



Cinnamaldehyde inhibit *Escherichia coli* associated with membrane disruption and oxidative damage

Tian-Fu He^{1,2} · Lang-Hong Wang^{1,2} · De-bao Niu^{1,2} · Qing-hui Wen^{1,2} · Xin-An Zeng^{1,2}

Received: 4 May 2018 / Revised: 29 July 2018 / Accepted: 9 September 2018 / Published online: 6 October 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

In this study, the antimicrobial mechanism of cinnamaldehyde (CIN) against Gram-negative *Escherichia coli* ATCC 25922 (*E. coli*) based on membrane and gene regulation was investigated. Treatment with low concentration (0, 1/8, 1/4, 3/8 MIC) of CIN can effectively suppress the growth of *E. coli* by prolonging its lag phase and Raman spectroscopy showed obvious distinction of the *E. coli* after being treated with these concentration of CIN. The determination of relative conductivity indicated that CIN at relatively high concentration (0, 1, 2, 4 MIC) can increase the cell membrane permeability, causing the leakage of cellular content. Besides, the content of malondialdehyde (MDA) and the activity of total superoxide dismutase (SOD) of *E. coli* increased with increasing treatment concentration of CIN, implying that CIN can cause oxidative damage on *E. coli* cell membrane and induce the increase of total SOD activity to resist this oxidative harm. Moreover, quantitative real-time RT-PCR (qRT-PCR) analysis revealed the relationship between expression of antioxidant genes (SODa, SODb, SODc) and treatment CIN concentration, suggesting that SOD, especially SODc, played a significant role in resistance of *E. coli* to CIN. The underlying inactivation processing of CIN on *E. coli* was explored to support CIN as a potential and natural antimicrobial agent in food industry.

Keywords Cinnamaldehyde · *Escherichia coli* · Membrane permeability · Oxidation · Gene regulation

Introduction

Escherichia coli is deemed as a kind of significant food-borne pathogens, which has caused serious disease outbreaks in recent years. Food may be contaminated with this microorganism by being tainted with animal waste in water or soil (Baskaran et al. 2010; Hong et al. 2016), so people try to find suitable disposing methods to solve this problem. However, traditional strategies such as sterilization of ultraviolet, thermal processing, and chemical technique have marked disadvantages, which may induce negative impacts on nutrition and even health problems (Hong

et al. 2016; Tian et al. 2012). Thus, natural and effective sterilization methods have received great attention with the aim to keep nutrition and control food-related undesirable microorganisms.

Essential oils were traditionally used to enhance flavor and preserve human foods as a group of natural food additives, which catered to people's pursuit of green and natural food (Boubaker et al. 2016; Raut and Karuppayil 2014). Importantly, essential oils are considered safe and approved to be used in foods, so they have gradually become a hot area of food preservation in recent years (Pérez-Alfonso et al. 2012; Zhou et al. 2014). Cinnamaldehyde (CIN), presenting as a major component of extraction of cinnamon bark (*Cinnamomum verum*), was reported that it was one of the most effective essential oils against undesirable food microorganisms (Wang et al. 2016a; Yin et al. 2015b) and can be a kind of feed additives to control microbial contamination (Yin et al. 2015a). Membrane fluidity is a basic condition to maintain normal cell activity (Tourdot-Maréchal et al. 2000; Wei et al. 2018). Shen et al. revealed that CIN can impact the morphology of cell, causing membrane disruption and leakage of cell

Communicated by Jorge Membrillo-Hernández.

✉ Xin-An Zeng
xazeng@scut.edu.cn

¹ School of Food Sciences and Engineering, South China University of Technology, Guangzhou 510641, China

² Overseas Expertise Introduction Center for Discipline Innovation of Food Nutrition and Human Health (111 Center), Guangzhou, China

content (Shen et al. 2015). Besides, our previous research has verified that *Escherichia coli* changed its fluidity to resist the harmful effect of CIN by decreasing the proportion of unsaturated fatty acids (He et al. 2017). Enzymes are essential components to maintain vital activities of microorganisms, and it was reported that CIN can drastically decrease the enzyme activities (Lee et al. 2002). Although the strong antimicrobial activity of CIN has been confirmed, its specific behavior of sterilization and the response of microbes to it are still lacking. Therefore, additional researches should be carried out for its wider and better application in future.

In this work, the disruption and oxidation of CIN on cytoplasmic membrane of *Escherichia coli* (*E. coli*) were explored. Moreover, to detect the resistance of *E. coli* to the oxidative damage, the expressions of antioxidant genes (SODa, SODb, SODc) were determined to identify the roles they played. In short, the antimicrobial mechanism of CIN on *E. coli* was detected more deeply to benefit that CIN can be commonly and safely used in food industry and agriculture.

Materials and methods

Materials

CIN (purity, 99.0%) was supplied by Aladdin Chemistry Co, Ltd. (Shanghai, China). It was dissolved in ethanol (purity, 99.7%) to achieve its stock solution (5.0 g/L) and then stored at 4 °C. The microbial strains, Gram-negative *E. coli* ATCC 25922 was obtained from Microbial Culture Collection Center of Guangdong Institute of Microbiology (Guangzhou, China).

