [34] reported a strategy for preventing lactic acid bacterial contamination by adding 50 $g \cdot kg^{-1}$ exogenous ethanol into the stock in a consolidated continuous solid-state bioethanol fermentation system. Analogously, based on acetate-tolerant or lactate-tolerant yeast, bioethanol production processes were constructed by adding exogenous acetate or lactate to prevent bacterial contamination [26,35]. It is worth noting that these types of additives can be recycled as a portion of the product.

Redesigning essential enzymes containing non-standard amino acids is a strategy to prevent the unintended proliferation of genetically modified organisms in natural ecosystems [42,43]. Analogously, metabolic pathways with preference for special substrates, such as special sulfur or nitrogen sources, could be established to prevent biocontamination of fermentation systems from natural ecosystems. This strategy requires a fermentation medium that uses unconventional sulfur or nitrogen sources as the sole source of nutrition.

For example, many strains lack metabolic pathways for the assimilation of formamide or phosphite and consequently suffer from impaired growth and reproduction when there are no additional nutrients. However, formamide could be decomposed to ammonium ions specifically by formamidase, and phosphite could be efficiently oxidized to phosphate by phosphite dehydrogenase [44,45]. By the introduction of these two enzymes, the engineered *Escherichia coli* could survive without any negative effect in the medium using formamide and

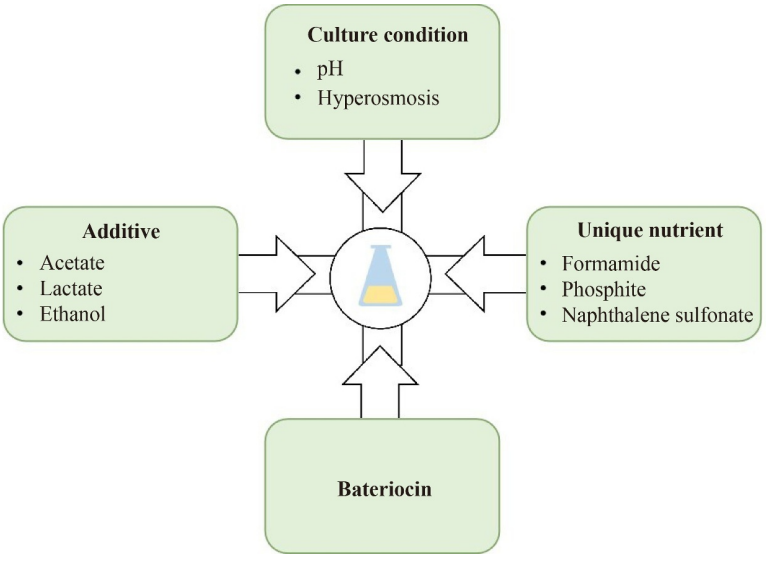

Fig. 2 Approaches to avoid microbial contamination.

phosphite as the sole sources of nitrogen and phosphate nutrition [46].

In addition to common sulfur- and nitrogen-containing compounds, complex compounds may have more stringent restrictions on bacterial contamination. In contrast to alkanesulfonates, the naphthalene sulfonates are resistant to bacterial degradation [47,48]. Brilon et al. [47] obtained two *Pseudomonas* strains that could degrade naphthalene sulfonate to sulfite and sulfate to meet the growth needs by prolonging strain selection under continuous culture conditions. Some coccal green algae, such as *Scenedesmus obliquus*, can also use naphthalene sulfonate as the sole sulfur source [42,49–51]. These strains, with the ability to metabolize naphthalene sulfonate, can be developed into powerful industrial workhorses. In addition, these studies demonstrate the possibility of identifying transferable pathways or enzymes to metabolize naphthalene sulfonate. However, the cost of unconventional sulfur sources should be carefully considered and evaluated.

In addition to optimizing culture conditions, the use of bacteriocins produced by gram-positive bacteria is another strategy for controlling microbial contamination [24]. Compared to antibiotics, bacteriocins have lower toxicity with both broad and narrow spectrum, which avoids collateral damage. Moreover, owing to their peptide nature, bacteriocins are more prone to engineering than classical antibiotics and can be produced *in situ* [52]. Thus, using bacteriocin producers as host strains or adding bacteriocin to the medium offers a potential approach to control microbial contamination. A *Pediococcus acidilactici* strain that can secrete bacteriocin was used to produce L-lactic acid in an open system without sterilization, and a high L-lactic acid titer was obtained without any contamination [53]. *Enterococcus faecium* RZS C5 and *Lactobacillus plantarum* SA6 are bacteriocin producers with the potential to be utilized in industrial fermentation [54].

2.2 Biophage contamination

Biophage contamination is another type of contamination observed during fermentation. Biophages show hostile relationships with industrial hosts and are usually lethal to bioprocesses [55,56]. The key to avoiding biophage contamination is to develop phage-resistant industrial hosts (Table 3), which can be achieved in two ways: random screening and rational design [56–59].

Random screening (also known as phage-induced screening) is achieved by culturing strains under phage suppression and screening surviving strains that undergo bacteriophage-insensitive mutations. In addition, phage-induced screening is usually implemented using physical, chemical, and biological mutagens to create large mutant libraries [58,59,70]. Mei et al. [59] obtained 21 stable phage-resistant *E. coli* strains after 72 h of cultivation by phage-induced screening and 39 stable phage-resistant strains by employing a 60-s UV exposure before cultivation. Transposon mutagenesis and insertional mutagenesis are alternative approaches to obtain mutant strains with desired features [68,71], which allow for the targeting of potential gene sites to enhance phage resistance of the resulting strains when combined with genomic sequencing [62,71].

Although random screening is useful and direct, it is usually labor-intensive and time-consuming. Some newly developed technologies and equipment, such as flow cytometry, are helpful for overcoming these obstacles [57,72]. Flow cytometry is an instrumental tool with high detection speed (up to 10^4 cells·s^{-1}) and excellent sensitivity (ability to select 2 out of 10^7 cells) [58,73]. Combined with immunoselection, flow cytometry can sort strains with or without binding to anti-phage antibodies and identify potentially phage-resistant strains, which shortens the screening period and improves efficiency [74,75]. Moreover, flow cytometry allows the identification of mutants resistant to multiple phages

Table 3 Approaches to enable phage-resistance

Species	Biophage	Approach description	Ref.
E. coli DH5*α*	Phage ΦTB16–25	A plasmid-encoded Abi (abortive infection) system, ToxIN, promotes cell death, and limits phage replication	[60]
*E. coli Gly*A	Phage ESP1	Phage-induced screen and UV-coupling phage-induced screen	[59]
E. coli K-12	Phage mEp213, phage *λ*	Phage-induced screen combining transposon mutagenesis	[61]
E. coli O157	Phage T4, phage T7	Phage-induced screen combining transposon mutagenesis	[62]
L. lactis	Phage Φmpl51, Φmpl86, Φmpl961, Φmpl1083	A plasmid-encoded protein to inhibit phage adsorption by masking phage receptor	[63]
L. lactis	Phage Tuc2009	A phage-encoded superinfection exclusion (Sie) to block phage DNA injection	[64]
S. thermophilus	Phage 2972	Combine clustered regularly interspaced short palindromic regions (CRISPR)-CRISPR-associated (Cas) and restriction−modification (R−M) systems	[65]
S. thermophilus	Phage κ1, κ3, κ4, κ5	Expression of Antisense RNA complementary to putative helicase gene to interfere phage transcription	[66]
S. thermophilus CNRZ368	Phage ΦST51	A novel type II R−M system, Sth368I, to cleave invade phage genome	[67]
S. thermophilus Sfi1	Phage Sfi19 and heterologous phages	Phage-induced screen combining thermolabile insertional vector	[68]
S. thermophilus NCK1125	Phage κ3	Expression of a mutant primase in trans to inhibit phage replication	[69]

based on their ability to distinguish different fluorochromes, making it possible to screen strains with multiple characteristics simultaneously.

Another effective approach for obtaining phage-resistant strains is rational design, which aims to manipulate industrial strains based on the molecular mechanisms of phage infection. High-throughput technologies are powerful tools for understanding phage infection mechanisms and providing novel and efficient strategies for the elimination of phage contamination. For example, using RB-TnSeq, Dub-seq, and CRISPRi technologies, Mutalik et al. [76] revealed multiple host genetic factors involved in phage resistance to 14 double-stranded DNA phages in *E. coli*. By constructing a well-classified library of 68 newly isolated *E. coli*-infecting phages, Maffei et al. [77] explored the phage-host interactions systematically, which provide a good example for understanding the molecular mechanisms of phage infection, even for many non-traditional systems. To avoid biophage attack and abort the infection process, specific defensive strategies can be utilized to target different stages, including phage adsorption, DNA injection, and the propagation cycle, as described below (Fig. 3) [56,78,79].

Phage adsorption. Phage adsorption is the first step in phage infection, during which the receptor in the cell envelope plays a pivotal role. Physical masking, changes in structure, or even the absence of the phage receptor are all beneficial for inhibiting phage adsorption [56,61].

Physical masking is always attributed to special proteins or carbohydrates produced by the acquired plasmids, which cover the receptor to lower phage sensitivity. A plasmid isolated from *Lactococcus lactis* subsp. *lactis* MPL56 synthesized a lectin-like protein that adsorbed specific monosaccharide components of polysaccharides in the cell wall [63]. Subsequently, this protein competes with phages for the specific recognition sites of receptors to inhibit phage adsorption. Studies have also demonstrated that mutations in genes involved in the synthesis of receptors (e.g., *fhuA*) and outer membrane lipopolysaccharides (e.g., *waaC* and *gmhD*) contribute to the acquisition of resistance to several phages, including mEp213 and λ [61].

DNA injection. After adsorption, the phage DNA was injected into the cells for amplification. At this stage, two approaches were used to inhibit phage propagation. The first approach involves blocking phage DNA injection into the host. Sie and Sie-like systems are thought to play a pivotal role in the injection blocking phenomenon, although the mechanism is poorly understood [80–82]. Most Sie genes are located within the prophage regions of chromosomes, except for the first lactococcal injection blocking system that was identified on the pNP40 plasmid and specifically blocks the DNA penetration of the lactococcal Φc2 phage group [64,83].

