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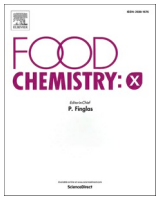

Isolation, characterization, and fermentation potential of coagulase-negative *Staphylococci* with taste-enhancing properties from Chinese traditional bacon

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ABSTRACT

No proprietary starter cultures for crafting Chinese bacon. This study aimed to isolate Coagulase-negative *Staphylococci* (CNS) from Chinese bacon, identify their species, and evaluate their ability to produce biogenic amines (BAs), peptides, free amino acids (FAAs), and degrade proteins. Twenty-one isolates were deficient in hemolysis, DNase, and coagulase activities, and exhibited low amino acid decarboxylase activity. Further characterization revealed 11 CNS species showing protease, lipase, or nitrate reductase activities. Specifically, *S. cohnii WX-M8* was able to degrade both sarcoplasmic and myofibrillar proteins, while *S. saprophyticus MY-A10* was found to only degrade myofibrillar proteins. Both were able to reduce the BAs and increase the content of peptides around day 3. The meat fermented by these two CNS contained FAAs that are more conducive to taste formation, such as Glu and Asp, and reduced the content of bitter FAAs. These findings will provide insights into the use of CNS for Chinese bacon.

Introduction

Chinese bacon, commonly known as “larou” is a traditional fermented food in China that has gained widespread popularity in the hot, humid south due to its ability to prolong the shelf life of pork while imparting a unique flavor (Wang, Wang, Pan, et al., 2021). Chinese bacon is processed via curing, drying (either in the sun or in the shade), smoking (or not smoking), and other processes using the sides, belly, or back of hog flesh, together with other auxiliary materials (Wang, Wang, Ji, et al., 2021). The production cycle is approximately 30 days, including approximately 7 days of curing and 3 weeks of drying (Wang, Wang, Pan, et al., 2021). According to the drying or smoking methods, Chinese bacon can be divided into two types: air-dried bacon and smoked bacon, both of which possess distinctive flavors and textures (Zhang et al., 2021). Although aroma and taste are the main sensations of flavor perception, the unique taste of Chinese bacon, as a non-ready-to-eat fermented meat product, is considered a key parameter associated with its acceptability. *Staphylococcus app.* plays a role in producing rich flavor compounds in Chinese bacon. Notably, *Staphylococcus* shapes the flavor of Chinese bacon by regulating and balancing the levels of key FAAs, such as glutamate, lysine, and alanine (Yang et al., 2023).

Staphylococcus spp. was the main microbial species engaged in the fermentation of various types of Chinese bacon, with relative abundances of 86.30 %, 79.72 %, 48.71 %, and 31.93 % observed in Guizhou bacon, Hunan bacon, Hubei bacon, and Sichuan-Chongqing bacon, respectively (Zhao et al., 2021). The production of coagulase distinguishes *Staphylococci aureus* from other *Staphylococcus*, and hence, bacteria with a deficiency in coagulase are classified as CNS spices (Khusro and Aarti, 2022). In Chinese bacon, the spices of CNS are considerable. In detail, CNS that are novobiocin-susceptible including *S.*

Abbreviations: BAs, biogenic amines; BB-M, Beibei smoked bacon; CD-M, Chengdu smoked bacon; CK-M, Chengkou smoked bacon; CNS, coagulase-negative *staphylococci*; GY-A, Guiyang air-dried bacon; GY-M, Guiyang smoked bacon; FAAs, free amino acids; FFAs, free fatty acids; FJ-A, Fengjie air-dried bacon; FJ-M, Fengjie smoked bacon; MN-A, Mianning air-dried bacon; MSA, mannitol salt agar; MY-A, Mianyang air-dried bacon; MY-M, Mianyang smoked bacon; PCA, Principal component analysis; PE-A, Pu′er air-dried bacon; QCS-M, Qingchengshan smoked bacon; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TSA, tryptic soy agar; TSB, tryptic soy broth; WX-M, Wuxi smoked bacon; XW-A, Xuanwei air-dried bacon.

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simulans, *S.xylosus*, *S.condimenti* and *S.epidermidis*, were found in Chinese bacon, while CNS that are novobiocin-resistant include *S.equorum*, *S.saprophyticus*, *S.vitulinus*, *S.warneri*, and *S.sciuri* (Hu et al., 2018; Wang et al., 2020; Zhao et al., 2020). Generally, CNS strains are used for the preparation of fermented sausages and meat products. Although some CNS, such as *S. saprophyticus* and *S. epidermidis*, occasionally produce enterotoxins and exhibit resistance to antibiotics, they are still recommended for use in the food industry due to their desirable technological and functional traits (Khusro and Aarti, 2022; Sanchez Mainar et al., 2017).

The metabolic characteristics of bacteria cause changes in the sensory properties of fermented foods (Khusro and Aarti, 2022). *Staphylococcus* reduces nitrate to nitrite by nitrate reductase, and promotes the formation of nitrosylmyoglobin, resulting in the characteristic color of fermented cured meat (Ras et al., 2018). At the same time, proteinase and lipase decompose proteins and fats into peptides, amino acids, and FFAs during fermentation, which are further transformed into flavor compounds through amino acid conversion and lipid β-oxidation (Ashaolu et al., 2023). Chen et al. (2022) screened 107 CNS strains from 30 spontaneously fermented meats in China, with most of them exhibiting nitrate reductase activity, protein and lipid hydrolysis capabilities. Specifically, *S.casei No.1* and *S.xylosus No.120* showed strong potential for flavor production. Li et al. (2022) identifird 114 CNS strains with positive catalase activity from nine traditional Chinese fermented meat products, of which 40 strains were able to decompose pork protein. Among them, *S.simulans QB7* exhibited the highest proteolytic activity. Semedo-Lemsaddek et al. (2016) discovered 104 CNS strains from Portuguese dry fermented sausages, among which *S.xylosus* and *S. equorum* were highly well suited to paiticular ecological niches.

Due to its non-infectious nature as a microorganism, CNS is a typical non-lactic acid bacteria that is commonly used in meat fermentation (Khusro and Aarti, 2022). However, during the screening of fermentation strains, each CNS needs to be tested for fermentation potential and safety due to the presence of species variation. Currently, the metabolic profile of specific microorganisms can be investigated through omics techniques, which seems to be more advantageous for finding suitable fermentation strains. However, even if a particular microbe is linked to the development of meat quality, many *Staphylococci* are non-culturable, thus purebred strains must be obtained through artificial isolation. This study aims to investigate the technological and safety characteristics of CNS isolated from various regions of China, as well as validate their ability for peptide production, protein degradation, and amino acid accumulation as a starter culture for Chinese bacon, laying the foundation for the development for screening *Staphylococci* with taste-enhancing properties for use as a starter cultures.

Materials and methods

Chinese bacon samples and isolation of bacteria

Chinese bacon sampling

Fourteen kinds of traditional Chinese bacon were collected from four provinces in southern China, including Pu′er air-dried bacon (PE-A), Xuanwei air-dried bacon (XW-A), Guiyang smoked bacon (GY-M), Guiyang air-dried bacon (GY-A), Beibei smoked bacon (BB-M), Fengjie smoked bacon (FJ-M), Fengjie air-dried bacon (FJ-A), Chengkou smoked bacon (CK-M), WuXi smoked bacon (WX-M), Qingchengshan smoked bacon (QCS-M), Mianning air-dried bacon (MN-A), Mianyang smoked bacon (MY-M), Mianyang air-dried bacon (MY-A), Chengdu smoked bacon (CD-M).

Isolation of bacteria

Twenty-five grams of Chinese bacon was chopped and placed into a sterile homogeneous bag with 225 mL of sterile saline solution. The mixture was beaten for 3.5 min using a SCIENT2-04 sterile homogenizer (Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China) to obtain a bacterial suspension. After diluting the bacterial suspension to an appropriate concentration, 0.1 mL was plated on MSA (Qingdao Hope Bio-Technology Co., Ltd, Qingdao, China). After being cultured at 37 °C for 48 h, colonies with round, convex, diameters of 1–4 mm with sharp borders, and presenting a white to yellow appearance were selected for continuous streak culture to obtain pure cultures (Chen et al., 2022).

Screening for Staphylococcus

Preliminary screening for Staphylococcus

The isolates were Gram-stained, and spherical cells with a blue-purple were selected for the subsequent experiments using a BX43 Olympus microscope (OM Digital Solutions Corporation). The catalase activity was determined with reference to the method of Chen et al. (2022) with some modifications as follows: isolated bacteria were cultured in TSB medium (Qingdao Hope Bio-Technology Co., Ltd., Qingdao, China) at 37 °C and 120 rpm for 24 h. The bacterial culture was then introduced in a volume of 100 μL to a slide that already contained 100 μL of hydrogen peroxide (5 %, V/V). The results were expressed as follows: "–" (no activity), "+-" (very weak activity), "+" (weak activity), "+ +" (moderate activity), and "+++" (strong activity). The furazolidone sensitivity of the purified strains was tested with reference to the method of Kayili and Sanlibaba. (2020) with some modifications. Briefly, TSA (Qingdao Hope Bio-Technology Co., Ltd, Qingdao, China) was coated with bacterial cultures that had been cultured in TSB at 37 °C and 120 rpm for 24 h. A 6-mm diameter antibiotic susceptibility disk containing 100 μg furazolidone was then placed on the plate and incubated at 37 °C for 24 h. The diameter of the clear zone around the disk was measured, and experiments were conducted three times.

Safety assessment

Based on the degree of erythrocyte lysis, the hemolytic reactions were divided into two main groups: β-hemolysis (complete lysis of erythrocytes) and α-hemolysis (partial lysis of erythrocytes), which were characterized by clear zones and greenish zones around the colonies, respectively (Ruaro et al, 2013). The hemolytic activity of isolated bacterial strains was confirmed by supplementing TSA with 5 % sterile defibrinated sheep blood (v/v). *S. aureus ATCC 25923* (China Center of Industrial Culture Collection) was used as the positive control. Experiments were conducted three times.

The DNase activity was conducted by DNase Agar (Qingdao Hope Bio-Technology Co., Ltd, Qingdao, China). After 48 h of incubation at 37 °C, purified and activated isolates were streaked onto a DNase plate.1 mol/L hydrochloric acid was then added around the colony. If it was DNase positive, an obvious clear transparent zone will appear around the colony. Experiments were conducted three times.

Coagulase activity was tested using the freeze-Dried Rabbit Plasma (Qingdao Hope Bio-Technology Co., Ltd, Qingdao, China). The activated purified strains were inoculated into an ampoule and incubated at 37 °C for 6 h. Every half-hour, it was checked, and if the clots or clotting volume were higher than half, it was recorded as positive "+". *S. aureus ATCC 25923* was used as the positive control.

Decarboxylase activity was referred to the method of Li et al. (2022) with some modifications. A medium containing 1 mL of 1.6 % bromocresol purple ethanol solution, 0.1 % glucose, and 0.5 % (w/v) each of lysine, arginine, histidine, cysteine, and ornithine was inoculated with purified and activated bacteria. The cultures were then incubated for 4 days at 37 °C, and the color change in the liquid medium during the incubation period was recorded.

