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CRITICAL REVIEW

Mechanisms of response to pH shock in microbial fermentation

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Abstract
The following review highlights pH shock, a novel environmental factor, as a tool for the improvement of fermentation production. The aim of this review is to introduce some recent original studies on the enhancement of microbial fermentation production by pH shock. Another purpose of this review is to improve the understanding of the processes that underlie physiological and genetic differences, which will facilitate future research on the improvement of fermentation production and reveal the associated molecular mechanisms. This understanding will simultaneously promote the application of this strategy to other microbial fermentation systems. Furthermore, improvement of the cellular tolerance of genetically engineered bacteria can also be a new field of research in the future to enhance fermentation production.

Keywords Environmental factor · pH shock · Physiological · Tolerance · Fermentation

Introduction

Currently, an increasing number of products are produced by microbial fermentation, such as antibiotics, proteins and biofuels. The growing demand for microbial fermentation products has stimulated research for the development of strain improvement methods for commercial application. For the development of a commercially feasible fermentation process, improvements in yield and overall productivity are essential. To date, researchers have established various efficient methods to obtain high-producing strains, including mutagenesis [1, 2], genome shuffling [3] and ribosome engineering [4]. However, the genetic instability of bacteria is a universal problem [5]. Therefore, it is essential to establish a strategy to improve fermentation yield without affecting genetic stability. The regulation of the fermentation process is usually an effective method to improve the products of the fermentation, such as dissolved oxygen (DO) and pH. The regulation of these fermentation parameters ultimately affects the microbial growth by environmental factors.

Environmental factors are very important for the growth of microorganisms. Any alteration in the environment will lead to a physiological or genetic adaptive response in microorganisms may be considered an environmental stressor. Microbes cannot grow normally when the stress is lethal but can survive under sublethal conditions by acquiring tolerance or generating an induced response to the environmental stress [6, 7]. In 1954, the concept of environmental stress was first proposed by Scherbaum, who used intermittent heat to treat mass cultures of *Tetrahymena* and found that simultaneous cell division was induced in 85% of the cells [8].

In recent decades, various environmental stress-based strategies have been applied to stimulate microbial growth and improve fermentation productivity. These stress-mediated bioprocesses gradually became novel strategies to enhance the production of metabolites (Table 1), such as osmotic stress [9–12], pH stress [13, 14], heat shock [15], ethanol stress [16], and oxidative stress [17, 18]. Among environmental stressors, pH stress was first reported in a study on methylenomycin production by *Streptomyces coelicolor* A3(2) in 1997 [19] and has been widely used in microbial fermentation relatively recently [20]. This strategy was designed based on previous studies [21], which showed that when the fermentation pH was lowered to the stress value, the production of methylenomycin was at its highest; this finding inspired the design of the subsequent fermentation strategy.

As one of the environmental factors, pH can affect the metabolism of cells. Long-term pH stress is harmful to cell growth, but short-term stress effects are likely to act as a

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Table 1 Different environmental stress strategies applied in the production of metabolites

Stress	Organism	Strategy of response	Fermentation production	Reference
Osmotic	*Streptomyces mobaraensis*	Stimulation of mycelium differentiation	Transglutaminase (TGase)	[11]
pH	*Streptomyces coelicolor A3(2)*	Regulators activation actinorhodin biosynthesis	Actinorhodin	[13]
Heat	*Saccharomyces cerevisiae*	Activated greater expression of triose phosphate isomerase	Glycerol	[15]
Ethanol	*Pseudomonas fluorescens S272*	LUXR system	Pyoluteorin	[16]
Oxidative	*Candida utilis*	Activities of catalase and GSH reductase	Glutathione	[17]

signal to stimulate cell metabolism and respond to other stresses [22]. Here, we define pH shock as a short-term stress, which means an abrupt change in pH during culture growth, followed by the pH returning to an appropriate value after a short period; pH shock can be alkaline or acidic. According to previous reports, pH shock has been widely accepted as a stress signal and successfully used to improve fermentation. An improved understanding of the signal transduction pathways that mediate pH shock and the enhancement of the productivity of these pathways are important for the optimization of commercial production.

The aim of this mini review is to summarize the advantages of pH shock as a novel strategy that can not only enhance microbial tolerance to alkaline or acid conditions but also increase the production of metabolites [19, 23–26]. Many signal transduction pathways involved in triggering fermentation production and responding to pH shock had been proposed [27–29]. Further analysis of the mechanism of this induction would help reveal the regulatory signaling pathways associated with development and metabolism in microbes.

Applications of pH shock in microbial fermentation

Generally, acidic or alkaline fermentation conditions are necessary for the production of secondary metabolites. Hence, microorganisms with high acid and alkali tolerance can ensure high yields of secondary metabolites due to their strong vitality and productivity in harsh environments [15, 30]. Encountering pH shock can endow microorganisms with high tolerance and adaptation capacity to successfully survive under harsh pH conditions combined with other stresses, which is considered paramount in the search for novel biotechnological applications for microorganisms in fermentation.

