




Transcriptome analysis of *Cyclocarya paliurus* flavonoids regulation of differently expressed genes in *Enterococcus faecalis* under low pH stress

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Abstract

Enterococcus faecalis (*E. faecalis*) is an indigenous intestinal bacterium and has potential to be applied as probiotic supplement. Low pH is one of the main stresses that *E. faecalis* has to deal with to colonize in the gastrointestinal tract. Previous study indicated low concentration of flavonoids may enhance the tolerance of probiotic to environmental stress. In the present research, transcriptome analysis was employed to investigate the influence of *Cyclocarya paliurus* flavonoids (CPF) on *E. faecalis* exposed to low pH environment. The results revealed that under the stress of low pH, genes related to cell wall and membrane, transmembrane transport, metabolism process, energy production, and conversion stress proteins were significantly differentially expressed. And certain undesired changes of which (such as genes for MFS transporter were downregulated) could be partially mitigated by CPF intervention, indicating their capacity to improve the low pH tolerance of *E. faecalis*. Results from this study deepened our understanding of the beneficial role of CPF on the probiotic in the gastrointestinal environment.

Keywords *Enterococcus faecalis* · Transcriptome · Low pH stress · *Cyclocarya paliurus* flavonoids

Introduction

Enterococcus faecalis (*E. faecalis*) belongs to lactic acid bacteria which naturally inhabits in the gut of humans and animals (Saito et al. 2014). *E. faecalis* contributes to the fermentation of some specific meat and vegetable products (Nueno and Narbad 2011). *E. faecalis* is generally considered to be a beneficial intestinal bacterium with the ability to regulate host immune responses and the activities of specific animal pathogens (such as *Listeria monocytogenes*) (Cebrián et al. 2012). Several strains of *E. faecalis* have been used as probiotics for the maintenance of a balanced intestinal flora and the treatment of gut diseases, such as irritable bowel

syndrome, and diarrhea (Cuiv et al. 2013). And some strains are widely applied in livestock and poultry farms, alone or in combination of multiple bacteria to improve the growth performance, gut health and immunity of animals (Shehata et al. 2020). Indigenous intestinal bacteria and probiotics are subject to a variety of stresses to survive in the gut, such as low pH and high bile salt concentration (Kaur et al. 2017). The pH of the stomach ranges from about 2.5 to 3.5, which is a barrier that prevents bacteria from entering the intestine (Tulini et al. 2013). Exposure to low pH may induce damages in bacterial membrane, and/or nucleic acids. It may also lead to protein misfolding or denaturing. (Salze et al. 2019). However, the impact of low pH on molecular pathways of *E. faecalis* remain largely unknown.

Gut microbiome with its vast repertoire of genes provide distinctive properties to the host by which they can degrade and utilize nutrients that otherwise pass the gastrointestinal tract unchanged (Chen and Yang 2019). Probiotics can exert beneficial effects on the host through several mechanisms, including the production of antimicrobial substances, competition for nutrients, adherence to the epithelium and stimulation of the immune system (Reis et al. 2016). The polyphenols in diet have selective growth promoting effects

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which is of utmost importance as the state of good health has been linked to dominance of particular microbial genera. The polyphenols in native form might more skilfully exert anti-oxidative and anti-inflammatory properties but in a living system, it is the microbial derivatives of polyphenol that play a critical role in determining health outcome (Banerjee and Dhar 2018). Polyphenols and probiotic may coexist in the intestine and exert synergistic health effects on the whole body (Peluso et al. 2014). For instance, flavonols have a promoting effect on the anti-inflammatory activity of *Bifidobacterium adolescentis* (Kawabata et al. 2013). It has been reported that glycosides and conjugated metabolites of flavonoids are hydrolyzed to aglycones by the enzymes produced by the intestinal bacteria, such as α -rhamnosidase, β -glucosidase and β -glucuronidase. The aglycones then undergo further bioconversion processes to exert biological activity (Aura et al. 2002). The promotional effect was sensitive to the concentration of flavonoids. Low to moderate concentrations of polyphenols could promote the growth of specific probiotics, while polyphenols at high concentrations could inhibit their growth (Banerjee and Dhar 2018). In addition, certain doses of polyphenols may help to develop and enhance microbial defense mechanisms (Bikels et al. 2010). Our previous study indicated that (–)-epigallocatechin 3-*O*-(3-*O*-methyl) gallate (EGCG3"Me) intervention (0.8%, w/v) can alleviate the negative effects of ethanol on *Saccharomyces cerevisiae* (Chen et al. 2019).