Culture conditions

A plate containing tryptic soy broth with 0.6% (w/v) of yeast extract (TSA-YE) was inoculated a loopful of strains onto and then incubated on an orbital shaker (120 rpm, HY-5, JinBo Equipment Industry Co., Jiangsu, China) at 37 °C for 18–24 h. A loopful of colony from the precultured plate was transferred to 200 mL fresh sterile TSA-YE liquid medium and cultured at 37 °C for 12 h with shaking at 120 rpm. 3 mL of this broth was added into sterile test tubes added with 1 mL, 25% glycerol, and stored at -80 °C. The suspension in test tubes was then transferred to 100 mL fresh sterile TSA-YE liquid mediums ($OD_{600} \approx 0.09$), and cultured with CIN at different concentrations [0, 0.29 mM (1/8 MIC), 0.59 mM (1/4 MIC) and 0.88 mM (3/8 MIC)] for the following antimicrobial tests.

Growth curves of *E. coli* with CIN treatment

The minimum inhibitory concentration (MIC) of CIN toward *E. coli* was indicated to be 0.31 g/L (2.35 mM) (Shen et al. 2015). Our research about the effect of CIN on growth curves of *E. coli* was consistent with a previous method (Zeng et al. 2012). The cultures in test tubes were transferred into 100 mL fresh sterile TSA-YE liquid mediums with equal volumes of ethanol (control) and CIN at different concentrations (1/8 MIC, 1/4 MIC, 3/8 MIC). These mediums were incubated on a rotary shaker at 37 °C, and measured every half hour for 4.5 h.

Raman spectroscopy analysis

The *E. coli* cell suspension was obtained according to the above conditions and treated with a series of concentrations of CIN (0, 1/8, 1/4, 3/8 MIC) as samples to prepare for the Raman measurement. In this work, a LabRAM HR Evolution spectrometer system (Horiba, Japan) using a 633-nm near-infrared diode laser source and equipped with a Leica DMLB microscope (Wetzlar, Germany) was used to detect the Raman scattering signals from these samples with range from 600 to 1800 cm^{-1} in extended mode. All samples were exposed 10 s and c. 10 mW Laser power according to a previous research (Liu et al. 2009). Then, the Raman data were obtained using the Delight version 3.2.1 (D-Squared Development Inc., LaGrande, OR, USA) software. Instrumental noises and polynomial subtract removes baseline offsets were eliminated smoothly in accordance with a previous study (He et al. 2008).

Principal component analysis (PCA) is a mathematical algorithm to analyze the connection among different data, indicating their similarities or differences. This technique focuses on the more significant factors, removing influence of random varieties (Goodacre et al. 1998). In this work, PCA was employed to suggest changes of cell compositions after treatment with different concentrations of CIN to exhibit their potential connection.

Relative conductivity determination

This experiment was based on a previous method using a conductivity meter (DDS-11A, Shanghai Leici Instrument Inc., Shanghai, China) to measure the electrolyte leakage (Wang et al. 2017). Briefly, the cells were first cultivated at the stationary phase. After these cell suspensions were centrifuged at $4000 \times g$ for 5 min, and washed twice with 5.0% glucose, the suspension was diluted and separated equally into four flasks. Then, CIN was added to the bacteria suspension to achieve different treatment concentrations

(0, 1/8, 1/4, 3/8, 1/2, 1, 2 and 4 MIC). A 5 mL sample from each flask was used for the measurement at 0, 0.5, 1, 1.5, 2, 2.5 and 6.5 h. The change of permeability of *E. coli* cell membrane influenced by different concentration of CIN was according to the measurement of relative conductivity.

$$\text{The relative electric conductivity (\%)} = 100\% \times \frac{E_2 - E_1}{E_0}, \quad (1)$$

where E_1 and E_2 represent the conductivity of the initial suspension and the suspension treated with different concentration of CIN for different treatment time, respectively. Additionally, E_0 is the conductivity of the sample in 5.0% glucose finally treated in boiling water for 10 min.

Determination of oxidation indexes

The content of malondialdehyde (MDA) and the activity of superoxide dismutase (SOD) are significant indexes to suggest the harm caused by the oxidative damage and the resistance of microorganisms to the oxidant harm from external environment (Yun et al. 2016a). The stationary-phase *E. coli* bacterial suspension was cultured with different concentrations of CIN (0, 1/2, 3/4, 1 MIC) and detected according to the instruction of Malondialdehyde (MDA) Assay Kit and Superoxide Dismutase (SOD) Assay Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Relevant RNA extraction and quantitative real-time RT-PCR (qRT-PCR) analysis

Following the instruction of FastQuantity RT Kit (KR106) (Tiangen biochemical technology Co., Ltd., Beijing, China), total RNA was extracted from the *E. coli* cells at stationary phase with different treated concentration of CIN (0, 1/8, 1/4, 3/8 MIC). Then the purity, concentration and integrity of RNA extraction were determined by Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., USA), Nanodrop 2000 (Thermo Scientific Inc., Wilmington, USA) and agarose gel electrophoresis, respectively.

