Proteomic Analysis Revealed Metabolic Inhibition and Elongation Factor Tu Deamidation by p-Coumaric Acid in Cronobacter sakazakii

Ping Lu1,2,3†, Xuemeng Ji4†, Juan Xue5, Yinping Dong6 and Xi Chen1,2,3,7*

1 Tianjin Key Laboratory of Ophthalmology and Visual Science, Tianjin Eye Institute, Tianjin, China, 2 Nankai University Affiliated Eye Hospital, Nankai University, Tianjin, China, 3 Clinical College of Ophthalmology, Tianjin Medical University, Tianjin, China, 4 Tianjin Key Laboratory of Food Science and Health, School of Medicine, Nankai University, Tianjin, China, 5 Institute of Infection and Immunity, Taihe Hospital, Hubei University of Medicine, Shiyan, China, 6 Institute of Radiation Medicine, Chinese Academy of Medical Science, Peking Union Medical College, Tianjin, China, 7 State Key Laboratory of Medicinal Chemical Biology, Nankai University, Tianjin, China

Screening drugs and compounds to fight against Cronobacter sakazakii (C. sakazakii), one of the most common pathogens that can cause fatal necrotizing enterocolitis, septicemia and meningitis, is still needed. We found that p-coumaric acid (pCA) has an inhibitory effect on C. sakazakii in vitro and in vivo. Proteomic changes of C. sakazakii BAA-894 exposed to pCA were studied to reveal the antibacterial mechanisms involved. A total of 1,553 proteins were identified in C. sakazakii BAA-894 by label-free proteomics analysis. Fuzzy cluster analysis showed that 33 were up-regulated, and 110 were down-regulated with pCA treatment. Gene Ontology (GO) analysis concluded that pCA caused the change of metabolic state of bacteria and generally in the state of metabolic inhibition. KEGG Enrichment Analysis (KEGG) analysis showed that pCA inhibited energy metabolism and distorted the balance of amino acid metabolism. Posttranslational modification analysis showed that pCA affected the deamidation of three proteins, including Elongation factor Tu, one of the vital proteins in bacteria. Molecular docking suggested the hydrogen bond between the pCA carboxyl group and Elongation factor Tu Asn-64 might contribute to deamidation. Overall, we found that pCA interfered with cellular energy and amino acid metabolism and promoted elongation factor Tu deamidation, suggesting that pCA can be an effective natural substitute to control C. sakazakii.

Keywords: p-coumaric acid, Cronobacter sakazakii, proteome, deamidation, elongation factor Tu

INTRODUCTION

Cronobacter sakazakii is a Gram-negative bacterium formerly known as "Enterobacter sakazakii". Cronobacter sakazakii can cause severe meningitis, sepsis, and necrotizing enterocolitis (Ji et al., 2021b). This microbe is ubiquitous in the environments and is often isolated from water, soil, kitchens, vegetables, and a variety of food samples, including powdered infant
formula (Mohammed et al., 2015; Li et al., 2016; Henry and Fouladkhah, 2019). Cronobacter sakazakii infections can occur in all age groups, and most, though usually not serious, infections occur in adults (Kadliecekova et al., 2018; Holý et al., 2019; Ohira et al., 2021). Nevertheless, immunocompromised patients, as well as premature, low-birth-weight babies were considered at higher risk (Stoll et al., 2004). Many cases of C. sakazakii infection have been reported worldwide in neonatal intensive care units. Mortality rates range from 40 to 80%, and meningitis survivors often suffer serious complications, including hydrocephalus, quadriplegia, and neural retardation (Nazarowec-White and Farber, 1997; Corti et al., 2007; Lachowska et al., 2021).