Cyclocarya paliurus (Batal.) Iljinskaja (*C. paliurus*) is a traditional plant that are both medicinal and edible, distributing in some southern provinces of China (Xie et al. 2015). *C. paliurus* flavonoids (CPF) have been shown various biological activities. Our previous study indicated that triterpenoids in *C. paliurus* may help improve ethanol tolerance of *Saccharomyces cerevisiae* (Chen et al. 2018). In addition, we have found that CPF is a potential prebiotic candidate with the ability to regulate the gut microbiota and affect certain metabolic pathways. CPF dramatically ameliorated the obesity-induced gut dysbiosis. A significant decrease was observed in the ratio of *Firmicutes/Bacteroidetes* after CPF treatment for 8 weeks. Moreover, Kyoto Encyclopedia of Genes and Genomes pathways of biosynthesis of amino acids, the two-component system and ATP-binding cassette transporters enriched the most differentially expressed genes after CPF intervention (Cheng et al. 2019). However, the information about the effects of CPF on probiotics and specific gut microbe that under gastrointestinal environmental stresses is limited.

RNA sequencing (RNA-seq), which can generate global transcription profiles, has been successfully applied to evaluate the response of gut microbe to environmental stress (Ma et al. 2018). In this study, we used RNA-seq to evaluate the influence of CPF on the gene expression profiles of *E. faecalis* under low pH stress. The result may deepen our

understanding of *E. faecalis* interacted with CPF in response to low pH from a molecular perspective. This may serve as a judgment on whether CPF is suitable as a prebiotic or as a potential functional additive for animal feed with *E. faecalis*.

Materials and methods

Chemicals and reagents

The leaves of *C. paliurus* were obtained from Quanshan Chinese herbal medicine planting Co., Ltd (Wenzhou, China). Polyamide resin was purchased from Ocean Chemical Co., Ltd. (Qingdao, China). All chemicals and reagents were analytical grade.

Preparation of CPF

The dried leaves of *C. paliurus* were smashed into powder by a grinder, and then sieved through a 60 mesh screen. According to our previous experiment, the powder of *C. paliurus* leaves was extracted with distilled water in a 35:1 liquid/material ratio (v/w) at 90 °C for 70 min. After removing the lipid and pigment by petroleum ether, the extract was centrifuged at 6000 g for 8 min followed by collection of the supernatant, and then the resulting insoluble residue was extracted again as above. The two supernatants collected were mixed and purified by polyamide column according to our previously reported method (Cheng et al. 2019). Finally, the resulting filtrate containing CPF was concentrated by rotary evaporator and preserved by lyophilization until future use.

Culture conditions of *E. faecalis* 131-2

E. faecalis 131-2 was acquired from China General Microbiological Culture Collection Center, Beijing, China. The strain of *E. faecalis* 131-2 was originally isolated from infant gut. The activated *E. faecalis* 131-2 were cultured in De Man, Rogosa, Sharpe (MRS) broth at 37 °C without shaking for 12 h. Four kinds of media [MRS broth (natural pH 6.3), MRS + CPF, acidified MRS (pH 3), acidified MRS + CPF] were prepared and pre-warmed. CPF was added at a final concentration of 0.1% (w/v), based on the effective ameliorate effect of CPF on the low pH stress for *E. faecalis* 131-2 from our previous study. Then, inoculated 1 mL of pre-cultured *E. faecalis* 131-2 into 50 mL above media in order, and divided them into 4 groups: control group, CPF group, acidified group, and acidified-CPF group. The *E. faecalis* 131-2 cells were grown at 37 °C and monitored by measuring the optical density of the culture medium at 600 nm (OD₆₀₀) for up to 24 h. Cells for scanning electron microscopy (SEM) analysis and RNA-seq analysis were collected

at 12 h of cultivation after cells reaching the stationary phase (Lee et al. 2012).

Scanning electron microscopy (SEM) analysis

E. faecalis 131-2 cells from the control group, acidified group, acidified-CPF group were collected by centrifugation at 5000 g, 4 °C for 8 min. And then they were washed 3 times with physiological saline. After that cells were resuspended in 2.5% glutaraldehyde at 4 °C for 4 h and washed 3 times with 0.1 M phosphate buffered solution for 15 min each time. The cells were eluted with a series of ethanol solutions (30, 50, 70, 80, 90, 95 and 100%) for 10 min per elution, and afterward with a gradient of tert-butanol and anhydrous ethanol mix mixtures (ratio 1:3, 1:1, 3:1 and 3:0) for 10 min in each mixture. Subsequently, the bacterial cells were lyophilized. Finally, they were coated with a 40:60 gold/palladium alloy and observed under a SEM system (Hitachi S3400N).