Activity of fermentation-related enzymes

The protease activity of the isolates was determined by referring to the method of Chen et al. (2022) with some modifications. The overnight cultures in TSB were adjusted to an OD_{600} of approximately 0.5 (about 2.5×10^{10} CFU/mL), and 10 μL of bacterial suspension was

transferred onto a sterilized 6-mm-diameter blank antibiotic test strips. After incubated at 37 °C for 48–72 h with the paper disks on TSA agar supplemented with 10 % skimmed milk and, the diameter of the clear zone around the antibiotic test strips was measured. Each experiment was repeated three times.

The Lipase activity of the isolates with reference to the method of Merchán et al. (2022) with some modifications. 10 μL of bacterial culture with OD_{600} of about 0.5 was added to sterilized 6-mm diameter blank antibiotic test strip, and then the strips were transferred to TSA medium (Each 100 mL of TSA medium contains 0.2 % rhodamine B polyvinylpyrrolidone olive oil emulsion). After incubating at 37 °C for 48–120 h, the diameter of the fluorescent circle was measured under a UV lamp. Each experiment was repeated three times.

10 μL of bacterial suspension was added to a nitrate reductase medium after the overnight bacterial culture's concentration was adjusted to an OD_{600} of approximately 0.5. And the nitrate reductase activity was referred to the method of Li et al. (2022). The intensity of "red" around the colony after 24–48 h of incubation at 37 °C the activity of nitrate reductase in the isolates, with darker colors hues denoting higher activity. Specifically, "+++" indicates strong activity, "++" indicates moderate activity, "+" indicates weak activity, "- " indicates no activity.

Bacterial identification

Bacterial identification was referred to Li et al. (2022) with some modifications. In brief, the bacterial genomic DNA of pure strains was extracted using the TSP701-50 DNA extraction kit (Beijing Tsingke Biotech Co., Ltd., Beijing, China) according to the manufacturer's instructions. The 16S rDNA gene was amplified using 2720 thermal cycler PCR (Applied Biosystems, USA) with the universal primers 27F (5′-GAGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′- TACGGCTACCTTGTTACGAC-3′). The amplification cycles were as follows: 98 °C for 3 min; 39 cycles (98 °C for 10 s, 55 °C for 15 s, 72 °C for 15 s/kb); and then extension at 72 °C for 5 min. The amplified PCR products were subjected to agarose gel electrophoresis (2 μL sample + 6 μL bromophenol blue) and run at 300 V for 12 min to obtain identification gel images. The prepared PCR products were sent to Beijing Tsingke Biotech Co., Ltd. for first-generation sequencing.

In vitro fermentation capacity test

Aseptic fermented meat model

Aseptic pork was prepared with reference to the method of Fang et al. (2022) and meat models were established with reference to the method of Stavropoulou et al. (2018), with some modifications. In brief, fresh pork ham (purchased from local super market) was repaired into 15 cm × 10 cm × 10 cm by removing the fat fraction and soaked in boiling water for 10 min for surface sterilization. After chopping the raw, sterile inside meat aseptically, 3.0 % NaCl, 150 mg/kg sodium nitrite, and 500 mg/kg ascorbic acid were added, and the mixture was then stirred. Each conical flask was dispensed with 40.0 g of sterile minced meat, and then inoculated with 2 mL of bacterial suspension at a concentration of 5.0×10^{10} CFU/mL. Fermentation was continued at 10 °C for 15 days under anaerobic conditions and samples were taken for analysis after 0, 3, 6, 9, 12, and 15 days of incubation. At each time point, three containers of each fermenting bacteria were randomly selected for analysis.

Determination of BAs

The extraction and derivatization of BAs in the samples were performed by the method of Li et al. (2023). The samples were analyzed by HPLC (LC-2010AHT, Shimadzu, Japan) equipped with an SPD-M20A ultraviolet detector. The separation was performed on a ChromplusTM C18 column (Swell scientific instruments Co., Ltd., 250 mm × 4.6 mm × 5 μm, Chengdu, China), which was maintained at 35 °C with a flow rate of 0.8 mL/min. The mobile phase and gradient elution procedures referred to the method of Chinese Standard GB5009.208–2016.

Determination of protein degradation

The extraction of sarcoplasmic and myofibrillar proteins from fermented meat was based on the method of Yu et al. (2020), and SDS-PAGE was used to visualize the degradation profiles of these two proteins, which were separated using 12 % and 15 % polyacrylamide gels (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China), respectively. Two volumes of sample at a concentration of 2 mg/mL were mixed with 1 vol of 3 × loading buffer in a boiling water bath for 5 min. Then, 15 μL of sample and 8 μL of standard protein marker (Sangon Biotech (Shanghai) Co., Ltd., Shanghai, China) were added to the gels and electrophoresed at 120 V, 80 mA for 3 h. After electrophoresis, the gels were stained in a 0.1 % (w/v) solution of Coomassie Brilliant Blue R-250 in 50 % methanol and 10 % acetic acid for 0.5 h and then decolorized until a clear background resulted.

Determination of peptides

The peptides of fermented meat were extracted using the method of Sun et al. (2023), with some modifications. 5 g minced fermented meat was homogenized with 25 mL of 0.05 mol/L phosphate buffer at pH 7.0 in an ice bath at 4900 rpm for 3 min. The homogenate was inactive for 2 h at 4 °C and then centrifuged at 10000 rpm for 20 min at 4 °C to obtain the supernatant. The supernatant was filtered through Whatman 4.0 filter paper and mixed with 15 % (w/v) trichloroacetic acid in equal volume, and then incubated for 30 min. The supernatant was centrifuged at 6500 rpm at 4 °C for 10 min and obtained the supernatant again. The peptides content was calculated by measuring the absorbance of the supernatant at a wavelength of 540 nm using Biuret method. Each experiment was repeated three times.

Determination of FAAs

FAAs in fermented meats were measured in accordance with the instruction outlined by our previous study (Yang et al., 2023). For hydrolysis, 2.5 g of chopped fermented meat was added to 10 mL of 5 % TCA. The mixture was then thoroughly mixed by rotating at 4700 rpm for 2 min. The first supernatant was obtained from the homogenized stuff by centrifugation (4 °C, 10 min, 10000 rpm). The second supernatant was obtained by adding the proper perchloric acid solution to the residual precipitate, mixing it, and repeating the steps above. The two supernatants were combined and the pH was adjusted to 2 with 5 % NaOH solution. Then, the volume was adjusted to 25 mL with distilled water. The mixture was shaken and passed through a 0.22 μm filter membrane, and then quantified using an automated amino acid analyzer (L-8900, Hitachi High Technologies Corp., Japan).

Statistical analysis

One-way analysis of variance was performed on the significance level of $P < 0.05$ in isolates of proteolytic activity, lipase activity, BAs, and FAAs using IBM SPSS Statistics (version 29.0, IBM Co., USA). Graphics were constructed using OriginPro 2023 software (Origin Lab Corp., MA, USA) and GraphPad Prism 9 (GraphPad Software, Boston, USA). Biochemical reactions, safety indices, enzymatic characterization and fermentation performance of *Staphylococci* were repeated three times, and all the analyses were performed in triplicate. Data were expressed as mean ± standard error (n = 3).

Results and discussion

Screening of Staphylococci

Staphylococcus spp. are non-motile, non-spore forming, facultative anaerobe, and catalase positive cocci. Their cell walls are dense and thick with peptidoglycan, which can be stained blue-purple by Gram staining solution (Khusro and Aarti, 2022). Simultaneously, due to the similarity in properties of *Staphylococcus* and *Micrococcus*, we used their sensitivity to furazolidone to distinguish them, where *Staphylococcus* is

positive and *Micrococcus* is negative (Kayili and Sanlibaba, 2020). In our research, 317 strains with a morphology of 1–4 mm round, convex, white, yellow, light yellow, and earthy yellow in color, with a smooth border, and Gram staining of blue-purple were isolated from 14 kinds of traditional Chinese bacon. The typical morphology of the isolates is shown in Fig. 1A. Additionally, the catalase activity test yielded a total of 119 strong (+++) catalase activity, 127 moderate (++) catalase activity, 27 weak (+) catalase activity, 35 very weak (+¯) catalase activity and 9 no (-) catalase activity. Among these bacteria, 21 strains were weakly viable (unable to survive for longer than one week on TSA medium), and 33 strains were mucoid colonies. The remaining strains were subjected to furazolidone sensitivity experiments, and the results are shown in Fig. 1B. Thirty-four (15.53 %) of the isolates lacked a distinct region, showing their insensitivity to furazolidone. In total, 66 strains made up around 30.14 % of the population, with the clear zone diameters between 16.0 and 17.0 mm. In summary, we obtained 185 isolates for further experimentation.

Analysis of bacterial safety

It is well known that microbial hemolytic activity is associated with anemia and edema in the host (Li et al., 2022). However, the hemolytic activity of microorganisms cannot be determined by detecting their hemolytic genes alone, because phenotypic hemolytic activity and genotype seldom match (Jeong et al., 2016). Therefore, we examined the phenotypic hemolytic activity of 185 strains of bacteria on blood agar plates. By using a hemolytic activity assay, we obtained 58 isolates (31.4 %) with neither β- nor α- hemolytic activity. On blood agar plates, their colonies lacked defined zones and abnormal color, and instead displayed a typical white and yellow tint. This detection rate is similar to the findings of Zell et al. (2008), who found that 330 CNS isolates from various origins had weak to moderate hemolytic activity in 59 % (with human blood) and 34 % (with sheep blood) of them.

DNase, an extracellular enzyme, is commonly considered a typical characteristic of *S. aureus*, *S. intermedius*, and *S, hyicus* (Subathra Devi et al., 2016). DNase will act by depolymerizing DNA, which will result in the colony in the culture media generating a translucent halo (Moraes et al., 2021). Thus, the DNase activity of the isolates was verified to obtain CNS. Experimentally, 94 (50.81 %) of the 185 isolates were found to be DNase-negative that were considered as CNS for further experiments.

Coagulase is an extracellular enzyme of some *Staphylococci,* and it is considered a virulence factor of this microorganism. When it contacts with blood plasma, coagulase reacts with prothrombin and converts fibrinogen to fibrin, causing the plasma to coagulate (Moraes et al., 2021). In this study, 11 of the 32 isolates were coagulase-positive bacteria by coagulase activity assay.

The biologically active BAs are low molecular weight, antinutritional nitrogenous components that are formed when microbial decarboxylases react with FAAs. They affect the food freshness and organoleptic properties and can even lead to food poisoning (Ashaolu et al., 2023). We qualitatively investigated the ability of 21 isolates to produce BAs in decarboxylase, and the experimental results were shown in Fig. 1C. Five of the 21 strains (XW-A7, XW-A9, WX-M3, WX-M16 and PE-A12) had a change in the color of the control tube, indicating that these strains could not produce acid by decomposing glucose under anaerobic conditions. Further verification is needed for their ability to produce BAs. The remaining isolates, which were all able to produce cadaverine and spermidine or spermine by utilizing lysine and arginine, continued to exhibit this phenomenon from the first day of the experiment until the fourth day. The ability of these bacteria to utilize cysteine, histidine, and ornithine to create other BAs varied between strains and over the course of culture. Especially, the color of the test tube of GY-M7 mutated at the fourth day when it utilized ornithine metabolism. This might be caused by the bacteria using ornithine decarboxylase to produce putrescine, which was further converted to spermidine or spermine (Gao et al., 2023). The BB-M12 isolate was unable to produce BAs from ornithine, and the MY-A32, QCS-M8, and MN-A5 isolates were able to produce BAs from cysteine, although this ability was quickly diminished over time. The ability of WX-M8, CK-M21, MY-A15, QCS-M8, and PE-A20 isolates to generate BAs using histidine also declined with the prolongation of cultivation time. These phenomena indicate that the content of BAs did not simply satisfy the cumulative effect during fermentation, they showed a certain regular dynamic change due to the metabolism of CNS. Comprehensive safety characteristics of the isolates were used to validate the functional characteristics of these 21 strains.