Cronobacter sakazakii is becoming a thorny problem, nearly half of the cases of C. sakazakii infection have been traced to contaminated infant formula, where the existence of C. sakazakii is now strictly limited (Iversen and Forsythe, 2003). However, given the wide distribution of C. sakazakii and its strong adaptability to the environment, it is challenging to control C. sakazakii and this may lead to occasional recalls of powdered infant formula (Var et al., 2021). Another reason is the emergence of drug-resistance of C. sakazakii, isolated in many countries, including environmental and clinical isolates, to vancomycin, penicillin, oxacillin, lincosamides, etc., details are as follows: in India, C. sakazakii isolated from newborns was resistant to cephalosporin, fluoroquinolones, and aminoglycosides (Kakatkar et al., 2017). Resistant C. sakazakii strains resistant to penicillin, tetracycline, ciprofloxacin, and nalidixic acid were isolated from kitchen samples in Tennessee, United States (Kilonzo-Nthenge et al., 2012), and multiple resistant strains were reported in China, causing a severe meningitis case in one neonate (Zeng et al., 2018).

The emergence of multidrug-resistant strains has prompted the researchers to turn their attention to traditional medicine to find alternatives. Volatile oils from leaves and flowers were reported to have antibacterial activity against C. sakazakii (Sharma and Prakash, 2013). The water-soluble muscadine seed extracts can inactivate C. sakazakii (Kim et al., 2009). Trans-cinnamaldehyde (TC), an ingredient in cinnamon, can control C. sakazakii (Amalaradjou and Venkitanarayanan, 2011). TC also inhibits biofilm synthesis and thus can be used to prevent C. sakazakii biofilm in infant formula feeding equipment.

A growing body of studies strongly suggest that phenolic compounds have antimicrobial activity (Basgedik et al., 2014; Ceylan and Alc, 2015; Călinoiu and Vodnar, 2020). Our previous studies have shown that p-coumaric acid (pCA), a phenolic acid of the hydroxycinnamic acid family, can inhibit growth and promote plasmid elimination of C. sakazakii (Ji et al., 2021a). However, the mechanism of pCA inhibiting the growth of C. sakazakii was lacking.

Our study used proteomics to characterize protein profile changes in C. sakazakii exposed to coumaric acid in an attempt to find the antibacterial mechanism of pCA. At the same time, postranslational modifications were analyzed to find a possible direct interaction between pCA and proteins. At last, molecular docking was performed to study the potential molecular interactions.

**MATERIALS AND METHODS**

**Bacterial Strains and Growth Conditions**

International standard strain C. sakazakii BAA-894 (ATCC, United States) was used in this study. Strain was stored in Luria-Bertani (LB) media (Oxoid, United Kingdom) containing 15% glycerol (Biosharp, China) at −80°C. To initiate all experiments, one loop of strain was innoculated in LB and cultured overnight. And then bacterium was diluted in LB media containing different concentrations of pCA to 10^6 CFU/mL; the growth or inactivation of bacteria was monitored by serial dilution of bacteria and plating on LB agar. The plates were incubated at 37°C for 24–48 h, and then colony forming unit (CFU) was numbered.

**In vivo Rat Virulence Assay**

Bacterial cells were washed and resuspended in phosphate buffered saline (PBS) (Vazyme, China). The bacterial suspension (5 × 10^9 CFU) were administered by gavage to 3-day-old female Sprague Dawley rats (6/group). Where appropriate, pCA was administered at a dose of 1 mg/g weight 2 h after bacteria challenging, while an equal volume of pCA was administered without bacterial inoculation as the negative infection control and bacterial inoculation but without pCA treatment as positive infection control. Rats were maintained in their home cages in the animal house at 24 ± 1°C and 55 ± 5% humidity, with a 12-h light-dark cycle (light on at 8:00 and off at 20:00) To analyze the colonization of bacteria in blood and organs, the rats were sacrificed after 24 h of injection; organs were homogenized in ice-cold PBS and serially diluted. Bacterial load was determined by plating the diluents on LB agar.

**Protein Extraction**

The bacterial suspension from in vitro experiment was centrifuged at 4°C for 2 min at 12,000g and washed twice with PBS. A protease inhibitor cocktail (Beyong, China) was added, and the bacteria were lysed by ultrasound (200 W, working 3 s and pausing 3 s) for 10 min. The protein extractions were centrifuged to obtain the supernatant (21,000g, 15 min at 4°C). Clean up the protein using a microporous filter (0.22 µm), and the extracted protein was stored at −80°C for subsequent MS analysis.