RNA preparation, library construction, and sequencing

E. faecalis 131-2 cells from each culture were collected by centrifugation at 5000 g, 4 °C for 8 min. Total RNA was extracted using the TRIzol (Invitrogen, Carlsbad, USA). After extraction, rRNA was removed by Ribo-Zero Magnetic kit (EpiCentre, Wisconsin, USA). mRNA was broken into short pieces using divalent cations under elevated temperature. Illumina Truseq™ RNA sample prep Kit was used to construct library. The first cDNA strand was synthesized using normal dTTP's and the second strand cDNA was subsequently synthesized using dUTP to replace dTTP, after that connected index joint and degraded the second strand cDNA using uracil-N-glycosylase. Finally, sequencing library was acquired by PCR amplification. Illumina HiSeq 4000 was applied to test and sequence the final library.

Reads mapping, annotation, and analysis

Illumina paired-end RNA-seq approach was used to sequence the transcriptome, which can generate billions of reads in a single run. To ensure the accuracy of subsequent biological information analysis, the original sequencing data were filtered to obtain high-quality clean data. The specific steps and sequence are as follows: remove the adapter sequence in reads and reads not inserted in fragments, trim off low-quality bases (quality score < 20) at the end of the sequence (end of 3'), remove reads containing N greater than 10%, discard sequences less than 75 bp in length after adaptor removal and quality trimming. Genes with ratio changed more than twofold ($P < 0.05$) were identified to have significantly different gene expression. The ratio changes

including the up- and down-fold change. Descriptions and annotations of genes were available in the Genome Database of *E. faecalis* (<https://www.ncbi.nlm.nih.gov/genome>). *E. faecalis* 131-2 genes annotations were subsequently applied to predict biochemical pathways by the pathway tools. GO terms and KEGG pathways were acquired from GO database (<http://geneontology.org>) and KEGG database (<http://www.kegg.jp/kegg>), respectively.

Defining differentially expressed genes

Repeat correlation of samples was evaluated to get comparison of gene expression levels in different libraries. Fragments Per Kilobase of exon model per Million mapped reads (FPKM) was used to normalize and calculate the transcript levels of expressed genes. Genes have a greater than twofold change in the two groups with P less than 0.05 were identified as differentially expressed genes (DEGs).

Quantitative real-time PCR validation

To determine the reliability of the transcriptome results, 10 representative DEGs were selected for quantitative analysis using real-time quantitative PCR. Total RNA of *E. faecalis* 131-2 was reverse-transcribed into cDNA by Fastquant RT Kit (with gDNase) (TIANGEN BIOTECH). Then PCR reactions were performed with a LightCycler96® (Roche Diagnostics GmbH, Mannheim, Germany) thermocycler following the procedures: 94 °C for 3 min, followed by 40 cycles of 95 °C for 3 s, 60 °C for 25 s, and a final extension of 72 °C for 2 min. The relative expression level of target genes was determined by the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

Three independent biological replicates were performed in each experiment. Data obtained were analyzed using SPSS (SPSS Inc., Chicago, IL, USA, V 17.0.0). Comparisons between groups were carried out by one-way analysis of variance (ANOVA) with the post hoc Tukey test for multiple comparisons, P values < 0.05 were considered significant.

Results and discussion

Effects of CPF on the growth of *E. faecalis*

Figure 1 showed that the growth of *E. faecalis* 131-2 was strongly inhibited in the acidified group compared to the control group, and CPF supplement could effectively alleviate the inhibition induced by low pH stress. The growth of *E. faecalis* 131-2 was not significantly affected by the addition of CPF when cultured without low pH stress.

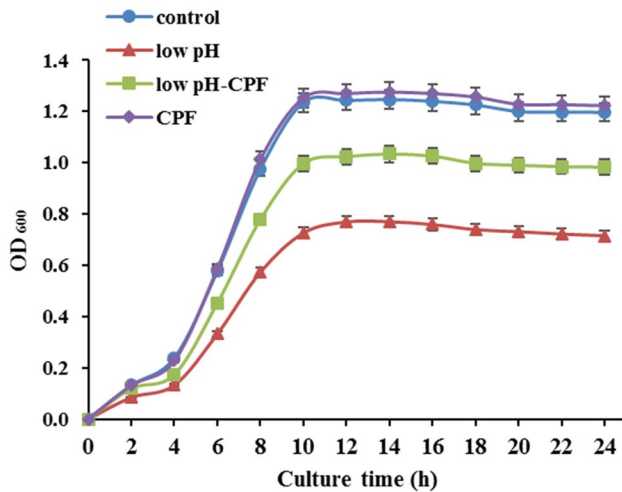


Fig. 1 Effects of CPF on the growth of *E. faecalis* 131-2. Results were obtained from three independent experiment and expressed as mean \pm SD ($n=3$)