Analysis of fermentation-related enzymes

The protease produced by CNS is effective in degrading peptides and

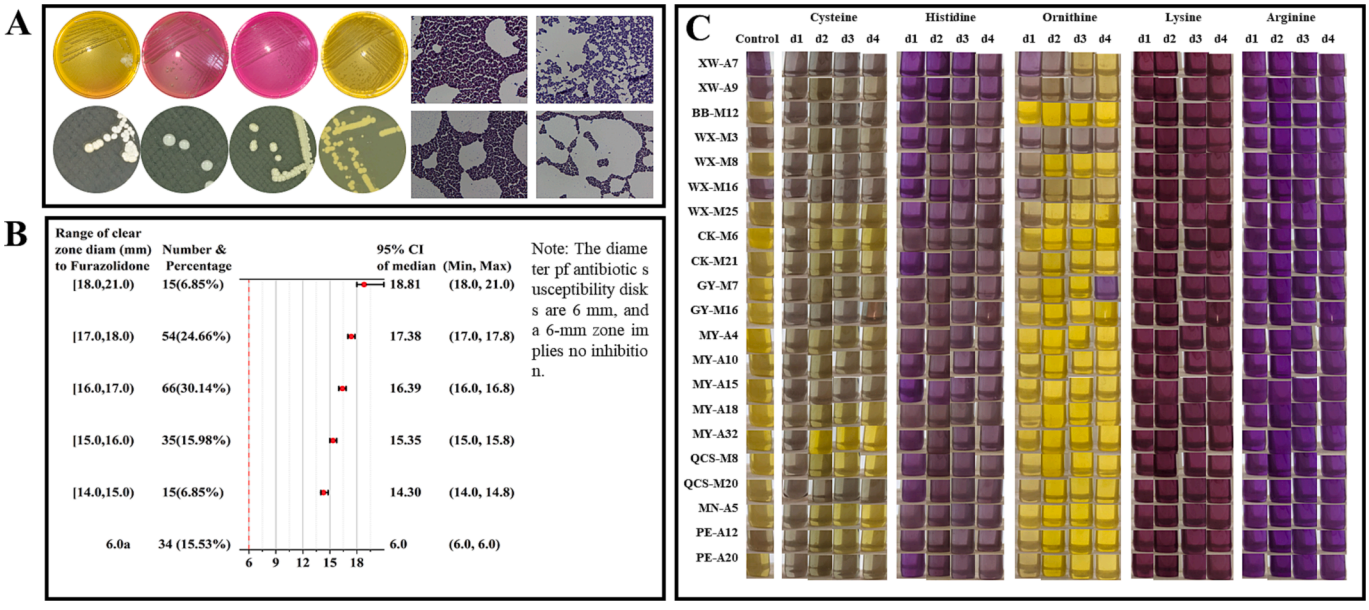

Fig. 1. Typical morphology and safety testing of the isolates. (A) Morphology, shape and color of colonies, (B) Sensitivity statistics of the isolates to furazolidone, (C) Decarboxylase activity experiment.

converting them into FAAs, and this protein degradation could reach 40 % during the fermentation process (Sanchez Mainar et al., 2017). Additionally, taste-active peptides, amino acids and amino acid derivatives are the main tastants in many fermented foods and impart bitter, umami or kokumi taste (Zhao et al., 2016). 13 out of the 21 isolates investigated in the experiment demonstrated protease activity, with the clear zone of the MY-A15 isolate exhibiting a much wider diameter than the others (22.2 ± 0.28 mm). In addition, XW-A7、WX-M3、GY-M16、MY-A18、MN-A5, and PE-A20 isolates all exhibited strong protease activity (Fig. 2A).

The lipase produced by CNS can degrade fatty acids through incomplete β-oxidation to produce FFAs (Sanchez Mainar et al., 2017). Although FFAs contribute less to the taste of fermented meat, they are the precursors of esters, aldehydes, ketones, lactones, and alcohols, which are beneficial for the formation of fermented meat flavor. Most of the isolates (15 out of 21) showed lipase activity, with the PE-A20 isolate exhibiting the strongest lipase activity ($P < 0.05$, with a clear area diameter of 19.75 ± 0.35 mm), followed by WX-M8 (17.00 ± 0.71 mm). Among these lipase positive strains, strains XW-A7, WX-M3, WX-M8, WX-M25, MY-A18, QCS-M8, PE-A12, and PE-A20 also exhibit protease activity (Fig. 2A). These strains may favor the development of the flavor of Chinese bacon.

Nitrate reductase in CNS is responsible for the production of nitrosomyoglobin, which gives fermented meat its red color. However, the nitrate reductase activity was not consistent for different species of CNS (Khusro and Aarti, 2022). Nineteen isolates of nitrate reductase-positive bacteria were selected from 21 isolates, among which all isolates except MY-A4, MY-A18, QCS-M8, and PE-A20 isolates showed strong activity of "+++" (Fig. 2A). These nitrate reductase positive strains can be used as a curing agent to promote the formation of flesh color in fermented meat with additional nitrate. In contrast, nitrate reductase negative strains can be added directly to meat products that contain only nitrite as a curing agent.

Generally, we selected 7 isolates with protease activity, lipase activity and nitrate reductase activity, namely XW-A7, WX-M3, WX-M8, WX-M25, MY-A18, QCS-M8, and PE-A20, 3 isolates with only protease activity and nitrate reductase activity, namely GY-M16, MY-A15, and MN-A5, and 1 isolate with only protease activity, namely MY-A10, for bacterial identification.

Identification of CNS strains

Table 1 lists the representative the NCBI accession numbers of isolates along with their percentage of identification. The 11 isolates, including 1 *S. equorum*, 1 *S. nepalensis*, 1 *S. cohnii*, and 8 *S. saprophyticus*, were identified as coagulase-negative novobiocin-resistant bacteria belonging to the *S. saprophyticus-group*. It is generally believed that only strains with intrinsic resistance or intrinsic resistance due to chromosomal genetic mutations and with a low horizontal transmission risk are acceptable (Sanchez Mainar et al., 2017). Antibiotic resistance is widespread in *Staphylococcus* and situated at the strain level. Some commonly starters, such as *S. xylosus*, *S. equorumh*, and *S. carnosus*, are

Table 1
Homology (%) of sequenced genes (16S rRNA) for isolates obtained from laboratory diagnosis and biochemical tests and the accession number of the entry with the highest homology.

Isolates	Colony morphology	Scientific Name	Identity/ %	NCBI Accession
XW-A7	1–2.5 mm round, convex, white, smooth	*Staphylococcus equorum*	100.00	NR_041926.1
WX-M3	2–3 mm round, convex, earthy yellow, smooth	*Staphylococcus nepalensis*	99.58	NR_028996.1
WX-M8	3–4 mm round, convex, light yellow, smooth	*Staphylococcus cohnii*	100.00	NR_036902.1
WX-M25	2.5–3 mm round, convex, light yellow, smooth	*Staphylococcus saprophyticus*	99.86	NR_114090.1
GY-M16	0.5–4 mm round, convex, yellow, smooth	*Staphylococcus saprophyticus*	99.93	NR_074999.2
MY-A10	2–4 mm round, convex, yellow, smooth	*Staphylococcus saprophyticus*	99.93	NR_114090.1
MY-A15	0.5–4 mm round, convex, yellow, smooth	*Staphylococcus saprophyticus*	100.00	NR_115607.1
MY-A18	1.5–4.5 mm round, convex, yellow, smooth	*Staphylococcus saprophyticus*	100.00	NR_074999.2
QCS-M8	0.5–3.5 mm round, convex, yellow, smooth	*Staphylococcus saprophyticus*	99.93	NR_074999.2
MN-A5	0.5–4 mm round, convex, light yellow, smooth	*Staphylococcus saprophyticus*	100.00	NR_074999.2
PE-A20	0.1–3 mm round, convex, white, smooth	*Staphylococcus saprophyticus*	99.86	NR_114090.1

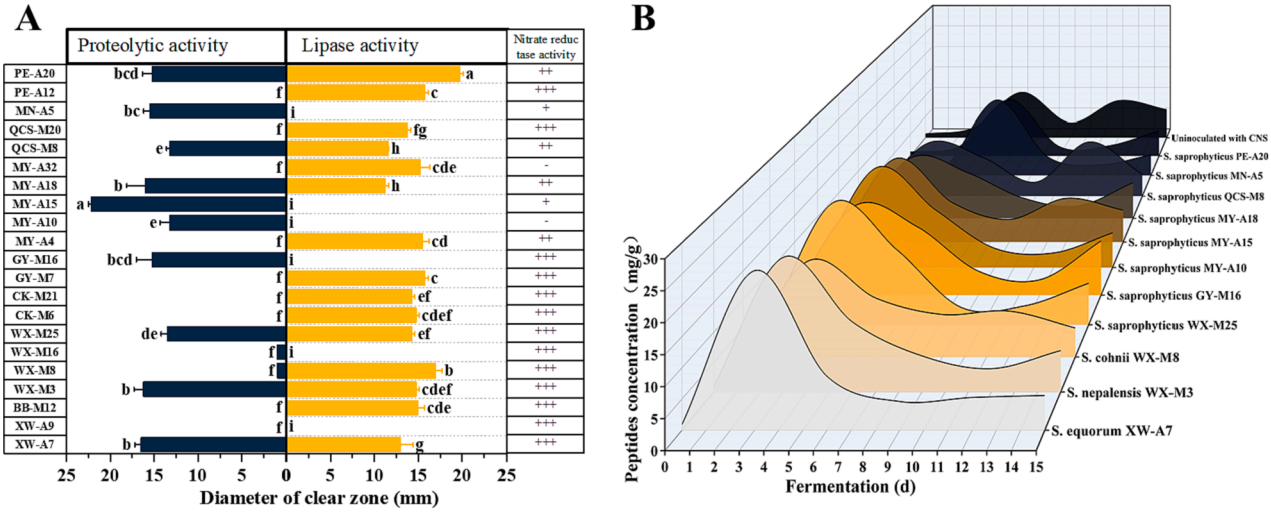

Fig. 2. Enzyme activity and peptide-producing characteristics of *Staphylococcus*. (A) Fermentation-related enzyme activities, (B) Changes in peptide concentration over fermentation time.

among the most frequently reported as antibiotic-resistant (Khusro and Aarti, 2022). Therefore, all 11 CNS strains can be further studied as potential starter cultures.

In vitro fermentation of 11 CNS strains

Bas during fermentation

Meat and meat products are rich in protein and amino acids, and are susceptible to the production of BAs by amino acid decarboxylases in microorganisms (Schirone et al., 2022). Several factors related to the raw material, pH and chemical composition, as well as some treatments such as manufacturing operations (e.g., fermentation and ripening, etc.), as in addition to temperature and storage time, influence the activity of microorganisms and thus the production of BAs. To investigate the changes in the content of BAs during CNS fermentation, we used the muscle fraction of pork as a fermentation substrate to simulate the fermentation of bacon. As shown in Table 2, tryptamine was not found and very small levels of 2-phenylethylamine were detected in pork inoculated without CNS. The total content of ABs was 174.04 ± 8.73 μg/g, with no significant difference between periods ($P > 0.05$, Table 2). As naturally occurring BAs in fresh meat, the contents of spermidine and spermine was at a low level indicating that the pork was fresh (Schirone et al., 2022). Putrescine was the most abundant BA, which may cause rapid spoilage of meat. The reason for this phenomenon may be that the content of putrescine in minced and reconstituted meat increases due to decomposition (Kalac, 2006).