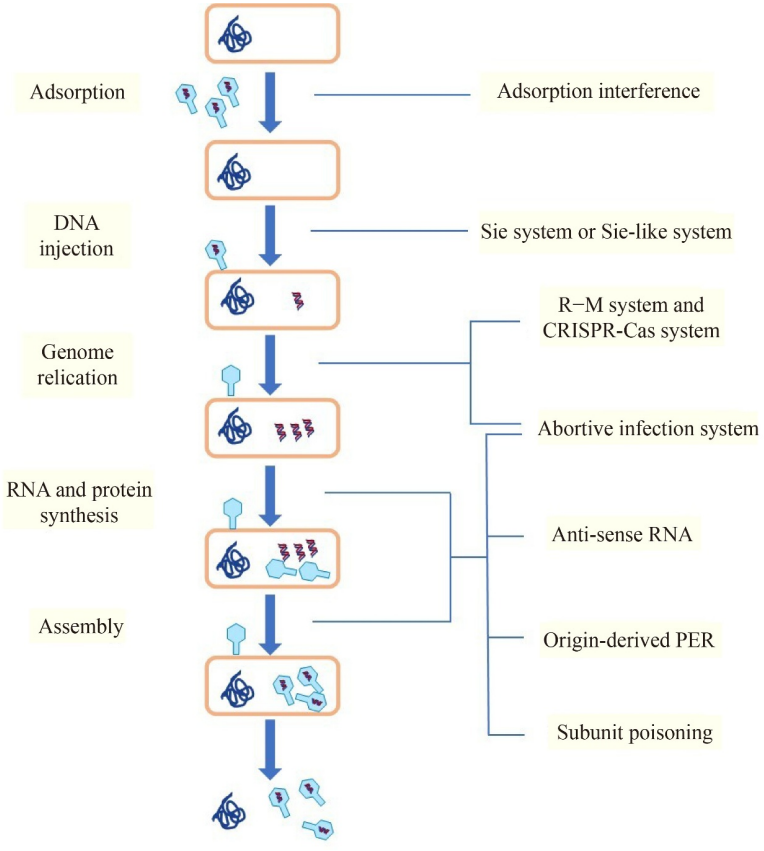

Fig. 3 Rational approaches to obtain phage-resistant microbes.

Another approach is to degrade invading DNA. Two systems, R−M and CRISPR-Cas, can be used against foreign DNA invasion [65,78,84]. The R−M system contains methyltransferase, which protects the host DNA from degradation by introducing a methyl group into a specific nucleotide of the target site, and endonuclease, which recognizes specific target sequences and degrades invading DNA lacking a unique methylation pattern [65]. For example, a LlaI R−M system that recognizes and cleaves the sequence 3′-GATC-5′ has been proven to endow strong phage resistance after being introduced into *Streptococcus thermophilus* and *L. lactis* [56,57]. Another tool to degrade foreign DNA is the CRISPR/Cas9 system, which is composed of CRISPR array and Cas proteins [65,78]. This system has been widely utilized for genome editing in bacteria, yeasts, and mammals. However, the nature of this system is a defense mechanism in prokaryotes against invading phages or plasmids [78,85,86]. Through careful design and control of expression, an artificial CRISPR/Cas9 system targeting multiple loci on the T7 phage genome efficiently protected *E. coli* BL21 from phage infection [87]. Recently, a novel anti-phage system, DISARM (defense island system associated with R−M), has been reported to widely exist in bacteria and archaea [88]. DISARM contains four genes encoding a DNA methylase, helicase domain, phospholipase D domain, DUF1998 domain, and a gene with unknown function, which modifies the host DNA with methylase as a marker for recognition and restricts invading phage DNA. This system has been successfully transferred to *Bacillus subtilis* for protection from all three major families of double-stranded DNA phages [88].

Notably, different anti-phage systems may be combined to improve defense efficiency. To demonstrate the compatibility of R−M and CRISPR/Cas9 systems, Dupuis et al. [65,78] constructed an *S. thermophilus* strain containing both systems by introducing a plasmid with genes coding for the LlaDCHI R−M system. It was found that the specific methylation of phage DNA caused by the R−M system does not impair the interference activity of the CRISPR/Cas9 system. More importantly, the phage resistance of bacteria can be further improved by combining the two systems owing to the reduction in leakiness. In addition, the existence of a pan-immune system was proposed, which was characterized by the sharing of various phage-immune systems via horizontal gene transfer between closely related strains [89]. The pan-immune system may benefit bacterial population fitness without causing an excessive metabolic burden imposed by multi-immune systems in a single cell [89]. Therefore, it is promising to study the interactions and co-utilization of distinct anti-phage systems in industrial strains.

DNA injection. If phage DNA invades the host successfully, the propagation process starts, during which the Abi system plays a primary role in providing population protection. Abi systems are found in both gram-positive and gram-negative species, including *E. coli*, *B. subtilis*, *Streptococcus pyogenes*, *Vibrio cholerae*, and *Lactococcus lactis* [60,90,91]. Abi systems can interrupt the propagation process at different points, such as phage DNA replication, transcription, protein synthesis, and phage particle assembly. Some Abi systems, such as AbiZ, can induce premature cell lysis [90,92]. To date, many Abi systems have been identified and designated into various groups, most of which are plasmid-encoded and simple genetic expression systems, providing them opportunities to be transferred and adapted to other strains [56,57].

Recently, Zou et al. [93,94] developed a DNA phosphorothioation-based Ssp defense module, SspABCD-SspE. SspABCD catalyzes the phosphorothioate modification between the two dCs in a 3-bp 5′-CCA-3′ sequence in which the non-bridging oxygen is replaced by sulfur. Thereafter, SspE recognizes sequence-specific phosphorothioate modifications and inhibits phage activity by introducing nicking damage. The SspABCD-SspE system showed no toxicity in hosts and no significant effect on recombinant protein production [94].

Antisense RNAs and origin-derived phage-encoded resistance have also been implemented to disturb phage propagation in *L. lactis* and *S. thermophilus* [66,79]. Subunit poisoning is another strategy to acquire phage resistance. The expression of mutant protein subunits in *trans*, such as putative helicases and primases, can suppress the function of native protein elements that are necessary for phage genome replication. Therefore, the phage propagation cycle is disturbed [57,69].

3 Strategies to prevent strain degeneration

Another challenge during continuous fermentation is the degeneration of industrial strains. Evolvability is an intrinsic feature of all living cells and is generally beneficial for the entire population to increase viability and adapt to various situations in the natural environment [95,96]. However, during the evolutionary process, most of the mutations were negative. For example, for *E. coli* K-12, the rate of deleterious mutations per genome per replication is approximately 2×10^{-4}–8×10^{-4}, which is 10000 times higher than that of beneficial mutations [95]. Since industrial workhorses are usually well-developed and carefully designed, they are more sensitive to most newly emerging mutations. Although the mutation is beneficial for cell growth, there is a trade-off between cell viability and industrial properties. In this study, we present approaches to prevent strain degradation from two aspects: genotype stability and phenotype stability (Fig. 4) [31,96,97].

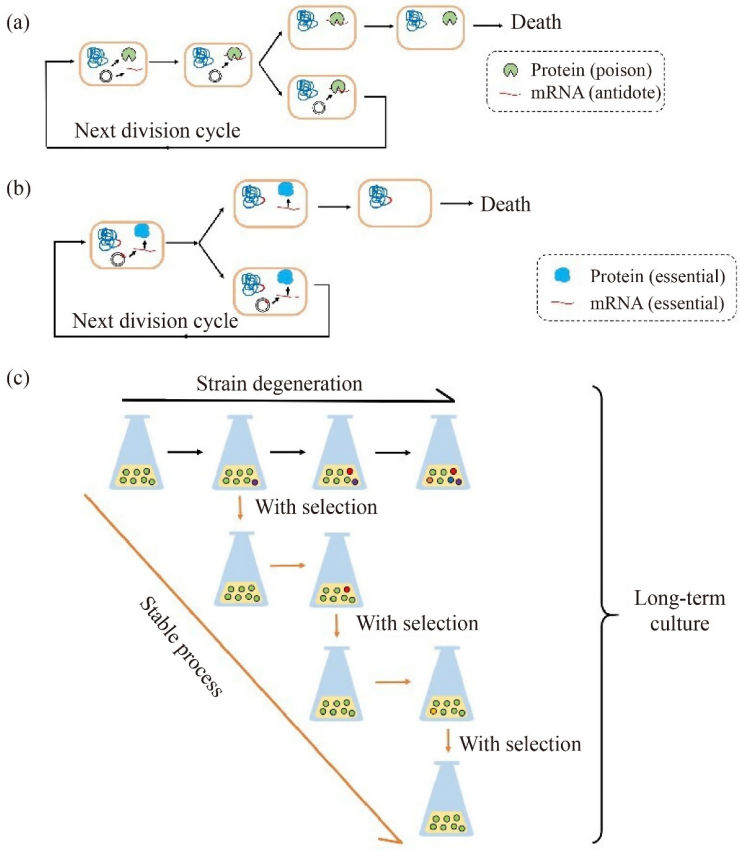

Fig. 4 Approaches to avoid strain degeneration. (a) Outline of the poison-antidote system to enable plasmid stability. If plasmid-free cells generate, the antidote degrades in a short time, while the poison exists stably. Then the cells without the plasmid will be killed. (b) Outline of essential-gene-deficiency approach to enable plasmid stability. The parental cells bear deficiency of certain essential genes, which is usually complemented by an expression vector. If the daughter cells lose the plasmid, a lack of the essential factors or proteins will occur, which is lethal to the plasmid-free cells. (c) Outline of phenotype selection to enable phenotype stability during continuous fermentation. The phenotype-monitoring system will kill the cells with targeted phenotype lower than the pre-set threshold, thus maintain the stability of bioprocesses.

3.1 Genotype stability

Genotype stability of industrial strains refers to plasmid stability and genome stability. In bioprocesses, plasmid-based expression systems are used to produce desired metabolites [6,7,98,99]. However, during cell propagation, plasmid loss occurs because of the uneven distribution of cell resources [100]. Owing to the burden of plasmid replication and gene expression on cell growth and metabolism, long-term cultivation without any selection will lead to an increase in plasmid-free cells in the population, causing a negative effect on productivity [97,101].

A conventional method to maintain the plasmids is using antibiotics as a selective pressure, which kills the plasmid-free daughter cells in each generation due to their lack of antibiotic resistance [97,101]. However, as mentioned above, antibiotics may increase economic costs and potential environmental hazards from both the antibiotic itself and resistance genes in industrial waste. Therefore, alternative methods are urgently required.

Other poison-antidote systems can also be applied to endow plasmid stability instead of antibiotics [30]. Gerdes et al. [102] demonstrated a *hok/sok* system to maintain plasmid stability in gram-negative bacteria, in which the Hok protein encoded by the *hok* gene is poisonous to the host, and the *sok* RNA encoded by the *sok* gene can repress the activity of the Hok protein to eliminate toxicity. In addition, *sok* RNA is unstable, with a half-life of less than 30 s, whereas *hok* mRNA and its truncated form are very stable, with half-lives in the order of hours. In this case, newly formed plasmid-free cells would lose the antidote in a short period but still endure the poison even if they lack the *hok* gene (Fig. 4(a)). When the *hok/sok* system was inserted into an unstable plasmid, the frequency of appearance of plasmid-free cells was reduced by a factor of 10^3 to 10^5. In addition, other *hok*-homologous genes, such as *flm*, *srnB*, *pnd*, *sef*, and *relF*, have been identified [30,102,103]. In contrast to the aforementioned systems, Smith et al. [30] presented a three-component system PasABC in pTF-FC2, which includes a PasC to enhance the ability of the antitoxin PasA to neutralize the toxin PasB. All of these systems are effective tools for maintaining plasmids in cells.