Comparison of the physiological phenotypes of *E. faecalis* 131-2

Since the OD₆₀₀ results showed that there was no significant difference in the growth of *E. faecalis* between CPF group and control group, SEM was conducted on the control, acidified group and acidified-CPF group. Fig. S1 exhibited the cell morphology of *E. faecalis* 131-2 in the control group (Fig. S1A), the acidified group (Fig. S1B), and the acidified-CPF group (Fig. S1C). Compared with *E. faecalis* 131-2 in the control group, bacteria grown in the acidified group showed a more rough and shrunken appearance, which may be attributed to a change in cell surface properties. Membrane vesicles were observed on the surface of *E. faecalis* 131-2 cells under low pH stress. For bacteria grown in the acidified-CPF group, fewer membrane vesicles and little cell morphology variation were observed. It indicated that CPF could maintain the integrity of the cell membrane and enhance the survival of *E. faecalis* 131-2 under low pH stress.

Illumina sequence data

Library representing transcriptome of each sample was established for RNA-seq. A total of 30.1 GB clean reads were acquired, and bases with an average quality value greater than 30 account for over 94% of the total bases. High-quality reads were aligned and mapped to *E. faecalis* 131-2 reference genome by Rockhopper software. The results revealed that over 83% reads of each library were matched to *E. faecalis* 131-2 genes.

Defining DEGs

Compared to the control group, 683 DEGs were identified in *E. faecalis* 131-2 under low pH stress, including 156 upregulated and 527 downregulated genes. Among 47 DEGs, 15 upregulated and 32 downregulated genes were screened out between the low pH-CPF and the acidified group.

GO classification analysis

To better understand the functional categories of the DEGs under low pH stress, they were classified into three different functional sub-groups GO categories. The main GO terms enriched in biological process consisted of “biological regulation”, “cellular process”, “metabolic process” and “response to stimulus”. For cellular component, the largest groups were “cell”, “cell part” and “membrane”. In molecular function domain, the main enriched GO terms including “binding”, “catalytic activity”, and “transport activity”. To evaluate the influence of CPF, we analyzed the DEGs between the acidified-CPF group and the acidified group. As shown in Fig. 2, when compared with the acidified group, the upregulated genes in the acidified-CPF group are represented by red bars and the downregulated genes are represented by blue bars. After CPF intervention, the DEGs in biological process were mainly belonged to “cellular process”, “localization” and “metabolic process”. High percentage of genes assigned to “cell”, “cell part” and “membrane” were observed in cellular component domain. With regard to molecular function, most genes distributed in “binding”, “catalytic activity”, and “transport activity”.

KEGG pathway analysis

KEGG pathway enrichment analysis for DEGs between the acidified group and the control group showed the complicated metabolic pathways in response to low pH stress. Figure 3a shows that DEGs were significantly enriched in pathways used for ABC transporters (ko02010, $P < 0.05$), lysine biosynthesis (ko00300, $P < 0.05$) and pantothenate and CoA biosynthesis (ko00770, $P < 0.05$). Pathways involved in biosynthesis of amino acids and amino sugar and nucleotide sugar metabolism also had high enrichment scores, but not considered significant. KEGG analysis of DEGs between the acidified-CPF group and the acidified group was exhibited in Fig. 3b. Selenocompound metabolism (ko00450, $P < 0.05$) and two-component system (ko02020, $P < 0.05$) were the most enriched pathway.