**Protein Digestion**

The protein samples for LC-MS/MS analysis were prepared according to the protocols described previously with minor modifications (Xue et al., 2022). Specifically, the protein sample (50 µg, qualified by optical density at 260 nm) was denatured by adding one-fourth 8M urea (Aladdin, China). Add 50 mM ammonium bicarbonate (Aladdin, China) to make the total volume reach 100 µL. Incubate the mixture at 37°C for 30 min. Subsequently, the protein sample was reduced by reacting with 1 µL 200 mM Dithiothreitol (DTT) (Aladdin,
When docking, the global search verbosity is set to 20, leaving the PDBQT format required for AutoDock Vina 1.1.2 docking. Convert all processed small molecules and receptor proteins into length set to 22.5 angstroms. In addition, use ADFRsuite 1.0 to active site residue as the center of the docking box with the side box using the PyMOL plug and define the center of mass of the and other small molecules (Skern, 2018). Then set up the docking receptor proteins, including removing water molecules, salt ions, (Gaillard, 2018). Before docking, PyMOL 2.5 was used to treat all proteins were predicted based on Alphafold2 (Jumper et al., 2021); the 3D structures of the small molecules were downloaded from the PubChem database (Kim et al., 2016), and the Chem3D V20 was used to minimize the energy in the MMFF94 field (Hajos, 2002). AutoDock Vina 1.1.2 was used to perform molecular docking (Gaillard, 2018). Before docking, PyMOL 2.5 was used to treat all receptor proteins, including removing water molecules, salt ions, and other small molecules (Skern, 2018). Then set up the docking box using the PyMOL plug and define the center of mass of the active site residue as the center of the docking box with the side length set to 22.5 angstroms. In addition, use ADFRsuite 1.0 to convert all processed small molecules and receptor proteins into the PDBQT format required for AutoDock Vina 1.1.2 docking. When docking, the global search verbosity is set to 20, leaving the rest of the parameters set by default. The output of the highest-scoring docking conformations is considered to be the binding conformations, and finally, PyMol is used for visual analysis of docking results.

**Statistical Analysis**

All experiments were conducted in triplicate, as independent experiments. Gene Ontology (GO) and KEGG Enrichment Analysis (KEGG) analysis was performed on DAVID’s online website 1 and enrichment scores were calculated automatically (Dennis et al., 2003). Fuzzy C-means (FCM) algorithm was conducted with R (Bezdek et al., 1984; Ihaka and Gentleman, 1996). Statistical analyses were performed with GraphPad Prism software or R as described in Experimental Procedures for individual analysis. For all, p < 0.05 was considered statistically significant.

**RESULTS**

**Bacteriostatic Effect of p-Coumaric Acid**

The effect of pCA on the growth of C. sakazakii is shown in Figure 1A. The bacteria in the control group began to grow exponentially after 20 mins and reached stationary phase at 6 h. Cronobacter sakazakii was inhibited at the concentration of 300 µg/mL pCA, and reached the stationary phase after 9 h. When the concentration of pCA reached 500 µg/mL, the growth of bacteria was inhibited entirely. These results indicated that pCA could inhibit the growth of C. sakazakii in vitro. We also studied the effect of pCA in vivo (Figure 1B). Rats were used as the infection model. In the pCA treatment group, the rats received gavage treatment of pCA (1 mg/g weight) 2 h after bacterial infection, while an equal volume of saline was administered as the control. The results showed that pCA could significantly improve the survival rate of infected rats. Moreover, pCA treatment significantly reduced the bacterial density of C. sakazakii in the blood, liver, and spleen of the rat sacrificed 1 day after infection (Figure 1C). Taken together, these results suggest that pCA can effectively inhibit bacterial growth in vivo and in vitro.