Total RNA extraction (200 ng) was used to carry out the first-strand cDNA synthesis reverse transcription according to the FastQuant RT Kit (with gDNase) (Tiangen biochemical technology Co., Ltd., Beijing, China). Then, ABI step one plus (ABI, USA) was used to detect the transcription levels of relevant genes (SODa, SODb, SODc) to process qRT-PCR analysis. Our complete sequences of genes were provided by NCBI database and all primers listed in Table 1 were obtained from Jidiao Biotech Co. Ltd. (Guangzhou, China). The relative expression was checked by 20 μL qPCR reaction mix, which consisted of 10 μL qPCR Master Mix, 0.6 μL of 10 μM forward primer, 0.6 μL of 10 μM reverse primer, 4 μL of template cDNA, and ddH₂O was used to

Table 1 Primers of relevant genes of SOD used in this study

Gene	Sequence (5'–3')	Product length (bp)
SODa	F: CGCAATAACGCTGGTGGTC	117
	R: ATCAACGGAACCGAAATCAC	
SODb	F: GCTGCTGACCGTTGATGT	84
	R: GCCCAGAAGTGCTCCAGATAG	
SODc	F: CAGCCAGCCACCAAAGATG	291
	R: TACACCACAGGCATAGCGTTC	

maintain the total volume to 20 μL . The conditions of qRT-PCR were as follows: 1 cycle at 95 °C for 90 s; 40 cycles at 95 °C for 5 s, 60 °C for 15 s and 72 °C for 20 s. The method of $2^{-\Delta\Delta C_t 162}$ was used to determine the relative change of gene expression (Livak and Schmittgen 2001).

Statistical analysis

All tests were measured in triplicates and all data were performed as mean \pm standard deviation, using statistical program SPSS (Statistical Package for the Social Sciences, version 22.0, IBM, NY, USA). One-way analysis of variance (ANOVA) was applied to analyze the comparison of difference between data groups. Significant differences were set if $P < 0.05$.

Results and discussion

The effect of CIN on the growth of *E. coli*

The negative influence of CIN on *E. coli*, performed by corresponding growth curves at different concentrations of CIN (0, 1/8, 1/4, 3/8 MIC), was investigated. As shown in Fig. 1, CIN has obviously inhibitory effect on the growth of *E. coli*, which can be inferred from these growth curves. Compared with the optical density at 600 nm of the control group at 2 h ($\text{OD}_{600} = 1.460 \pm 0.035$), the value of OD_{600} at 2 h decreased from 1.250 ± 0.001 to 0.529 ± 0.003 with the concentration of CIN increasing from 1/8 MIC to 3/8 MIC. Besides, the lag phase was gradually prolonged when *E. coli* was exposed to increasing concentration of CIN. The lag phase of *E. coli* with absence of CIN was nearly 1.5 h, while it was extended to 2 h with 1/8 MIC concentration of CIN, further longer lag phase when the concentration of CIN was 1/4 and 3/8 MIC. A previous research has found that the growth of microorganism can be significantly inhibited by thymol (Wang et al. 2016b), and our result obviously verified the similar depression effect of CIN on *E. coli* growth.

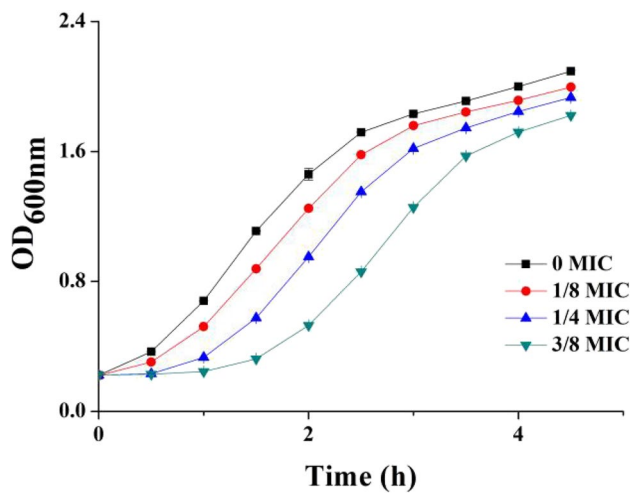


Fig. 1 The growth curve of *E. coli* impacted by different concentrations of CIN (0, 1/8, 1/4, 3/8 MIC), measured according to its optical density at 600 nm (OD_{600})

Raman spectroscopy analysis

This spectra showed different absorption bands from 600 to 1800 cm^{-1} , suggesting the change of cell compositions. Previous studies have indicated these bands represented different cell compositions, including lipids, proteins, carbohydrates and nucleic acids (Liu et al. 2009). For instance, absorption bands at 977 and 1453 cm^{-1} represent lipids. Bands around 853, 1005, 1252 and 1665 cm^{-1} are consistent with proteins. Those bands around 1035 cm^{-1} and Raman swifts (726, 783, 936, 1101, 1340 and 1577 cm^{-1}) are carbohydrates and nucleic acids, respectively. As shown in Fig. 2a, the shape of Raman spectroscopy of the cells with absence of CIN was in good agreement with others in different concentrations (1/8, 1/4, 3/8 MIC). However, the messages in Fig. 2b illustrated that with increasing concentration of CIN, the Raman spectral intensity of nearly all kinds of cell substances increased, suggesting that CIN had negative impact on *E. coli* membrane, causing the slight leakage of cellular content and the effect was more obvious as concentration of CIN increasing. PCA analysis was performed to process the data of Raman spectroscopy and the score plot was generated to show their potential connection. As shown in Fig. 3c, the first two principal components accounted for 90% and 6% of total variance in *E. coli* cell, respectively. Clear segregation was observed among the samples treated with different concentration of CIN, which suggested that the exposure of *E. coli* to CIN has caused systematic changes in cell compositions.