Significant changes in the content of BAs occurred when CNS were inoculated into the meat. Apparently, the level of BAs does not always increase during fermentation. For pork inoculated with CNS, there were five tendencies for the total amount of BAs during fermentation (Table 2): 1) increase followed by decrease, including *S. equorum XW-A7*, *S. saprophyticus GY-M16*, *S. saprophyticus MY-A18*, *S. saprophyticus QCS-M8*, *S. saprophyticus MN-A5*, and *S. saprophyticus PE-A20*; 2) increase followed by decrease and then increase, including *S. nepalensis WX-M3* and *S. saprophyticus MY-A15*; 3) increase followed by decrease and then increase followed by decrease, including *S. saprophyticus WX-M25*; 4) decrease followed by increase and then decrease, including *S. saprophyticus MY-A10*; 5) decrease followed by increase and then decrease followed by increase, including *S. cohnii WX-M8*. A similarly non-accumulative trend was observed for the content of the eight measured BAs, which fluctuated dynamically with the duration of fermentation. Among them, spermine, tyramine, putrescine, histamine and cadaverine were the most prevalent and showed the most pronounced trends. Tyramine, putrescine, histamine and cadaverine were commonly detected in meat products (Gao et al., 2023). Notably, spermine is known to react with nitrite in meat to form carcinogenic nitrosamines, leading to serious health risks.

The type of BAs produced from CNS is species or strain dependent (Khusro and Aarti, 2022). Some CNS with strong protease activity can participate more quickly in the process of protein degradation in meat, generating peptides and FAAs. Subsequently, amino acid decarboxylases produced by the microorganisms decarboxylate the amino acids to produce BAs. However, the specificity of amino acid decarboxylases in *Staphylococcus* is uncritical, leading to a more complex composition of BAs (Gao et al., 2023). Therefore, a discussion of the composition of BAs at particular period is not an adequate way to assess the toxicity of BAs in fermented meat products. In traditional processing, Chinese bacon is cured at lower oxygen concentrations for 2–10 days (Ruan et al., 2023), so CNS with lower BA content is preferable in terms of BAs production potential. In our study, *S. cohnii WX-M8* and *S. saprophyticus MY-A10* belonged to the fourth and fifth cases mentioned above, they possessed the ability to diminish the content of BAs, and they possessed the lowest BAs content on the third day, 35.23 ± 7.93 μg/g and 61.62 ± 9.49 μg/g, respectively. The reason for this phenomenon may be that at the beginning of CNS colonization, there were few FAAs in meat and more BAs could be degraded than could be produced. With the prolongation of fermentation, the content of FAAs increased and the BAs production capability of CNS was much higher than that of degradation. Nevertheless, the protease activity of *S. saprophyticus MY-A10* was greater than that of *S. cohnii WX-M8*, and the capacity of BAs accumulation was significantly inferior to that of *S. cohnii WX-M8*, which seemed to indicate that the capacity of BAs degradation of *S. saprophyticus MY-A10* was active throughout the fermentation process. In addition to the two types of CNS, *S. equorum XW-A7*, *S. saprophyticus MY-A18*, and *S. saprophyticus PE-A20* were able to generate lower contents of BAs during the first six days of curing. They were all CNS with proteases and lipases that could also act as potential CNS starters (The ability of *S. saprophyticus MY-A18* to produce BAs was well predicted in the qualitative experiments described in Fig. 1C). We found that most CNS (*S. cohnii WX-M8* on day 6) accumulated the highest levels of BAs on day 9, which may be caused by the conversion of BAs into other substances, but the reasons are complex and no similar studies have been reported.

Peptides during fermentation

The protein in pork consists mainly of connective-tissue proteins (3–15 %), sarcoplasmic proteins (30–40 %), and myofibrillar proteins (around 55 %). The content of connective tissue remains basically unchanged within 5 months after slaughter, whereas sarcoplasmic and myofibrillar proteins are broken down due to pH, storage temperature, storage time and endogenous enzymes (Khan, 1977). The concentration of peptides in meat uninoculated with CNS reached a maximum level of 10.76 ± 0.57 mg/g on day 6, then decreased and increased again to 8.73 ± 3.75 mg/g on day 12 (Fig. 2B). It was shown that during meat fermentation, the activities of dipeptidyl peptidase I, alanyl aminopeptidase, leucyl aminopeptidase, prolyl aminopeptidase, and lysyl aminopeptidase remained essentially constant, which could promote the accumulation of small molecular peptides, especially the types with molecular weights less than 3 KDa. Some peptides with molecular weights of 1 to 2 KDa were the most abundant species, and they showed higher levels in both the late curing and maturation stages (Zhou, 2020)). In our study, this phenomenon showed a more pronounced trend because of the inoculation with *Staphylococcus*. In particular, in all meat inoculated with CNS, the peptide concentrations peaked twice along with higher levels, but at inconsistent times. Besides *S. saprophyticus MN-A5* and *S. saprophyticus PE-A20* where the first peak appeared on day 6, the first peak of peptide concentration appeared around day 3 in all the CNS-inoculated meat. Among them, *S. equorum XW-A7* appeared at less than 3 days and showed the highest peptide concentration of 25.52 ± 0.15 mg/g. On day 3, concentrations of the peptides were also greater in *S. nepalensis WX-M3* and *S. saprophyticus WX-M25*, 22.52 ± 0.26 mg/g and 22.27 ± 1.26 mg/g, respectively. Meanwhile, the peptide concentrations of *S. cohnii WX-M8* and *S. saprophyticus MY-A10* remained a higher level of 16.85 ± 0.58 and 19.63 ± 1.66 mg/g, respectively. The patterns of peptide content formation could help us find CNS that shorten the fermentation period and optimize the fermentation process of Chinese bacon.

Protein degradation during fermentation

We selected *Staphylococci* with low levels of BAs and high levels of peptides, namely *S. cohnii WX-M8*, *S. equorum XW-A7*, *S. saprophyticus MY-M10* and *S. saprophyticus MY-M18*, observing their ability to degrade sarcoplasmic proteins (Fig. 3A) and myofibrillar proteins (Fig. 3B) during the fermentation process. The protein profile of sarcoplasmic proteins (Fig. 3A) consists of a series of molecular weight proteins of approximately 55 (glucose phosphate isomerase), 48 (enolase), 45 (creatine phosphate kinase), 42 (aldolase), 37 (glyceraldehyde phosphate dehydrogenase), 35, and 28 kDa (Cachaldora et al., 2013). Similar to previous studies (Drosinos et al., 2007; Ju et al., 2022), we found that *S. cohnii WX-M8* and *S. saprophyticus MY-A18* exhibited a weak degradation activity of sarcoplasmic protein, as evidenced by the fading of the 35 KDa band with fermentation time. The protein profile of myofibrillar proteins (Fig. 3B) consists of a series of molecular weight proteins of

Table 2
Changes in the content of individual and total BAs during the fermentation of pork inoculated 11 different CNS strains.