Microorganisms can survive in a certain environmental pH range, but the metabolites produced vary with pH conditions. After the discovery that fermentation products can be regulated by adjusting the pH, different fermentation processes associated with pH regulation were established [31, 32]. Subsequently, the common pH shock strategy was proposed and has been used mainly in the fermentation of *Streptomyces* (Table 2). pH shock was first identified as an environmental signal and applied in the fermentation of methylenomycin in 1997 [19]. The researchers demonstrated that increased methylenomycin production occurred independently of the nutritional status of the growth environment by using

Table 2 Applications of pH shock in the fermentation

Organism	Types	Strategy of response	Fermentation production	Reference
Streptomyces coelicolor A3(2)	Acid	Obtained adaptation to stress	Methylenomycin	[19]
Streptomyces kasugaensis	Acid	Not mentioned	Kasugamycin	[26]
Streptomyces hygroscopicus subsp. duamyceticus JCM4427	Acid	Enhanced nutrients flux for geldanamycin biosynthesis	Geldanamycin	[25]
Streptomyces coelicolor A3(2)	Acid	Regulates activation of actinorhodin biosynthesis	Actinorhodin	[13]
Streptomyces sp. CK4412	Acid	Sigma factors and shock-related proteins were enhanced	Tautomycetin	[90]
Streptomyces albulus M-Z18	Acid	Obtained adaptation to stress	Epsilon-poly-lysine	[30]
Streptomyces coelicolor M511	Acid	Genes encoding transcriptional activators redD for prodiginine upregulation	Prodiginine	[24]
Streptomyces hygroscopicus 5008	Alkaline	Enhanced proton transport and respiratory activity of cells	Validamycin A	[14]

chemostat cultures. By testing gene transcription, the researchers found that the transcript levels of the methylenomycin biosynthetic gene (*mmy*) were enhanced after artificial acidic pH shock. The signal transduction pathway involved in triggering antibiotic production and the responses to acidic pH shock were of great interest to the researchers.

pH shock was also applied in the production of kasugamycin by *Streptomyces kasugaensis* [26]. After sequentially changing the pH from neutral to acidic and then back to neutral, the biosynthesis of kasugamycin was greatly enhanced. As a non-nutritional stressor, the duration of pH shock is important for the recovery of cells, thus an optimal duration of pH shock is crucial for efficient production. With pH control systems being part of most industrial-scale fermenters, researchers believe that the proposed pH shock method is readily applicable in industrial fermentation and that such systems can be easily operated.

The application of alkaline pH shock has not been as widespread as that of acidic pH shock. Although some metabolites are produced under alkaline conditions, there is little information regarding the increase in yield due to alkaline pH shock. Chen and Zhou reported that the production of poly-γ-glutamic acid (γ-PGA) [23] and validamycin A (Val-A) [14] could be enhanced with alkaline pH shock, respectively. When studying the effects of multiple physicochemical stresses on the production of γ-PGA and the transcription of the associated synthetase genes in *Bacillus licheniformis* WX-02, the authors found that the γ-PGA yields increased by 133% under alkaline stress, and the transcription levels of the γ-PGA synthetase gene *pgsC* were upregulated under the stress. Val-A is produced by *Streptomyces* as a secondary metabolite and has a wide range of agricultural applications, such as in the control of rice sheath blight, false smut and damping off. By optimization experiments to determine the pH, time and duration of pH shock, the authors established an optimal alkaline pH shock strategy, which increased Val-A production by 27.43%.

Based on previous studies, our laboratory had also proposed a fermentation strategy, using acidic pH shock to enhance the production of ε-poly-lysine (ε-PL) in *Streptomyces albulus* M-Z18 [33]. After that, we have modified the fermentation process with pH shock and divided it into two stages: the first stage (cultivation stage) comprised mycelia growth, pH shock and mycelia viability recovery and was performed in one fermenter, the second stage (fermentation stage) only included ε-PL production at a constant pH 3.8 and was performed in the other fermenter. Finally, the production of ε-PL was enhanced by 33.4% in two-stage fermentation using this pH shock strategy [30].

Mechanisms of response to pH shock

Generally, the exposure of organisms to adverse environmental conditions, especially the abrupt pH changes discussed in this review, has detrimental effects, such as disruption of intracellular pH homeostasis and cell membrane, protein and DNA damage, eventually leading to cell death. To cope with such changes in the environment, a microbial cell must acquire some adaptive mechanisms that will enable it to respond to the effect of pH shock. The microbial response to pH shock occurs via the evolution of varying morphological, physiological and molecular mechanisms of adaptation. Numerous morphological features, such as size, shape and pigmentation, are involved in the cellular response to pH shock and play a significant role in cell survival under stress [34]. In addition, some main physiological responses to abrupt changes in pH have been identified, including changes in membrane composition, translocation and energy metabolism. Moreover, molecular responses reflect the changes in gene expression associated with some physiological and signal transduction-based responses to enhanced fermentation production. In the following sections, we will discuss these adaptive mechanisms in detail.