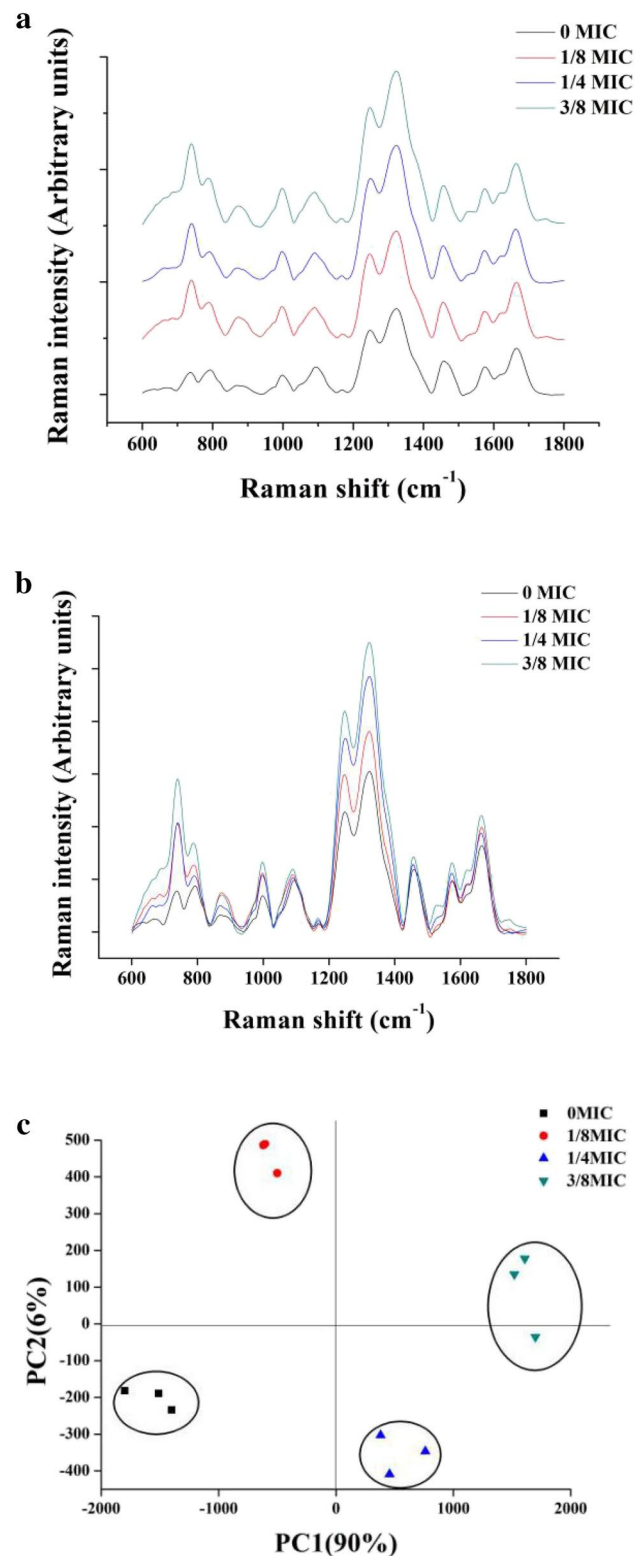


Fig. 2 Raman spectra of *E. coli* cells treated with different concentrations of CIN (0, 1/8, 1/4, 3/8 MIC). The Raman shift of measurement was in range from 600 to 1800 cm^{-1} . **a** Separated curves; **b** aggregated curves; **c** principal component analysis (PCA) based on the data of Raman spectra of *E. coli* cells treated with different conditions

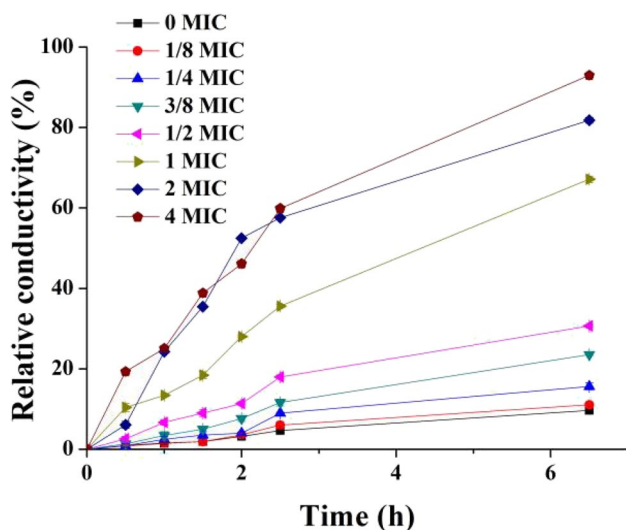


Fig. 3 Effects of CIN with different concentrations (0, 1/8, 1/4, 3/8, 1/2, 1, 2, 4 MIC) on the relative extracellular conductivity of *E. coli* cells

