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RESEARCH

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# The mechanisms of condensed tannins inhibit *Pediococcus pentosaceus*

Rongzheng Huang[1], Fanfan Zhang[1], Xuzhe Wang[1], Chunhui Ma[1*] and Mingxin Ma[2*]

**Abstract**

**Background** The antibacterial mechanisms of action of condensed tannins (CTs) obtained from tea are well known. However, the antibacterial mechanism of CTs from legumes, such as sainfoin, against to *Pediococcus pentosaceus* was still unclear. Using *Pediococcus pentosaceus* SF11 as a model organism, this study investigated the antibacterial mechanism of CTs (extract from sainfoin by 70% acetone aqueous solution).

**Methods** The mechanism of CTs against *Pediococcus pentosaceus* was investigated though determined the minimal inhibitory concentration (MIC) of CTs, effects of CTs on cell membrane, scanning and transmission electron microscopy analysis and global transcriptome analysis, et al.

**Results** The results showed that CTs decreased the activities of enzymes such as lactic dehydrogenase, and inhibited the pentose phosphate (PP)/glycolytic pathway. The content of hydrogen peroxide produced by CTs was increased in *P. pentosaceus* SF11, and antibacterial activity partly occurred due to this hydrogen peroxide. The global transcriptome analysis showed that CTs upregulated the expression of 187 genes, most of which were involved in hypothetical protein, followed by the PTS (phosphotransferase system) system, while three genes were involved in oxidative stress. The expression of 161 genes was downregulated, most of which were involved in the phosphate ABC transporter system.

**Conclusion** These findings suggest that the mechanism of antibacterial action of sainfoin CTs mainly operates through the inhibition of protease activity, and is partly associated with oxidative stress induced by hydrogen peroxide.

**Keywords** Condensed tannins, *Pediococcus pentosaceus*, Hydrogen peroxide, Antibacterial activity

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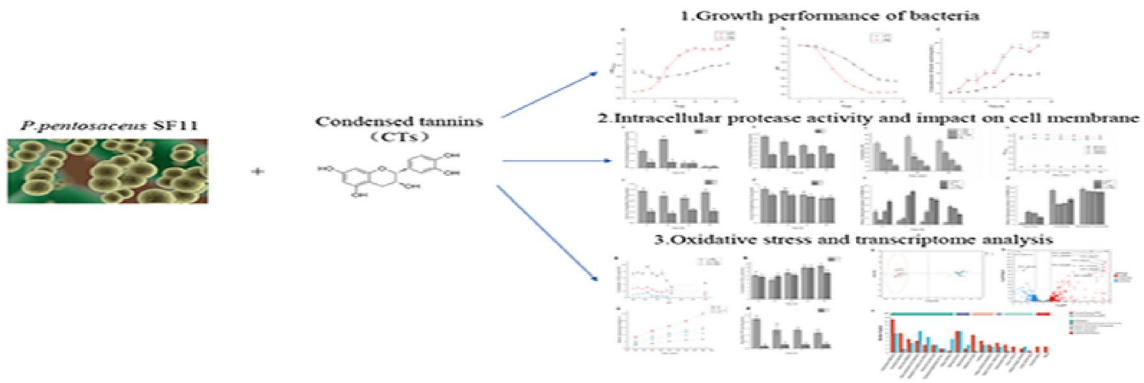
**Graphical Abstract**




## Introduction

Condensed tannins (CTs) are secondary metabolites in plants that are composed of diverse mixtures of oligomeric and polymeric substances, namely flavan-3-ols. Catechin (C), epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC), epicatechin gallate (ECG), gallocatechin gallate (GCG), and epigallocatechin gallate (EGCG) are the subunits of CTs [1]. CTs from green tea extracts contain EGCG, EGC, ECG, and EC and show antibacterial activity against a broad range of gram-positive and gram-negative bacteria [2]. The biological activity of CTs has been shown to be correlated with the presence of galloyl and gallic moieties in flavan-3-ol units. Among these CTs, EGCG has drawn the most interest from microbiologists because it contains both galloyl and gallic moieties in flavan-3-ol units, resulting in EGCG having the strongest ability to inhibit bacteria [3]. The mechanisms of antibacterial action of green tea CTs, such as the inhibition of bacterial toxins, the extracellular matrix, plasmid transfer, cell wall synthesis, and membrane disruption have been reported. However, as far as we know, none of study have focused on the mechanism of CTs from legume against to *Pediococcus pentosaceus*. Various legume plants, such as sainfoin, contain CTs with a different composition of flavan-3-ols compared to green tea [1].

Previous research found that CTs only inhibited *Pediococcus* activity during the ensiling stage of sainfoin [4]. The relative abundance of *Pediococcus acidilactici* decreased as the ratio of sainfoin increased during mixed alfalfa and sainfoin ensiling [5]. Therefore, CTs can directly inhibit *Pediococcus* activity during fermentation. *Pediococcus pentosaceus* has several beneficial effects, such as improving the taste of food, enhancing the growth of plants and animals, and inhibiting inflammation, which have allowed it to play an increasingly pivotal role in industrial LAB applications [6]. Thus, considering that CTs are naturally found in numerous plants, clarifying the mechanism of how CT inhibits *Pediococcus* could help researchers better utilize *P. pentosaceus* as an additive or probiotic in the food industry. However, to the best of our knowledge, few studies have focused on the inhibition of *Pediococcus* by CTs.

The present study aimed to determine the mechanism of how CTs inhibit the activity of *P. pentosaceus*. Considering that EGCG is not found in sainfoin CTs, this work hypothesized that sainfoin CTs show lower antibacterial activity than green tea and that the mechanism may also be different. The findings of the present study could facilitate the elucidation of the inhibition mechanisms of CTs against *P. pentosaceus*, providing an opportunity for the development of probiotics that can better tolerate CTs during food industry processes.

## Material and methods

### Strain

In this study, the strain *P. pentosaceus SF11* was isolated from sainfoin silage. After 14 days of sainfoin ensiling, 10 g samples with the addition of 90 mL sterile water were serially diluted by $10^2$–$10^8$. One milliliter of each concentration was inoculated onto a De Man–Rogosa–Sharpe (MRS) agar plate after shaking for 1 h at 37 °C. After incubation for 24 h at 37 °C, a single colony was selected for purification and cultivation, and the selected colony was inoculated into MRS broth for 24 h at 37 °C [7]. The bacterial fluids were collected for 16S rRNA sequence analysis by Sangon Biotechnology Co., Ltd. (Shanghai, China). The primers used for polymerase chain reaction (PCR) amplification were 27F: 5′GCAGAGTTCTCG GAGTCACGAAGAGTTTGATCCTGGCTCAG-3′ and

1492R: 5′-AGCGGATCACTTCACACAGGACTACGG GTACCTTGTTACGA-3′. The nucleotide sequence data of *P. pentosaceus* SF11 were deposited in the National Center for Biotechnology Information (NCBI) under the number "ON866519" (accessed on July 3, 2022). The phylogenetic evolution tree of *P. pentosaceus SF11* is shown in Additional file 1: Fig. S1. All reagents described below were purchased from Sigma-Aldrich Co., Ltd., Germany unless otherwise specified.

#### Extraction and identification of CT composition

The extraction and purification of CTs from sainfoin was performed according to methods [8]. Briefly, 50 g samples were freeze-dried (FD-1B-50, Jipu Electronic Technology Co., Ltd., Shanghai, China) and added to 500 mL extraction solution (7:3, acetone: water, v/v) containing 0.1% ascorbic acid, followed by extraction in a homogenizer (L-1BA, Kuansons Biotechnology Co. Ltd, shanghai, PR china) for 1 h. The sample was extracted twice, then ether was added until the solution became layered. The aqueous phase was collected and residual acetone was removed using a rotary evaporation apparatus (R-1020, Great Wall Scientific Industrial and Trade Co., Ltd., Zhengzhou, China) at 120 rpm and 45 °C under a vacuum using a vacuum pump. The solution was then freeze-dried to obtain the crude CT extract. Fifty milligrams of crude CT was resolved in 50 mL methanol and the Sephadex LH-20 (Sigma-Aldrich Co., Ltd., Germany) was used to filter the solution. Next, 50% methanol–water solution was used to wash the column, and elution was performed with 100 mL of 70% acetone water–solution. The final solution obtained after elution consisted of purified CTs.