**Proteomic Analysis of Cronobacter sakazakii Exposure to p-Coumaric Acid**

Using label-free relative quantitative mass spectrometry, we systematically monitored the protein expression profiles of C. sakazakii at 2 mins, 30 mins, 1 h, and 2 h after pCA exposure (Figure 2A). C. sakazakii not exposed to pCA at the initial stage and after 2 h of normal growth were set as controls. We obtained 15,621 unique peptides that matched 1,553 proteins. Among them, 905 proteins met the quantitative requirements (Figure 3A). T-test (p < 0.05) was used to determine the up-and-down-regulated peptides according to the criterion that the folding change of differential expressed proteins was more than two times (Figure 2B and Supplementary Table 1). Compared with the control group, 2 mins of pCA exposure could significantly change 39 protein abundances, and

---

1 https://david.ncifcrf.gov
abundance changes increased over prolonged exposure time to pCA (Figure 2C). After 2 h of pCA treatment, there were 90 up-regulated proteins and 113 down-regulated proteins. Surprisingly, however, the bacterial proteome showed even more changes after 2 h in standard growth control. It is suggested that prolonged time and drug treatment can cause changes in the proteome.

To distinguish the pCA-induced proteome changes from time-induced proteome changes, fuzzy C-means (FCM) algorithm was used to cluster the protein expression profiles at different time stages (Figure 3 and Supplementary Table 2). In general, we observed eight different time-pattern clusters representing different regulatory proteins, indicating different expression dynamics. Clusters 2 and 4 represent down-regulated proteins, clusters 6 and 7 represent up-regulated proteins, and clusters 1, 3, and 5 represent proteins showing bimodal expression patterns, and cluster 8 characterizes proteins which expression does not change much. When time interference was excluded by superimposing the protein expression 2 h after normal growth, some proteins were continuously upregulated or downregulated, clustered in clusters 7 and 8, respectively. The up- and down-regulated proteins accounted for 11 and 17% of the total 905 proteins. Among the core-altered proteins (membership > 0.58), 40% were downregulated, 3.3 times as many as the upregulated. Overall, we found that pCA caused protein expression changes and more downregulated proteins than upregulated proteins, consistent with the observed inhibition of bacterial growth exposed to pCA.

**Fuzzy Cluster and Gene Ontology Analysis**

GO analysis revealed that the altered proteins were enriched in the molecular function (Figure 4 and Supplementary Table 3). The NADP binding and glycine decarboxylation via glycine cleavage system were significantly enriched among molecular function clusters. The upregulated protein was significantly enriched in NADP binding. NADP-binding proteins are associated with the metabolic status of bacteria, so we hypothesize that pCA causes changes in the metabolic status of bacteria. In addition, pCA-induced up-regulated proteins were enriched in the biological process of the Protoporphyrinogen IX biosynthesis, and Protoporphyrinogen IX was reported to play an essential role in the biosynthesis of heme, suggesting that the bacteria were in an iron-deficient state. It is well documented that polyphenols can chelate iron ions, and perhaps pCA can competitively chelate environmental iron ions, making it difficult for bacteria to obtain iron. Additionally, pCA-induced down-regulated proteins were enriched in seven biological-function-related clusters and one cellular component-related cluster. However, pCA-up-regulated proteins were enriched in only two clusters. Both up-regulated and down-regulated proteins were
found in three clusters: the plasma membrane, ATP binding, four iron, and four sulfur cluster binding. Still, the number of down-regulated proteins was more than up-regulated in the same cluster. Collectively, GO analysis infers that pCA leads to alterations in the bacterial metabolic state and an overall metabolically repressed state.

**KEGG Enrichment Analysis Pathway Analysis**

By performing KEGG pathway enrichment analysis, the down-regulated proteins were significantly enriched in seven pathways ($p < 0.05$), and the up-regulated proteins were enriched in only one pathway (Figure 5 and Supplementary Table 3). pCA distorted l-phenylalanine, tyrosine, and tryptophan biosynthesis. Of the seven pathways down-regulated, “metabolism” was the most common, including several subclasses: “glyoxylxlic and dicarboxylic acid metabolism,” “fructose and mannose metabolism,” “arginine and proline metabolism,” “glycine, serine and threonine metabolism.” In addition, some important categories were also significantly enriched (e.g., “phosphotransferase system,” “oxidative phosphorylation”). These results illustrated that pCA might inhibit cellular energy metabolism while the balance of amino acid metabolism might be disturbed.