Cell membrane permeability

Microorganism cell membrane plays an important role in maintaining cell morphology, information exchange and material movement in and out of cell, so it is an essential part for the maintenance of life activities. The damage of cell membrane will cause leakage of cellular content, leading to the value of electric conductivity increasing (Wang et al. 2016b). Therefore, the measurement of relative electrical conductivity is a reliable method to explore the permeability of cell membrane. As shown in Fig. 3, relative electrical conductivity of bacterial suspension with 1, 2 and 4 MIC of CIN at 2.5 h were 35.537 ± 0.242 , 58.093 ± 0.477 and $59.904 \pm 0.256\%$, respectively, which were obviously higher than that of the control group with 0 MIC CIN ($4.672 \pm 0.674\%$) and gradually increased with the extension of processing time, implying the increase of cell membrane permeability at higher treated concentration of CIN. Shen et al. used SEM and TEM techniques to exhibit the morphology of *E. coli* after CIN treatment, showing that CIN can cause distortion and rupture of *E. coli* cell membrane (Shen et al. 2015). Xu et al. verified that the integrity of membrane of *A. alternata* was destroyed after being treated with CIN (Xu et al. 2018). However, CIN at relatively low concentration (1/8, 1/4, 3/8, 1/2 MIC) induced slight increase of relative electrical conductivity, suggesting that these concentrations of CIN cannot lead to serious membrane disruption. In short, it can be inferred that high concentration of CIN had significant influence on cell membrane, leading to increase of membrane permeability and rupture of cell membrane.

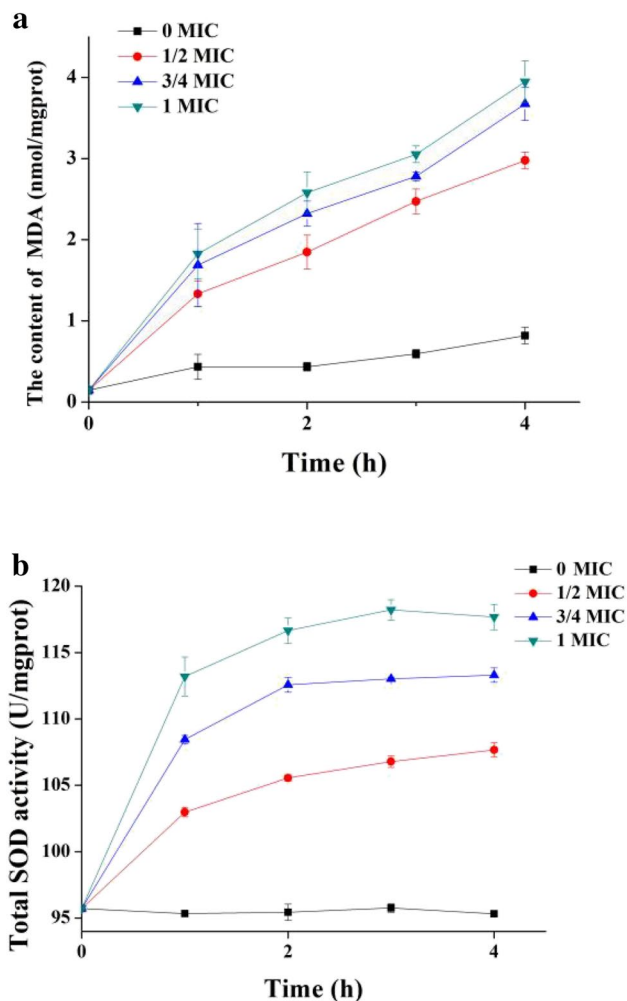


Fig. 4 The effect of CIN on the content of MDA and the activity of SOD. **a** The content of MDA of *E. coli* treated with different concentration of CIN (0, 1/2, 3/4, 1 MIC). **b** The activity of SOD of *E. coli* treated with above conditions

Oxidation damage of CIN in *E. coli*

Malondialdehyde (MDA), is normally generated as a by-product during the oxidation of cell membrane, and its content reflects the peroxidation degree of cell membrane lipid (Yun et al. 2016b). As shown in Fig. 4a, the content of MDA exhibited an increasing trend with the increase of CIN concentration and the treatment time. For example, the content of MDA was 1.848 ± 0.210 nmol/mgprot after the cells were treated with 1/2 MIC CIN for 2 h, which was obviously higher than the content of MDA from samples with the absence of CIN (0.435 ± 0.051 nmol/mgprot), and it generally increased to 2.322 ± 0.154 and 2.580 ± 0.256 nmol/mgprot when *E. coli* being treated with 3/4 and 1 MIC CIN, respectively. Besides, the MDA content exhibited a marked increase with increasing treatment time. Our result illustrated that CIN can cause oxidative injury in *E. coli*