Morphological responses to pH shock

In nature, microbes exhibit morphological diversity. During fermentation, environmental conditions change over time, which could change the morphology, for example, from filamentous to spherical [35]. A previous study demonstrated that morphology and product formation are closely associated [36]. Reportedly, pellet size distribution in a pH shock group was polydisperse and rarely changed throughout the fermentation process, whereas severe pellet disintegration was observed in a group that was not subjected to pH shock, resulting in decreased pellet sizes and amount (Fig. 1); this result was consistent with the ε-PL productivity observed in our group [34]. Therefore, morphological differentiation of microorganisms can also be used to monitor fermentation processes [37]. Another study reported that spores exhibited dark blue pigmentation after 5 days of cultivation in solid medium with pH shock, and this effect was apparently associated with actinorhodin synthesis [13].

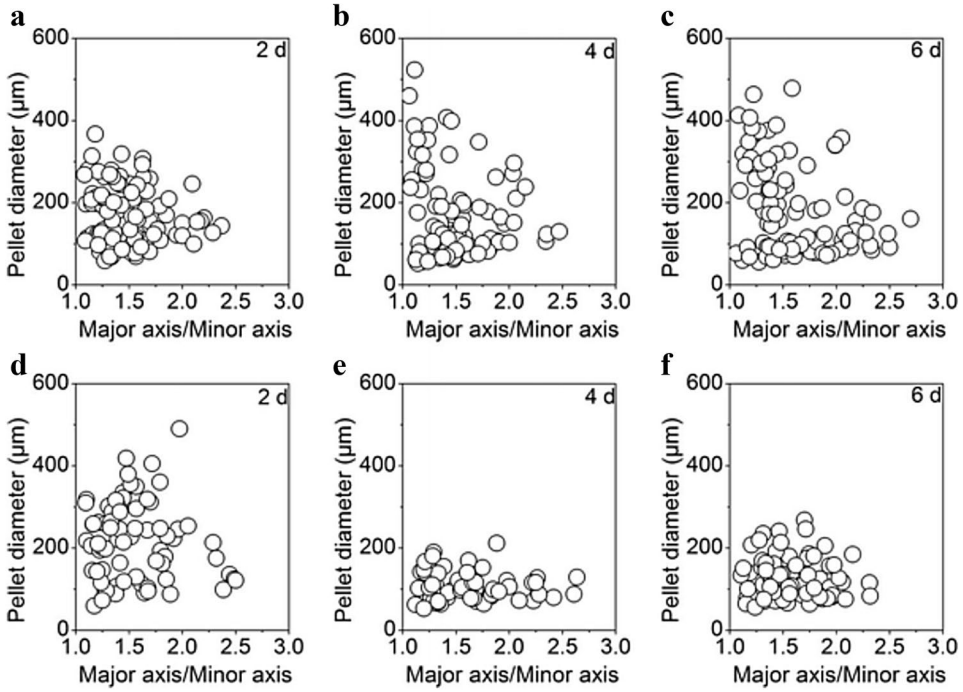

Fig. 1 Distribution of pellet size and shape in fed-batch fermentations for ε-PL production by *Streptomyces* sp. M-Z18 with the pH shock and no pH shock [34]. pH shock (**a**–**c**); no pH shock (**d**–**f**)

Physiological responses to pH shock

Response of cellular membranes to pH shock

Cell membranes are natural barriers that microbes use to protect themselves from external damage. The importance of the role of cell membranes is demonstrated by the changes in membrane fatty acid profiles in response to a pH shock to protect DNA and protein from overall damage. Many of the genes involved in cell membrane biogenesis, assembly and maintenance have been shown to be upregulated by pH shock.

Biological membranes are composed of structured phospholipid assemblies that, under physiological conditions, form a layer of bimolecular thickness with integrated proteins. Saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs) are important components of membrane phospholipids and determine the fluidity and deformability of cell membranes. Phospholipids with unsaturated fatty acids were more elastic than phospholipids with saturated fatty acids [39]. Changes in the environment induce variations in membrane fluidity and even in membrane lipid structure. In response to pH shock, phospholipids can alter their chain structures in various ways, such as by changing the ratio of saturation to unsaturation, the degree of unsaturation, the ratio of branched to unbranched structures, and the chain length [38]. These alterations can occur simultaneously to achieve the desired level of fluidity [39].

In our previous research, the fatty acids components of *S. albulus* M-Z18′s membrane were analyzed and shown in Fig. 2a, b. With the treatment of pH shock, tetradecanoic acid (C14:0), hexadecanoic acid (C16:0) and heptadecanoic acid (C17:0) in *S. albulus* M-Z18 were significantly decreased, whereas tetradecenoic acid (C14:1), hexadecenoic acid (C16:1) and heptadecenoic acid (C17:1) were increased, especially hexadecenoic acid (C16:1), which only existed after pH shock. The change of fatty acid composition in cell membrane not only leads to the decrease of average chain length, but also increased the ratio of unsaturated to saturated fatty acids in the membrane. The increase in the unsaturation of the cell membrane increases the fluidity of the membrane, which is conducive to the transport of material into the cell and intracellular energy metabolism. To a certain extent, this change enhanced the tolerance of the cells to acidic conditions. This speculation was also confirmed by Foster that *S. typhimurium* could obtain stronger viability after an acid shock in log-phase [40]. Consequently, at the end of fermentation, the group subjected to pH shock exhibited high dry cell weight (DCW). Similarly, studies in *Lactobacillus casei* have shown that the cytoplasmic membrane fatty acid (CMFA) content was altered, exhibiting an increase in the proportion of C18:1 and cyclopropane fatty acid (C19:0), in response to acid stress [41].