**Deamidation of Proteins by p-Coumaric Acid**

Three proteins were identified that were affected by pCA to increase deamidation significantly. These include: A7MKI5, which has 64 amino acid l-asparagine deamidation, A7MGU5:
FIGURE 3 | Clustering the protein expression profiles at different stages using Fuzzy C-means (FCM) algorithm. The expression profiles were clustered into eight different regulatory patterns, indicating different expression dynamics. See also Supplementary Table 2.

FIGURE 4 | GO analysis on the up-regulated and down-regulated proteins. GO analysis was performed on DAVID's online website (https://david.ncifcrf.gov) and enrichment scores were calculated automatically. \( p < 0.05 \) was considered statistically significant.
43 amino acid L-asparagine deamidation, A7MKM7:379 amino acid L-asparagine deamidation. Notably, all three proteins have important effects on the physiological functions of bacteria. A7MKI5 is a crucial protein of bacteria, termed Elongation factor Tu (EF-Tu), a G protein with the intrinsic ability to hydrolyze GTP into GDP, contributes to the overall translation fidelity, and plays a vital role in the elongation process of extension of protein biosynthesis. And A7MGU5 is an ancillary SecYEG translocon subunit, which is thought to act as a molecular chaperone, helps maintain the typical conformation of proteins in bacteria. A7MKM7 is a Peptidase M3 domain-containing protein with zinc ion metal-binding ability and metallopeptidase activity. In summary, we found that the effect of pCA on posttranslational modifications mainly affected the deamidation of bacteria, including three critical proteins.

**DISCUSSION**

Previous studies have shown that pCA can kill bacteria through a dual damage mechanism, increasing membrane permeability and binding to DNA phosphate anions (Lou et al., 2012). These mechanisms affect replication, transcription, and protein expression. Here we report the effect of pCA on the proteomic changes of *C. sakazakii*.

In this study, label-free protein mass spectrometry was used to investigate the proteomic changes caused by pCA. pCA caused most downregulation of core-altered proteins and a few proteins upregulated. This result is not surprising because pCA suppresses bacterial growth. The reduced proteins caused by pCA were significantly enriched in the glycine decarboxylation via glycine cleavage system, which widely exists...
In addition to the regulation of biological metabolic pathways, protein post-translational modifications by chemicals are also important but often overlooked. Protein posttranslational modification includes many kinds, such as acetylation, methylation, etc.; some of these modifications may even affect host-bacteria interactions, such as the recently reported CSPC acetylation modification that enhances the presence of Pseudomonas aeruginosa in the host (Li et al., 2021). We characterized the post-translational effects of pCA, including acetylation, phosphorylation, methylation, and deamidation. However, except for changes in deamidation, we did not identify any other type of significant modification changes. Our study found that pCA induced deamidation of elongation factor Tu. These results suggest that the antibacterial action of pCA on C. sakazakii is multi-target (Zhang et al., 2021). But whether C. sakazakii is easy to develop resistance to pCA needs further study.

Our posttranscriptional modification analysis combined with molecular docking revealed that pCA has a targeted deamidation effect on the elongation factor Tu. The A7MKI5/p-Coumaric acid complex predicts the hydrogen bonding of the pCA carboxyl group to the amide bond on the ASN-64 backbone, which is consistent with the deamidation sites analyzed by the proteomic data, which further confirms the potential interaction between Elongation factor Tu and pCA. Considering the critical role of elongation factor Tu for microorganisms, the deamidation of the factor by pCA may cause the growth inhibition of C. sakazakii. This is the first report investigated the deamidation effect of bacteriostatic drugs on proteins in organisms, and also the first report on the deamidation effect of bacteriostatic drugs on elongation factor Tu. The importance of the elongation factor Tu for microorganisms has long been recognized, and research has been underway to develop antibiotics that inhibit its effects. As far in 1974, the inhibitory effect of kirromycin on the bacterial elongation factor Tu has been found (Wolf et al., 1974). Over the past few decades, a great deal of biological information has been accumulated about Elongation factor Tu inhibitors. Recently, the Elongation factor-Tu has developed into an attractive antibacterial target for rational drug discovery (Harvey et al., 2019). However, to sum up, the currently reported drugs, including rationally designed drugs, are primarily targeted into the pocket of the elongation factor Tu (Parmeggiani and Nissen, 2006; Parmeggiani et al., 2006; Kumar and Garg, 2022). Deamidation seems not to be the active modification of bacteria but the degradation process of proteins. For example, active protein drugs will undergo spontaneous deamidation during storage, and deamidation may affect the normal function of drugs, but the deamidation in organisms is still lacking research (Liu, 1992; Krause and Sahin, 2019). Our study suggests that the asparagine deamidation of the elongation factor Tu may also be a target for antimicrobial agents.