Moreover, a deficiency of essential genes can also be employed to eliminate plasmid-free cells (Fig. 4(b)). Nilsson and Skogman [31] replaced the essential gene *val* encoding valyl-tRNA synthetase in *E. coli* with a temperature-sensitive allele that is only functional under a certain temperature. By introducing a normal wild-type allele into the heterologous plasmid, the plasmid could be maintained for over 150 generations. Terrinoni et al. [104] targeted another essential gene, *lgt*, encoding prolipoprotein glyceryl transferase, which was deleted from the genome of *E. coli* and complemented by an expression vector carrying the *V. cholerae*-derived *lgt* gene. There was no significant difference in the growth rate or the final optical density between the new strain and the wild type, and the plasmid retention rate was 100% after 40 generations. In addition, the *ssb* gene, encoding the SSB protein required for DNA replication and cell viability, and the *infA* gene, encoding the translation-initiation factor required for cell viability, are alternative targets for plasmid maintenance [100,101].

Furthermore, genome stability can be enhanced by genome modifications. Classical fluctuation assays have been conducted to analyze mutations in growing populations of *E. coli* MG1655 by selecting for D-cycloserine-resistant mutants. It was revealed by PCR that point mutations and small indels accounted for 74.3% of the total mutations, insertion sequence (IS) transpositions accounted for 24.2%, and deletions accounted for the rest [105]. Thus, different approaches for these mutation types may be used to enhance genome stability.

First, a stronger and more powerful DNA-repairing system might reduce bacterial mutation rates. The methyl-directed mismatch repair (MMR) system plays an important role in the repair of post-replicative errors and oxidative DNA lesions [106]. Enhancement of the MMR system by overexpressing *mutL*, *mutH*, and *mutA* reduced the mutation rate by more than 50% [107].

In addition, a simplified "clean" strain with the elimination of nonessential genes, including recombinogenic or mobile DNA, and cryptic virulence genes may reduce the mutations induced by IS transpositions [96,105,108]. For an MG1655-derived strain whose genome size was deleted by 8.11%, no IS-related mutations were found in the fluctuation assay, and the total mutation rate decreased by 21.2% compared to MG1655 [105]. Furthermore, by eliminating the error-prone DNA polymerase enzyme from the strain lacking all genomic IS elements, a more stable strain was obtained that could serve as a host for molecular biology, synthetic biology, and industrial applications [108].

3.2 Phenotype stability

Phenotype stability can be used as a direct indicator of plant productivity. In addition to genotype degradation, a deteriorative phenotype can also arise from non-genetic cell-to-cell variation [29] due to uneven cell division, variations in gene copy number and epigenetic modification, stochastic gene expression, variable mRNA stability, and protein activity [29,109]. These factors can generate a broad range of protein and metabolite concentration variations. Selection based on phenotype by directly monitoring the performance of the strains can identify and eliminate variation with low productivity, thus avoiding strain degeneration and enhancing productivity without considering genotype and non-genetic cell-to-cell variation (Fig. 4(c)). Intracellular transcription factor-based biosensors offer the opportunity to establish these methods [109,110].

Xiao et al. [29] developed an *in vivo* population quality control system to execute continuous phenotype selection for high-performance variants to produce free fatty acids (FFAs). The vital part of this system is the fatty acid-responsive biosensor. Specifically, an FFA-responsive transcription factor FadR, correlated to FFAs via the FFA precursor acyl-CoA, and a synthetic promoter P_{AR} repressed by FadR were employed to regulate the expression of a tetracycline efflux protein. Under the selection pressure of tetracycline, this system could continuously screen for high-performing variants and increased the FFA titer from 4.8 to 21.5 $g \cdot L^{-1}$ [29]. Similarly, Snoek et al. [110] constructed a biosensor system in *Saccharomyces cerevisiae* based on the LysR-type transcriptional regulator family. They used the transcriptional regulator BenM, which correlates with *in vivo cis*,*cis*-muconic acid concentration, to control the expression of an antibiotic resistance gene, thus coupling muconic acid production to cell fitness. Under G418 pressure, the enriched best-performing yeast strain produced 2 $g \cdot L^{-1}$ of muconic acid in 120 h.

The *in vivo* biosensor is a rapid and high-throughput screening tool; however, there are still some barriers to overcome. First, it is not prone to finding a strictly correlating transcription factor or promoter to a certain product. Therefore, many pre-experiments and rational designs are required. Second, many effectors may transfer from cell to cell, interfering with the responses of the biosensor [110]. Finally, the biosensors mentioned above still involve the use of antibiotics and antibiotic-resistance genes, which could be replaced by poison-antidote systems for better industrial application.

4 Strategies to increase productivity

Generally, continuous processes have lower product titers than fed-batch fermentation processes because of a shorter retention time, which increases the cost of downstream processes. Metabolic engineering and synthetic biology offer the opportunity to obtain powerful workhorses to produce desired chemicals and increase productivity.

However, the overall efficiency of bioprocesses is usually limited by the cell growth rate and substrate consumption rate [111,112]. Hosts with high growth rates, substrate consumption rates, and metabolic synthetic capacity can ameliorate these issues, thus improving process productivity and economic competitiveness.

4.1 Metabolic productivity

Several efforts have been made to improve the metabolic productivity of industrial strains. Enhancing rate-limiting reactions of cellular metabolism is a generally applied strategy. Enzymes in central metabolic pathways, including glycolysis, the pentose phosphate cycle, and the tricarboxylic acid cycle, are often explored to increase metabolic productivity [33,113–117]. For example, a *Corynebacterium glutamicum* strain overexpressing phosphofructokinase, pyruvate kinase, glyceraldehyde-3-phosphate dehydrogenase, and glucose-6-phosphate isomerase showed 5.0- and 7.0-fold increases in glucose consumption and alanine productivity, respectively, compared to the parental strain [114].

Another approach to accelerate substrate consumption is to introduce ATP deficiency in bioproduction systems. Several studies have shown that glycolytic flux is limited by ATP utilization and ADP availability during the oxidative metabolism of glucose rather than by ATP generation [118–120]. Based on this conclusion, a mutation that deleted the *atpFH* gene was introduced into *E. coli*, resulting in disrupted oxidative phosphorylation while retaining the hydrolytic activity of the F_1-ATPase [119,120]. With this mutation, a maximum of only four ATP molecules can be produced per glucose molecule (instead of a theoretical maximum of 36 ATP molecules for wild-type), promoting glucose consumption to provide sufficient ATP for biomass formation and maintenance requirements. The resulting strain also showed a 2-fold increase in glycolytic flux compared with the wild-type strain [119]. Moreover, *atp*FH deletion was conducted in an *E. coli* strain to increase glycolytic flux and mevalonate productivity, resulting in a 2.1-fold improvement in maximum productivity [121]. Similarly, reducing the activity of *atpG*, which encodes a subunit of H^+-ATPase in *C. glutamicum*, resulted in an improved glucose consumption rate of 10.5% and (2R,3R)-2,3-butanediol productivity of 10.9% [122]. In addition, Luo et al. [123] constructed an ATP-futile cycle system (ATP-FCS) in *Candida glabrata*, a dominant yeast strain for pyruvate production. As a result, the intracellular ATP level was decreased by 51% without destroying the F_0F_1-ATPase function, leading to improved glycolysis flux and pyruvate production. With further optimization of ATP-FCS, an ultimate increase in pyruvate titer and substrate conversion rate of 98.5% and 160%, respectively, were achieved.

In addition, substrate transport systems play an important role in improving metabolic rates [124–126]. Engineering of sugar transport systems is widely used to fine-tune the balance between production and growth to meet diverse production requirements [124,125]. In many microorganisms, the major type of glucose transport system is the phosphoenolpyruvate (PEP)/carbohydrate phosphotransferase system (PTS), which is composed of soluble and non-sugar-specific protein enzymes I and HPr (Fig. 5) [127,128]. This system makes the greatest contribution to growth and metabolism but consumes a large quantity of PEP, the precursor of succinate, malate, and aromatic compounds [126,127], resulting in competition between productivity and metabolic capacity. To increase the PEP pool by tuning the glucose transport process, Hao et al. and Michalowski et al. [129,130] deleted the GTP pyrophosphokinase and introduced a ppGpp 3′-pyrophosphohydrolase mutant. The glucose uptake rate and product L-valine titer increased by 16.7% and 22.2%, respectively. Although strains independent of PTS are ideal hosts for some specific products, they often show limited growth on glucose [127]. A non-PTS glucose system (Fig. 5) in *E. coli*, which is active upon nutrient starvation, is composed of the galactose proton symporter GalP encoded by *galP* and glucokinase encoded by *glk* [127,128]. By inactivation of PTS and combinatorial modulation of *galP* and *glk* expression, Lu et al. [128] obtained a strain with the highest succinate production rate (1.64 $g \cdot L^{-1} \cdot h^{-1}$), which was 10-fold higher than that of the PTS^- strain and 39% higher than that of the wild-type *E. coli* under anaerobic conditions. Similar to *E. coli*, *C. glutamicum* also possesses both PTS and non-PTS, which expands the options for the development of more efficient production strains for succinate, malate, amino acids, and aromatic compounds [125,126,131]. For example, with the deletion of *iolR* and overexpression of *iolT1* and *ppgk* in PTS-defective *C. glutamicum*, succinic acid production increased by 11.6% [126]. The glucose transport system has also been successfully engineered in *Bacillus licheniformis* and *B. subtilis* for the improved production of 2-phenylethanol and *N*-acetylglucosamine, respectively [132,133].