According to Gea et al. (2011), benzyl mercaptan was applied to induce the thermal decomposition of CTs under acidic conditions, and the end unit produced corresponding flavanol molecules, while the extension unit produced benzyl sulfide derivatives. Briefly, 200 mg of CT was taken and 1.6 mL methanol, 800 μL of 3.3% hydrochloric acid, and 80 μL benzyl mercaptan were added. After being placed in a homogenizer at 1000 rpm and 40 °C for 1 h, the solution was placed into an ice bath for 10 min. The supernatant was collected following centrifugation at 3000 rpm for 10 min. The solution was filtered through the Sephadex LH-20, elution was performed with 50% methanol–water solution, and the solution was collected and stored at − 4 °C for high-performance liquid chromatography (HPLC) (Agilent Technologies, Inc., Waldbronn, Germany) analysis. Before HPLC analysis, 100 μL of dihydroquercetin (0.384 mg/mL) was added as an internal standard substance for each solution [9].

The Titank C18 column (3 μm; 150 × 4.6 mm; FMF-5559-EONU; FLM Scientific Instrument Co., Ltd., Guangzhou, China) was used for the HPLC analysis. The HPLC analysis procedure was as follows: flow rate 0.5 mL/min; mobile phase A consisted of 1% acetic acid aqueous solution, and mobile phase B consisted of methanol, following the order of 0–5 min, 20% B; 5–40 min, 20–70% B linear; 40–45 min, 70–90% B linear; 45–50 min, 90% B; 50–55 min, 90–20% B linear; and 55–60 min, 20% B, with an injection volume of 20 μL. The ultraviolet detection wavelength was 280 nm [9].

#### Determination of the minimal inhibitory concentration (MIC) of CT

The broth dilution method [10] was used to determine the MIC of CTs against *P. pentosaceus SF11*. Briefly, CTs were diluted to 125–3000 mg/L after filter membrane sterilization (aperture, 0.45 μm). One milliliter of bacterial solution (MRS broth, SF11 growth for 3.5–4 h) was taken and the concentration was diluted to $10^6$ colony-forming units (CFU)/mL. Next, 50 μL bacteria solution with and without the addition of 50 μL of CT solution was added to 96 microporous plates (the final concentration of bacteria was approximately $5 \times 10^5$ CFU/mL), followed by incubation at 37 °C for 24 h.

#### Effect of CTs on the growth of *P. pentosaceus* SF11

Bacteria were cultured on MRS broth with and without the addition of CT at a concentration of 1500 mg/L. The absorbance was measured at 600 nm using a spectrophotometer (Multiskan Go, Thermo Fisher Scientific Inc., USA; same as below), and the pH and lactate acid (LA) production after 0, 2, 4, 6, 8, 12, 14, 16, 18, 20, and 24 h of incubation were determined. HPLC (Agilent Technologies, Inc., Waldbronn, Germany) was used to analyze the LA content in the bacterial solution according to the methods described above [4].

Bacteria were cultured on xylose-based MRS broth [11] with or without the addition of CTs at a concentration of 1500 mg/L. Cells were collected after 8, 24, 32, and 48 h of incubation. The cells were harvested through centrifugation at 8000 rpm for 5 min and washed twice using 0.1 M phosphate-buffered saline (PBS) solution (pH = 7.0). Broken cells according to the methods described above [11] and the supernatant was collected for protease activity analysis. The activities of four enzymes involved in xylose metabolism, namely lactate dehydrogenase (LDH), phosphoketolase (PK), transketolase (TKT), and transaldolase (TAL), were analyzed according to the methods described above [11].

#### Effect of CTs on the cell membrane of *P. pentosaceus SF11*

The cells were collected after overnight incubation on MRS broth. Cells were harvested through centrifugation at 8000 rpm for 5 min and washed twice in 0.1 M

PBS solution (pH = 7.0). The concentration of cells was adjusted to $OD_{600} = 0.5$.

To analyze the efflux of potassium ions in cells, 0.5 mL cell solution was taken and 0.5 mL CT at concentrations of 0.5 × MIC (750 mg/L), 1 × MIC (1500 mg/L), and 2 × MIC (3000 mg/L) was added. The same volume of sterilized distilled water was added to the cell solution to serve as a control group. The potassium ion content in the supernatant (collected via centrifugation at 8000 rpm for 5 min) was measured after 30, 60, and 90 min of incubation at 37 °C following the methods described above [12].

For 260 nm absorbing materials of cells analysis, the procedure for bacteria incubation was same as potassium efflux (CT concentrations of 200, 500, 1000, and 1500 mg/L). The absorbance was determined at 280 nm at intervals of 20 min for 2 h.

For the determination of glucose intake, according to the methods described above [13], a fluorescence probe of 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino)-2-deoxyglucose (2-NBDG) was used as a glucose analog (synthetic). During this procedure, 0.5 mL cell solution was taken and 0.5 mL CT at concentrations of 0.25 × MIC (375 mg/L), 0.5 × MIC (750 mg/L), and 1 × MIC (1500 mg/L) was added, with the same volume of sterilized distilled water added to the control group. Cells were collected after incubation for 0.5, 1, 2, and 4 h. Cells were lysed using 1% Triton X-100. The fluorescence intensity of the cell solution was determined at Ex 466 nm/Em 587 nm using the Synergy 2 detector (BioTek Instruments, Inc., USA).

#### Scanning and transmission electron microscopy analysis

Bacteria were cultured on MRS broth with and without the addition of 750 mg/L CTs for 8 h at 37 °C. The bacterial cells were collected after centrifugation (8000 rpm for 5 min) and washed twice in 0.1 M PBS (pH = 7.0). The producer of fixation and dehydration for bacteria followed previously described methods [14, 15]. For transmission electron microscopy analysis, the samples were embedded in ultramicrotome (EM-UC7, Leica Co., Ltd., Germany) to obtain 70–90 nm thin sections, and then double-strained with uranyl acetate and lead citrate. The cell morphology of bacterial cells was analyzed using a scanning electron microscope (SU8100, Hitachi Co., Ltd., Japan) and transmission electron microscope (HT7800, Hitachi Co., Ltd., Japan.).

#### Intracellular concentration of adenosine triphosphate in *P. pentosaceus SF11*

Bacteria were cultured on MRS broth with and without the addition of CTs (1500 mg/L) for 2, 4, 6, and 8 h at 37 °C. The intracellular ATP content was determined using an ATP assay kit (Elabscience Biotechnology Co., Ltd., Wuhan, China). The luminescence intensity of chemiluminescence by mixed was determined using a Synergy 2 detector (BioTek Instruments, Inc., USA), and the same detector was applied for reactive oxygen species (ROS) analysis (Ex 485 nm/Em 528 nm).

#### Determination of the $H_2O_2$ content generated by CTs

PBS solutions with different pH values (4, 4.2, 4.4, 4.6, 4.8, 5, 5.2, 5.4, 5.8, and 7) were established. Next, 0.5 mL PBS solution with the addition of 0.5 mL CT (concentrations of 1 × MIC, 0.5 × MIC, and 0.25 × MIC) was incubated for 30 min, and the $H_2O_2$ content of each solution was determined.

The concentration of bacteria was adjusted to $10^6$ CFU/mL and CT (1 × MIC) was added. The $H_2O_2$ content in the bacterial solution was determined after incubation for 2, 4, 6, 8, and 24 h. The content of $H_2O_2$ in the solution was obtained according to the methods described above [16].

#### Determined catalase on the inhibited activity of CT against *P. pentosaceus SF11*

According to the methods described above [17], 100 μL CT with different concentrations of 1 × MIC, 0.5 × MIC, and 0.25 × MIC was taken and incubated for 30 min at 37 °C. Then, different concentrations of CAT (10 U/mL and 100 U/mL) were added to each CT solution, followed by incubation for 30 min at 37 °C. Then, the CT solutions were added to 850 μL bacterial solution (the concentration of bacteria was approximately $1 \times 10^6$ CFU/mL) after boiling the CT solutions for 5 min and cooling the CT solutions to room temperature before use, followed by incubation for 8 h at 37 °C. After incubation, 100 μL bacterial solution was taken and added to MRS agar, followed by incubation for 24 h at 37 °C.

#### Reactive oxygen species (ROS) activity of cells under CT exposure

Bacteria were cultured on MRS broth with and without the addition of CT (1500 mg/L), CAT (100 U/mL), and CAT + CT for 40, 80, 120, 160, and 200 min at 37 °C. The fluorescent probe 2,7-dichlorofluorescein diacetate (DCFH-DA) was used following the methods described above [18].