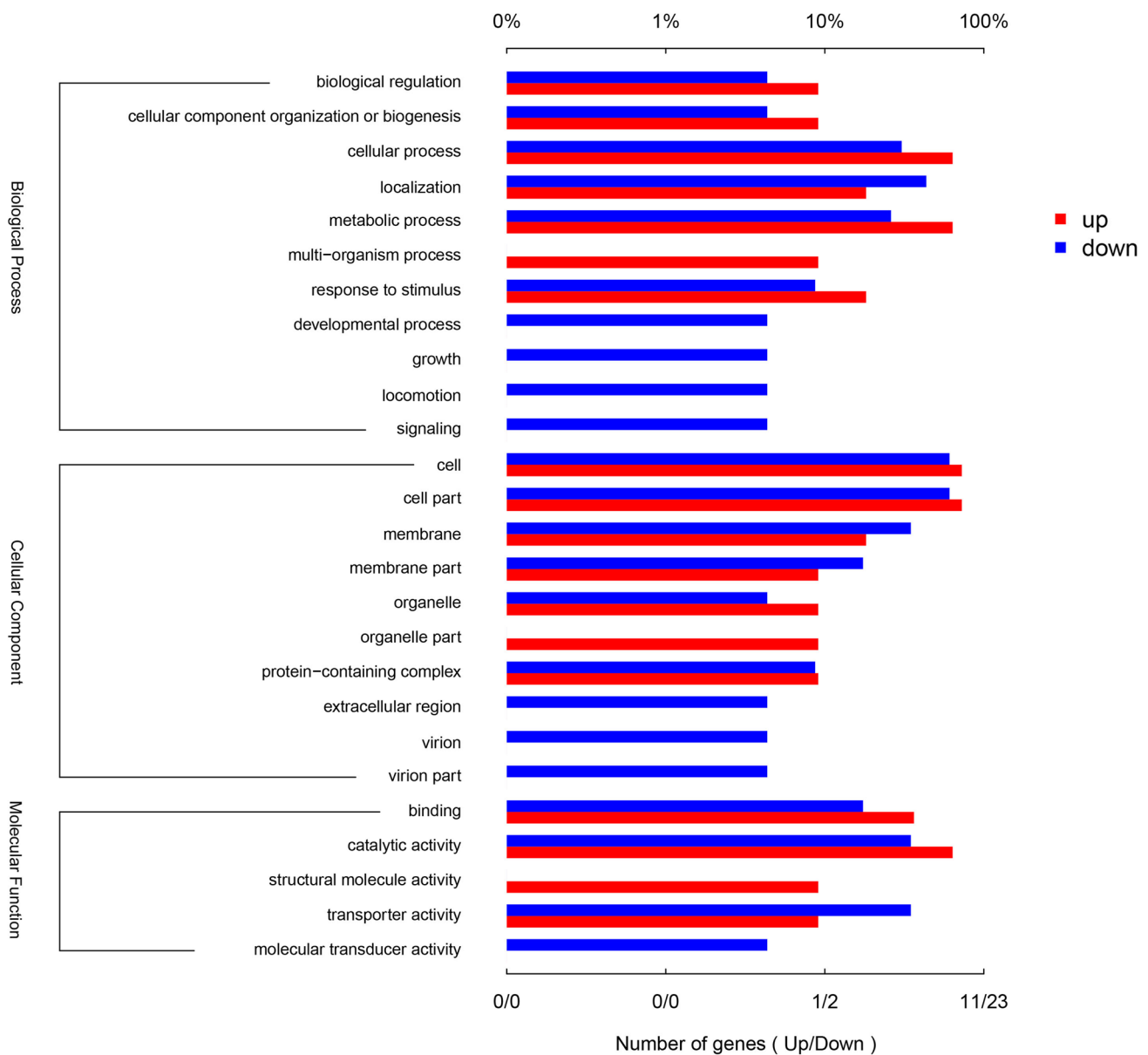


Fig. 2 GO analysis of DEGs between the acidified-CPF group and the acidified group. Red bars represent upregulated genes, blue bars represent downregulated genes

RNA-seq expression validation by quantitative real-time PCR

To validate the RNA-seq sequencing, we detected the relative expression of 10 randomly selected DEGs by RT-qPCR (Fig. 4). The candidate DEGs included 5 up- and 5 downregulated genes. The upregulation or downregulation of the tested genes is consistent with the results of transcriptome analysis. A high consistency was showed between the RNA-seq and RT-qPCR data ($P > 0.05$), indicating the reliability of the transcriptome results.

Cell wall and membrane

The cell wall of *Enterococcus* is a complex arrangement of glycopolymers and proteins. It consists of a thick peptidoglycan sacculus which surrounds the cytoplasmic membrane and is decorated with other glycopolymers, including proteins, polysaccharides, and teichoic acids. During bacterial growth, the cell wall is essential to maintain bacterial cell shape and integrity, as well as resist external and internal stress. As the interface between the bacterium and its surroundings, the cell wall mediates the interactions between