Furthermore, a number of genes have been implicated in the response to pH shock via changes in membrane stability. The gene *ffh* has been identified in *Bacillus subtilis* and *Escherichia coli* and is involved in protein translocation and membrane biogenesis [42]. Another protein, SGP (a *Streptococcus* GTP-binding protein) is associated with the membrane when *S. mutans* is grown under acidic pH

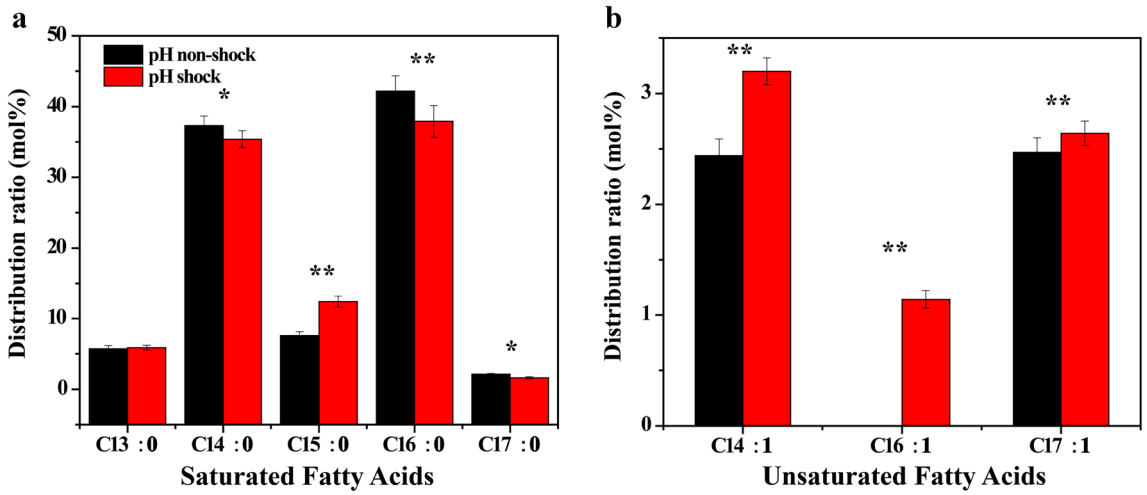

Fig. 2 Fatty acid composition analysis of cellular membrane (**a** saturated fatty acids, **b** unsaturated fatty acids)

conditions [43]. In *Lactobacillus plantarum* ZDY2013, the gene encoding phospholipid synthesis protein (*plsX*) was upregulated after acid treatment. In addition, genes encoding fatty acid elongation proteins (*fabF, fabZ, fabG*) were activated and exhibited increased expression at pH 3.0. These results indicate that *Lb. plantarum* ZDY2013 adapted its cell membrane structure by adjusting the transcription levels of genes involved in CMFA synthesis in response to acid stress [44]. Additional, by DNA chip analysis of the response of *S. coelicolor* A3(2) to pH shock, a group of genes encoding several enzymes involved in fatty acid metabolism were up- or downregulated; however, the function of each gene remains unclear [13].

Response of translocators to pH shock

Unfavorable environmental pH values can have negative effects on bacterial cells and can result in severe damage. However, within a certain range, cells can adapt to environmental changes via self-regulation. Maintenance of cellular pH homeostasis is a critical stress response of cells to pH shock and may affect the absorption and utilization of nutrients and the synthesis of proteins and nucleic acids. Therefore, pH homeostasis is crucial for cell growth and metabolism [20]. The maintenance of pH homeostasis is dependent on the interactions of multiple ion transport systems [45]. According to previous research, the cation/H^+ antiporter regulates the intracellular cation and pH levels [46]. The Na^+/H^+ antiporter [47, 48], K^+/H^+ antiporter [49] and proton pump [20] have all been demonstrated to be involved in transport. Among these systems, K^+ transporters are the most effective in the formation of chemical osmotic gradients, using reverse membrane potentials to regulate the influx of protons. The respiratory chain-related proton pump of *Sulfolobus spp.* has also been shown to play an important role [50]. Notably, proton pumps, the activity of which is based on proton motive force (PMF), are necessary for transmembrane transport processes.