#### RNA isolation and global transcriptome analysis of *P. pentosaceus SF11*

*P. pentosaceus SF11* was cultured following the same procedure used in growth profile studies (the CT concentration was set at 750 mg/L and cells were harvested after 8 h of incubation). The total RNA was extracted from the bacterial solution using TRIzol® Reagent according to the

manufacturer's instructions (Invitrogen, ThermoFisher Scientific Ltd., Shanghai, China), and genomic DNA was removed using DNase I (Takara Co., Ltd., Tokyo, Japan). The RNA quality was determined using the 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) and quantified using the ND-2000 (ThermoFisher Scientific Co., Ltd., Shanghai, China). Only high-quality RNA samples (OD 260/280 = 1.8–2.0, OD 260/230 ≥ 2.0, RIN ≥ 6.5, 28S:18S ≥ 1.0, ≥ 100 ng/μL, ≥ 2 μg) were used to construct the sequencing library. Illumina HiSeq × TEN (Illumina Inc., San Diego, CA, USA) was employed for RNA sequencing (RNA-seq). The raw sequences of bacteria were deposited in the NCBI sequence read archive (SRA) under accession number "PRJNA1014208". The transcriptomic analysis pipeline followed the methods described above [19], except the data were aligned and mapped against the genome sequences of *P. pentosaceus* ATCC 25745 (NCBI assembly, ASM1450v1). Transcripts with > 2 (upregulated) or < 0.5 (downregulated) fold change (FC) in abundance with $P < 0.05$ were regarded as differentially expressed genes. For the discussion of the differences in transcripts among the control and treatment groups, transcripts expressed at $\log_2$ fold change (FC), $\geq 2|\log_2|$ fold change were taken at highly biologically relevant with each sample [20].

### Gene expression evaluation using reverse-transcription real-time quantitative PCR

RNA extraction followed the procedure used for RNA-seq. Complimentary DNA (cDNA) was synthesized using PrimeScript™ RT Master Mix (Takara Ltd., Tokyo, Japan). RT-qPCR was performed on the LightCycler® 96 Real-Time PCR System (Roche Ltd., Basel, Switzerland) using TB Green™ Premix Ex Taq™ II (Takara Ltd., Tokyo, Japan). Ten genes were selected, including one for the normalization of gene expression data (the most highly expressed gene in both groups), with no significant differences between the control and CT treatments), and nine genes that represented a range of RNA-seq results (genes with significant differences in expression between the control and CT treatments) (Additional file 1: Tables S5 and S6). Primers were designed for each gene using the NCBI Primer-BLAST tool. The primers for selected genes are shown in Additional file 1: Table S8. The gene expression levels were presented as $\log_2$-fold changes following the ΔΔCT methodology with normalization to 16S rRNA [19].

### Statistical analysis

The bacterial characteristic data were subjected to a two-way analysis of variance using a two factorial (treatment × time) complete randomized design. Data were analyzed using IBM SPSS 22 Statistics (IBM Corp., Armonk, NY, USA). Significant differences between treatments were determined using Tukey's test at $P < 0.05$.

## Results

### Effects of CTs on *P. pentosaceus SF11* growth

As shown in Fig. 1, there were significant differences between the CT-treated and control groups. The absorbance at 600 nm was higher under CT treatment than in the control group during 6 h of incubation ($P < 0.05$); however, the absorbance at 600 nm under CT treatment was lower than that of the control after 8 h of incubation ($P < 0.05$; Fig. 1a). The pH decreased rapidly in both groups after 4 h of incubation. The CT group had a higher pH level than the control groups from 4 to 24 h of incubation ($P < 0.05$; Fig. 1b). The content of LA was lower in the CT group than in the control group from 4 to 24 h of incubation ($P < 0.05$; Fig. 1c).

As shown in Fig. 2a, the LDH activity was highest in the control after 24 h of incubation ($P < 0.05$), while the

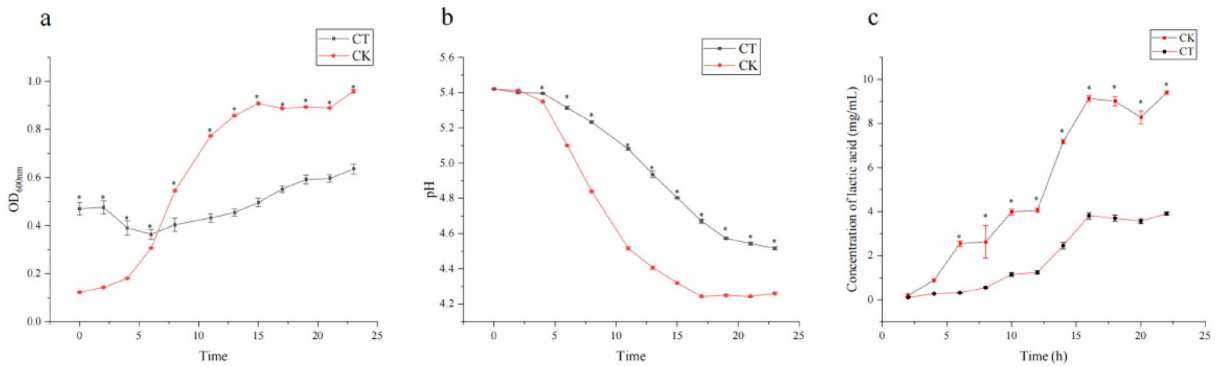



**Fig. 1** Growth of *Pediococcus pentosaceus SF11* with and without the addition of 1500 mg/L condensed tannins (CTs). In the figure, CK represents the control group, while CT represents the addition of CTs at a minimum inhibitory concentration (MIC) of 1500 mg/L. **a** $OD_{600nm}$. **b** pH. **c** Lactic acid production. "*" means $P < 0.05$

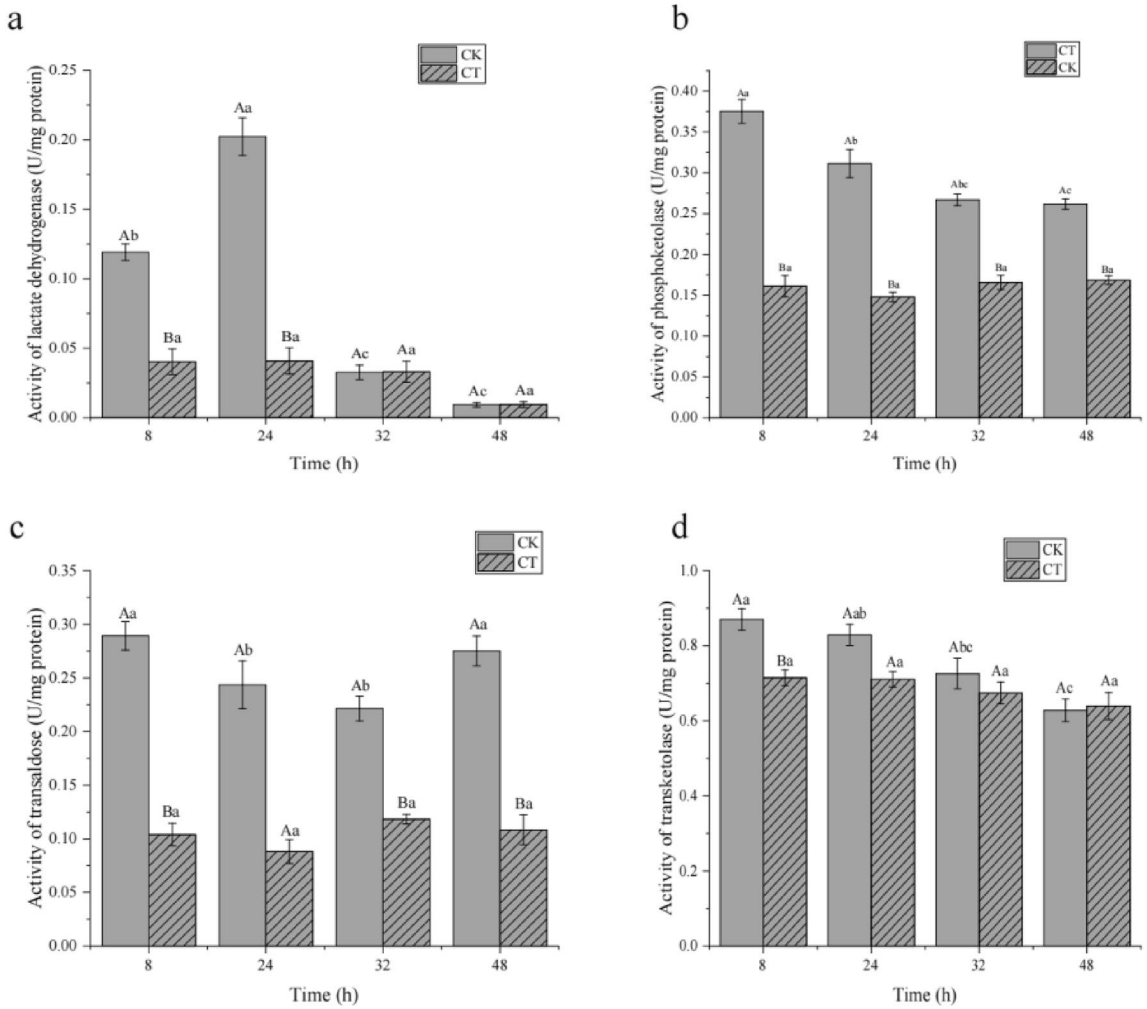



**Fig. 2** Four enzyme activities involved in the xylose metabolism of Pediococcus pentosaceus *SF11* addition with and without the addition of condensed tannins (CTs). CK: control group. CT: the addition of CTs at a minimum inhibitory concentration (MIC) of 1500 mg/L. **a** Lactate dehydrogenase (LDH). **b** Phosphoketolase (PK). **c** Transaldolase (TAL). **d** Transketolase (TKT). Different capital letters on columns represent significant differences with treatment ($P<0.05$), while different lowercase letters on the columns represent significant differences with time ($P<0.05$)