CNS	Fermentation time/(d)	The content of biogenic amines/(μg/g)								
		TRP	PEA	PUT	CAD	HIS	TYR	SPD	SPM	Total BAs
Without microbial inoculation	0	n. d	2.83 ± 1.61^{Eb}	61.47 ± 2.35^{Abc}	54.66 ± 3.33^{Ba}	28.17 ± 0.38^{Ca}	14.69 ± 0.30^{Dcd}	15.33 ± 1.87 ^{Da}	2.54 ± 0.63^{Eb}	179.68 ± 10.52^{a}
	3	n. d	7.26 ± 0.64^{Ea}	61.05 ± 1.87^{Ac}	53.22 ± 2.83^{Bab}	22.00 ± 3.06^{Ccd}	17.25 ± 0.26^{Db}	7.06 ± 0.28^{Ed}	6.05 ± 1.15^{Ea}	173.88 ± 10.14^{a}
	6	n. d	n. d	66.53 ± 3.22^{Aa}	58.80 ± 2.34^{Ba}	20.29 ± 1.57^{Cd}	16.64 ± 1.12^{Db}	11.94 ± 0.04^{Eb}	7.25 ± 1.38^{Fa}	181.45 ± 9.95^{a}
	9	n. d	n. d	60.72 ± 1.51^{Ac}	51.89 ± 0.87^{Bab}	23.99 ± 1.56^{Cbc}	14.10 ± 1.12^{Dd}	10.71 ± 0.01^{Eb}	7.56 ± 0.81^{Fa}	168.97 ± 6.13^{a}
	12	n. d	n. d	65.62 ± 1.13^{Aab}	48.96 ± 1.55^{Bb}	25.22 ± 0.92^{Cab}	25.81 ± 0.42^{Ca}	8.57 ± 0.04^{Dc}	n. d	174.17 ± 4.16^{a}
	15	n. d	n. d	64.04 ± 3.21^{Ac}	51.64 ± 1.78^{Bab}	22.84 ± 1.47^{Ccd}	16.52 ± 1.25^{Dbc}	7.30 ± 0.40^{Ecd}	3.76 ± 0.04^{Fb}	166.10 ± 8.43^{a}
S. equorum XW-A7	0	n. d	2.83 ± 1.61^{Eb}	61.47 ± 2.35^{Ab}	54.66 ± 3.33^{Ba}	28.17 ± 0.38^{Cb}	14.69 ± 0.36^{Db}	15.33 ± 1.87^{Db}	2.54 ± 0.63^{Ed}	179.68 ± 10.52^{b}
	3	59.97 ± 7.05^{A}	17.88 ± 2.75^{Ca}	41.55 ± 6.91^{Bc}	11.80 ± 2.60^{CDc}	12.73 ± 1.61^{CDc}	6.50 ± 0.04^{Dc}	7.91 ± 0.07^{Dc}	66.39 ± 8.02^{Ab}	224.71 ± 29.04^{b}
	6	n. d	n. d	28.13 ± 4.06^{Bd}	13.12 ± 6.52^{Cc}	n. d	18.69 ± 3.97^{BCb}	23.69 ± 5.32^{Ba}	113.71 ± 10.65^{Aa}	197.33 ± 30.51^{b}
	9	n. d	n. d	105.81 ± 10.76^{Ba}	58.30 ± 4.46^{Ca}	40.22 ± 13.85 ^{Da}	61.10 ± 7.91^{Ca}	24.39 ± 6.43 ^{Da}	128.99 ± 15.42^{Aa}	418.81 ± 58.81^{a}
	12	n. d	n. d	100.56 ± 5.69^{Aa}	42.00 ± 4.96^{Bb}	24.13 ± 2.88^{Cbc}	14.28 ± 3.92^{Db}	4.07 ± 0.04^{Ec}	40.34 ± 4.44^{Bc}	225.3721.91^{b}
	15	n. d	n. d	32.53 ± 8.86^{Bcd}	8.14 ± 1.30^{CDc}	35.46 ± 3.93^{Bab}	16.82 ± 2.75^{Cb}	4.43 ± 0.07^{Dc}	73.75 ± 10.83^{Ab}	171.1220.71^{b}
S. nepalensis WX-M3	0	n. d	2.83 ± 1.61^{Ccd}	61.47 ± 2.35^{Aa}	54.66 ± 3.33^{Bc}	28.17 ± 0.38^{Cd}	14.69 ± 0.36^{Dd}	15.33 ± 1.87^{Db}	2.54 ± 0.63^{Cf}	179.68 ± 10.52^{f}
	3	32.10 ± 4.24^{C}	14.98 ± 0.02 ^{Da}	52.55 ± 3.04^{Ab}	33.23 ± 3.98^{Cd}	56.57 ± 2.96^{Ac}	10.61 ± 0.03^{Dd}	5.05 ± 0.00^{Ec}	44.96 ± 0.64^{Be}	250.05 ± 14.90^{e}
	6	n. d	11.97 ± 1.27^{Db}	46.70 ± 0.31^{Cc}	54.60 ± 6.87^{Bc}	50.45 ± 6.81^{BCc}	16.50 ± 1.53^{Dd}	16.46 ± 0.15^{Db}	129.23 ± 1.64^{Ac}	325.90 ± 18.58^{d}
	9	n. d	3.83 ± 0.00^{Gc}	43.67 ± 1.86^{Ec}	95.50 ± 1.86^{Cb}	54.92 ± 1.99^{Dc}	115.75 ± 5.30^{Bb}	28.45 ± 1.86^{Fa}	180.37 ± 10.24^{Aa}	522.51 ± 23.10^{a}
	12	n. d	3.99 ± 1.23^{Fc}	41.32 ± 0.31^{Ed}	92.17 ± 4.75^{Cb}	75.19 ± 4.75^{Db}	125.16 ± 7.90^{Aa}	5.12 ± 0.02^{Fc}	99.46 ± 1.06^{Bd}	442.41 ± 20.01^{c}
	15	n. d	1.23 ± 0.00^{Ed}	38.60 ± 1.03^{Dd}	147.53 ± 7.09^{Aa}	85.32 ± 3.09^{Ba}	63.05 ± 1.30^{Cc}	4.42 ± 1.25^{Ec}	147.10 ± 9.21^{Ab}	487.25 ± 22.97^{b}
S. cohnii WX-M8	0	n. d	2.83 ± 1.61^{Eb}	61.47 ± 2.35^{Ad}	54.66 ± 3.33^{Bd}	28.17 ± 0.38^{Cc}	14.69 ± 0.36^{De}	15.33 ± 1.87^{Dc}	2.54 ± 0.63^{Ee}	179.68 ± 10.52^{d}
	3	n. d	5.03 ± 130^{Ba}	3.36 ± 3.76^{Bf}	26.84 ± 2.88^{Ae}	n. d	n. d	n. d	n. d	35.23 ± 7.93^{e}
	6	n. d	n. d	145.19 ± 6.80^{Ba}	253.58 ± 18.52^{Aa}	29.54 ± 5.19^{Dc}	54.97 ± 8.12^{Cc}	53.78 ± 3.22^{Ca}	135.98 ± 10.18^{Bb}	673.04 ± 52.01^{a}
	9	n. d	n. d	44.78 ± 3.09^{De}	86.70 ± 3.17^{Bc}	63.98 ± 4.36^{Ca}	39.73 ± 0.61^{Dd}	26.47 ± 8.87^{Eb}	196.93 ± 16.97^{Aa}	458.58 ± 37.07^{c}
	12	n. d	n. d	100.01 ± 8.71^{Ac}	97.31 ± 10.97^{Ac}	54.28 ± 1.85^{Bb}	89.62 ± 6.08^{Ab}	23.20 ± 4.10C^{bc}	54.98 ± 3.21^{Bd}	419.39 ± 34.91^{c}
	15	n. d	n. d	122.23 ± 14.69^{Bb}	186.55 ± 8.13^{Ab}	60.77 ± 10.44^{Dab}	123.36 ± 7.79^{Ba}	15.81 ± 2.71^{Ec}	89.45 ± 3.04^{Cc}	598.18 ± 46.80^{b}
S. saprophyticus WX-M25	0	n. d	2.83 ± 1.61^{Ec}	61.47 ± 2.35^{Ac}	54.66 ± 3.33^{Bb}	28.17 ± 0.38^{Cc}	14.69 ± 0.36^{Dc}	15.33 ± 1.87^{Dd}	2.54 ± 0.63^{Ee}	179.68 ± 10.52^{d}
	3	n. d	26.29 ± 0.83^{Ea}	49.68 ± 2.87^{Cd}	84.69 ± 3.49^{Aa}	48.04 ± 4.12^{Cb}	34.24 ± 1.74 ^{Da}	46.86 ± 6.76^{Ca}	73.98 ± 5.96^{Bd}	363.77 ± 25.77^{b}
	6	n. d	15.19 ± 4.10^{Cb}	24.94 ± 10.06^{Ce}	23.42 ± 1.51^{Cd}	20.29 ± 6.39^{Cc}	21.72 ± 0.38^{Cb}	40.00 ± 4.34^{Bb}	125.29 ± 10.87^{Ab}	270.85 ± 37.65^{c}
	9	n. d	n. d	74.94 ± 4.72^{Bb}	40.32 ± 10.44^{Cc}	75.08 ± 4.72^{Ba}	18.24 ± 6.43^{Db}	27.77 ± 2.85^{Dc}	179.77 ± 7.74^{Aa}	416.11 ± 36.89^{a}
	12	n. d	n. d	123.49 ± 8.84^{Aa}	28.54 ± 4.07^{Dd}	39.20 ± 7.86^{Cb}	11.11 ± 1.25^{Ecd}	5.37 ± 1.29^{EFe}	89.57 ± 4.72^{Bc}	297.27 ± 28.01^{c}
	15	n. d	n. d	33.04 ± 5.68^{Ce}	13.44 ± 2.71^{De}	44.57 ± 6.91^{Bb}	7.35 ± 0.10^{DEd}	4.04 ± 2.49^{Ee}	74.46 ± 5.83^{Ad}	176.90 ± 23.71^{d}
S. saprophyticus GY-M16	0	n. d	2.83 ± 1.61^{Eb}	61.47 ± 2.35^{Ab}	54.66 ± 3.33^{Bb}	28.17 ± 0.38^{Cc}	14.69 ± 0.36^{Dcd}	15.33 ± 1.87 ^{Da}	2.54 ± 0.63^{Ef}	179.68 ± 10.52^{c}
	3	62.94 ± 7.67^{B}	19.66 ± 2.75^{Ca}	89.27 ± 7.17^{Aa}	60.66 ± 4.58^{Bb}	24.89 ± 4.09^{Ccd}	6.54 ± 0.07^{Dd}	7.45 ± 0.02^{Dbc}	55.03 ± 7.77^{Be}	326.43 ± 34.11^{b}
	6	n. d	n. d	46.56 ± 7.77^{Bc}	37.83 ± 2.87^{Cb}	n. d	25.22 ± 5.28^{Dc}	18.78 ± 2.75 ^{Da}	142.93 ± 9.87^{Ab}	271.32 ± 28.53^{b}
	9	n. d	n. d	35.79 ± 5.06^{Dd}	130.39 ± 10.44^{Ba}	37.13 ± 1.67^{Db}	57.20 ± 10.72^{Cb}	18.75 ± 4.10^{Ea}	167.15 ± 11.47^{Aa}	446.40 ± 43.44^{a}
	12	n. d	n. d	23.71 ± 6.55^{Ce}	74.26 ± 4.38^{Bb}	22.00 ± 3.84^{Cd}	87.83 ± 12.20^{Aa}	9.19 ± 2.55^{Db}	85.83 ± 5.91^{Ad}	302.82 ± 35.43^{b}
	15	n. d	n. d	17.86 ± 2.60^{Ee}	32.69 ± 2.87^{Db}	45.44 ± 5.55^{Ca}	60.72 ± 9.53^{Bb}	4.34 ± 0.04^{Fc}	119.65 ± 9.62^{Ac}	280.69 ± 30.21^{b}

(*continued on next page*)

Table 2 (*continued*)