Adaptive laboratory evolution (ALE) can also be used to improve the fitness and productivity of microorganisms under certain environmental conditions [134,135]. Based on different cultivation methods, ALE can be divided into serial batch cultivation and chemostat cultivation [135,136]. In ALE processes, microorganisms are cultivated under artificially defined conditions for several weeks or even years, and strains with the desired phenotypes can be obtained [135–139]. Deletions of *ptsH*, *ptsI*, and *crr* resulted in a PTS-deficient *E. coli* strain that showed 79% loss in fitness and a high level of PEP accumulation. The strain was subsequently evolved on glucose minimal media using an automated ALE platform, resulting in a 259% improvement in fitness on average and high accumulation of aromatic amino acid precursors, thus

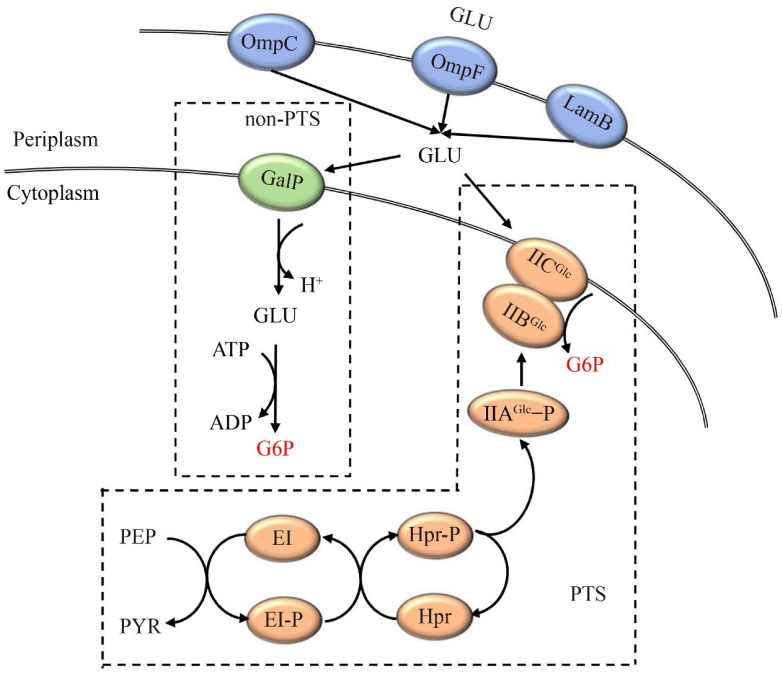

Fig. 5 Proteins involved in glucose transport and phosphorylation in *E. coli*. PTS glucose transport system and non-PTS glucose transport system (GLU: glucose; G6P: glucose 6-phosphate; PYR: pyruvate).

providing a fast-growing host for aromatic metabolite bio-production [140]. Similarly, ALE was applied to increase ethanol production from xylose by an engineered *S. cerevisiae* strain, which showed an 8.5-fold increase in xylose consumption rate [137]. In addition to the growth and metabolic rates, phenotypes, including stress tolerance and production yields, can be improved by ALE under proper conditions with the aid of biosensors and genome-editing methods [141–144]. For example, Chen et al. [145] designed an efficient continuous ALE system utilizing CRISPR-Cas9-facilitated *in vivo* mutagenesis and real-time monitoring of cell growth and tryptophan-induced fluorescence intensity using a tryptophan biosensor. After evolving the PTS^- and GalP/Glk-dependent tryptophan-producing strains, the specific production rate of tryptophan was improved by 52.93% in fed-batch fermentation.

4.2 Strain robustness

High titers of products could simplify the separation processes and improve the economics of bioprocesses, which, in contrast, require the strains in the bioreactor to survive under high titers of metabolites for a long time. However, during long-term fermentation, stress caused by high osmotic pressure and high concentrations of products or by-products is usually harmful to microorganisms by damaging biological molecules, membranes, or disrupting biological processes [8,146,147]. Consequently, an improvement in stress tolerance is important for production [148].

Different biological components have been identified and introduced in microbes to regulate and maintain intracellular turgor pressure and improve their osmotic tolerance and survival ability, including aquaporin encoded by *aqpZ* and potassium transporters encoded by *kup*, *kdpFABC*, and *trkA* [120,149–151]. In addition, global transcriptional regulators, such as sigma S (σ^S, rpoS) from gram-negative bacteria and sigma B (σ^B) from gram-positive bacteria, also play important roles in osmotolerance [152–154]. IrrE, a global regulator of *Deinococcus radiodurans*, can act in both *E. coli* and yeast to improve osmotic tolerance [152,153]. In addition, osmoprotectants, such as glycine betaine, trehalose, proline, and heat-shock proteins, such as GroESL and Dnak, can also be added or synthesized *in situ* to alleviate osmotic stress [155–161]. However, it is often challenging to improve osmotic tolerance and cellular production capacity simultaneously.

Directed evolution is a simple and powerful approach for improving the overall performance of strains, which combines diversified mutant libraries with high-throughput screening (Fig. 6) [162–164]. For example, genome shuffling and recursive protoplast fusion are often used to perform mutagenesis [164]. Yu et al. [163] improved the osmotic pressure tolerance of *Lactobacillus rhamnosus* ATCC 11443 to enhance L-lactic acid production by combining ultraviolet irradiation, nitrosoguanidine mutagenesis, and recursive protoplast fusion. The lactic acid production, cell growth, and glucose consumption of the best-performing strain from the second-round genome-shuffled populations were 71.4%, 44.9%, and 62.2% higher than those of the wild-type at high osmotic pressure, respectively.

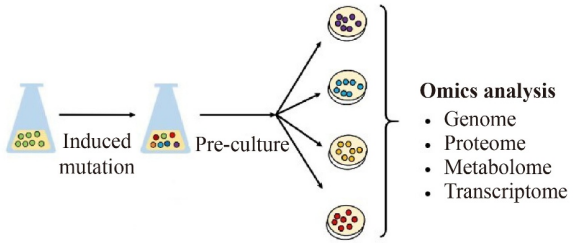

Fig. 6 Directed evolution combined with induced mutagenesis and omics analysis. Directed evolution and high throughput screening from the induced mutagenesis library according to the desired phenotypes. Omics analysis can be used to analyze the obtained strains for deep study of the mechanism.

With the development of omics techniques, many unexpected modifiable genes can be targeted to improve cellular tolerance to osmotic pressure. Based on the genome sequencing of a high-succinate-producing and osmotolerant *E. coli* strain obtained by genetic engineering and metabolic evolution, Xiao et al. and Zhu et al. [165,166] found a mutation in *cusS* (G629T) that activated the expression of *CusCFBA* to transport Ag(I) and Cu(I) out of the cells to reduce the toxicity of Cu(I). The introduction of a *cusS* mutation into a wild-type strain led to a 21% increase in cell mass and a 40% increase in succinate titer [165]. Analogously, combining transposon (Tn) mutagenesis with genome-wide next-generation sequencing-based Tn insertion site determination, some novel genes in *E. coli* have been identified to improve osmotic tolerance, including *rfe*, *typA*, *yciW*, *ptsP*, *evgA*, *ackA*, and *yobF* [167,168].

Compared to genome and transcriptome analyses, proteome and metabolome analyses can provide more direct information in response to high osmotic pressure. Yang et al. [169] quantitatively analyzed differential intracellular protein levels of *Yarrowia lipolytica* cultured under low (3.17 osmol·kg^{-1}) and high (4.21 osmol·kg^{-1}) osmotic pressures. Under hyperosmotic stress, proteins related to nucleotide biosynthesis, energy generation, metabolism, cell rescue, and stress response were significantly inhibited, reflecting the growth arrest of *Y. lipolytica*. These results provide guidelines for improving osmotic tolerance. Cheng et al. [146] conducted a comparative proteomic analysis of *S. cerevisiae* in different fermentation processes and identified eight oxidative responses and heat-shock proteins important for cellular redox and osmotic state adjustment. Sevin et al. [151] analyzed the global metabolic response of *E. coli* to sustained hyperosmotic stress using non-targeted mass spectrometry. The abundance of 52% of the 1071 detected metabolites changed with increasing salt concentrations. In addition to known osmoprotectants, ubiquinone-8 increased by more than 100 times with increasing salt concentration, which could reside flat in the center of the lipid bilayer and increase the hydrophobic thickness of the cytoplasmic membrane to enhance its cytoplasmic membrane stability [151].

Instead of enhancing osmotic tolerance through gene modification, extremophiles can provide a shortcut to achieve this purpose. *Halomonas* spp. are halophilic bacteria that can be developed into industrial strains to produce bulk chemicals, including polymeric materials and biofuels [38]. A series of genome-editing tools based on *Halomonas* spp. have been developed. Moreover, fermentation processes can be executed with a very high cell density of over 80 g·L^{-1} (CDW) under high NaCl concentration and non-sterile conditions [23,38,170]. *Vibrio natriegens* is another attractive halophilic microorganism with a minimal generation time between 7 and 10 min, which is more than two times faster than *E. coli* in rich media and 2–4 times faster in minimal media. Moreover, *V. natriegens* has an exceptionally high glucose uptake rate in minimal media under both aerobic (~3.9 g·g^{-1}·h^{-1}) and anaerobic conditions (~7.8 g·g^{-1}·h^{-1}) [170–172]. These features ensure that these two strains are remarkably superior for development as industrial hosts.

For the bioproduction of organic acids, oxidative and acidic stresses also significantly affect strain performance during long-term fermentation. Fungi are often selected as the chassis for organic acid fermentation at low pH. For instance, *Aspergillus terreus* has been used for itaconic acid fermentation because of its high metabolic capacity and acid tolerance [173,174]. Alternative NADH dehydrogenases in many fungi protect the cell from oxidative stress [175]. With peptide supplementation during fumaric acid fermentation by *Rhizopus oryzae*, productivity was elevated by nearly 100% owing to the improvement in cell stress defense systems and enhanced glycolytic flux [176].

5 Conclusions and prospect

Advances in metabolic engineering and synthetic biology have provided great opportunities to improve the performance of industrial strains for chemical and biofuel production. With more advanced genetically modified strains, updating process engineering is necessary to increase the economic competitiveness of bioproduction systems [5,8,11,18,146]. Continuous fermentation offers competitive advantages over classical batch/fed-batch fermentation processes, including high productivity, low operating costs, and high economic efficiency. In this study, we summarized the key challenges in continuous fermentation and demonstrated several approaches to overcome barriers to microbial/phage contamination, strain degeneration, and low product titers.

To date, most of the contamination-preventing approaches for continuous fermentation have been developed against one type of contamination. Thus, it is necessary to

combine versatile approaches to meet industrial requirements. Moreover, the development of continuous fermentation processes requires precise equipment, process design, and control systems. For example, although several halophilic microorganisms, such as *Halomonas* spp. and *V. natriegens*, exhibit strong tolerance to high osmotic pressure, excellent growth, and metabolic rates [171,177], the implementation of these organisms in continuous culture requires the utilization of specific equipment to cope with high-salt media. In addition, recently developed biotechnological techniques, such as ALE together with high-throughput methods and multi-omics analysis, may provide optimized industrial strains and help discover previously unknown mechanisms of contamination and strain degeneration, which can be used for process improvement of continuous fermentation. For example, fluorescence-activated cell sorting has been widely applied for high-throughput screening of bacteria, yeasts, and fungi with the desired phenotypes [178]. Furthermore, the combination of immobilization technology with optimized strains is important for continuous fermentation. In summary, continuous fermentation should be further optimized using various approaches for a wide range of industrial applications.

Acknowledgements This work was supported by the National Key R&D Program of China (Grant No. 2021YFC2100900), the National Natural Science Foundation of China (Grant Nos. 21938004, 22078172, and 21878172), and DongGuan Innovative Research Team Program (Grant No. 201536000100033).