lowest values were found at 32 and 48 h of incubation. CT treatment showed lower activity compared to the control after 8 and 24 h of incubation ($P<0.05$). The PK activity was decreased in both the CT-treated and control groups after 24 h of incubation compared with 8 h incubation ($P<0.05$). The CT-treated group showed lower PK activity during the entire incubation period compared with the control (Fig. 2b). The activity of TAL decreased in the control after 24 and 32 h of incubation ($P<0.05$), and the CT-treated group showed lower TAL activity compared with the control after 8, 32, and 48 h incubation (Fig. 2c). The TKT activity was higher in the control after 8 h and 24 h of incubation ($P<0.05$), but lower after 32 and 48 h of incubation ($P<0.05$). The CT treatment showed lower TKT activity compared with the control after 8 h of incubation ($P<0.05$; Fig. 2d).

### Effect of CTs on the cell membrane of *P. pentosaceus SF11*

As shown in Fig. 3a, the extracellular concentration of $K^+$ showed no differences in all groups between different incubation times ($P>0.05$), but the addition of CTs decreased the concentration of $K^+$ during 90 min of incubation. The lowest concentration appeared in the treatment with 2×MIC CT, followed by 1×MIC and 0.5×MIC ($P<0.05$). There were no differences in all CT-treated groups in the absorbance at 280 nm between different incubation times ($P>0.05$; Fig. 3b). The relative fluorescence intensity of 2-NBDG was higher in the

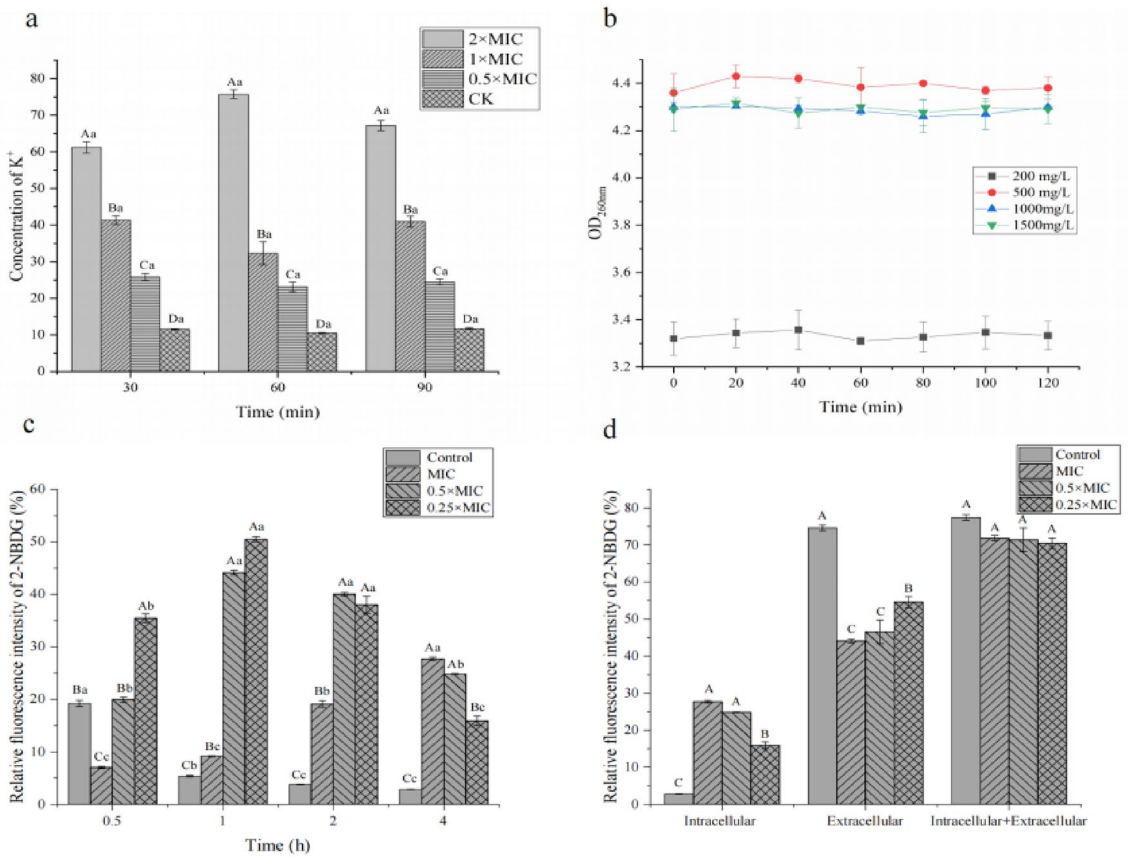



**Fig. 3** Effect of condensed tannins (CTs) on the cell membrane of *Pediococcus pentosaceus SF11*. Minimum inhibitory concentration (MIC): addition of CTs at the MIC concentration of 1500 mg/L. **a** Potassium ion efflux in cells. **b** Molecular absorbance at 260 nm in cells. **c** Changes in the intracellular fluorescence intensity of 2-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino)-2-deoxyglucose (2-NBDG) in cells. **d** Changes in the fluorescence intensity of 2-NBDG after incubation for 4 h. Different uppercase letters on the columns represent significant differences between the treatments ($P<0.05$), and different lowercase letters on the columns represent significant differences over time ($P<0.05$)

0.25×MIC of CT treatment and lower in the 1×MIC of CT treatment compared with the control after 30 min of incubation ($P<0.05$). All CT-treated groups showed higher relative fluorescence intensity of 2-NBDG compared with the control from 1 to 4 h of incubation ($P<0.05$; Fig. 3c). The relative fluorescence intensity of 2-NBDG in the intracellular space was lower in the control than in the CT-treated groups after 4 h of incubation ($P<0.05$), while it was higher in the extracellular space ($P<0.05$; Fig. 3d).

### Effect of CTs on induced oxidative stress in *P. pentosaceus SF11*

As shown in Fig. 4a, the content of $H_2O_2$ was the highest in the three CT-treated groups at pH 4.6 ($P<0.05$). The content of $H_2O_2$ was increased with prolonged incubation time in the CT-treated groups ($P<0.05$); CT treatment showed higher $H_2O_2$ content compared with the control after 24 h of incubation ($P<0.05$; Fig. 4b). The relative fluorescence intensity of 2,7-dichlorofluorescein (DCF) was increased in both the control and treatment groups with prolonged incubation time; CT treatment showed the highest relative fluorescence intensity compared with the control during the entire incubation period ($P<0.05$), followed by the CT + CAT-treated groups ($P<0.05$; Fig. 4c). The content of intracellular ATP was lower in the CT-treated groups compared with the control during 8 h of incubation ($P<0.05$).

### Effect of CT on the transcriptome of *P. pentosaceus SF11*

As shown in Fig. 5a, there were clear differences between the CT-treated and control groups. In total, there were 348 differently expressed genes between the CT-treated and control groups, among which 187 genes were upregulated, while 161 genes were downregulated. Most differently expressed genes were involved in carbohydrate metabolism (27 genes), followed by membrane transport (22 genes) and glycan biosynthesis and metabolism (17 genes).