CNS	Fermentation time/(d)	The content of biogenic amines/(μg/g)								
		TRP	PEA	PUT	CAD	HIS	TYR	SPD	SPM	Total BAs
S. saprophyticus MY-A10	0	n. d	2.83 ± 1.61^{E}	61.47 ± 2.35^{Ab}	54.66 ± 3.33^{Ba}	28.17 ± 0.38^{Cb}	14.69 ± 0.36^{Dbc}	15.33 ± 1.87^{Db}	2.54 ± 0.63^{Ee}	179.68 ± 10.52^{c}
	3	n. d	n. d	n. d	61.62 ± 9.49^{a}	n. d	n. d	n. d	n. d	61.62 ± 9.49^{d}
	6	n. d	n. d	42.22 ± 3.98^{Bc}	33.42 ± 2.83^{Bb}	1.20 ± 0.64^{Cd}	8.36 ± 0.71^{Ccd}	33.31 ± 6.41^{Ba}	138.42 ± 13.74^{Ab}	256.92 ± 28.32^{b}
	9	n. d	n. d	83.22 ± 9.62^{Ba}	34.16 ± 10.10^{Db}	27.17 ± 2.84^{Db}	56.71 ± 8.12^{Ca}	29.88 ± 7.61 ^{Da}	228.09 ± 12.26^{Aa}	459.24 ± 50.53^{a}
	12	n. d	n. d	80.92 ± 3.47^{Aa}	28.64 ± 0.38^{Bb}	19.30 ± 3.98^{Cc}	16.96 ± 5.16^{Cb}	4.56 ± 0.64^{Dc}	84.03 ± 6.84^{Ac}	234.41 ± 27.83^{b}
	15	n. d	n. d	32.64 ± 10.19^{Bc}	11.13 ± 4.44^{Cc}	35.90 ± 6.64^{ABa}	10.77 ± 1.39^{Cbc}	1.79 ± 1.26^{Cc}	44.70 ± 11.80^{Ad}	136.93 ± 35.69^{c}
S. saprophyticus MY-A15	0	n. d	2.83 ± 1.61^{Eb}	61.47 ± 2.35^{Aab}	54.66 ± 3.33^{Bd}	28.17 ± 0.38^{Ce}	14.69 ± 0.36^{Dd}	15.33 ± 1.87^{Dbc}	2.54 ± 0.63^{Ee}	179.68 ± 10.52^{e}
	3	49.05 ± 4.31^{C}	18.90 ± 7.74^{Ea}	69.29 ± 4.12^{Aa}	31.26 ± 6.51^{Dd}	51.65 ± 2.88^{BCbc}	11.15 ± 1.29^{Ed}	9.95 ± 4.92^{Ecd}	60.31 ± 7.78^{ABd}	301.56 ± 39.53^{d}
	6	n. d	n. d	51.88 ± 5.36^{Cbc}	292.65 ± 12.69^{Aa}	50.43 ± 1.62^{Cc}	36.81 ± 5.73^{Dc}	20.71 ± 6.55^{Eab}	141.69 ± 8.95^{Bb}	594.16 ± 40.89^{a}
	9	n. d	n. d	30.59 ± 6.43^{Dd}	178.40 ± 14.96^{Ab}	50.70 ± 5.20^{Cc}	104.75 ± 6.79^{Ba}	27.37 ± 8.84 ^{Da}	193.66 ± 14.94^{Aa}	585.47 ± 57.15^{a}
	12	n. d	n. d	62.25 ± 10.60^{Cab}	113.15 ± 11.07^{Ac}	57.90 ± 4.42^{Cb}	65.30 ± 3.98^{Cb}	2.66 ± 6.03^{Dd}	81.89 ± 7.17^{Bc}	383.14 ± 37.22^{c}
	15	n. d	n. d	40.06 ± 7.99^{Dcd}	174.41 ± 10.10^{Ab}	70.93 ± 5.20^{Ca}	105.01 ± 9.27^{Ba}	5.60 ± 6.02^{Ecd}	95.83 ± 4.45^{Bc}	491.84 ± 43.50^{b}
S. saprophyticus MY-A18	0	n. d	2.83 ± 1.61^{Eb}	61.47 ± 2.35^{Ac}	54.66 ± 3.33^{Bb}	28.17 ± 0.38^{Cc}	14.69 ± 0.36^{Dc}	15.33 ± 1.87^{Db}	2.54 ± 0.63^{Ee}	179.68 ± 10.52^{d}
	3	56.45 ± 9.36^{Ab}	10.73 ± 3.77^{Ca}	37.31 ± 5.32^{Bd}	39.71 ± 3.97^{Bc}	8.63 ± 1.29^{Cd}	7.09 ± 0.29^{Cd}	7.52 ± 0.44^{Cc}	14.10 ± 1.38^{Cd}	181.54 ± 25.79^{d}
	6	n. d	n. d	31.88 ± 3.94^{Bd}	14.85 ± 2.72^{De}	0.00 ± 0.02^{Fe}	10.05 ± 0.44^{Ed}	23.03 ± 1.74^{Ca}	133.01 ± 5.28^{Aa}	212.82 ± 14.12 ^{cd}
	9	n. d	n. d	137.25 ± 6.83^{Aa}	76.46 ± 9.62^{Ca}	71.71 ± 3.03^{CDa}	65.40 ± 4.72 ^{Da}	25.43 ± 1.67^{Ea}	124.70 ± 8.53^{Ba}	500.95 ± 34.38^{a}
	12	n. d	n. d	93.64 ± 9.62^{Ab}	26.20 ± 5.32^{Cd}	26.49 ± 1.65^{Cc}	40.11 ± 3.20^{Bb}	24.80 ± 1.42^{Ca}	96.43 ± 5.94^{Ab}	307.65 ± 27.12^{b}
	15	103.69 ± 3.09^{Aa}	n. d	20.35 ± 5.33^{De}	18.28 ± 3.98^{De}	36.63 ± 5.45^{Cb}	17.48 ± 1.57^{Dc}	4.91 ± 1.26^{Ec}	50.13 ± 3.23^{Bc}	251.47 ± 23.91^{c}
S. saprophyticus QCS-M8	0	n. d	2.83 ± 1.61^{Eb}	61.47 ± 2.35^{Aa}	54.66 ± 3.33^{Bb}	28.17 ± 0.38^{Cd}	14.69 ± 0.36^{Df}	15.33 ± 1.87^{Dab}	2.54 ± 0.63^{Ee}	179.68 ± 10.52^{e}
	3	n. d	13.55 ± 0.03^{Ea}	61.22 ± 4.27^{Aa}	40.31 ± 3.98^{Cc}	52.61 ± 4.11^{Bc}	25.56 ± 0.39^{De}	7.54 ± 2.55^{Fc}	41.97 ± 0.27^{Cd}	242.76 ± 15.59^{d}
	6	25.10 ± 1.86^{D}	n. d	64.59 ± 2.75^{Ba}	13.65 ± 0.29^{Ed}	35.19 ± 1.74^{Cd}	32.95 ± 1.63^{Cd}	13.02 ± 1.64^{Eb}	131.30 ± 2.74^{Ab}	315.81 ± 13.65^{c}
	9	n. d	n. d	59.46 ± 1.97^{BCa}	69.18 ± 5.95^{Ba}	56.27 ± 5.82^{Cc}	60.77 ± 0.64^{BCc}	19.04 ± 3.98 ^{Da}	165.43 ± 13.09^{Aa}	430.16 ± 31.45^{a}
	12	n. d	n. d	54.53 ± 2.76^{Db}	56.23 ± 5.82^{Db}	66.87 ± 6.89^{Cb}	86.25 ± 2.37^{Bb}	4.14 ± 0.07^{Ec}	120.77 ± 3.93^{Ab}	388.79 ± 20.44^{b}
	15	n. d	n. d	42.90 ± 0.64^{Cc}	9.16 ± 0.10^{Dd}	88.43 ± 2.75^{Ba}	109.30 ± 5.16^{Aa}	4.95 ± 2.48^{Dc}	88.92 ± 3.99^{Bc}	343.66 ± 15.12^{c}
S. saprophyticus MN-A5	0	n. d	2.83 ± 1.61^{Eb}	61.47 ± 2.35^{Ab}	54.66 ± 3.33^{Ba}	28.17 ± 0.38^{Cc}	14.69 ± 0.36^{Dd}	15.33 ± 1.87 ^{Da}	2.54 ± 0.63^{Ee}	179.68 ± 10.52^{d}
	3	43.63 ± 5.67^{Ba}	16.68 ± 2.75^{Ca}	57.42 ± 8.02^{Ab}	38.47 ± 5.29^{Bb}	42.72 ± 4.32^{Babc}	3.54 ± 0.44^{Dd}	4.32 ± 0.04^{Dc}	47.87 ± 8.00^{Bd}	254.66 ± 34.52^{c}
	6	n. d	n. d	49.18 ± 3.98^{Bb}	23.66 ± 3.97^{Cc}	46.14 ± 10.19^{Bab}	9.04 ± 2.60^{Dd}	11.26 ± 1.41^{Db}	141.22 ± 11.02^{Ab}	280.49 ± 33.17^{bc}
	9	37.49 ± 2.00^{Cb}	n. d	57.92 ± 9.32^{Bb}	12.11 ± 3.83^{Dd}	41.16 ± 3.94^{Cbc}	62.76 ± 10.87^{Bb}	13.90 ± 1.25^{Dab}	202.11 ± 16.62^{Aa}	427.45 ± 43.82^{a}
	12	n. d	n. d	99.25 ± 10.19^{Aa}	21.24 ± 6.39^{DEc}	48.52 ± 9.62^{Cab}	30.58 ± 7.74^{Dc}	13.19 ± 2.53^{Eab}	65.46 ± 10.58^{Bcd}	278.23 ± 47.04^{bc}
	15	n. d	n. d	22.95 ± 3.20^{DEc}	29.19 ± 4.10^{Db}	57.45 ± 11.77^{Ca}	132.83 ± 8.64^{Aa}	14.50 ± 2.52^{Eab}	72.14 ± 7.99^{Bc}	329.06 ± 38.21^{b}
S. saprophyticus PE-A20	0	n. d	2.83 ± 1.61^{Ec}	61.47 ± 2.35^{Aa}	54.66 ± 3.33^{Ba}	28.17 ± 0.38^{Cc}	14.69 ± 0.36^{Dc}	15.33 ± 1.87^{Dab}	2.54 ± 0.63^{Ef}	179.68 ± 10.52 ^{cd}
	3	n. d	16.42 ± 2.60^{Db}	56.55 ± 3.53^{ABa}	30.57 ± 4.42^{Cb}	50.66 ± 3.61^{Bab}	5.13 ± 0.03^{Ed}	7.13 ± 0.09^{Ec}	59.98 ± 5.75^{Ad}	226.45 ± 23.94^{bc}
	6	n. d	n. d	43.38 ± 2.46^{Bb}	16.04 ± 3.22^{Cd}	47.40 ± 4.51^{Bb}	2.27 ± 0.01^{Dd}	11.86 ± 1.12^{Cb}	140.85 ± 6.25^{Ac}	261.79 ± 21.20^{b}
	9	n. d	20.88 ± 1.67^{DEa}	39.33 ± 8.45^{Bb}	26.70 ± 2.35^{CDc}	43.73 ± 5.61^{Bb}	35.66 ± 4.42^{BCb}	13.56 ± 2.10^{Eb}	192.50 ± 9.46^{Ab}	372.36 ± 41.32^{a}
	12	17.28 ± 0.03^{D}	n. d	28.22 ± 2.35^{Cc}	31.26 ± 4.42^{Cb}	50.75 ± 3.45^{Bab}	46.75 ± 6.52^{Ba}	19.21 ± 3.22 ^{Da}	206.44 ± 2.18^{Aa}	399.90 ± 27.14^{a}
	15	n. d	n. d	25.40 ± 2.22^{Bc}	14.58 ± 2.25^{Cd}	59.23 ± 7.85^{Aa}	7.59 ± 1.05^{Dd}	14.48 ± 2.21^{Cb}	22.86 ± 5.36^{Be}	144.13 ± 25.65^{d}

Note: Tryptamine (TRP), 2-phenylethylamine (PEA), Putrescine (PUT), Cadaverine (CAD), Histamine (HIS), Tyramine (TYR), Spermidine (SPD), Spermine (SPM). Different letters (A–D and a–d) within a column indicate significant differences among the BAs and fermentation times ($P < 0.05$). n. d: not detected.