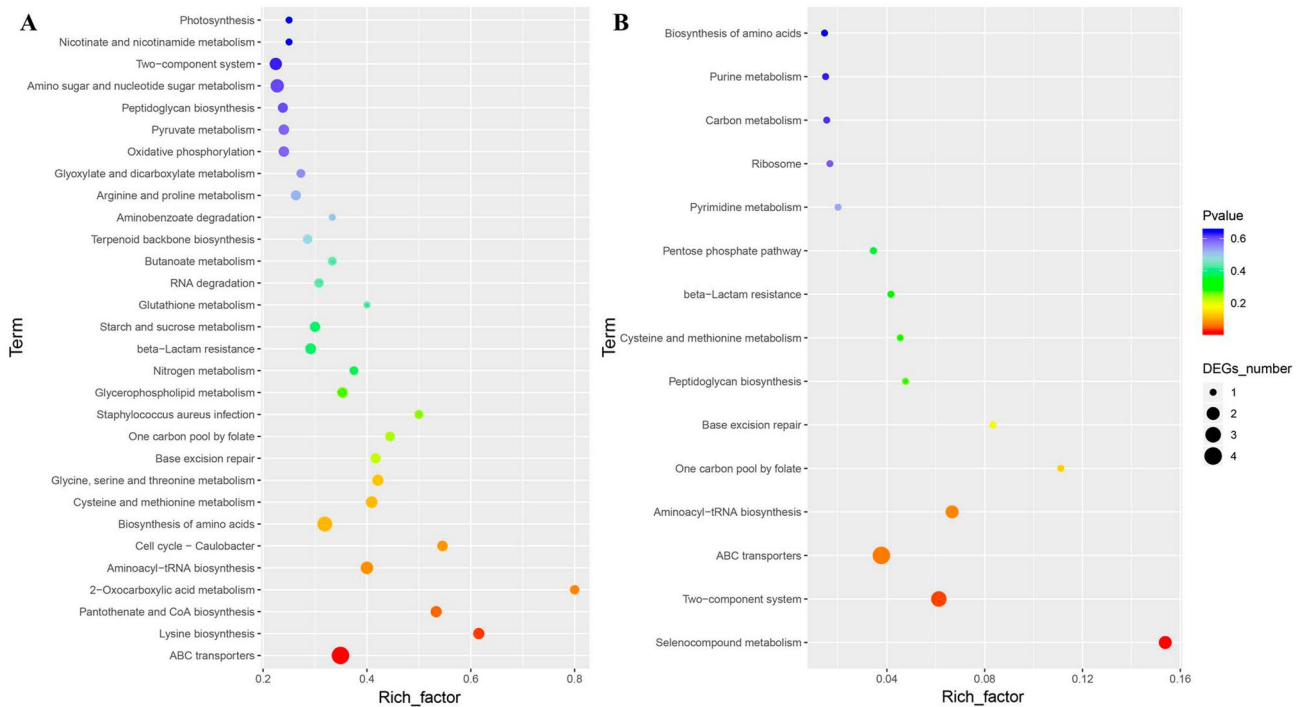
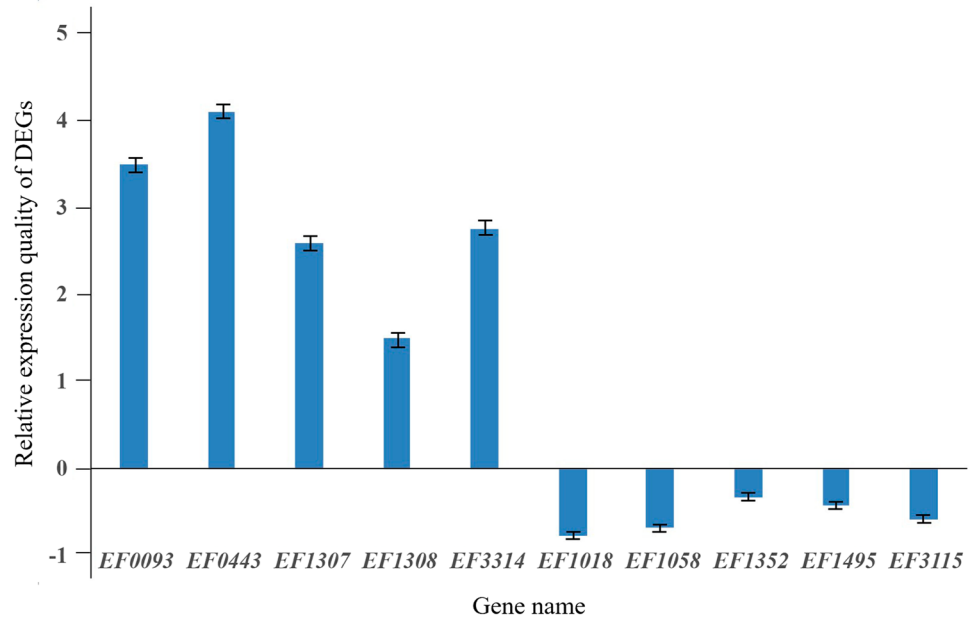


Fig. 3 KEGG analysis of DEGs between the control group and the acidified group (a). KEGG analysis of DEGs between the acidified-CPF group and the acidified group (b). The diameter of the circle

is proportional the number of DEGs enriched in each pathway. The color of circle represents the *P* value for enrichment