In conclusion, our study demonstrates on the one hand that pCA interferes with cellular energy and amino acid metabolism, thus inhibiting bacterial growth. On the other hand, we found that pCA promoted L-asparagine deamidation of the bacterial elongation factor Tu, which hints at the great potential of deamidation of elongation factor Tu as an antimicrobial target.
FUNDING
This work was supported by the Open Project Program of State Key Laboratory of Medicinal Chemical Biology, Nankai University (No. 2018065), Talent Cultivation Project of Health Commission of Tianjin (No. RC20147), Talent Cultivation Project of Tianjin Eye Hospital (No. YQKQN1912), and Tianjin Key Medical Discipline (Specialty) Construction Project.

ACKNOWLEDGMENTS
We are grateful to PL (Tianjin University of Science and Technology, China) for her technical assistance.

SUPPLEMENTARY MATERIAL
The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.888103/full#supplementary-material
Krause, M. E., and Sahin, E. (2019). Chemical and physical instabilities in manufacturing and storage of therapeutic proteins. *Curr. Opin. Biotechnol.* 60, 159–167. doi: 10.1016/j.copbio.2019.01.014

Kumar, N., and Garg, P. (2022). Probing the Molecular Basis of Cofactor Affinity and Conformational Dynamics of Mycobacterium tuberculosis Elongation Factor Tu: an Integrated Approach Employing Steered Molecular Dynamics and Umbrella Sampling Simulations. *J. Phys. Chem. B* 126, 1447–1461. doi: 10.1021/acs.jpcb.c009438

Lachowska, M., Izdebski, R., Urbanowicz, P., ˙Zabicka, D., and Królak-Olejnik, Li, S., Weng, Y., Li, X., Yue, Z., Chai, Z., Zhang, X., et al. (2021). Acetylation of Lysine and GlcNAcylation and Activity Regulation of PhoP by a Type III Secretion System Effector in *Pseudomonas aeruginosa*. *Nucleic Acids Res.* 49, 6756–6770. doi: 10.1093/nar/gkah506

Li, Z., Ge, W., Li, K., Gan, J., Zhang, Y., Zhang, Q., et al. (2016). Prevalence and characterization of Cronobacter sakazakii in retail milk-based infant and baby foods in Shaanxi, China. *Foodborne Pathog. Dis.* 13, 221–227. doi: 10.1089/fpd.2015.2074

Liu, D. T.-Y. (1992). Deamidation: a source of microheterogeneity in pharmaceutical proteins. *Trends Biotechnol.* 10, 364–369. doi: 10.1016/0167-7799(92)90269-2

Lou, Z., Wang, H., Rao, S., Sun, J., Ma, C., and Li, J. (2012). p-Coumaric acid kills bacteria through dual damage mechanisms. *Food Control* 25, 550–554. doi: 10.1016/j.foodcont.2011.11.022

Ma, J., Chen, T., Wu, S., Yang, C., Bai, M., Shu, K., et al. (2019). iProX: an integrated proteome resource. *Nucleic Acids Res.* 47, D1211–D1217. doi: 10.1093/nar/gky869

Mohammed, M. A., Sallam, K. I., and Tamura, T. (2015). Prevalence, identification and molecular characterization of Cronobacter sakazakii isolated from retail meat products. *Food Control* 53, 206–211. doi: 10.1016/j.foodcont.2015.01.010

Nazarowec-White, M., and Farber, J. M. (1997). Enterobacter sakazakii: a review. *Int. J. Food Microbiol.* 34, 103–113.