References

1. Clomburg J M, Crumbley A M, Gonzalez R. Industrial biomanufacturing: the future of chemical production. Science, 2017, 355(6320): aag0804
2. Formenti L R, Nørregaard A, Bolic A, Hernandez D Q, Hagemann T, Heins A L, Larsson H, Mears L, Mauricio-Iglesias M, Krühne U, Gernaey K V. Challenges in industrial fermentation technology research. Biotechnology Journal, 2014, 9(6): 727–738
3. Gu Y, Jiang Y, Wu H, Liu X, Li Z, Li J, Xiao H, Shen Z, Dong H, Yang Y, Li Y, Jiang W, Yang S. Economical challenges to microbial producers of butanol: feedstock, butanol ratio and titer. Biotechnology Journal, 2011, 6(11): 1348–1357
4. Li T, Chen X B, Chen J C, Wu Q, Chen G Q. Open and continuous fermentation: products, conditions and bioprocess economy. Biotechnology Journal, 2014, 9(12): 1503–1511
5. Verbelen P J, De Schutter D P, Delvaux F, Verstrepen K J, Delvaux F R. Immobilized yeast cell systems for continuous fermentation applications. Biotechnology Letters, 2006, 28(19): 1515–1525
6. Talebnia F, Taherzadeh M J. *In situ* detoxification and continuous cultivation of dilute-acid hydrolyzate to ethanol by encapsulated *S. cerevisiae*. Journal of Biotechnology, 2006, 125(3): 377–384
7. Mussatto S I, Dragone G, Guimaraes P M, Silva J P, Carneiro L M, Roberto I C, Vicente A, Domingues L, Teixeira J A. Technological trends, global market, and challenges of bio-ethanol production. Biotechnology Advances, 2010, 28(6): 817–830
8. Ghose T K, Tyagi R D. Rapid ethanol fermentation of cellulose hydrolysate. I. Batch versus continuous systems. Biotechnology and Bioengineering, 1979, 21(8): 1387–1400
9. Ding S F, Tan T W. L-lactic acid production by *Lactobacillus casei* fermentation using different fed-batch feeding strategies. Process Biochemistry, 2006, 41(6): 1451–1454
10. Chen Y, Liu Q, Zhou T, Li B, Yao S, Li A, Wu J, Ying H. Ethanol production by repeated batch and continuous fermentations by *Saccharomyces cerevisiae* immobilized in a fibrous bed bioreactor. Journal of Microbiology and Biotechnology, 2013, 23(4): 511–517
11. Yue H T, Ling C, Yang T, Chen X B, Chen Y L, Deng H T, Wu Q, Chen J C, Chen G Q. A seawater-based open and continuous process for polyhydroxyalkanoates production by recombinant *Halomonas campaniensis* LS21 grown in mixed substrates. Biotechnology for Biofuels, 2014, 7(1): 1–12
12. Mitsumasu K, Liu Z S, Tang Y Q, Akamatsu T, Taguchi H, Kida K. Development of industrial yeast strain with improved acid- and thermo-tolerance through evolution under continuous fermentation conditions followed by haploidization and mating. Journal of Bioscience and Bioengineering, 2014, 118(6): 689–695
13. Branyik T, Vicente A, Cruz J M, Teixeira J. Continuous primary beer fermentation with brewing yeast immobilized on spent grains. Journal of the Institute of Brewing, 2002, 108(4): 410–415
14. Wang J, Lihan Z I, Bai F. Co-production of ethanol and yeast during continuous fermentation using self-flocculating fusant *SPSC*01. Journal of Chemical Industry and Engineering, 2004, 55(6): 1024−1027 (in Chinese)
15. Ren N Q, Li J Z, Li B K, Wang Y, Liu S R. Biohydrogen production from molasses by anaerobic fermentation with a pilot-scale bioreactor system. International Journal of Hydrogen Energy, 2006, 31(15): 2147–2157
16. Gao M T, Koide M, Gotou R, Takanashi H, Hirata M, Hano T. Development of a continuous electrodialysis fermentation system for production of lactic acid by *Lactobacillus rhamnosus*. Process Biochemistry, 2004, 40(3–4): 1033–1036
17. Hirao T, Nakano T, Azuma T, Sugimoto M, Nakanishi T. L-Lysine production in continuous culture of an L-lysine hyperproducing mutant of *Corynebacterium glutamicum*. Applied Microbiology and Biotechnology, 2004, 32: 269–273
18. Yan Q, Zheng P, Tao S T, Dong J J. Fermentation process for continuous production of succinic acid in a fibrous bed bioreactor. Biochemical Engineering Journal, 2014, 91: 92–98
19. Yen H W, Li R J, Ma T W. The development process for a continuous acetone-butanol-ethanol (ABE) fermentation by immobilized *Clostridium acetobutylicum*. Journal of the Taiwan Institute of Chemical Engineers, 2011, 42(6): 902–907
20. Chatzifragkou A, Papanikolaou S, Dietz D, Doulgeraki A I,

Nychas G J, Zeng A P. Production of 1,3-propanediol by *Clostridium butyricum* growing on biodiesel-derived crude glycerol through a non-sterilized fermentation process. Applied Microbiology and Biotechnology, 2011, 91(1): 101–112

21. Huang W C, Ramey D E, Yang S T. Continuous production of butanol by *Clostridium acetobutylicum* immobilized in a fibrous bed bioreactor. Applied Biochemistry and Biotechnology, 2004, 113–116(1–3): 887–898
22. Alfenore S, Molina Jouve C, Guillouet S E, Uribelarrea J L, Goma G, Benbadis L. Improving ethanol production and viability of *Saccharomyces cerevisiae* by a vitamin feeding strategy during fed-batch process. Applied Microbiology and Biotechnology, 2002, 60(1–2): 67–72
23. Tan D, Xue Y S, Aibaidula G, Chen G Q. Unsterile and continuous production of polyhydroxybutyrate by *Halomonas* TD01. Bioresource Technology, 2011, 102(17): 8130–8136
24. Nishie M, Nagao J, Sonomoto K. Antibacterial peptides "bacteriocins": an overview of their diverse characteristics and applications. Biocontrol Science, 2012, 17(1): 1–16
25. Kivistö A, Santala V, Karp M. Non-sterile process for biohydrogen and 1,3-propanediol production from raw glycerol. International Journal of Hydrogen Energy, 2013, 38(27): 11749–11755
26. Saithong P, Nakamura T, Shima J. Prevention of bacterial contamination using acetate-tolerant *Schizosaccharomyces pombe* during bioethanol production from molasses. Journal of Bioscience and Bioengineering, 2009, 108(3): 216–219
27. Sanchez C. Bacterial evolution: phage resistance comes at a cost. Nature Reviews Microbiology, 2011, 9(6): 398–399
28. Scanlan P D, Buckling A, Hall A R. Experimental evolution and bacterial resistance: (co)evolutionary costs and trade-offs as opportunities in phage therapy research. Bacteriophage, 2015, 5(2): e1050153
29. Xiao Y, Bowen C H, Liu D, Zhang F. Exploiting nongenetic cell-to-cell variation for enhanced biosynthesis. Nature Chemical Biology, 2016, 12(5): 339–344
30. Smith A S, Rawlings D E. The poison-antidote stability system of the broad-host-range *Thiobacillus ferrooxidans* plasmid pTF-FC2. Molecular Microbiology, 2010, 26(5): 961–970
31. Nilsson J, Skogman S G. Stabilization of *Escherichia coli* tryptophan-production vectors in continuous cultures: a comparison of three different systems. Nature Biotechnology, 1986, 4(10): 901–903
32. Jojima T, Fujii M, Mori E, Inui M, Yukawa H. Engineering of sugar metabolism of *Corynebacterium glutamicum* for production of amino acid L-alanine under oxygen deprivation. Applied Microbiology and Biotechnology, 2010, 87(1): 159–165
33. Emmerling M, Bailey J E, Sauer U. Glucose catabolism of *Escherichia coli* strains with increased activity and altered regulation of key glycolytic enzymes. Metabolic Engineering, 1999, 1(2): 117–127
34. Katakura Y, Moukamnerd C, Harashima S, Kino-oka M. Strategy for preventing bacterial contamination by adding exogenous ethanol in solid-state semi-continuous bioethanol production. Journal of Bioscience and Bioengineering, 2011, 111(3): 343–345
35. Watanabe I, Nakamura T, Shima J. A strategy to prevent the occurrence of *Lactobacillus* strains using lactate-tolerant yeast *Candida glabrata* in bioethanol production. Journal of Industrial Microbiology & Biotechnology, 2008, 35(10): 1117–1122
36. Solomon E B, Okull D. Utilization of bacteriophage to control bacterial contamination in fermentation processes. US Patent, 20090104157A1, 2009-04-23
37. Tiquia S M, Davis D, Hadid H, Kasparian S, Ismail M, Sahly R, Shim J, Singh S, Murray K S. Halophilic and halotolerant bacteria from river waters and shallow groundwater along the Rouge River of southeastern Michigan. Environmental Technology, 2007, 28(3): 297–307
38. Chen G Q, Jiang X R. Next generation industrial biotechnology based on extremophilic bacteria. Current Opinion in Biotechnology, 2018, 50: 94–100
39. Zhang D X, Cheryan M. Direct fermentation of starch to lactic acid by *Lactobacillus amylovorus*. Biotechnology Letters, 1991, 13(10): 733–738
40. Zhang D X, Cheryan M. Starch to lactic acid in a continuous membrane bioreactor. Process Biochemistry, 1994, 29(2): 145–150
41. Meng W, Zhang Y, Ma L, Lu C, Xu P, Ma C, Gao C. Non-sterilized fermentation of 2,3-butanediol with seawater by metabolic engineered fast-growing *Vibrio natriegens*. Frontiers in Bioengineering and Biotechnology, 2022, 10: 955097
42. Linder T. Assimilation of alternative sulfur sources in fungi. World Journal of Microbiology & Biotechnology, 2018, 34(4): 51
43. Mandell D J, Lajoie M J, Mee M T, Takeuchi R, Kuznetsov G, Norville J E, Gregg C J, Stoddard B L, Church G M. Biocontainment of genetically modified organisms by synthetic protein design. Nature, 2015, 518(7537): 55–60
44. Johannes T W, Woodyer R D, Zhao H. Efficient regeneration of NADPH using an engineered phosphite dehydrogenase. Biotechnology and Bioengineering, 2007, 96(1): 18–26
45. Hung C L, Liu J H, Chiu W C, Huang S W, Hwang J K, Wang W C. Crystal structure of *Helicobacter pylori* formamidase AmiF reveals a cysteine-glutamate-lysine catalytic triad. Journal of Biological Chemistry, 2007, 282(16): 12220–12229
46. Ou X Y, Wu X L, Peng F, Zeng Y J, Li H X, Xu P, Chen G, Guo Z W, Yang J G, Zong M H, Lou W Y. Metabolic engineering of a robust *Escherichia coli* strain with a dual protection system. Biotechnology and Bioengineering, 2019, 116(12): 3333–3348
47. Brilon C, Beckmann W, Hellwig M, Knackmuss H J. Enrichment and isolation of naphthalenesulfonic acid-utilizing pseudomonads. Applied and Environmental Microbiology, 1981, 42(1): 39–43
48. Luther M, Soeder C J. 1-Naphthalenesulfonic acid and sulfate as sulfur sources for the green ALGA *Scenedesmus obliquus*. Water Research, 1991, 25(3): 299–307
49. Soeder C J, Hegewald E, Kneifel H. Green microalgae can use naphthalenesulfonic acids as sources of sulfur. Archives of Microbiology, 1987, 148(4): 260–263
50. Dopson M, Johnson D B. Biodiversity, metabolism and applications of acidophilic sulfur-metabolizing microorganisms.