membrane, resulting in the increase of MDA content with the concentration of CIN increasing. Moreover, a previous study has revealed that MDA was a significant product of the oxidation of unsaturated fatty acids (Lefèvre et al. 1998) and our previous study (He et al. 2017) has verified the decrease of the ratio of unsaturated fatty acids in *E. coli* cell membrane when it was exposed to CIN, so it can be inferred that the unsaturated fatty acids of membrane was one of important targets when CIN impacted on *E. coli*. Besides, it was reported that microorganism resisted the harm from external environment by adjusting the ratio of unsaturated fatty acids in cell membrane, which changed the fluidity of cell membrane to protect itself (Burns et al. 1979). The fluidity of cell membrane decreased when the ratio of unsaturated fatty acid decreased, which enhanced its resistance (Denich et al. 2003). Thus, except the stress response of microorganism itself (He et al. 2017), the oxidative damage of CIN may also be one of the important reasons for the change of the fluidity of *E. coli* cell membrane when it was exposed to CIN.

Superoxide dismutase (SOD) can effectively remove the excessive active oxygen to keep the balance of active oxygen metabolism, and its activity is commonly used to detect the resistance of microorganisms to the external oxidative harm (Knight and Knight 2001; Rohrdanz et al. 2001). As shown in Fig. 4b, the activity of total SOD without treatment of CIN for 1 h was 95.718 ± 0.090 U/mgprot, and increased to 102.983 ± 0.354 , 108.460 ± 0.298 , 113.192 ± 1.469 U/mgprot with 1/2, 3/4, 1 MIC CIN, respectively. Besides, it had a rapid increase during the first hour and then increased smoothly, exhibiting a total increase trend with the extension of treatment time. However, there was a slight decrease after the samples were treated in 1 MIC CIN for 3 h, which may be due to the production of free radicals exceeding the scavenging ability of total SOD, leading to negative impacts on total SOD and decrease of its activity. It was reported that active oxygen radicals can effectively induce the activity of total SOD increasing (Greenberg et al. 1990), and it will be produced when the unsaturated fatty acids of the cell membrane are oxidized (Lefèvre et al. 1998). Therefore, it can be inferred that the mechanism of CIN oxidizing *E. coli* cell membrane is that CIN oxidizes the unsaturated fatty acids of the cell membrane, causing generation of large number of active oxygen radicals, which induces increasing SOD activity to resist this harm.

The relationship between regulation of SOD genes and the resistance of *E. coli* to CIN

To detect the relationship between the expression of antioxidant genes and the resistance of *E. coli* to CIN, SOD relevant genes (SODa, SODb, SODc) in *E. coli* were selected and their transcription levels were investigated after being treated with different concentration of CIN (0, 1/8, 1/4, 3/8

MIC). Previous studies have indicated that SOD was divided into Mn-SOD (SODa), Fe-SOD (SODb) and CuZn-SOD (SODc) according to its combination with metal ions and they played significant roles in different locations to maintain normal life activities of microbes (Battistoni 2003).

As shown in Fig. 5, the relative expression of SODc gradually increased with the concentration of CIN increasing (from 1 ± 0.05 - to 3.78 ± 0.05 -fold), showing up-regulated relationship between them. Meanwhile, for SODa and SODb, there was no clear relationship between their relative expression and CIN concentration. It was reported that microorganism can generate superoxide dismutase (SOD), catalasein (CAT), peroxidase (PO) and other protein to eliminate the oxidative damage of external environment (Knight and Knight 2001; Rohrdanz et al. 2001). Moreover, previous researches illustrated that SODc was revolved in the resistance of *E. coli* to H_2O_2 , aiming to protect cell against the oxidative damage (Scotti et al. 2015), and SODc mutant strains suffered more serious damage compared to the normal strains (Pacello et al. 2008). Therefore, it can be inferred that the activity of SODc of *E. coli* was promoted and had a gradually increased trend with the increase of CIN concentration. Besides, SODc played a significant role in resistance of *E. coli* to the oxidative damage of CIN when *E. coli* was against to the harm of CIN according to our present work.

What's more, SODc may also have a great significance to protect *E. coli* cell membrane from CIN. It was reported that SOD was divided into three kinds of enzymes (SODa, SODb, SODc). SODa and SODb mainly played important roles in protecting inside cells while SODc cleared free radicals on or outside membrane (Battistoni 2003). Previous study suggested that SODc gene was revolved in the

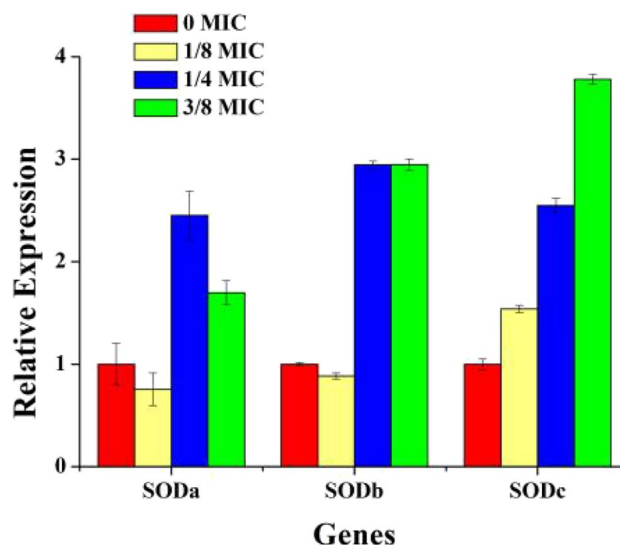


Fig. 5 The relative expression of antioxidant genes (SODa, SODb, SODc) of *E. coli* cultured with 0, 1/8, 1/4, 3/8 MIC of CIN