Ohira, S., Ikeda, E., Kamijo, K., Nagai, T., Tsunemi, K., Uchiyama, N., et al. (2021). Pyosalpinx due to Cronobacter sakazakii in an elderly woman. *BMC Womens Health* 21:136. doi: 10.1186/s12955-021-01283-8

Parmeggiani, A., Krab, M., Watanabe, T., Nielsen, R. C., Dahlberg, C., Nyborg, J., et al. (2006). Enacyloxin IIA pinpoints a binding pocket of elongation factor Tu for development of novel antibiotics. *J. Biol. Chem.* 281, 2893–2900. doi: 10.1074/jbc.M505955200

Parnegjani, A., and Nissen, P. (2006). Elongation factor Tu-targeted antibiotics: four different structures, two mechanisms of action. *FEBS Lett.* 580, 4576–4581. doi: 10.1016/j.febslet.2006.07.039

Sharma, G., and Prakash, A. (2013). Susceptibility of Cronobacter sakazakii to plant products, antibiotics, and to lactic acid bacteria. *Int. J. Nutr. Pharmacol. Neurol. Dis.* 3:263. doi: 10.4103/2231-0738.114847

Shi, C., Sun, Y., Zheng, Z., Zhang, X., Song, K., Jia, Z., et al. (2016). Antimicrobial activity of syringic acid against Cronobacter sakazakii and its effect on cell membrane. *Food Chem.* 197, 100–106. doi: 10.1016/j.foodchem.2015.10.100

Skern, T. (2018). An Archive and a Tool: pdb and pymol. Exploring Protein Structure: Principles and Practice. Berlin: Springer. 7–28.

Stoll, B. J., Hansen, N., Fanaroff, A. A., and Lemons, J. A. (2004). Enterobacter sakazakii is a rare cause of neonatal septicemia or meningitis in VLBW infants. *J. Pediatr.* 144, 821–823. doi: 10.1016/j.jpeds.2004.02.045

Tezuka, T., and Ohnishi, Y. (2014). Two glycine riboswitches activate the gyAC cleavage system essential for gyA gene detoxification in Streptomyces griseus. *J. Bacteriol.* 196, 1369–1376. doi: 10.1128/JB.01480-13

Var, I., Özakmak, S., Tekin, A., Yılmaz, S., Heshmati, B., Üçkun, O., et al. (2021). Evaluation of Food Safety of Commercial Baby Foods according to Legal Regulations. *Eur. J. Agric. Food Sci.* 3, 72–80. doi: 10.24018/ejfood.2021.3.5.373

Wolf, H., Chinali, G., and Parmeggiani, A. (1974). Kirromycin, an inhibitor of protein biosynthesis that acts on elongation factor Tu. *Proc. Natl. Acad. Sci. U.S.A.* 71, 4910–4914. doi: 10.1073/pnas.71.12.4910

Xue, J., Huang, Y., Zhang, H., Hu, J., Pan, X., Peng, T., et al. (2022). Arginine GlcNacylation and Activity Regulation of PhoP by a Type III Secretion System Effector in *Salmonella*. *Front. Microbiol.* 12:852743. doi: 10.3389/fmicb.2021.825743

Zeng, H., Lei, T., He, W., Zhang, J., Liang, B., Li, C., et al. (2018). Novel multidrug-resistant Cronobacter sakazakii causing meningitis in neonate, China, 2015. *Emerg. Infect. Dis.* 24:2121. doi: 10.3201/eid2411.180718

Zhang, Y., Liu, Y., Tang, Y., Zhang, D., He, H., Wu, J., et al. (2021). Antimicrobial u-defensins as multi-target inhibitors against amyloid formation and microbial infection. *Chem. Sci.* 12, 9124–9139. doi: 10.1039/d1sc01133b

**Conflict of Interest:** 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.

**Publisher’s Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Lu, Ji, Xue, Dong and Chen. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.