The association between the putative Na^+/H^+ antiporter (*sha*) genes and pH shock in *S. coelicolor* was also investigated by Chang et al. [51]. By transcriptional analysis and overexpression experiments on eight *sha* genes, the researchers observed that expression of most of the *sha* genes was promoted by acidic pH shock. They further confirmed that *sha8* participates in pH recovery via Na^+ extrusion after pH shock. Subsequently, these researchers reported the effect of the *sha* gene on the response to acidic pH shock in *Escherichia*. They found that the tolerance to acidic environments was enhanced by upregulation of the *sha* gene after pH shock [52]. These results are consistent with those of a previous report from Efstathios S. Giotis' group. The expression of genes encoding antiporters of Na^+/H^+ and other cations was significantly upregulated in *Listeria* after alkaline pH shock, most likely to minimize the increase in intracellular pH [53]. In addition, *Bifidobacterium* and *Listeria* species, which possess acid and alkali tolerance response systems, respectively, can survive under acidic and alkaline conditions by maintaining their cytoplasmic pH at near-neutral levels. The main mechanism for this pH modulation is via proton translocation by F1F0-ATPase, which is a multisubunit enzyme whose expression can be up- or downregulated in response to acidic or alkaline pH shock [53, 54].

Response of metabolic pathways to pH shock

Most microorganisms prefer neutral environments, but many fermentation products are synthesized in only acidic or alkaline environments, which are unfavorable for the growth of

bacteria compared to neutral environments. pH shock is known to act as an environmental signals that can stimulate and promote the biosynthesis of secondary metabolites by strengthening the metabolic pathways or inducing metabolic pathways in response to pH shock. These pathways for the alleviation of pH stress can be roughly divided into energy metabolism or cellular metabolism pathways.

Energy metabolism is crucial for responding to environmental changes, and enhanced microbial respiration increase the amount of energy available for vital activities. By daily monitoring of respiratory activity during the fermentation of ε-PL, we found that the respiratory activity was distinctly higher with pH shock than without pH shock (Fig. 3a). Similarly, in the study by Zhou [14], the respiratory activity of mycelia from both strategies decreased after inoculation, but the downward trend in pH shock was slower than that without pH shock (Fig. 3b); in addition, the genes *SHJG0557* (cod cytochrome BD oxidase) and *SHJG7814* (cod iron sulfur oxidoreductase), which are basic components of the respiratory chain, were upregulated. The authors speculated that enzyme activity in the respiratory electron transfer chain was significantly enhanced after pH shock, so electron transfer and respiration were accelerated to increase energy production. In a study by Wan, genes involved in ATP synthesis (*atpA* and *atpD*) were upregulated in response to acid stress in *Lb. plantarum* ZDY2013 [44]. The enhancement of energy metabolism by pH shock is beneficial to the growth of bacteria and the improvement of fermentation production.

Cellular metabolism is a main method for the alleviation of pH stress via the synthesis of a series of alkaline or acidic metabolites in cells. In a study on *S. albulus* M-Z18 by Ren [34], physiological responses to acidic pH were examined. The aspartic acid, glutamic acid, arginine, serine and glycine levels exhibited increasing trends with decreasing environmental pH, indicating that these molecules are involved in the response to acidic environments. The glutamic acid decarboxylase (GAD), arginine deiminase (ADI) and urease systems have been identified in a variety of bacteria, including *Halobacteria*, *Pseudomonas*, *Bacillus*, *Lactobacillus* and *Staphylococcus aureus* to alleviate the effect of acid shock [55–59]. It has been widely reported that a number of genes (*arcA*, *arcB*, and *es*) participate in ADI systems [60]. ArcD, an arginine–ornithine transporter, has been identified in *Lactobacillus sakei*, while ArcT, found in *L. sakei* and *S. gordonii*, appears to be a dipeptidase; the expression of the genes encoding these proteins was affected by environmental pH [61]. Urease could generate NH_3 and CO_2 from urea, is key to pH homeostasis in bacterial pathogens under acidic stress. Over 90% of *S. aureus* strains are urease producing [62], which is encoded by the urease gene cluster *ureABCEFGD*. Urease had been confirmed to be involved in the acid-tolerant response network of *S. aureus* and regulate intracellular metabolic homeostasis [58]. A recent study showed that glycolytic pathways and the synthesis of glutamic acid were enhanced after alkaline pH shock in *Streptomyces hygroscopicus* [14]; the researchers found that the intracellular glutamate concentration in the alkaline pH shock group was 282% higher than that in the control. Glycolysis is known to lead to overall acidification, and increased glutamate levels can relieve alkaline pressure. Therefore, the pH fluctuations in the pH shock group may have been the overall results of rapid consumption of carbohydrates and amino acid metabolism.