Fig. 4 PCR validations of RNA-seq data



them (Chapot and Kulakauskas 2014). Transcriptome analysis indicated that the genes related to cell wall structure were significantly differentially expressed under low pH stress. Compared with the control group, significantly upregulated genes in the acidified group related to cell wall component

included *EF0093* and *EF3314* for cell wall surface anchor protein, as well as genes (*EF0355*, *EF1992* and *EF2802*) for LysM peptidoglycan-binding domain-containing protein. It has been reported that cell wall surface proteins involved in bacterial motility, adherence, enzymatic activity and

protection from phagocytosis (Navarre and Schneewind 1999). The significant differential expression of these genes may be attributed to the compensatory mechanism to offset low pH stress and protect the bacterial cell wall from destruct induced by low pH environment (Solheim et al. 2014). *EF1194* for cell wall metabolism sensor histidine kinase WalK, *EF2713* for LPXTG cell wall anchor domain-containing protein and *EF2486* for teichoic acids export ATP-binding protein TagH were significantly downregulated under low pH stress.

Bacterial cell membranes play an important role in nutrients uptake, homeostasis, as well as the capability to adapt and respond to the external environment (Cathro et al. 2016). Membrane proteins are responsible for various functions, including nutrient transport, adhesion, response to environmental stress, biofilm formation, virulence, and antibiotic resistance. In addition, integral membrane proteins are crucial in the initiation of signal transduction pathways, which allows the bacterial cells to adjust their physiology in response to changes in the extracellular environment (Solis and Cordwell 2011). Paulsen et al. genomic study predicted 50 membrane proteins were involved in the stress response of *E. faecalis* (Paulsen et al. 2003). Acids can damage cell membranes of *E. faecalis* and increase their permeability, which may lead to cell compound leakage (Wang et al. 2018). Present study showed genes (*EF0025*, *EF0397*, *EF0399*, *EF0943*, *EF1189*, *EF1910*, *EF2771*, *EF2794*) for membrane protein and *EF1913* encoding membrane protein insertion efficiency factor YidD were significantly downregulated, which suggested that low pH had a damaging effect on the protein composition of the *E. faecalis* cell membrane. Genes (*EF2489*, *EF0994*, *EF0993*, *EF2585*) encoding peptidoglycan biosynthesis and genes (*EF0365*, *EF0282*) involved in fatty acid biosynthesis were observed to be significantly downregulated. Changes in these genes might imply that the cell membrane of *E. faecalis* was restructured to cope with low pH environment.

We did not observe significant changes in the expression of genes related to cell wall and membrane of *E. faecalis* between the acidified group and the acidified-CPF group. This might imply that the cell wall and membrane of *E. faecalis* were not the main sites of action of CPF, nor they were damaged by CPF.

Transmembrane transport

The major facilitator superfamily (MFS) is the largest group of secondary active transporters on the cell membrane, characterized by a common 12-transmembrane helix motif which can selectively transport a variety of substrates across the membrane. MFS transporters are crucial in many physiological processes, as transporting various ligands, including nucleotides, peptides, monosaccharides, oligosaccharides,

enzyme cofactors cations and anions (Joanna et al. 2016). In this study, we found *EF1042* and *EF2773* for MFS transporter in the acidified group were both downregulated compared with the control group, while *EF2773* in the acidified-CPF group was significantly upregulated compared with the acidified group. It indicated that addition of CPF might improve the expression of MFS transporters, which was conducive to the active transport of *E. faecalis* under low pH stress. The upregulated expression of MFS transporters-related genes was probably helpful for compensating for the inadequate capacity of *E. faecalis* cell under environmental stress by strengthening H⁺ efflux (Lv et al. 2016).

Metabolism process

A lot of DEGs related to metabolism process in *E. faecalis* were identified in the present study. Amino acids are considered to be the main metabolites involved in the biosynthesis and metabolism of *E. faecalis*. Previous researches on the nutritional requirements and biosynthetic capacities of *E. faecalis* showed that isoleucine, histidine, tryptophan and methionine were necessary in the growth of all strains (Murray et al. 1993). Besides, *E. faecalis* can use certain amino acids as carbon and energy source (Deibel 1964). Under low pH stress, genes for amino acid or peptide ABC transporter (*EF0243*, *EF0760*, *EF1117*, *EF1791*) which related to amino acid transport and metabolism were downregulated. It suggested that the transport and supply of amino acids might be decreased under acidic stress, which might inhibit the growth and reproduction of *E. faecalis* (Ran et al. 2015). Interestingly, *EF0243*, *EF0760* were significantly upregulated in the acidified-CPF group compared with the acidified group, which indicated CPF might partially relieve low pH-induced inhibition of amino acid transport and metabolism.