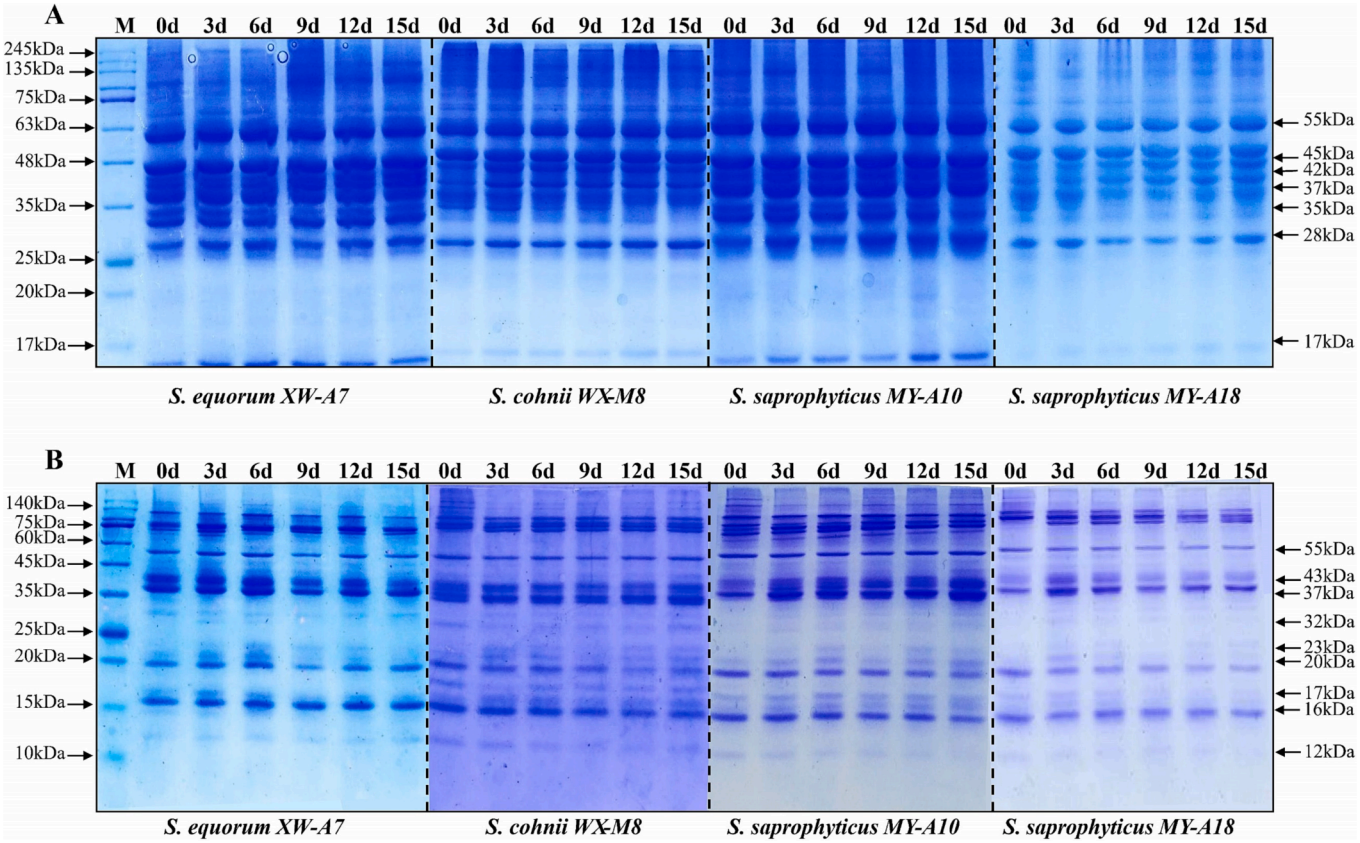

Fig. 3. SDS-PAGE profile of sarcoplasmic proteins (A) and myofibrillar proteins (B) extracted from *Staphylococcus*-inoculated pork. Line M: the molecular weights of a protein standard. Line 0d ~ 15d: Protein samples were fermented for 0, 3, 6, 9, 12 and 15 days after inoculation with *Staphylococci*.

approximately 200 (myosin) and 43 (actin) and other molecular weights of 98, 91, 79, 69, 64, 55, 37, 35 (tropomyosin) and 28 kDa, together with several polypeptides with molecular weights less than 12 kDa (Drosinos et al., 2007). In our tests, all four CNS strains were able to degrade myofibrillar proteins, as shown by an increase in bands around 32 KDa, 23 KDa, 20 KDa, 17 KDa and 16 KDa, implying the production of polypeptides. The highest number of bands and the darkest color were found in *S. cohnii WX-M8* and *S. saprophyticus MY-A10*.

Our studies showed that the profile of myofibrillar protein became darker around the 23 KDa band from the 3 to the 6 day, while this band became lighter on the 9 day and darker again on the 12 and 15 day as the fermentation time increased. This trend may be related to the phenomenon that while cathepsin activity is still quite high in meat, calpains' activity is declining. The endogenous enzymes associated with protein degradation, such as calpain and cathepsin, and extracellular enzymes secreted by microorganisms, could facilitate the degradation of proteins in the early stages of curing. Later, due to collapse of cells, the flow of cellular solutes led to changes in osmotic pressure, which inhibited the activity of calpain, while the activity of cathepsin and extracellular enzymes were not significantly affected (Zhou, 2020)). Thus, the poor degradation of sarcoplasmic proteins may also result from changes in protease activity caused by changes in osmotic pressure.

Formation of FAAs during fermentation

Inoculation of CNS in the sterile meat was characterized by significantly different types and contents of FAAs (Fig. 4). The PCA revealed that meat uninoculated with CNS was farther apart from meat inoculated with CNS and formed a separate region on the score plot (Fig. 4A), where meat inoculated with *S. saprophyticus MY-A10* and *S. saprophyticus MY-A18* showed similar composition of FAAs and a close score region; The meat inoculated with *S. equorum XW-A7* and *S. cohnii WX-M8* was characterized by different FAAs. Consistent with the findings of Wang et al. (2022), the meat showed a reduced content of FAAs in the late stages of fermentation (Fig. 4B). This phenomenon may be related to the conversion of FAAs to other VOCs such as aldehydes, ketones, and alcohols. In the meat inoculated with CNS, the diminishing of FAAs was even greater, mainly in amino acids such as Ser, Gly, Ala, Ile, Leu, and Lys. These amino acids are subject to transamination, decarboxylation, and microbial metabolism to form important VOCs in meat (Flores, 2018). For example, Leu is a precursor for the formation of butanal,3-methyl (Merlo et al., 2021). Meanwhile, some FAAs that facilitate the taste formation of bacon were increased, such as Glu (umami) and Asp (sour), etc (Fig. 4C). Glu and Asp may be the key FAAs for the taste formation of fermented meat (Yang et al., 2023). Apparently, the meat fermented by CNS contained lower levels of bitter and sweet FAAs (Fig. 4D). In terms of taste performances of FAAs, meat inoculated with *S. cohnii WX-M8* and *S. equorum XW-A7* showed an increase in umami and a decrease in sweetness; meat inoculated with *S. saprophyticus MY-A10* and *S. saprophyticus MY-A18* showed a relatively higher sweetness. Therefore, in term of the role of FAAs in shaping the taste of bacon, CNS fermentation facilitates the formation of taste.

Conclusion

In this study, 317 primary isolates were isolated from 14 types of Chinese bacon from four provinces in southwestern China, and 21 of them showed typical *Staphylococcal* biochemical reactions, as well as hemolysis-negative, DNase-negative, coagulase-negative, and low-amino acid decarboxylase activities. Eleven of the CNS strains possessed protease activity, and either lipase activity or nitrate reductase activity. Among them, *S. cohnii WX-M8* contained all three enzyme activities simultaneously, while *S. saprophyticus MY-A10* possessed only protease activity. It was demonstrated by simulated fermentation experiments that *S. cohnii WX-M8* and *S. saprophyticus MY-A10* could

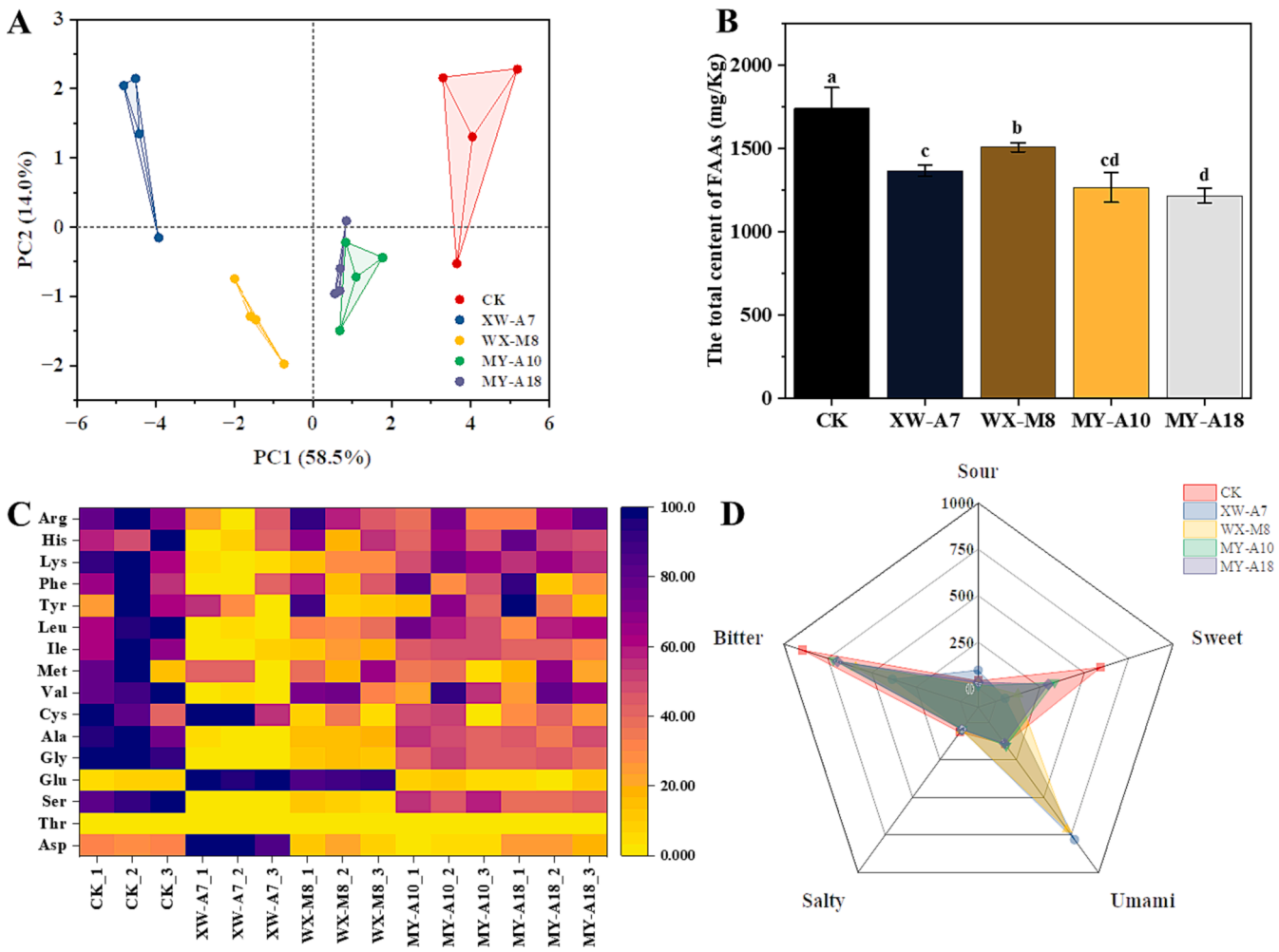

Fig. 4. Composition of FAAs of fermented meat. (A) PCA analysis of FAAs; (B) Total content of FAAs; (C) Composition and content of FAAs; (D) Taste contribution of fermented meat based on FAAs.

significantly reduce the content of BAs on the third day of fermentation and showed stronger degradation of myofibrillar proteins on days 3–6 and 12–15, resulting in more soluble polypeptides and FAAs that were beneficial to taste formation. However, the fermentation characteristics of a single strain can only be used to evaluate the fermentation potential of a specific bacteria strain and are not responsive to biochemical changes in the actual fermentation environment. In order to produce Chinese bacon with a traditional aroma, it is necessary to incorporate different strains for multi-fermentation, which can make up for the fermentation deficiencies of single strains and is more adaptable to the complex fermentation environment. In consequence, the enzymatic and fermentation properties of CNS identified in this study show a bright future for its application in Chinese bacon fermentation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Ashaolu, T. J., Khalifa, I., Mesak, M. A., Lorenzo, J. M., & Farag, M. A. (2023). A comprehensive review of the role of microorganisms on texture change, flavor and biogenic amines formation in fermented meat with their action mechanisms and safety. *Critical Reviews in Food Science and Nutrition*, 63 (19), 3538-355. 10.1080/10408398.2021.1929059.