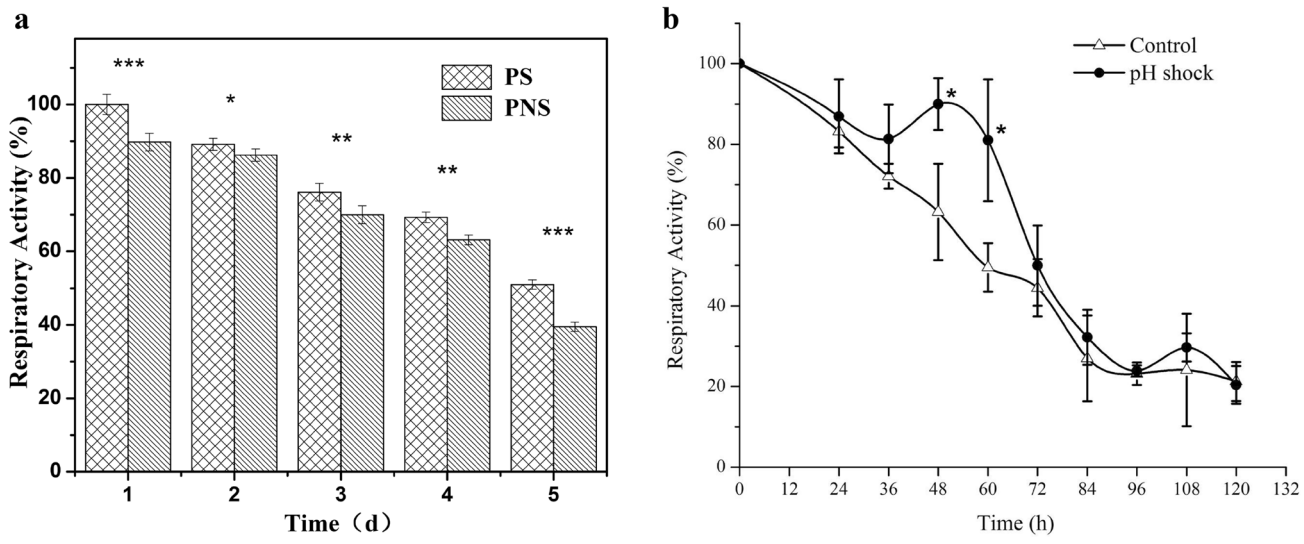

Fig. 3 Change of respiratory activity in fermentations. **a** *Streptomyces* sp. M-Z18 with acid pH shock; **b** *S. hygroscopicus* 5008 with alkaline pH shock [14]

Response of the secretion system to pH shock

As mentioned above, acidic or alkaline pH shock can lead to changes in metabolic pathways. Some metabolites are used to maintain intracellular stability, while others undergo extracellular transport to alleviate environmental stress or for self-protection; therefore, many proteins associated with the secretory system are essential [63, 64]. The effects of pH shock on the secretory system of *S. coelicolor* A3(2) were investigated. Actinorhodin secretion was observed to be highly enhanced when the cells were subjected to acidic pH shock. In addition, the gene *actVA-orf1*, encoding a putative actinorhodin transporter protein with the function of an efflux pump, was strongly upregulated under acidic pH shock, which was associated with the secretion of actinorhodin [65]. Similar results had also been reported in *B. licheniformis*, in which alkaline pH shock improved the γ-PGA yield via the induction of secretion [23].

Signal transduction pathways induced by pH shock

Regulation of gene expression in response to the extracellular environment is an adaptive response that is required for bacterial replication and survival. A complex regulatory network is required to respond to environmental stimuli or stresses, such as nutrient depletion, salt shock, oxidative stress, osmotic stress, acidic shock, and alkaline shock. When the cell membrane is stimulated by the external environment, the stress signal received by the cell wall is transduced via a two-component system (TCS), leading to the initiation of intracellular signal transduction and to the transfer of extracellular signals across the membrane and into the intracellular space. pH shock is known to be an environmental signal that can be transmitted via a series of signal transduction systems. Sigma factors [66] and TCSs [67] are integral components of signal transduction and have been demonstrated to play an important role in signal transmission and in the regulation of gene expression in bacteria undergoing pH shock. The transcription of genes in prokaryotes requires RNA polymerase, which is composed of a core enzyme and sigma factors. Environmental stress signals are transduced by TCSs combined with promoters of sigma factors, and then, transcription is activated via interactions with the core enzyme. Thus, sigma factors regulated by TCSs act on downstream genes together with the core enzyme and activate the cellular response system [14]. Figure 4 depicts the complex pathways involved in signal transduction described in previous studies.

Bacteria can sense and respond to environmental changes via TCSs. Normally, a TCS is composed of a signal–ligand-responsive histidine protein kinase (sensor kinase) and a response regulator. The mechanisms