Genes involved in nucleotide transport and metabolism exhibited differential expression in low pH environment. The genes (*EF0002*, *EF0039*, *EF0186*, *EF0285*, *EF2549*, *EF2764*) involved in pyrimidine metabolism were significantly downregulated in acidic stress. Genes (*EF0104*, *EF0105*, *EF0106*, *EF1415*) responsible for proline and arginine metabolism also appeared significant down-regulation, which have been reported to be positively related to thymidine production (Ran et al. 2015). This might indicate the nucleotide transport and metabolism of *E. faecalis* was inhibited in pH 3 media. The survival of *E. faecalis* not only relies on balanced generation of genetic variation but also on the protection of the genome from changes that result cytopathic and fitness reduction. Base excision repair (BER) is generally considered to be the primary defense against DNA damage (Hilde et al. 2001). *EF0879* for BER were observed significantly downregulated under low pH environment, while *EF0879* were highly upregulated in the acidified-CPF group compared with the acidified group, suggesting CPF

intervention might relieve this negative impact of low pH on *E. faecalis* and enhance their acid tolerance.

Nine genes (*EF0019*, *EF0020*, *EF0021*, *EF0717*, *EF1017*, *EF1018*, *EF1019*, *EF1829*, *EF1830*) encoding in the IIABCD component of phosphotransferase system (PTS) were significantly downregulated in acidified group. These included the predicted mannose, fructose, sorbose, lactose and cellobiose PTS systems. Interestingly, a previous study has found the genes for PTS were also downregulated under alkaline stress (Ran et al. 2015). PTS is a unique method employed by bacteria to uptake sugar where the source of energy is phosphoenolpyruvate. It is participated in transporting various sugars into bacteria cell, including fructose, mannose, glucose, and cellobiose. PTS sugars may vary between different bacterial groups, with each group is evolving to reflect the most appropriate carbon source available in the substrate. This suggested that substrates other than glucose might also contribute to the growth of *E. faecalis* in low pH stress (Ran et al. 2015). Moreover, *EF0718* encoding 1-phosphofructokinase, *EF1034* encoding aminoglycoside phosphotransferase family protein, *EF1049* encoding phosphogluconate dehydrogenase and *EF2425* encoding phospho-sugar mutase were downregulated in low pH stress. This indicated that the glycolytic pathway of *E. faecalis* was suppressed in acidic environment and the bacteria began to use of less preferred carbon and energy sources, especially fructose.

Energy production and conversion

In this study, four significantly downregulated genes (*EF1493*, *EF2612*, *EF2613*, *EF2614*) encoding ATP synthase were detected in the acidified group compared with the control group. *EF1046* encoding pyruvate kinase which is responsible for converting phosphoenolpyruvate to pyruvate was downregulated. Pyruvate is a metabolic key molecule which can be utilized in many different reaction pathways to elevate the ATP levels (BøHle et al. 2010). In addition, genes (*EF0237*, *EF0238*, *EF1304*, *EF1352*, *EF1495*) encoding ATPase in the acidified group, which couples ATP hydrolysis to the translocation of protons across bacterial cell membranes, were also downregulated when comparing with the control group. Compared with the acidified group, the genes (*EF149*, *EF0237*, *EF0238*, *EF1304*) were upregulated in the acidified-CPF group. It indicated that the generation and conversion of ATP in *E. faecalis* might become slack in low pH environment, which could be partially improved by CPF (Li et al. 2020).

Stress proteins

Genes *EF1307* and *EF1308* encoding two general stress proteins (heat-shock protein GrpE and the chaperone DnaK)

were upregulated under low pH stress, which might be a self-protection mechanism of *E. faecalis* to cope with stress conditions (BøHle et al. 2010). However, *EF1058* encoding universal stress protein were observed to be downregulated. Inactivation of Gls24 has been reported to have a significant impact on stress tolerance and cell morphology of *E. faecalis* (Giard et al. 2000). In the present study, we observed *EF0976* and *EF3115* which encoding Asp23/Gls24 family envelope stress response protein was downregulated in the acidified group compared with the control group. When comparing the acidified group and the acidified-CPF group, no significant changes in the expression of genes related to stress proteins of *E. faecalis* were observed.

Conclusion

In this study, RNA-seq analysis revealed the response of *E. faecalis* 131-2 cells under low pH stress. The effect of CPF on *E. faecalis* under low pH stress was also investigated. KEGG pathways for ABC transporters, lysine biosynthesis, as well as pantothenate and CoA biosynthesis were enriched the most DEGs in response to low pH stress, while KEGG analysis of DEGs between the acidified-CPF group and the acidified group suggested the most enriched metabolic pathways were selenocompound metabolism and Two-component system. The results may contribute to an enhanced understanding of the influence of CPF on *E. faecalis* exposed to low pH, and promote the potential applications of *E. faecalis* and CPF in the animal foods, fermented foods, and probiotic supplements.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00203-021-02215-8>.

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Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest.

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