synthesis of cell membrane (Kim et al. 2006), and according to the SEM images showing the difference between SODc mutants and normal strains when they suffered from the harm of H₂O₂, the result indicated that SODc probably played a significant role on the surface of cell membrane (Scotti et al. 2015). In our present work, it was found that one of the impacting locations of CIN on *E. coli* was cell membrane, which led to cell membrane distortion and oxidation. Then, the activity of total SOD, especially SODc, which mainly played an important role on the cell membrane or outside the cell, will increase to protect cell membrane from the oxidative damage caused by CIN.

Conclusions

This work has shown two key mechanisms of CIN impacting on *E. coli* membrane to exert its antibacterial behaviors: increasing the permeability of cell membrane and oxidizing the cell membrane. CIN at lower levels can effectively suppress the growth of *E. coli* cell while CIN at relative higher concentration can increase the permeability of cell membrane, leading to the leakage of cellular content. Analysis of the content of MDA and SOD indicated that cell membrane of *E. coli* would be oxidized when the cells were exposed to CIN and SOD would be produced increasingly to resist its oxidative harm. Besides, according to qRT-PCR analysis of SOD genes, it was explored that total SOD, especially SODc, played a significant role in resistance to the oxidative harm of CIN. In conclusion, CIN had excellent antibacterial effect against *E. coli*, especially its cell membrane and the corresponding genes were regulated to resist this damage. Moreover, this result implied that CIN may be a potential natural preservative and may get commonly used to enhance the safety in food industry and agriculture.

Acknowledgements This research was supported by the National “Thirteenth Five” Key Research Project (2017YFD0400502), the 111 Project (B17018), the National Natural Science Foundation of China (21576099) and the S&T projects of Guangdong Province (2017B020207001 and 2015A030312001).

References

- Baskaran SA, Amalaradjou MAR, Hoagland T, Venkitanarayanan K (2010) Inactivation of *Escherichia coli* O157:H7 in apple juice and apple cider by trans-cinnamaldehyde. *Int J Food Microbiol* 141:126–129
- Battistoni A (2003) Role of prokaryotic Cu,Zn superoxide dismutase in pathogenesis. *Biochem Soc Trans* 31:1326–1329
- Boubaker H et al (2016) Chemical characterization and antifungal activities of four *Thymus* species essential oils against postharvest fungal pathogens of citrus. *Industrial Crops Products* 86:95–101
- Burns CP, Luttenegger DG, Dudley DT, Buettner GR, Spector AA (1979) Effect of modification of plasma membrane fatty acid composition on fluidity and methotrexate transport in L1210 Murine leukemia cells I. *Can Res* 39:1726–1732
- Denich TJ, Beaudette LA, Lee H, Trevors JT (2003) Effect of selected environmental and physico-chemical factors on bacterial cytoplasmic membranes. *J Microbiol Methods* 52:149–182
- Goodacre R, Timmins EM, Burton R, Kaderbhai N, Woodward AM, Kell DB, Rooney PJ (1998) Rapid identification of urinary tract infection bacteria using hyperspectral whole-organism fingerprinting and artificial neural networks. *Microbiology* 144(Pt 5):1157
- Greenberg JT, Monach P, Chou JH, Josephy PD, Demple B (1990) Positive control of a global antioxidant defense regulon activated by superoxide-generating agents in *Escherichia coli*. *Proc Natl Acad Sci USA* 87:6181
- He L, Kim NJ, Li H, Hu Z, Lin M (2008) Use of a fractal-like gold nanostructure in surface-enhanced raman spectroscopy for detection of selected food contaminants. *J Agric Food Chem* 56:9843–9847
- He TF, Zhang ZH, Zeng XA, Wang LH, Brennan CS (2017) Determination of membrane disruption and genomic DNA binding of cinnamaldehyde to *Escherichia coli* by use of microbiological and spectroscopic techniques. *J Photochem Photobiol B Biol* 178:623–630
- Hong J, Chen R, Zeng XA, Han Z (2016) Effect of pulsed electric fields assisted acetylation on morphological structural functional characteristics of potato starch. *Food Chem* 192:15–24
- Kim YH et al (2006) The role of periplasmic antioxidant enzymes (superoxide dismutase and thiol peroxidase) of the Shiga toxin-producing *Escherichia coli* O157:H7 in the formation of biofilms. *Proteomics* 6:6181–6193
- Knight H, Knight MR (2001) Abiotic stress signalling pathways: specificity and cross-talk. *Trends Plant Sci* 6:262
- Lee HS, Kim BS, Kim MK (2002) Suppression effect of *Cinnamomum cassia* bark-derived component on nitric oxide synthase. *J Agric Food Chem* 50:7700–7703
- Lefèvre G, Beljeanleymarie M, Beyerle F, Bonnefontrousselot D, Cristol JP, Théron P, Torrelles J (1998) Evaluation of lipid peroxidation by measuring thiobarbituric acid reactive substances. *Annales De Biologie Clinique* 56:305
- Liu Y, He L, Mustapha A, Li H, Hu ZQ, Lin M (2009) Antibacterial activities of zinc oxide nanoparticles against *Escherichia coli* O157:H7. *J Appl Microbiol* 107:1193–1201
- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2^{(-Delta Delta C(T))} Method. *Methods* 25:402–408
- Pacello F, Ceci P, Ammendola S, Pasquali P, Chiancone E, Battistoni A (2008) Periplasmic Cu, Zn superoxide dismutase and cytoplasmic Dps concur in protecting *Salmonella enterica* serovar Typhimurium from extracellular reactive oxygen species. *Biochim Biophys Acta* 1780:226
- Pérez-Alfonso CO, Martínez-Romero D, Zapata PJ, Serrano M, Valero D, Castillo S (2012) The effects of essential oils carvacrol and thymol on growth of *Penicillium digitatum* and *P. italicum* involved in lemon decay. *Int J Food Microbiol* 158:101–106
- Raut JS, Karuppaiyl SM (2014) A status review on the medicinal properties of essential oils. *Ind Crops Prod* 62:250–264
- Rohrdanz E, Schmuck G, Ohler S, Kahl R (2001) The influence of oxidative stress on catalase and MnSOD gene transcription in astrocytes. *Brain Res* 900:128–136
- Scotti R, Nicolini L, Stringaro A, Gabbianelli R (2015) A study on prophagic and chromosomal sodC genes involvement in *Escherichia coli* O157:H7 biofilm formation and biofilm resistance to H₂O₂. *Annali Dellistituto Superiore Di Sanità* 51:62–66
- Shen S, Zhang T, Yuan Y, Lin S, Xu J, Ye H (2015) Effects of cinnamaldehyde on *Escherichia coli* and *Staphylococcus aureus* membrane. *Food Control* 47:196–202