Environmental Microbiology, 2012, 14(10): 2620–2631
51. Luther M, Soeder C J. Some naphthalenesulfonic acids as sulfur sources for the green microalga. Chemosphere, 1987, 16(7): 1565–1578
52. Cotter P D, Ross R P, Hill C. Bacteriocins—a viable alternative to antibiotics? Nature Reviews Microbiology, 2013, 11(2): 95–105
53. Qureshi A S, Zhang J, da Costa Sousa L, Bao J. Antibacterial peptide secreted by *Pediococcus acidilactici* enables efficient cellulosic open L-lactic acid fermentation. ACS Sustainable Chemistry & Engineering, 2017, 5(10): 9254–9262
54. Leroy F, Moreno M R F, De Vuyst L. Enterococcus faecium RZS C5, an interesting bacteriocin producer to be used as a co-culture in food fermentation. International Journal of Food Microbiology, 2003, 88(2−3): 235–240
55. Likhacheva N A, Samsonov V V, Samsonov V V, Sineoky S P. Genetic control of the resistance to phage C1 of *Escherichia coli* K-12. Journal of Bacteriology, 1996, 178(17): 5309–5315
56. Szczepankowska A K, Gorecki R K, Koakowski P, Bardowski J K. Lactic Acid Bacteria—R & D for Food, Health and Livestock Purposes. London: IntechOpen, 2013, 23–72
57. Sturino J M, Klaenhammer T R. Engineered bacteriophage-defence systems in bioprocessing. Nature Reviews Microbiology, 2006, 4(5): 395–404
58. Viscardi M, Capparelli R, Di Matteo R, Carminati D, Giraffa G, Iannelli D. Selection of bacteriophage-resistant mutants of *Streptococcus thermophilus*. Journal of Microbiological Methods, 2003, 55(1): 109–119
59. Mei Y J, Liu H. Selection of phage-resistant strains from *Escherichia coli gly*A genetic engineering bacteria. Agricultural Biotechnology, 2012, 1(2): 35–37
60. Fineran P C, Blower T R, Foulds I J, Humphreys D P, Lilley K S, Salmond G P. The phage abortive infection system, ToxIN, functions as a protein-RNA toxin-antitoxin pair. Proceedings of the National Academy of Sciences of the United States of America, 2009, 106(3): 894–899
61. Reyes-Cortes R, Martinez-Penafiel E, Martinez-Perez F, de la Garza M, Kameyama L. A novel strategy to isolate cell-envelope mutants resistant to phage infection: bacteriophage mEp213 requires lipopolysaccharides in addition to FhuA to enter *Escherichia coli* K-12. Microbiology, 2012, 158(Pt 12): 3063–3071
62. Cowley L A, Low A S, Pickard D, Boinett C J, Dallman T J, Day M, Perry N, Gally D L, Parkhill J, Jenkins C, Cain A K. Transposon insertion sequencing elucidates novel gene involvement in susceptibility and resistance to phages T4 and T7 in *Escherichia coli* O157. mBio, 2018, 9(4): e00705–e00718
63. Tuncer Y, Akcelik M. A protein which masks galactose receptor mediated phage susceptibility in *Lactococcus lactis* subsp *lactis* MPL56. International Journal of Food Science & Technology, 2002, 37(2): 139–144
64. McGrath S, Fitzgerald G F, van Sinderen D. Identification and characterization of phage-resistance genes in temperate lactococcal bacteriophages. Molecular Microbiology, 2002, 43(2): 509–520
65. Dupuis M E, Villion M, Magadan A H, Moineau S. CRISPR-Cas and restriction-modification systems are compatible and increase phage resistance. Nature Communications, 2013, 4(1): 2087
66. Sturino J M, Klaenhammer T R. Expression of antisense RNA targeted against *Streptococcus thermophilus* bacteriophages. Applied and Environmental Microbiology, 2002, 68(2): 588–596
67. Burrus V, Bontemps C, Decaris B, Guedon G. Characterization of a novel type II restriction-modification system, Sth368I, encoded by the integrative element ICESt1 of *Streptococcus thermophilus* CNRZ368. Applied and Environmental Microbiology, 2001, 67(4): 1522–1528
68. Lucchini S, Sidoti J, Brussow H. Broad-range bacteriophage resistance in *Streptococcus thermophilus* by insertional mutagenesis. Virology, 2000, 275(2): 267–277
69. Sturino J M, Klaenhammer T R. Inhibition of bacteriophage replication in *Streptococcus thermophilus* by subunit poisoning of primase. Microbiology, 2007, 153(Pt 10): 3295–3302
70. Xue Y P, Shen Q, Zhou X T, Guo Q, Zheng Y G. Potential of the signal peptide derived from the PAS_chr3_0030 gene product for secretory expression of valuable enzymes in *Pichia pastoris*. Applied and Environmental Microbiology, 2021, 88(9): e0029622
71. Reyes-Cortes R, Arguijo-Hernandez E S, Carballo-Ontiveros M A, Martinez-Penafiel E, Kameyama L. Random transposon mutagenesis for cell-envelope resistant to phage infection. Methods in Molecular Biology, 2016, 1440: 71–83
72. Wang M S, Nitin N. Rapid detection of bacteriophages in starter culture using water-in-oil-in-water emulsion microdroplets. Applied Microbiology and Biotechnology, 2014, 98(19): 8347–8355
73. deMello A, Rane A, Holzner G, Stavrakis S. Ultra-high-throughput multi-parametric imaging flow cytometry. EPJ Web of Conferences, 2019, 215: 10001
74. Edgar R H, Cook J, Noel C, Minard A, Sajewski A, Fitzpatrick M, Fernandez R, Hempel J D, Kellum J A, Viator J A. Bacteriophage-mediated identification of bacteria using photoacoustic flow cytometry. Journal of Biomedical Optics, 2019, 24(11): 1–7
75. Viscardi M, Capparelli R, Iannelli D. Rapid selection of phage-resistant mutants in *Streptococcus thermophilus* by immunoselection and cell sorting. International Journal of Food Microbiology, 2003, 89(2−3): 223–231
76. Mutalik V K, Adler B A, Rishi H S, Piya D, Zhong C, Koskella B, Kutter E M, Calendar R, Novichkov P S, Price M N, Deutschbauer A M, Arkin A P. High-throughput mapping of the phage resistance landscape in *E. coli*. PLoS Biology, 2020, 18(10): e3000877
77. Maffei E, Shaidullina A, Burkolter M, Heyer Y, Estermann F, Druelle V, Sauer P, Willi L, Michaelis S, Hilbi H, Thaler D S, Harms A. Systematic exploration of *Escherichia coli* phage-host interactions with the BASEL phage collection. PLoS Biology, 2021, 19(11): e3001424
78. Barrangou R, Horvath P. CRISPR: new horizons in phage resistance and strain identification. Annual Review of Food Science and Technology, 2012, 3(1): 143–162

79. Chung D K, Chung S K, Batt C A. Antisense RNA directed against the major capsid protein of *Lactococcus lactis* subsp. cremoris bacteriophage 4–1 confers partial resistance to the host. Applied Microbiology and Biotechnology, 1992, 37(1): 79–83
80. Mahony J, McGrath S, Fitzgerald G F, van Sinderen D. Identification and characterization of lactococcal-prophage-carried superinfection exclusion genes. Applied and Environmental Microbiology, 2008, 74(20): 6206–6215
81. Ventura M, Canchaya C, Pridmore R D, Brussow H. The prophages of *Lactobacillus johnsonii* NCC 533: comparative genomics and transcription analysis. Virology, 2004, 320(2): 229–242
82. Sun X, Gohler A, Heller K J, Neve H. The *ltp* gene of temperate *Streptococcus thermophilus* phage TP-J34 confers superinfection exclusion to *Streptococcus thermophilus* and *Lactococcus lactis*. Virology, 2006, 350(1): 146–157
83. Garvey P, Hill C, Fitzgerald G F. The lactococcal plasmid pNP40 encodes a third bacteriophage resistance mechanism, one which affects phage DNA penetration. Applied and Environmental Microbiology, 1996, 62(2): 676–679
84. Kim J W, Dutta V, Elhanafi D, Lee S, Osborne J A, Kathariou S. A novel restriction-modification system is responsible for temperature-dependent phage resistance in *Listeria monocytogenes* ECII. Applied and Environmental Microbiology, 2012, 78(6): 1995–2004
85. Cong L, Zhang F. Genome engineering using CRISPR-Cas9 system. Methods in Molecular Biology, 2015, 1239: 197–217
86. Hsu P D, Lander E S, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell, 2014, 157(6): 1262–1278
87. Liu L, Zhao D, Ye L, Zhan T, Xiong B, Hu M, Bi C, Zhang X. A programmable CRISPR/Cas9-based phage defense system for *Escherichia coli* BL21(DE3). Microbial Cell Factories, 2020, 19(1): 136
88. Ofir G, Melamed S, Sberro H, Mukamel Z, Silverman S, Yaakov G, Doron S, Sorek R. DISARM is a widespread bacterial defence system with broad anti-phage activities. Nature Microbiology, 2018, 3(1): 90–98
89. Bernheim A, Sorek R. The pan-immune system of bacteria: antiviral defence as a community resource. Nature Reviews Microbiology, 2020, 18(2): 113–119
90. Chopin M C, Chopin A, Bidnenko E. Phage abortive infection in lactococci: variations on a theme. Current Opinion in Microbiology, 2005, 8(4): 473–479
91. Webster R E, Cashman J S. Abortive infection of *Escherichia coli* with the bacteriophage f1: cytoplasmic membrane proteins and the f1 DNA-gene 5 protein complex. Virology, 1973, 55(1): 20–38
92. Durmaz E, Klaenhammer T R. Abortive phage resistance mechanism AbiZ speeds the lysis clock to cause premature lysis of phage-infected *Lactococcus lactis*. Journal of Bacteriology, 2007, 189(4): 1417–1425
93. Xiong X, Wu G, Wei Y, Liu L, Zhang Y, Su R, Jiang X, Li M, Gao H, Tian X, Zhang Y, Hu L, Chen S, Tang Y, Jiang S, Huang R, Li Z, Wang Y, Deng Z, Wang J, Dedon P C, Chen S, Wang L. SspABCD-SspE is a phosphorothioation-sensing bacterial defence system with broad anti-phage activities. Nature Microbiology, 2020, 5(7): 917–928
94. Zou X, Xiao X, Mo Z, Ge Y, Jiang X, Huang R, Li M, Deng Z, Chen S, Wang L, Lee S Y. Systematic strategies for developing phage resistant *Escherichia coli* strains. Nature Communications, 2022, 13(1): 4491
95. Denamur E, Matic I. Evolution of mutation rates in bacteria. Molecular Microbiology, 2006, 60(4): 820–827
96. Umenhoffer K, Feher T, Baliko G, Ayaydin F, Posfai J, Blattner F R, Posfai G. Reduced evolvability of *Escherichia coli* MDS42, an IS-less cellular chassis for molecular and synthetic biology applications. Microbial Cell Factories, 2010, 9(1): 38
97. Vidal L, Pinsach J, Striedner G, Caminal G, Ferrer P. Development of an antibiotic-free plasmid selection system based on glycine auxotrophy for recombinant protein overproduction in *Escherichia coli*. Journal of Biotechnology, 2008, 134(1−2): 127–136
98. Zhang Y, Liu D, Chen Z. Production of C2–C4 diols from renewable bioresources: new metabolic pathways and metabolic engineering strategies. Biotechnology for Biofuels, 2017, 10(1): 299
99. Chen Z, Geng F, Zeng A P. Protein design and engineering of a de novo pathway for microbial production of 1,3-propanediol from glucose. Biotechnology Journal, 2015, 10(2): 284–289
100. Hagg P, de Pohl J W, Abdulkarim F, Isaksson L A. A host/plasmid system that is not dependent on antibiotics and antibiotic resistance genes for stable plasmid maintenance in *Escherichia coli*. Journal of Biotechnology, 2004, 111(1): 17–30
101. Porter R D, Black S, Pannuri S, Carlson A. Use of the *Escherichia coli* SSB gene to prevent bioreactor takeover by plasmidless cells. Biotechnology, 1990, 8(1): 47–51
102. Gerdes K, Poulsen L K, Thisted T, Nielsen A K, Martinussen J, Andreasen P H. The hok killer gene family in gram-negative bacteria. New Biology, 1990, 2(11): 946–956
103. Afif H, Allali N, Couturier M, Van-Melderen L. The ratio between CcdA and CcdB modulates the transcriptional repression of the ccd poison-antidote system. Molecular Microbiology, 2010, 41(1): 73–82
104. Terrinoni M, Nordqvist S L, Kallgard S, Holmgren J, Lebens M. A novel nonantibiotic, *lgt*-based selection system for stable maintenance of expression vectors in *Escherichia coli* and *Vibrio cholerae*. Applied and Environmental Microbiology, 2018, 84(4): e02143–e02117
105. Posfai G, Plunkett G III, Feher T, Frisch D, Keil G M, Umenhoffer K, Kolisnychenko V, Stahl B, Sharma S S, de Arruda M, Burland V, Harcum S W, Blattner F R. Emergent properties of reduced-genome *Escherichia coli*. Science, 2006, 312(5776): 1044–1046
106. Wyrzykowski J, Volkert M R. The *Escherichia coli* methyl-directed mismatch repair system repairs base pairs containing oxidative lesions. Journal of Bacteriology, 2003, 185(5): 1701–1704
107. Galan J C, Turrientes M C, Baquero M R, Rodriguez-Alcayna M, Martinez-Amado J, Martinez J L, Baquero F. Mutation rate is reduced by increased dosage of *mutL* gene in *Escherichia coli* K-12. FEMS Microbiology Letters, 2007, 275(2): 263–269