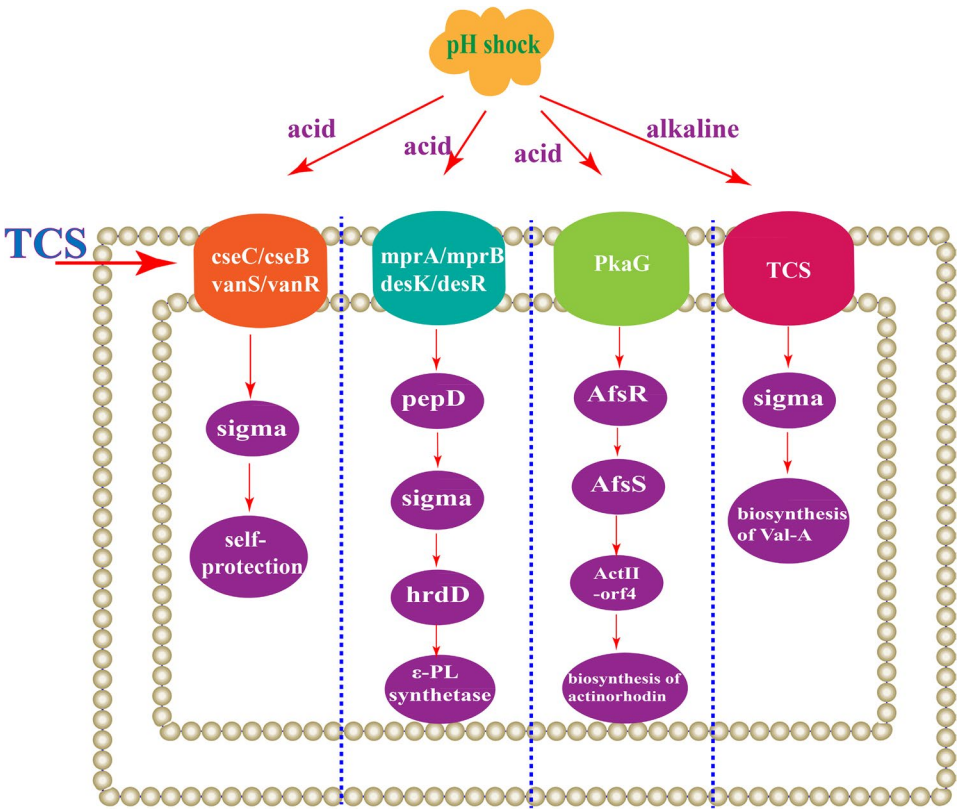

Fig. 4 Pathways involved in signal transduction

involved in signal transduction by TCSs have been well described elsewhere [68, 69]. TCSs function in bacterial adaptation, survival, and virulence by sensing environmental parameters and providing bacteria with a response mechanism [70]. In a study by Chang, the effects of acidic pH shock on TCSs were confirmed in *S. coelicolor* A3(2). The researchers found that the *cseC/cseB* [71] and *vanS/vanR* [72] TCSs, which are known to be closely associated with self-protection against cell wall hydrolysis, were upregulated by pH shock. In addition, *chiS/chiR*, *afsQ2/afsQ1*, *ecrA2/ecrA1*, *bldM*, *ramC/ramR*, and *ragK/ragR*, which are known to be positively associated with the initiation of secondary metabolism, were also upregulated. Upregulation of TCSs by acidic pH shock might have contributed in a concerted manner to the enhancement of secondary metabolite production [73]. Two pairs of TCS genes, namely, *mprA-mprB* and *desK-desR*, were upregulated together with the serine protease *pepD* after acidic pH shock in *S. albulus* M-Z18, possibly to promote ε-PL synthesis and bacterial growth. In addition, the expression of genes encoding two membrane-bound serine/threonine kinases, namely, *pkaG* [74] and *afsK* [75], was observed to be enhanced by pH shock, and two downstream involved in actinorhodin biosynthesis regulators, namely, *afsR* and *afsS*, were subsequently activated [13].

Sigma factors (σ factors) are proteins that are needed for only the initiation of transcription [76]. There proteins are bacterial transcription initiation factors that enable specific binding of RNA polymerase to gene promoters. The specific sigma factor used to initiate the transcription of a given gene varies depending on the gene and the environmental signals needed to initiate the transcription of that gene [77]. According to the results of transcriptional analyses, a wide range of sigma factor-encoding genes, including *sigH* [78, 79] (heat shock), *sigK* [80] (salt stress), *sigB* [81] (osmotic shock), and *hrdD* [82], were upregulated by acidic pH shock in *S. coelicolor* A3(2). The upregulation of sigma factors that were already known to be associated with actinorhodin biosynthesis was considered to have contributed to enhanced actinorhodin productivity by transducing the pH shock signal to regulators or biosynthetic genes for actinorhodin production [27]. Among these genes, *hrdD*, which can specifically bind to the promoter of *pls* (ε-PL synthetase), exhibited the most sensitive response to changes in pH [83]. In our recent study, expression of the gene *hrdD* was also upregulated in *S. albulus* M-Z18 after acidic pH shock which was considered to upregulate the gene *pls* and enhance the production of ε-PL. In *S. hygroscopicus* 5008, the gene *SHJG6022*, encoding an RNA polymerase ECF-subfamily sigma factor, was significantly upregulated after alkaline pH shock, in turn regulating the expression of genes involved in the biosynthesis of Val-A. Additionally, it had been demonstrated that the sigma factors *sigF* of *Mycobacterium* and *rpoS* (σ^S) of *Salmonella typhimurium* were required for tolerance to acidic pH shock [29, 84].