- Tian J, Huang B, Luo X, Zeng H, Ban X, He J, Wang Y (2012) The control of *Aspergillus flavus* with *Cinnamomum jensenianum* Hand.-Mazz essential oil and its potential use as a food preservative. *Food Chem* 130:520–527
- Tourdot-Maréchal R, Gaboriau D, Beney L, Diviès C (2000) Membrane fluidity of stressed cells of *Oenococcus oeni*. *Int J Food Microbiol* 55:269–273
- Wang LH, Wang MS, Zeng XA, Gong DM, Huang YB (2016a) An in vitro investigation of the inhibitory mechanism of β -galactosidase by cinnamaldehyde alone and in combination with carvacrol and thymol. *Biochim Biophys Acta* 1861
- Wang LH, Zhang ZH, Zeng XA, Gong DM, Wang MS (2016b) Erratum to: Combination of microbiological, spectroscopic and molecular docking techniques to study the antibacterial mechanism of thymol against *Staphylococcus aureus*: membrane damage and genomic. *DNA Bind Anal Bioanal Chem* 409:3055–3055
- Wang LH, Wang MS, Zeng XA, Xu XM, Brennan CS (2017) Membrane and genomic DNA dual-targeting of citrus flavonoid naringenin against *Staphylococcus aureus*. *Integr Biol* 9
- Wei JN, Zeng XA, Tang T, Jiang Z, Liu YY (2018) Unfolding and nanotube formation of ovalbumin induced by pulsed electric field. *Innovative Food Sci Emerg Technol* 45:249–254
- Xu L, Tao N, Yang W, Jing G (2018) Cinnamaldehyde damaged the cell membrane of *Alternaria alternata* and induced the degradation of mycotoxins in vivo *Industrial Crops Products* 112:427–433
- Yin HB, Chen CH, Kollanoor-Johny A, Darre MJ, Venkitanarayanan K (2015a) Controlling *Aspergillus flavus* and *Aspergillus parasiticus* growth and aflatoxin production in poultry feed using carvacrol and trans-cinnamaldehyde. *Poult Sci* 94:2183
- Yin HB, Chen CH, Kollanoorjohny A, Darre MJ, Venkitanarayanan K (2015b) Controlling *Aspergillus flavus* and *Aspergillus parasiticus* growth and aflatoxin production in poultry feed using carvacrol and trans-cinnamaldehyde. *Poult Sci* 94:2183–2190
- Yun O, Liu ZW, Zeng XA, Han Z (2016a) Salmonella typhimurium resistance on pulsed electric fields associated with membrane fluidity and gene regulation. *Innov Food Sci Emerg Technol* 36:252–259
- Yun O, Zeng XA, Brennan CS, Han Z (2016b) Effect of pulsed electric field on membrane lipids and oxidative injury of *Salmonella typhimurium*. *Int J Mol Sci* 17
- Zeng WC, He Q, Sun Q, Zhong K, Gao H (2012) Antibacterial activity of water-soluble extract from pine needles of *Cedrus deodara*. *Int J Food Microbiol* 153:78–84
- Zhou H, Tao N, Jia L (2014) Antifungal activity of citral, octanal and α -terpineol against *Geotrichum citri-aurantii*. *Food Control* 37:277–283