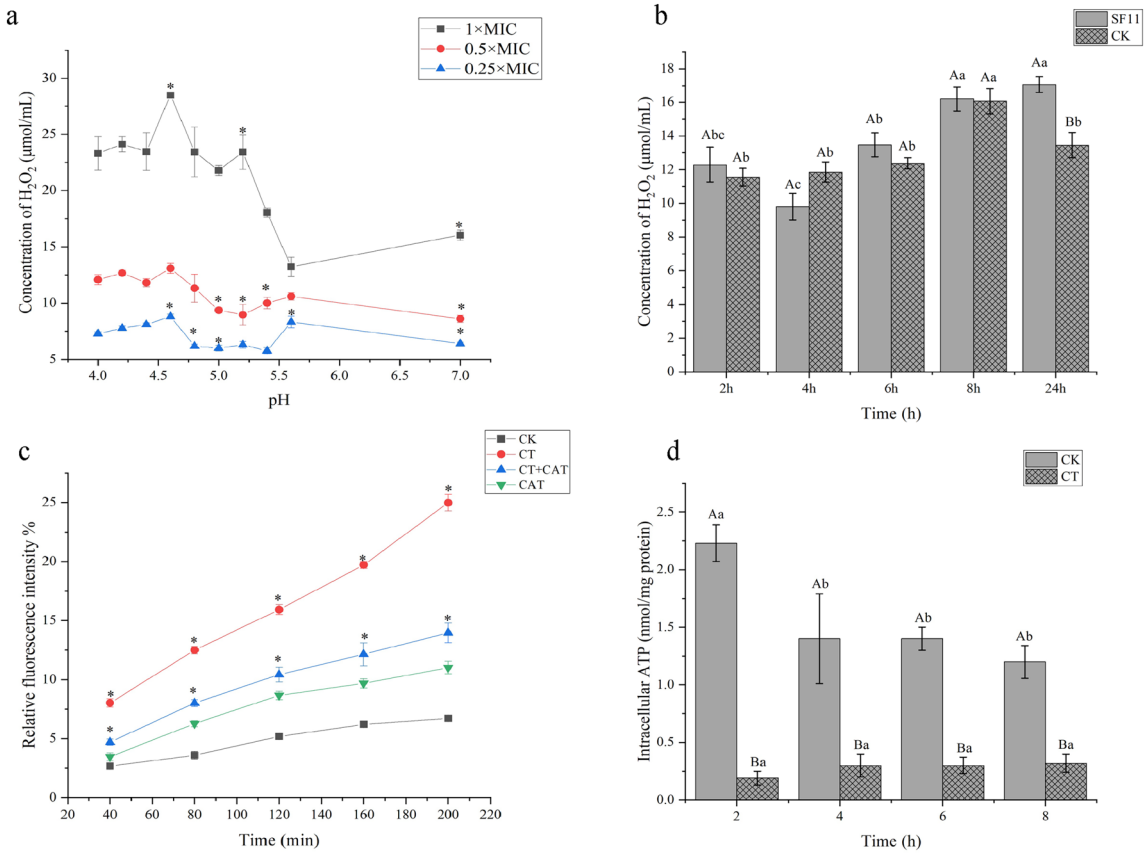



**Fig. 4** Effect of condensed tannins (CTs) on induced oxidative stress in *Pediococcus pentosaceus SF11*. MIC: minimum inhibitory concentration of CT (1500 mg/L); CK: control; CAT: catalase-treated group (100 U/mL). **a** Effect of pH on hydrogen peroxide in the CT solution. **b** Effect of bacteria (De Man–Rogosa–Sharpe (MRS) broth) on hydrogen peroxide in the CT solution. **b** Effect of bacteria (MRS broth) on hydrogen peroxide in the CT solution. **c** Intracellular reactive oxygen species (ROS) level. **d** Intracellular ATP content. "*" means $P < 0.05$ (at the pH level for **a** and the treatment level for **c**). Different uppercase letters indicate significant differences with treatments ($P < 0.05$), and different lowercase letters indicate significant differences with time ($P < 0.05$)

## Discussion

### Effect of CTs on *P. pentosaceus SF11* growth

The sainfoin CTs were mainly composed of EGC and GC, which contributed 65.33% and 25.54%, respectively (Additional file 1: Table S1). The MIC of CTs for *P. pentosaceus SF11* was 1500 mg/L. CT addition disrupted the cell wall of strain SF11, but the cells retained a coccus-likes shape (Additional file 1: Figs. S4 and S5a, b), indicating that the deformation of the cell did not occur during CT treatment [14]. The results showed that CT inhibited the growth of strain SF11, leading to the reduction of LA content and increased pH during incubation. Researchers have found that *P. pentosaceus* can utilize pentoses (D-ribose, L-arabinose, and D-xylose) through the PK pathway [21]. As shown in Additional file 1: Fig. S2, CT inhibited the growth of strain SF11 on xylose-based culture, indicating that the ability of CT to inhibit growth was not affected by different carbon sources. However, the LA and AA content showed no differences between the CT-treated and control groups after 8 h of incubation ($P > 0.05$). Significant differences appeared after 24 h of incubation ($P < 0.05$). Compared to glucose-based culture, a difference in LA production appeared after 6 h of incubation. The results probably correlated with a previous study that reported lower growth for xylose-based culture compared with glucose-based culture [21]. Two pathways are involved in xylose metabolism: the pentose phosphate (PP)/glycolytic pathway, which produces LA only, and the PK pathway, which produces equimolar amounts of LA and AA [11]. The present study showed that the ratio of LA to AA increased up to 1 as the incubation time was prolonged in both the control and CT-treated groups. Considering that two pathways are involved in LA production, but one pathway is involved in AA production, this finding indicated that the activity of the PK pathway exceeded that of the PP pathway during incubation. The PK pathway becomes predominant in xylose metabolism as this pathway yields much

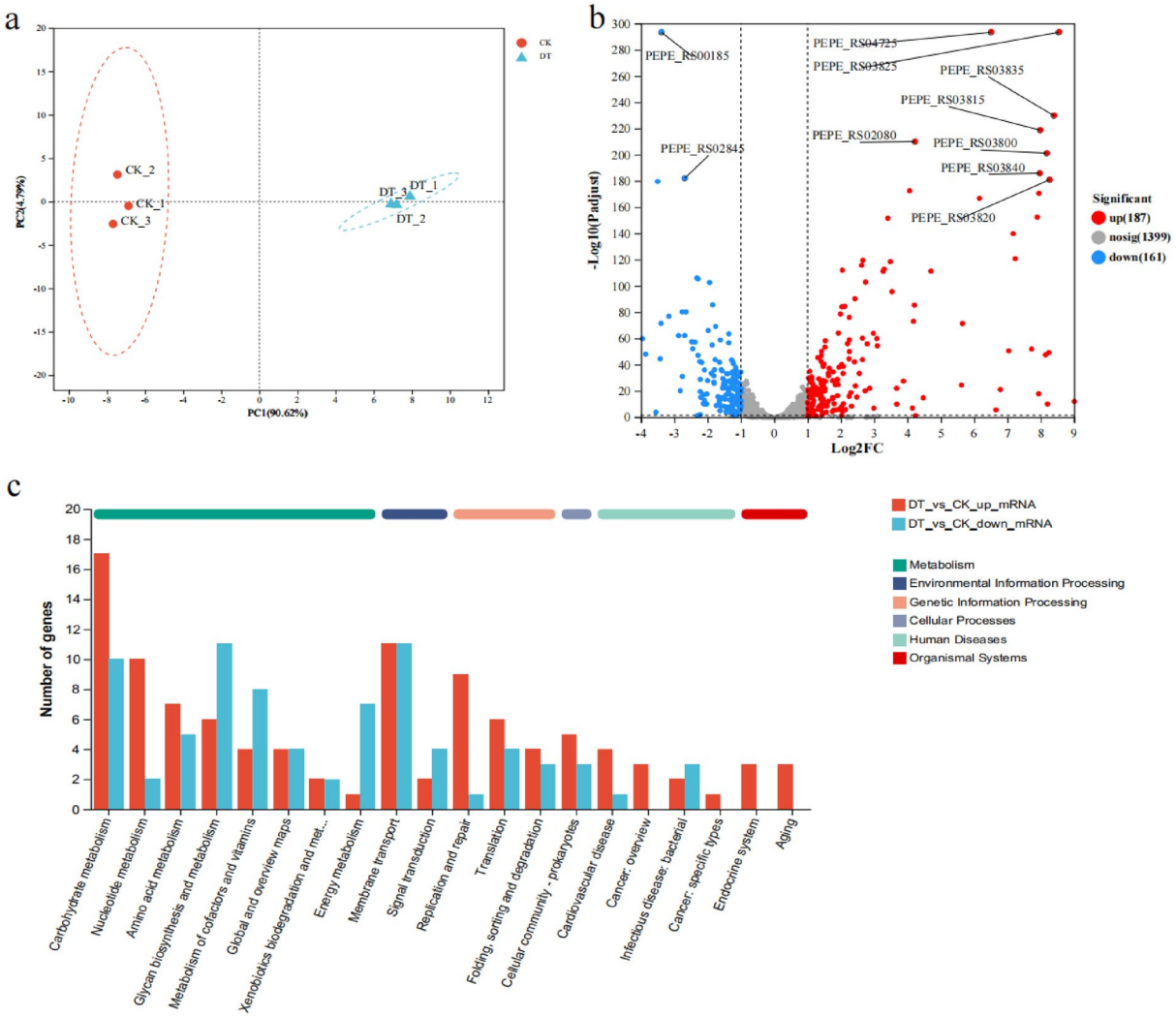



**Fig. 5** Comprehensive RNA sequencing (RNA-seq) analysis of *Pediococcus pentosaceus SF11* with and without the addition of condensed tannins (CTs). **a** Principal component analysis (PCA). **b** Volcano plot representing transcriptional levels. **c** Differently expressed genes between the DT-treated and control groups as annotated to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. CT. CK_1: Biological duplication of the control group. DT_1: Biological duplication of the CT-treated group