108. Csorgo B, Feher T, Timar E, Blattner F R, Posfai G. Low-mutation-rate, reduced-genome *Escherichia coli*: an improved host for faithful maintenance of engineered genetic constructs. Microbial Cell Factories, 2012, 11(1): 11
109. Michener J K, Thodey K, Liang J C, Smolke C D. Applications of genetically-encoded biosensors for the construction and control of biosynthetic pathways. Metabolic Engineering, 2012, 14(3): 212–222
110. Snoek T, Romero-Suarez D, Zhang J, Ambri F, Skjoedt M L, Sudarsan S, Jensen M K, Keasling J D. An orthogonal and pH-tunable sensor-selector for muconic acid biosynthesis in yeast. ACS Synthetic Biology, 2018, 7(4): 995–1003
111. Crook N, Abatemarco J, Sun J, Wagner J M, Schmitz A, Alper H S. *In vivo* continuous evolution of genes and pathways in yeast. Nature Communications, 2016, 7(1): 13051
112. Zhang X, Jantama K, Moore J C, Jarboe L R, Shanmugam K T, Ingram L O. Metabolic evolution of energy-conserving pathways for succinate production in *Escherichia coli*. Proceedings of the National Academy of Sciences of the United States of America, 2009, 106(48): 20180–20185
113. Hauf J, Zimmermann F K, Muller S. Simultaneous genomic overexpression of seven glycolytic enzymes in the yeast *Saccharomyces cerevisiae*. Enzyme and Microbial Technology, 2000, 26(9−10): 688–698
114. Yamamoto S, Gunji W, Suzuki H, Toda H, Suda M, Jojima T, Inui M, Yukawa H. Overexpression of genes encoding glycolytic enzymes in *Corynebacterium glutamicum* enhances glucose metabolism and alanine production under oxygen deprivation conditions. Applied and Environmental Microbiology, 2012, 78(12): 4447–4457
115. Ma W, Wang J, Li Y, Hu X, Shi F, Wang X. Enhancing pentose phosphate pathway in *Corynebacterium glutamicum* to improve L-isoleucine production. Biotechnology and Applied Biochemistry, 2016, 63(6): 877–885
116. Irani N, Beccaria A J, Wagner R. Expression of recombinant cytoplasmic yeast pyruvate carboxylase for the improvement of the production of human erythropoietin by recombinant BHK-21 cells. Journal of Biotechnology, 2002, 93(3): 269–282
117. Wang Z, Chen T, Ma X, Shen Z, Zhao X. Enhancement of riboflavin production with *Bacillus subtilis* by expression and site-directed mutagenesis of *zwf* and *gnd* gene from *Corynebacterium glutamicum*. Bioresource Technology, 2011, 102(4): 3934–3940
118. Koebmann B J, Westerhoff H V, Snoep J L, Nilsson D, Jensen P R. The glycolytic flux in *Escherichia coli* is controlled by the demand for ATP. Journal of Bacteriology, 2002, 184(14): 3909–3916
119. Causey T B, Shanmugam K T, Yomano L P, Ingram L O. Engineering *Escherichia coli* for efficient conversion of glucose to pyruvate. Proceedings of the National Academy of Sciences of the United States of America, 2004, 101(8): 2235–2240
120. Causey T B, Zhou S, Shanmugam K T, Ingram L O. Engineering the metabolism of *Escherichia coli* W3110 for the conversion of sugar to redox-neutral and oxidized products: homoacetate production. Proceedings of the National Academy of Sciences of the United States of America, 2003, 100(3): 825–832
121. Wang J, Niyompanich S, Tai Y S, Wang J, Bai W, Mahida P, Gao T, Zhang K. Engineering of a highly efficient *Escherichia coli* strain for mevalonate fermentation through chromosomal integration. Applied and Environmental Microbiology, 2016, 82(24): 7176–7184
122. Kou M, Cui Z, Fu J, Dai W, Wang Z, Chen T. Metabolic engineering of *Corynebacterium glutamicum* for efficient production of optically pure (2R,3R)-2,3-butanediol. Microbial Cell Factories, 2022, 21(1): 150
123. Luo Z, Zeng W, Du G, Chen J, Zhou J. Enhanced pyruvate production in *Candida glabrata* by engineering ATP futile cycle system. ACS Synthetic Biology, 2019, 8(4): 787–795
124. Fuentes L G, Lara A R, Martinez L M, Ramirez O T, Martinez A, Bolivar F, Gosset G. Modification of glucose import capacity in *Escherichia coli*: physiologic consequences and utility for improving DNA vaccine production. Microbial Cell Factories, 2013, 12(1): 42
125. Ikeda M. Sugar transport systems in *Corynebacterium glutamicum*: features and applications to strain development. Applied Microbiology and Biotechnology, 2012, 96(5): 1191–1200
126. Zhou Z, Wang C, Xu H, Chen Z, Cai H. Increasing succinic acid production using the PTS-independent glucose transport system in a *Corynebacterium glutamicum* PTS-defective mutant. Journal of Industrial Microbiology & Biotechnology, 2015, 42(7): 1073–1082
127. Hernandez-Montalvo V, Martinez A, Hernandez-Chavez G, Bolivar F, Valle F, Gosset G. Expression of *galP* and *glk* in a *Escherichia coli* PTS mutant restores glucose transport and increases glycolytic flux to fermentation products. Biotechnology and Bioengineering, 2003, 83(6): 687–694
128. Lu J, Tang J, Liu Y, Zhu X, Zhang T, Zhang X. Combinatorial modulation of *galP* and *glk* gene expression for improved alternative glucose utilization. Applied Microbiology and Biotechnology, 2012, 93(6): 2455–2462
129. Hao Y, Ma Q, Liu X, Fan X, Men J, Wu H, Jiang S, Tian D, Xiong B, Xie X. High-yield production of L-valine in engineered *Escherichia coli* by a novel two-stage fermentation. Metabolic Engineering, 2020, 62: 198–206
130. Michalowski A, Siemann-Herzberg M, Takors R. *Escherichia coli* HGT: engineered for high glucose throughput even under slowly growing or resting conditions. Metabolic Engineering, 2017, 40: 93–103
131. Zhang X, Lai L, Xu G, Zhang X, Shi J, Koffas M A G, Xu Z. Rewiring the central metabolic pathway for high-yield L-serine production in *Corynebacterium glutamicum* by using glucose. Biotechnology Journal, 2019, 14(6): e1800497
132. Zhan Y, Shi J, Xiao Y, Zhou F, Wang H, Xu H, Li Z, Yang S, Cai D, Chen S. Multilevel metabolic engineering of *Bacillus licheniformis* for de novo biosynthesis of 2-phenylethanol. Metabolic Engineering, 2022, 70: 43–54
133. Gu Y, Deng J, Liu Y, Li J, Shin H D, Du G, Chen J, Liu L. Rewiring the glucose transportation and central metabolic pathways for overproduction of *N*-acetylglucosamine in *Bacillus subtilis*. Biotechnology Journal, 2017, 12(10): 170020