Conclusions and perspectives

As an environmental stressor, pH shock is a promising and valuable tool with biotechnological applications in fermentation. As an abiotic stressor, pH shock can significantly influence the performance and productivity of industrial microorganisms. Bacteria use a number of mechanisms to respond to acidic or alkaline pH shock, such as the modulation of physiological parameters, the transcriptome, or the proteome. In this mini review, we summarized the primary microbial stress responses, including morphological alteration, alteration of cell membrane composition, translocation, alteration of metabolic pathways, and signal transduction (Fig. 5). The main purpose of these physiological changes is to synergistically protect cells in an effort to maintain intracellular homeostasis for a sufficient length of time to permit the induction of adapted systems.

Most fermentation was not carried out in neutral environments but in acidic or alkaline environments. pH shock usually consists of an abrupt change in pH that lasts for a certain duration, after which the pH stabilizes at an optimal value, either artificially or spontaneously. The adapted systems mentioned above are the tolerance mechanisms gained during pH shock, which ensure the robustness of the bacteria in subsequent acidic or alkaline fermentation environments. There have been many reports on the enhancement of tolerance after pH shock. The acid tolerance and genetic traits of *S. albulus* M-Z18 following pH shock were evaluated in our previous study. We demonstrated that *S. albulus* M-Z18 developed strong acid tolerance after pH shock, which might be responsible for the high ε-PL production by this strain. The acquisition of acid tolerance might be the result of signal transduction due to low pH-induced environmental stress and not a result of genetic changes [30]. The tolerance acquired by cells due to pH shock manifests in response to not only environmental pH but also heat, oxidative stress and osmotic stress. For instance, acid adaptation of *Cronobacter sakazakii* can enhance the tolerance of the cells against subsequent environmental stress, such as acidic pH, mild heat, and organic acids. This tolerance is a result of the activation of physiological mechanisms such as the production of acid shock proteins or changes in the fatty acid composition of the cell membrane [40, 85]. The improvement of environmental tolerance mainly manifests in the following aspects: respiratory vitality, biomass and fermentation yield [14]. In the middle and late stages of industrial bacterial fermentation, there exist many factors that are not conducive to cell growth, such as oxidative stress, acidic conditions, alkaline conditions and osmotic stress. Therefore, increased cell tolerance is essential for increasing the production and yield

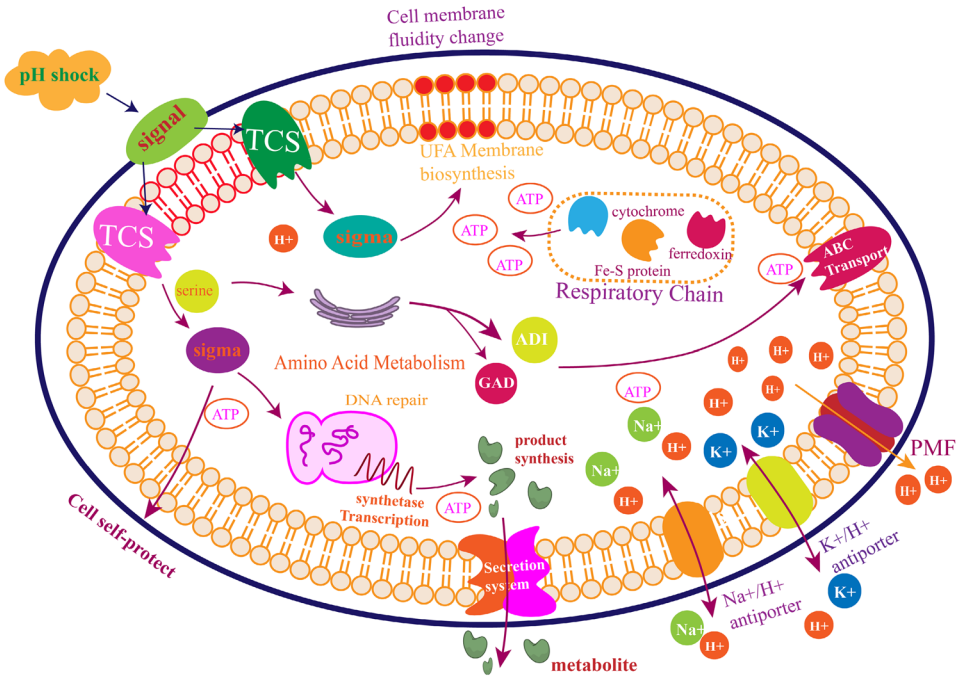

Fig. 5 Mechanisms that respond to pH shock by bacteria

[22, 44, 86]. As reported, a number of genetic modification techniques are currently being explored for the improvement of stress tolerance to enhance fermentation production [87–89].

The pH shock strategy discussed has been applied in a variety of microbial fermentation systems. Here, we systematically discussed the mechanisms by which pH shock leads to high yields by altering the levels of physiological parameters, gene expression and signal transduction. This information will be useful for widespread application of the pH shock strategy in other microbial fermentation systems. In addition, the improvement of cellular tolerance can also be a new field of research for the development of genetically engineered bacteria to enhance fermentation production.

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Compliance with ethical standards

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

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