more ATP content than the PP pathway [11]. There are four enzymes involved in xylose metabolism: TKT, TAL, and LDH, which are involved in the PP pathway, and PK, which is involved in the PK pathway [11]. In the present study, the CT average decreased the activity of four enzymes, LDH, PK, TAL, and TKL, by 72.97, 45.76, 57.10, and 17.86%, respectively. For TKL activity, because the only significant difference between the CT treatment and the control occurred after 8 h of incubation ($P < 0.05$). As shown in Additional file 1: Fig. S2, the LA:AA ratio was lower under CT treatment compared with the control ($P < 0.05$), which indicated that the speed of AA production was higher under CT treatment than that in the control as a result of the higher activity of the PK pathway. Overall, the contents of LA and AA were both lower in the CT-treated group than in the control. Thus, the results suggested that CT showed the highest ability to decrease the activity of LDH, and this ability was probably more efficient for the PP pathway than the PK pathway.

### Effect of CT on the cell membrane of *P. pentosaceus SF11*

In general, intracellular substances are released into the extracellular space when the bacterial cell membrane is damaged by bacteriostatic substances. Ions such as potassium tend to be released first, followed by large molecular substances such as DNA and RNA, which have the maximum absorbance at 260 nm [22]. Previous

researchers observed that no large molecular substances were released from *Pseudomonas aeruginosa* after incubation with tea polyphenols (TPs), but TPs showed a dose-dependent ability to inhibit this bacterium [14]. As shown in Fig. 3a, b, no large molecules were released during the present study, but the release of small ions (potassium) in the CT-treated groups was increased in a dose-dependent manner by a maximum of five-fold compared with the control. Punicalagin was found to inhibit *Staphylococcus aureus*, resulting in the increased release of potassium by a maximum of two-fold compared with the control; the mechanism was mainly changes in cell membrane permeability or interference with the transmembrane proton gradient due to the interaction between punicalagin and cell membrane [12]. The findings of the present study suggested that CTs influenced the cell membrane permeability, but the cell membrane maintained its integrity (consistent with the findings of transmission electron microscopy, Additional file 1: Fig. S5a, b). In addition, in bactericidal substances such as enterocin CRL3, a low dose (0.5 mg/L) caused the release of small molecules such as potassium ions, while a high dose (8 mg/L) caused holes in the cell membrane and cell wall, and the release of large molecules such as proteins [23]. The present results showed that the release of potassium exhibited no difference between different incubation times in all treatment groups ($P > 0.05$). Thus, the results indicated that CTs exerted more bacteriostatic activity than bactericidal activity.

The phosphoenolpyruvate: glycose phosphotransferase system (PTS) in the cell membrane is essential for the uptake of glucose by microbes [24]. EGCG decreased the protein units of PTS on the cell membrane of *Bacillus subtilis*, resulting in reduced glucose uptake [25]. In the present study, the CT concentration at the MIC level inhibited 2-NBDG uptake but increased uptake at the 0.25 × MIC level after 30 min of incubation. However, CT application increased 2-NBDG uptake at all concentration levels during 1–4 h of incubation. The results suggest that the ability of CT to inhibit glucose uptake by the cell may be reversible; although higher concentrations of CT at or exceeding the MIC level can inhibited the glucose uptake of cells in the short term, CT may increase glucose uptake over the long term.

### Effect of CTs on induced oxidative stress in *P. pentosaceus* SF11

Hydrogen peroxide is a small molecular substance that carries no charge under physiological pH conditions (p*K*a = 11.6) and can pass through the cell membrane, leading to cellular oxidative stress when the extracellular hydrogen peroxide concentration is > 0.2 μmol/L [26]. The pyrogallol group on CTs (B ring) is essential for the production of hydrogen peroxide. EGCG can generate the most hydrogen peroxide content, followed by EGC [27]. In previous work, the content of hydrogen peroxide was stabilized when the pH ranged from 2 to 6 but increased exponentially when the pH ranged from 6 to 8 [17]. The present study showed that the content of hydrogen peroxide produced by CT tended to increase first and then decrease at pH ranging from 4.0 to 7.0, and exhibited dose dependence at the same pH level. The maximum content of hydrogen peroxide produced by CT was observed when the pH was 4.6. Notably, the maximum concentration of hydrogen peroxide (28.50 μmol/L) was produced by CTs at 1500 mg/L, which was lower than the concentration produced by EGCG under the same conditions (over 100 μmol/L) [17]. In addition, the concentration of hydrogen peroxide in the CT-treated group was higher than that in the control after 24 h of incubation ($P < 0.05$), indicating that *P. pentosaceus* SF11 promoted the production of hydrogen peroxide by CT.

The bactericidal activity of EGCG disappears after the addition of CAT to culture, indicating that the main factor underlying its bactericidal activity is hydrogen peroxide [17]. The present study showed that the sterilizing rate of CTs against *P. pentosaceus* SF11 was 8.73% at the MIC level, and the sterilizing rate decreased with CAT addition (Additional file 1: Table S2). The reduction of the sterilizing rate under CAT addition (100 U/mL) was 46.95% (maximum). Compared with EGCG, the reduction of the 98% (maximum) sterilizing rate of EGCG was attributed to CAT, and the results suggested that hydrogen peroxide was partly correlated with the antibacterial activity of CTs.

The ROS level induced by CTs was 3.72-fold compared to the control, but this level was reduced to two-fold by CAT addition after 200 min of incubation. EGCG enhanced intracellular ROS activity, as the bactericidal activity of EGCG was not attributed to hydrogen peroxide, which made EGCG play an endogenous oxidative stress role for *Escherichia coli* OP50 [15]. The addition of CAT to CTs condition a reduction of 46.23% in ROS level (induced by CT). This data were consistent with the reduction of the sterilizing rate of CT attributed to CAT addition and indicated that hydrogen peroxide was probably the main factor for the induction of ROS in *P. pentosaceus* SF11 [27]. Therefore, sainfoin CTs play an exogenous oxidative stress role attributed to the production of hydrogen peroxide, partly resulting in antibacterial activity.

### Effect of CTs on the global transcriptome of *P. pentosaceus SF11*

RNA-seq analysis (Additional file 1: Table S3) yielded a total of 24,455,531 bp and 24,228,616 bp of transcripts

(mRNA) sequences isolated from the control and CT-treated groups, respectively; the genome mapped ratio (compared with *P. pentosaceus* ATCC 25745) were 96.19% and 81.74%, respectively. A total of 348 genes (Additional file 2: Table S9) showed significant differences between the control and CT-treated groups ($P < 0.05$); among them, 187 genes were upregulated ($\log_2$ FC > 2.00), while 161 genes were downregulated ($\log_2$ FC < −1.00).