134. Long C P, Gonzalez J E, Feist A M, Palsson B O, Antoniewicz M R. Fast growth phenotype of *E. coli* K-12 from adaptive laboratory evolution does not require intracellular flux rewiring. Metabolic Engineering, 2017, 44: 100–107
135. Dragosits M, Mattanovich D. Adaptive laboratory evolution—principles and applications for biotechnology. Microbial Cell Factories, 2013, 12(1): 64
136. Portnoy V A, Bezdan D, Zengler K. Adaptive laboratory evolution—harnessing the power of biology for metabolic engineering. Current Opinion in Biotechnology, 2011, 22(4): 590–594
137. Shen Y, Chen X, Peng B, Chen L, Hou J, Bao X. An efficient xylose-fermenting recombinant *Saccharomyces cerevisiae* strain obtained through adaptive evolution and its global transcription profile. Applied Microbiology and Biotechnology, 2012, 96(4): 1079–1091
138. Guimaraes P M, Francois J, Parrou J L, Teixeira J A, Domingues L. Adaptive evolution of a lactose-consuming *Saccharomyces cerevisiae* recombinant. Applied and Environmental Microbiology, 2008, 74(6): 1748–1756
139. Radek A, Tenhaef N, Muller M F, Brusseler C, Wiechert W, Marienhagen J, Polen T, Noack S. Miniaturized and automated adaptive laboratory evolution: evolving *Corynebacterium glutamicum* towards an improved D-xylose utilization. Bioresource Technology, 2017, 245(Pt B): 1377–1385
140. McCloskey D, Xu S, Sandberg T E, Brunk E, Hefner Y, Szubin R, Feist A M, Palsson B O. Adaptive laboratory evolution resolves energy depletion to maintain high aromatic metabolite phenotypes in *Escherichia coli* strains lacking the Phosphotransferase System. Metabolic Engineering, 2018, 48: 233–242
141. Reyes L H, Gomez J M, Kao K C. Improving carotenoids production in yeast via adaptive laboratory evolution. Metabolic Engineering, 2014, 21(1): 26–33
142. Mahr R, Gatgens C, Gatgens J, Polen T, Kalinowski J, Frunzke J. Biosensor-driven adaptive laboratory evolution of L-valine production in *Corynebacterium glutamicum*. Metabolic Engineering, 2015, 32: 184–194
143. Niu F X, He X, Wu Y Q, Liu J Z. Enhancing production of pinene in *Escherichia coli* by using a combination of tolerance, evolution, and modular co-culture engineering. Frontiers in Microbiology, 2018, 9: 1623
144. Tuyishime P, Wang Y, Fan L, Zhang Q, Li Q, Zheng P, Sun J, Ma Y. Engineering *Corynebacterium glutamicum* for methanol-dependent growth and glutamate production. Metabolic Engineering, 2018, 49: 220–231
145. Minliang C, Chengwei M, Lin C, Zeng A P. Integrated laboratory evolution and rational engineering of GalP/Glk-dependent *Escherichia coli* for higher yield and productivity of L-tryptophan biosynthesis. Metabolic Engineering Communications, 2021, 12: e00167
146. Cheng J S, Qiao B, Yuan Y J. Comparative proteome analysis of robust *Saccharomyces cerevisiae* insights into industrial continuous and batch fermentation. Applied Microbiology and Biotechnology, 2008, 81(2): 327–338
147. Nicolaou S A, Gaida S M, Papoutsakis E T. A comparative view of metabolite and substrate stress and tolerance in microbial bioprocessing: from biofuels and chemicals, to biocatalysis and bioremediation. Metabolic Engineering, 2010, 12(4): 307–331
148. Jia K, Zhang Y, Yin L. Systematic engineering of microorganisms to improve alcohol tolerance. Engineering in Life Sciences, 2010, 10(5): 422–429
149. Calamita G, Bishai W R, Preston G M, Guggino W B, Agre P. Molecular cloning and characterization of AqpZ, a water channel from *Escherichia coli*. Journal of Biological Chemistry, 1995, 270(49): 29063–29066
150. Laimins L A, Rhoads D B, Epstein W. Osmotic control of *kdp* operon expression in *Escherichia coli*. Proceedings of the National Academy of Sciences of the United States of America, 1981, 78(1): 464–468
151. Sevin D C, Sauer U. Ubiquinone accumulation improves osmotic-stress tolerance in *Escherichia coli*. Nature Chemical Biology, 2014, 10(4): 266–272
152. Ma R, Zhang Y, Hong H, Lu W, Lin M, Chen M, Zhang W. Improved osmotic tolerance and ethanol production of ethanologenic *Escherichia coli* by IrrE, a global regulator of radiation-resistance of *Deinococcus radiodurans*. Current Microbiology, 2011, 62(2): 659–664
153. Pan J, Wang J, Zhou Z, Yan Y, Zhang W, Lu W, Ping S, Dai Q, Yuan M, Feng B, Hou X, Zhang Y, Ruiqiang M, Liu T, Feng L, Wang L, Chen M, Lin M. IrrE, a global regulator of extreme radiation resistance in *Deinococcus radiodurans*, enhances salt tolerance in *Escherichia coli* and *Brassica napus*. PLoS One, 2009, 4(2): e4422
154. Stasic A J, Lee Wong A C, Kaspar C W. Osmotic and desiccation tolerance in *Escherichia coli* O157:H7 requires *rpoS* (sigma(38)). Current Microbiology, 2012, 65(6): 660–665
155. Grothe S, Krogsrud R L, McClellan D J, Milner J L, Wood J M. Proline transport and osmotic stress response in *Escherichia coli* K-12. Journal of Bacteriology, 1986, 166(1): 253–259
156. Jr M T R, Courtenay E S, Cayley D S, Guttman H J. Responses of *E. coli* to osmotic stress: large changes in amounts of cytoplasmic solutes and water. Trends in Biochemical Sciences, 1998, 23(4): 143–148
157. Weymarn N V, Nyyssola A, Reinikainen T, Leisola M, Ojamo H. Improved osmotolerance of recombinant *Escherichia coli* by de novo glycine betaine biosynthesis. Applied Microbiology and Biotechnology, 2000, 55(2): 214–218
158. Aranda A, Querol A, del Olmo M. Correlation between acetaldehyde and ethanol resistance and expression of HSP genes in yeast strains isolated during the biological aging of sherry wines. Archives of Microbiology, 2002, 177(4): 304–312
159. Jiang C, Xu J, Zhang H, Zhang X, Shi J, Li M, Ming F. A cytosolic class I small heat shock protein, RcHSP17.8, of *Rosa chinensis* confers resistance to a variety of stresses to *Escherichia coli*, yeast and *Arabidopsis thaliana*. Plant, Cell & Environment, 2009, 32(8): 1046–1059
160. Seydlova G, Halada P, Fiser R, Toman O, Ulrych A, Svobodova J. DnaK and GroEL chaperones are recruited to the *Bacillus subtilis* membrane after short-term ethanol stress. Journal of Applied Microbiology, 2012, 112(4): 765–774
161. Zingaro K A, Terry Papoutsakis E. GroESL overexpression

imparts *Escherichia coli* tolerance to *i*-, *n*-, and 2-butanol, 1,2,4-butanetriol and ethanol with complex and unpredictable patterns. Metabolic Engineering, 2013, 15: 196–205

162. Sridhar M, Sree N K, Rao L V. Effect of UV radiation on thermotolerance, ethanol tolerance and osmotolerance of *Saccharomyces cerevisiae* VS1 and VS3 strains. Bioresource Technology, 2002, 83(3): 199–202
163. Yu L, Pei X, Lei T, Wang Y, Feng Y. Genome shuffling enhanced L-lactic acid production by improving glucose tolerance of *Lactobacillus rhamnosus*. Journal of Biotechnology, 2008, 134(1−2): 154–159
164. Zheng D Q, Wu X C, Tao X L, Wang P M, Li P, Chi X Q, Li Y D, Yan Q F, Zhao Y H. Screening and construction of *Saccharomyces cerevisiae* strains with improved multi-tolerance and bioethanol fermentation performance. Bioresource Technology, 2011, 102(3): 3020–3027
165. Xiao M, Zhu X, Fan F, Xu H, Tang J, Qin Y, Ma Y, Zhang X. Osmotolerance in *Escherichia coli* is improved by activation of copper efflux genes or supplementation with sulfur-containing amino acids. Applied and Environmental Microbiology, 2017, 83(7): e03050–e03016
166. Zhu X, Tan Z, Xu H, Chen J, Tang J, Zhang X. Metabolic evolution of two reducing equivalent-conserving pathways for high-yield succinate production in *Escherichia coli*. Metabolic Engineering, 2014, 24: 87–96
167. Jensen S I, Lennen R M, Herrgard M J, Nielsen A T. Seven gene deletions in seven days: fast generation of *Escherichia coli* strains tolerant to acetate and osmotic stress. Scientific Reports, 2015, 5(1): 17874
168. Lennen R M, Herrgard M J. Combinatorial strategies for improving multiple-stress resistance in industrially relevant *Escherichia coli* strains. Applied and Environmental Microbiology, 2014, 80(19): 6223–6242
169. Yang L B, Dai X M, Zheng Z Y, Zhu L, Zhan X B, Lin C C. Proteomic analysis of erythritol-producing *Yarrowia lipolytica* from glycerol in response to osmotic pressure. Journal of Microbiology and Biotechnology, 2015, 25(7): 1056–1069
170. Chen X, Yin J, Ye J, Zhang H, Che X, Ma Y, Li M, Wu L P, Chen G Q. Engineering *Halomonas bluephagenesis* TD01 for non-sterile production of poly(3-hydroxybutyrate-co-4-hydroxybutyrate). Bioresource Technology, 2017, 244(Pt 1): 534–541
171. Hoffart E, Grenz S, Lange J, Nitschel R, Müller F, Schwentner A, Feith A, Lenfers-Lücker M, Takors R, Blombach B. High substrate uptake rates empower *Vibrio natriegens* as production host for industrial biotechnology. Applied and Environmental Microbiology, 2017, 83(22): e01614–e01617
172. Weinstock M T, Hesek E D, Wilson C M, Gibson D G. *Vibrio natriegens* as a fast-growing host for molecular biology. Nature Methods, 2016, 13(10): 849–851
173. Saha B C, Kennedy G J. Efficient itaconic acid production by *Aspergillus terreus*: overcoming the strong inhibitory effect of manganese. Biotechnology Progress, 2020, 36(2): e2939
174. Tevz G, Bencina M, Legisa M. Enhancing itaconic acid production by *Aspergillus terreus*. Applied Microbiology and Biotechnology, 2010, 87(5): 1657–1664
175. Voulgaris I, O'Donnell A, Harvey L M, McNeil B. Inactivating alternative NADH dehydrogenases: enhancing fungal bioprocesses by improving growth and biomass yield? Scientific Reports, 2012, 2(1): 322
176. Zhang J, Wu N, Ou W, Li Y, Liang Y, Peng C, Li Y, Xu Q, Tong Y. Peptide supplementation relieves stress and enhances glycolytic flux in filamentous fungi during organic acid bioproduction. Biotechnology and Bioengineering, 2022, 119(9): 2471–2481
177. Tschirhart T, Shukla V, Kelly E E, Schultzhaus Z, NewRingeisen E, Erickson J S, Wang Z, Garcia W, Curl E, Egbert R G, Yeung E, Vora G J. Synthetic biology tools for the fast-growing marine bacterium *Vibrio natriegens*. ACS Synthetic Biology, 2019, 8(9): 2069–2079
178. Gao F, Hao Z, Sun X, Qin L, Zhao T, Liu W, Luo H, Yao B, Su X. A versatile system for fast screening and isolation of *Trichoderma reesei* cellulase hyperproducers based on DsRed and fluorescence-assisted cell sorting. Biotechnology for Biofuels, 2018, 11(1): 261