Most of the top 30 upregulated genes (based on FC value, Additional file 1: Table S5) were involved in hypothetical protein (11), thus observably playing an important role in *P. pentosaceus* SF11 under CT stress. As shown in Additional file 1: Table S7, based on the Blast database, the PEPE_RS03875 and PEPE_RS03820 genes encoding hypothetical protein showed 88.98–94.09% and 88.36–99.66% identity with other strains of *P. pentosaceus* or *P. acidilactici*, which encode amino acid description as phage tail protein and phage capsid protein, respectively. In addition, five genes (PEPE_RS03800, PEPE_RS03890, PEPE_RS03840, PEPE_RS03795, and PEPE_RS03880) involved in encode with phage protein were among the top 30 most upregulated genes. Various CTs such as C and commercial tannins at concentrations of 3–500 mg/L showed the capacity to inhibit bacteriophage PL-1 from forming plaques on *Lactobacillus casei* strain S [28]. A higher number of upregulated phage genes is probably correlated with a stress response upon oxygen exposure [29]. Thus, in the present study, the upregulation of gene expression correlated with phage protein was probably due to CT stress. The PEPE_RS04980 gene showed 96.77 and 82.26% identity with other strains of *P. pentosaceus* and *P. acidilactici*, respectively, and encodes an amino acid described as an XRE family transcriptional regulator. The *XtrSs* encode the XRE family transcriptional regulator involved in the fitness of *Streptococcus suis* under hydrogen peroxide stress [30]. As the present study showed that *P. pentosaceus SF11* increased the capacity of CT to produce hydrogen peroxide, this indicated that PEPE_RS04980 was mainly upregulated in response to hydrogen peroxide stress generated by CTs. The PEPE_RS03740 gene showed 91.56–92.21% identity with another strain of *P. pentosaceus* and encodes an amino acid described as a helix-turn-helix transcriptional regulator. The PEPE_RS06010 gene showed 95.41–100% identity with another strain of *P. pentosaceus* and encodes an amino acid described as acetyl-CoA carboxylase (ACC). The second most abundant differentially expressed gene was PEPE_RS 03825, which has been described as an Ig-like domain-containing protein. Furthermore, PEPE_RS07925 (*arc*C) expression as carbamate kinase was involved in the third enzymatic step of the arginine dihydrolase pathway for ATP formation [31]. The expression of *arc*C was upregulated due to ethanol treatment, but the consumption of arginine was decreased, which indicated that the upregulation of *arc*C primarily occurred in response to ethanol stress [32]. The present study showed that the content of intracellular ATP decreased and the *arc*C gene was upregulated after treatment with CTs, which indicated that this difference in gene expression primarily occurred in response to CT stress. The PEPE_RS05335 gene, which is related to the diacylglycerol kinase family of lipid kinases, was upregulated. Diacylglycerol kinase (DGK, EC 2.7.1.107) can phosphorylate diacylglycerol (DAG) to generate phosphatidic acid (PA) [33]. Three PAs were upregulated due to CT activity after 60 days of sainfoin ensiling [34]. The results suggest that CTs probably facilitate the generation of PA through the upregulated expression of DGK genes.

Among the 30 top downregulated genes (based on the FC value, Additional file 1: Table S6), *pst*B was the higher FC value of downregulated gene, followed by *pho*U and PEPE_RS08470, which are described as a phosphate ATP-binding cassette (ABC) transporter, a phosphate signaling complex protein, and an ABC transporter, respectively. Of the six genes involved in ABC transport, three (*pst*A, *pst*B, and *pst*C) are described as phosphate ABC transporters. Phosphate-specific ABC transporter (Pst) is involved in phosphate transport in bacteria, and the overexpression of this gene results in the strong uptake of phosphate by bacteria [35]. The results of the present study suggested that CTs showed a greater ability to inhibit the expression of genes involved in the Pst system. In addition, the Pst system is involved in conferring drug resistance to bacteria. For example, the overexpression of *pst*B genes involved in the Pst system results in a high level of ciprofloxacin resistance in bacteria [35]. Thus, CTs may have the ability to reduce the drug resistance of *P. pentosaceus SF11*.

KEGG analysis showed that most of these differently expressed genes between the control and CT treatment were involved in carbohydrate metabolism, among which 17 genes were upregulated (Fig. 5c). Six genes out of these 17 genes encoded PTS transporter protein, with $\log_2$ FC values ranging from 1.09 to 3.09. The gene with the highest fold change ($\log_2$FC = 3.09) encoded fructose-specific PTS transporter subunit EIIC (Additional file 2: Table S9). CTs increased the intracellular 2-NDBG content when the incubation time was prolonged from the starting period of 1 h of incubation with a CT concentration of 1500 mg/L. This increased effect of CTs on sugar uptake by cells was probably associated with the upregulation of genes related to the PTS protein system. In addition, PEPE_RS04675 (*pgm*B) and PEPE_RS04680 showed highest FC values ($\log_2$FC = 4.14 and 3.67, respectively) among these 17 genes, which encoded beta-phosphoglucomutase (PGM) and glycoside hydrolase family 65

protein, respectively. PGM involved in the transition between β-glucose 1-phosphate and glucose 6-phosphate (G6P), and the G6P involved in glycolysis pathway [36]. Based on the results that content of intracellular ATP in SF11 were decreased by CT. Up-regulated expression of these genes involved in PTS system and glycolysis pathway were mainly respond for energy decreased in cell. Altogether, the results showed that CTs could better inhibit the PP pathway than the PK pathway.

Only four differently expressed genes were oxidoreductases, among which three upregulated genes (PEPE_RS05050, PEPE_RS08595, and PEPE_RS04445, with values of $\log_2$FC = 2.47, 1.75, and 1.14, respectively) were described as an NAD(P)H-dependent oxidoreductase, a short-chain dehydrogenases/reductases (SDR) family oxidoreductase, and an LLM class flavin-dependent oxidoreductase, respectively; while the single downregulated gene (PEPE_RS00170) was an FAD-dependent oxidoreductase. The SDR family is composed of NAD(P)(H)-dependent oxidoreductases, which showed similar mechanisms and share sequence motifs [37]. In a previous work, CTs obtained from tea induced oxidative stress in *E. coli*. SoxRS (*sod*A, *sox*R, and *sox*S) and OxyR genes (*oxy*R, *ahp*C, *oxy*S, *dps*, *gor*, *kat*G, and *ahp*F), which are induced by superoxide radicals and peroxide stress, respectively, are involved in oxidative stress. As a result, CT application induced the upregulation of *sod*A, *sox*R, *oxy*R, *oxy*S, and *kat*G [15]. However, these genes were not detected in *P. pentosaceus SF11.* The results probably suggest that the CT-induced oxidative stress of strain SF11 not be the mainly reason for its antibacterial activity.

Sixteen differently expressed genes between the control and CT-treated groups were involved in the lipid metabolism pathway, among which 15 genes were downregulated and one gene was upregulated (Additional file 1: Table S9). Among the 15 downregulated genes, *fab*G, which encodes β-ketoacyl-ACP reductase (FabG), is widely distributed in bacteria, and FabG is a key rate-limiting enzyme for fatty acid synthesis. The structure and active center of this enzyme show high similarity among different bacterial species [38]. The inhibition of FabG activity results in a synthesis system blocked of bacteria such as quorum sensing, cell membrane, and cell wall [26]. The present results (Additional file 1: Fig. S3) showed that CTs decreased the activity of FabG by 65.95% compared with the control after 2 h of incubation and decreased the activity of FabG by 37.55% after 6 h, but this effect disappeared with prolonged incubation time. Thus, the results probably suggest that the capacity of CT to inhibit FabG activity was associated with downregulated relative gene expression. One upregulated gene was *dha*M, code by PEPE_RS08075 ($\log_2$FC = 1.13). DhaM was phosphoprotein subunits of dihydroxyacetone kinase (DhaK) as a phosphoryl donor, involved in dihydroxyacetone (DHA) production [39]. Consider of intracellular ATP in *P. pentosaceus SF11* were decreased by CT, the gene of *dha*M was mainly respond for insufficient energy due to CT impact.

## Conclusion

In this study, CTs showed bacteriostatic activity (MIC = 1500 mg/L) against *P. pentosaceus SF11*. This effect was attributed to the fact that CTs bound with enzymes involved in carbohydrate metabolism, such as LDH, PK, and TAL, resulting in decreased enzyme activity, and was partly associated with the oxidative stress induced by the hydrogen peroxide produced by CTs. At the global transcriptome level, CTs upregulated genes involved in the hypothetical protein, phage protein system, and PTS system of *P. pentosaceus SF11*, indicating that bacteria responded to CT stress, such as through the inhibition of enzyme activity and increased cell membrane permeability; most of the downregulated genes were involved in ABC transport, especially for phosphate, which probably decreased the ability of bacteria to resist antibiotic stress. Only four differently expressed genes were involved in the expression of oxidoreductase, indicating that CT-induced oxidative stress was probably not the dominant reason for its bacteriostatic activity. Overall, CTs obtained from sainfoin inhibited *P. pentosaceus SF11* growth primarily via decreased enzyme activity and the expression changes of certain genes, partly due to oxidative stress induced by hydrogen peroxide.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-023-00527-z.

**Additional file 1.** Characteristics of *Pediococcus pentosaceus* under condensed tannins stress.

**Additional file 2.** Genes expression of *Pediococcus pentosaceus* under condensed tannins stress.

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**Author contributions**
RH: Conceptualization, Writing-original draft, Data curation, Investigation, Methodology. CM: Supervision, Project administration, Funding acquisition. MM: Review and Editing, Supervision. FZ: Review and Editing. XW: Formal analysis, Software.

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**Availability of data and materials**
The data supporting the bacterial RNA-seq of the Sequence Read Archive (SRA) can be found at the National Center for Biotechnology Information (NCBI) under SRA number "PRJNA1014208" (accessed on September 09, 2023).

The sequences of the strain used for this study were uploaded to the NCBI under number "ON866519" (accessed on July 3, 2022). The data used in the study analyses can be made available by the corresponding author upon reasonable request.

## Declarations

### Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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### References

1. Zeller WE. Activity, purification, and analysis of condensed tannins: current state of affairs and future endeavors. Crop Sci. 2019;59:886–904.
2. Reygert WC. Green tea catechins: their use in treating and preventing infectious diseases. Biomed Res Int. 2018;2018:9105261.
3. Ikigai H, Nakae T, Hara Y, Shimamura T. Bactericidal catechins damage the lipid bilayer. Biochem Biophys Acta. 1993;1147:132–6.
4. Huang RZ, Zhang FF, Wang T, Zhang YL, Li X, Chen YC, et al. Effect of intrinsic tannins on the fermentation quality and associated with the bacterial and fungal community of sainfoin silage. Microorganisms. 2022;10:844.
5. Wang MS, Chen MY, Bai J, Zhang JY, Su R, Franco M, et al. Ensiling characteristics, in vitro rumen fermentation profile, methane emission and archaeal and protozoal community of silage prepared with alfalfa, sainfoin and their mixture. Anim Feed Sci Technol. 2022;284:115154.
6. Jiang SM, Cai LZ, Lv LX, Li LJ. Pediococcus pentosaceus, a future additive or probiotic candidate. Microb Cell Fact. 2021;20:45.
7. Adhikari TB, Joseph CM, Yang GP, Phillips DA, Nelson LM. Evaluation of bacteria isolated from rice for plant growth promotion and biological control of seedling disease of rice. Can J Microbiol. 2001;47:916–24.
8. Terrill TH, Windham WR, Evans JJ, Hoveland CS. Condensed tannin concentration in sericea lespedeza as influenced by preservation method. Crop Sci. 1990;30:219–24.
9. Gea A, Elisabetta S, Ron HB, Irene M-H. In situ analysis and structural elucidation of sainfoin (*Onobrychis viciifolia*) tannins for high-throughput germplasm screening. J Agric Food Chem. 2011;59:495–503.
10. Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc. 2008;3:163–75.
11. Tanaka K, Komiyama A, Sonomoto K, Ishizaki A, Hall SJ, Stanbury R. Two different pathways for D-xylose metabolism and the effect of xylose concentration on the yield coefficient of L-lactate in mixed-acid fermentation by the lactic acid bacterium *Lactococcus lactis* IO-1. Appl Microbiol Biotechnol. 2002;60:160–7.
12. Xu YF, Shi C, Wu Q, Zheng ZW, Liu PF, Li GH, et al. Antimicrobial activity of punicalagin against staphylococcus aureus and its effect on biofilm formation. Foodborne Pathog Dis. 2017;14:282–7.
13. Lee E, Jung SR, Lee SY, Lee NK, Paik HD, Lim SI. Lactobacillus plantarum strain Ln4 attenuates diet-induced obesity, insulin resistance, and changes in hepatic mRNA levels associated with glucose and lipid metabolism. Nutrients. 2018;10:643.
14. Yi SM, Zhu JL, Fu LL, Li JR. Tea polyphenols inhibit *Pseudomonas aeruginosa* through damage to the cell membrane. Int J Food Microbiol. 2010;144:111–7.
15. Xiong L-G, Chen Y-J, Tong J-W, Huan J-A, Li J, Gong Y-S, et al. Tea polyphenol epigallocatechin gallate inhibits *Escherichia coli* by increasing endogenous oxidative stress. Food Chem. 2017;217:196–204.
16. Eisenberg G. Colorimetric determination of hydrogen peroxide. Ind Eng Chem. 1943;15:327–8.
17. Arakawa H, Maeda M, Okubo, Shimamura T. Role of hydrogen peroxide in bactericidal action of catechin. Biol Pharmaceut Bull. 2004;27:227–81.
18. Fathima A, Rao JR. Selective toxicity of Catechin—a natural flavonoid towards bacteria. Appl Microbiol Biotechnol. 2016;100:6395–402.
19. Han D, Shi R, Yan QJ, Shi YQ, Ma JW, Jiang ZQ. Global transcriptomic analysis of functional oligosaccharide metabolism in *Pediococcus pentosaceus*. Appl Environ Microbiol. 2021;105:1601–14.
20. Bergsveinson J, Friesen V, Ziola B. Transcriptome analysis of beer-spoiling *Lactobacillus brevis* BSO 464 during growth in degassed and gassed beer. Int J Food Microbiol. 2016;235:28–35.
21. Dobrogosz WJ, DeMoss RD. Pentose utilization by *Pediococcus pentosaceus*. J Bacteriol. 1963;85:1356–64.
22. Chen CZS, Cooper SL. Interactions between dendrimer biocides and bacterial membranes. Biomaterials. 2002;23:3359–68.
23. Minahk CJ, Farias ME, Sesma F, Morero RD. Effect of enterocin CRL35 on Listeria monocytogenes cell membrane. FEMS Microbiol Lett. 2000;192:79–83.
24. Meadow ND, Fox DK, Roseman S. The bacterial phosphoenolpyruvate: glycose phosphotransferase system. Annu Rev Biochem. 1990;50:497–542.
25. Nakayama M, Shimatani K, Ozawa T, Shigemune N, Tomiyama D, Yui K, et al. Mechanism for the antibacterial action of epigallocatechin gallate (EGCg) on *Bacillus subtilis*. Biosci Biotechnol Biochem. 2015;79:845–54.
26. Renzetti A, Betts JW, Fukumoto K, Rutherford RN. Antibacterial green tea catechins from a molecular perspective: mechanisms of action and structure–activity relationships. Food Funct. 2020;11:9370–96.
27. Nakayama T, Ichiba M, Kumazawa S. Mechanisms and structural specificity of hydrogen peroxide formation during oxidation of catechins. Food Sci Technol Res. 2002;8:261–7.
28. Lee A, Eschenbruch R, Waller J. Effect of phenolic compounds, ethyl alcohol, and sodium metabisulphite on the lytic activity of phage PL-1 on a *Lactobacillus casei* S strain. Can J Microbiol. 1985;31:873–5.
29. Eikmeyer FG, Heinl S, Marx H, Puhler A, Grabherr R, Schluter A. Identification of oxygen-responsive transcripts in the silage inoculant *Lactobacillus buchneri* CD034 by RNA sequencing. PLoS ONE. 2015;10:1.
30. Zhang YM, Lliang S, Pan ZH, Yu Y, Yao HC, Liu YJ, et al. XRE family transcriptional regulator XtrSs modulates *Streptococcus suis* fitness under hydrogen peroxide stress. Arch Microbiol. 2022;204:244.
31. Dimopoulos M, Bagnara AS, Edwards MR. Characterisation and sequence analysis of a carbamate kinase gene from the diplomonad *Hexamita inflata*. J Eukaryot Microbiol. 2000;47:499–503.
32. Araque I, Bordons A, Reguant C. Effect of ethanol and low pH on citrulline and ornithine excretion and arc gene expression by strains of Lactobacillus brevis and *Pediococcus pentosaceus*. Food Microbiol. 2013;33:107–13.
33. Luo B, Regier DS, Prescott SM, Topham MK. Diacylglycerol kinases. Cell Signal. 2004;16:983–9.
34. Huang RZ, Wang XZ, Ma CH, Zhang FF. Effects of intrinsic tannins on proteolysis dynamics, protease activity, and metabolome during sainfoin ensiling. Front Microbiol. 2022;13:976118.
35. Chakraborti PK, Bhatt K, Banerjee SK, Misra P. Role of an ABC importer in mycobacterial drug resistance. Biosci Rep. 1999;19:293–300.
36. Andersson U, Radstrom P. β-Glucose 1-phosphate-interconverting enzymes in maltose- and trehalose-fermenting lactic acid bacteria. Environ Microbiol. 2002;4:81–8.
37. Kavanagh L, Jornvall H, Persson B, Oppermann U. The SDR superfamily: functional and structural diversity within a family of metabolic and regulatory enzymes. Cell Mol Life Sci. 2008;65:3895–960.
38. Oppermann U, Filling C, Hult M, Shafqat N, Wu XQ, Lindh M, et al. Short-chain dehydrogenases/reductases (SDR): the 2002 update. Chem Bilog Interact. 2003;143:247–53.
39. Gutknecht R, Beutler R, Garcia-Alles LF, Baumann U, Erni B. The dihydroxyacetone kinase of *Escherichia coli* utilizes a phosphoprotein instead of ATP as phosphoryl donor. EMBO J. 2001;20:2480–6.

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