Cachaldora, A., Fonseca, S., Franco, I., & Carballo, J. (2013). Technological and safety characteristics of *Staphylococcaceae* isolated from Spanish traditional dry-cured sausages. *Food Microbiology, 33*(1), 61–68. https://doi.org/10.1016/j.fm.2012.08.013

Chen, J., Zhang, J., Yang, Z., Niu, Y., Cai, Z., Wang, J., … Lu, X. (2022). Characterization of indigenous coagulase-negative *Staphylococci* isolated from Chinese spontaneously fermented meat products. *Microbiological Research, 263*, Article 127160. https://doi.org/10.1016/j.micres.2022.127160

Drosinos, E. H., Paramithiotis, S., Kolovos, G., Tsikouras, I., & Metaxopoulos, I. (2007). Phenotypic and technological diversity of lactic acid bacteria and *Staphylococci* isolated from traditionally fermented sausages in southern Greece. *Food Microbiology, 24*(3), 260–270. https://doi.org/10.1016/j.fm.2006.05.001

Fang, J., Feng, L., Lu, H., & Zhu, J. (2022). Metabolomics reveals spoilage characteristics and interaction of *Pseudomonas lundensis* and *Brochothrix thermosphacta* in

refrigerated beef. *Food Research International, 156*, Article 111139. https://doi.org/10.1016/j.foodres.2022.111139

Flores, M. (2018). Understanding the implications of current health trends on the aroma of wet and dry cured meat products. *Meat Science, 144*, 53–61. https://doi.org/10.1016/j.meatsci.2018.04.016

Gao, X., Li, C., He, R., Zhang, Y., Wang, B., Zhang, Z., & Ho, C. (2023). Research advances on biogenic amines in traditional fermented foods: Emphasis on formation mechanism, detection and control methods. *Food Chemistry, 405*, Article 134911. https://doi.org/10.1016/j.foodchem.2022.134911

Hu, M., Yu, J., Yu, J., Pan, Y., & Ou, Y. (2018). Isolation and Screening of *Staphylococcus Xylosus P2* from Chinese Bacon: A Novel Starter Culture in Fermented Meat Products. *International Journal of Food Engineering, 15*, 1–2. https://doi.org/10.1515/ijfe-2018-0021

Jeong, D. W., Lee, B., Her, J. Y., Lee, K. G., & Lee, J. H. (2016). Safety and technological characterization of coagulase-negative *Staphylococci* isolates from traditional Korean fermented soybean foods for starter development. *International Journal of Food Microbiology, 236*, 9–16. https://doi.org/10.1016/j.ijfoodmicro.2016.07.011

Ju, M., Piao, C., Zhang, J., Mu, B., Li, G., & Zhang, W. (2022). Hydrolysis of pork sarcoplasmic protein extracts by unique *Staphylococci* isolated from low-salt dry-cured ham. *LWT - Food Science and Technology, 164*, Article 113639. https://doi.org/10.1016/j.lwt.2022.113639

Kalac, P. (2006). Biologically active polyamines in beef, pork and meat products: A review. *Meat Science, 73*(1), 1–11. https://doi.org/10.1016/j.meatsci.2005.11.001

Kayili, E., & Sanlibaba, P. (2020). Prevalence, characterization and antibiotic resistance of *Staphylococcus aureus* isolated from traditional cheeses in Turkey. *International Journal of Food Properties, 23*(1), 1441–1451. https://doi.org/10.1080/10942912.2020.1814323

Khan, A. W. (1977). Some factors causing variation in the texture of similar muscles from comparable animals. *Meat Science, 1*(3), 169–176. https://doi.org/10.1016/0309-1740(77)90034-1

Li, H., Zhu, Q., Chen, X., Zhou, J., & Wu, J. (2022). Isolation and characterization of coagulase negative *Staphylococci* with high proteolytic activity from dry fermented sausages as a potential starter culture. *Food Research International, 162*(Pt A), Article 111957. https://doi.org/10.1016/j.foodres.2022.111957

Li, Y., Geng, Y., Shi, D., Li, R., Tang, J., & Lu, S. (2023). Impact of Coreopsis tinctoria Nutt. Essential oil microcapsules on the formation of biogenic amines and quality of smoked horsemeat sausage during ripening. *Meat Science, 195*, Article 109020. https://doi.org/10.1016/j.meatsci.2022.109020

Merchán, A. V., Ruiz-Moyano, S., Hernández, M. V., Martín, A., Lorenzo, M. J., & Benito, M. J. (2022). Characterization of autochthonal *Hafnia spp.* strains isolated from Spanish soft raw ewe's milk PDO cheeses to be used as adjunct culture. *International Journal of Food Microbiology, 373*, Article 109703. https://doi.org/10.1016/j.ijfoodmicro.2022.109703

Merlo, T. C., Lorenzo, J. M., Saldana, E., Patinho, I., Oliveira, A. C., Menegali, B. S., … Contreras-Castillo, C. J. (2021). Relationship between volatile organic compounds, free amino acids, and sensory profile of smoked bacon. *Meat Science, 181*, Article 108596. https://doi.org/10.1016/j.meatsci.2021.108596

Moraes, G. F. Q., Cordeiro, L. V., & Júnior, F. P.d A. (2021). Main laboratory methods used for the isolation and identification of *Staphylococcus spp*. *Revista Colombiana de Ciencias Químico Farmacéuticas, 50*(1), 5–28. https://doi.org/10.15446/rcciquifa.v50n1.95444

Ras, G., Bailly, X., Chacornac, J. P., Zuliani, V., Derkx, P., Seibert, T. M., … Leroy, S. (2018). Contribution of nitric oxide synthase from coagulase-negative *Staphylococci* to the development of red myoglobin derivatives. *International Journal of Food Microbiology, 266*, 310–316. https://doi.org/10.1016/j.ijfoodmicro.2017.11.005

Ruan, J., Wu, Z., Xu, J., Yu, Y., Tang, Y., Xie, X., … Li, H. (2023). Effects of replacement partial sodium chloride on characteristic flavor substances of bacon during storage based on GC×GC-MS and non-targeted metabolomics analyses. *Food Chemistry, 428*, Article 136805. https://doi.org/10.1016/j.foodchem.2023.136805

Ruaro, A., Andrighetto, C., Torriani, S., & Lombardi, A. (2013). Biodiversity and characterization of indigenous coagulase-negative *Staphylococci* isolated from raw milk and cheese of North Italy. *Food Microbiology, 34*(1), 106–111. https://doi.org/10.1016/j.fm.2012.11.013

Sanchez Mainar, M., Stavropoulou, D. A., & Leroy, F. (2017). Exploring the metabolic heterogeneity of coagulase-negative *Staphylococci* to improve the quality and safety of fermented meats: A review. *International Journal of Food Microbiology, 247*, 24–37. https://doi.org/10.1016/j.ijfoodmicro.2016.05.021

Schirone, M., Esposito, L., D'Onofrio, F., Visciano, P., Martuscelli, M., Mastrocola, D., & Paparella, A. (2022). Biogenic Amines in Meat and Meat Products: A Review of the Science and Future Perspectives. *Foods, 11*(6), 788. https://doi.org/10.3390/foods11060788

Semedo-Lemsaddek, T., Carvalho, L., Tempera, C., Fernandes, M. H., Fernandes, M. J., Elias, M., Barreto, A. o. S., & Fraqueza, M. J. (2016). Characterization and Technological Features of Autochthonous Coagulase-Negative *Staphylococci* as Potential Starters for Portuguese Dry Fermented Sausages. *Journal of Food Science*, 84 (Nr. 5), M1197-M1202. 10.1111/1750-3841.13311.

Stavropoulou, D. A., Filippou, P., De Smet, S., De Vuyst, L., & Leroy, F. (2018). Effect of temperature and pH on the community dynamics of coagulase-negative *Staphylococci* during spontaneous meat fermentation in a model system. *Food Microbiology, 76*, 180–188. https://doi.org/10.1016/j.fm.2018.05.006

Subathra Devi, C., Mohanasrinivasan, V., Subramaniam, V., Parashar, M., Vaishnavi, B., & Jemimah Naine, S. (2016). Molecular Characterization and Potential Assessment of Extracellular DNase Producing *Staphylococcus aureus VITSV4* Isolated from Bovine Milk. *Iranian Journal of Science and Technology, Transactions A: Science, 40*(3), 191–199. https://doi.org/10.1007/s40995-016-0090-z

Sun, F., Wang, H., Liu, Q., Xia, X., Chen, Q., & Kong, B. (2023). Proteolysis and quality characteristics of Harbin dry sausages caused by the addition of *Staphylococcus xylosus* protease. *Food Chemistry, 404*(Pt B), Article 134692. https://doi.org/10.1016/j.foodchem.2022.134692

Wang, H., Xu, J., Liu, Q., Xia, X., Sun, F., & Kong, B. (2022). Effect of the protease from *Staphylococcus carnosus* on the proteolysis, quality characteristics, and flavor development of Harbin dry sausage. *Meat Science, 189*, Article 108827. https://doi.org/10.1016/j.meatsci.2022.108827

Wang, S., Gao, K., Tang, L., Yang, Y., & Liu, S. (2020). Research progress on application of coagulase-negative *Staphylococci* in fermen- ted meat products. *Food and Fermentation Industries, 46*(23), 293–300. https://doi.org/10.13995/j.cnki.11-1802/ts.024527 (In Chinese with English abstract).

Wang, S., Wang, X., Pan, W., Liu, A., Liu, S., Yang, Y., & Zou, L. (2021). Evaluation of Bacterial Diversity and Quality Features of Traditional Sichuan Bacon from Different Geographical Region. *Applied Sciences, 11*(20). https://doi.org/10.3390/app11209738. Article 9738.

Wang, Z., Wang, Z., Ji, L., Zhang, J., Zhao, Z., Zhang, R., … Chen, L. (2021). A Review: Microbial Diversity and Function of Fermented Meat Products in China. *Frontiers in Microbiology, 12*, Article 645435. https://doi.org/10.3389/fmicb.2021.645435

Yang, L., Li, H., Wu, H., Su, C., & He, Z. (2023). Quality relationship between smoked and air-dried bacon of Sichuan-Chongqing in China: Free amino acids, volatile compounds, and microbial diversity. *Food Research International, 164*, Article 112274. https://doi.org/10.1016/j.foodres.2022.112274

Yu, D., Feng, M. Q., Sun, J., Xu, X. L., & Zhou, G. H. (2020). Protein degradation and peptide formation with antioxidant activity in pork protein extracts inoculated with *Lactobacillus plantarum* and *Staphylococcus simulans*. *Meat Science, 160*, Article 107958. https://doi.org/10.1016/j.meatsci.2019.107958

Zell, C., Resch, M., Rosenstein, R., Albrecht, T., Hertel, C., & Gotz, F. (2008). Characterization of toxin production of coagulase-negative *Staphylococci* isolated from food and starter cultures. *International Journal of Food Microbiology, 127*(3), 246–251. https://doi.org/10.1016/j.ijfoodmicro.2008.07.016

Zhang, J., Zhang, W., & Xing, L. (2021). Effects of ultrasound on the taste components from aqueous extract of unsmoked bacon. *Food Chemistry, 365*, Article 130411. https://doi.org/10.1016/j.foodchem.2021.130411

Zhao, C. J., Schieber, A., & Ganzle, M. G. (2016). Formation of taste-active amino acids, amino acid derivatives and peptides in food fermentations - A review. *Food Research International, 89*(Pt 1), 39–47. https://doi.org/10.1016/j.foodres.2016.08.042

Zhao, G., Li, S., Cui, W., Zhu, C., Wang, H., Yin, F., Jiao, Y., Li, J., & Han, M. (2021). Correlation analysis of bacterial community structure and flavor in different Chinese bacon. *Food and Fermentation Industries*, 47 (13), 246-253. 10. 13995/j. cnki.11-1802/ts.026439.

Zhao, R., Shao, C., Gao, S., Liu, G., & Yang, F. (2020). High-throughput sequencing analysis of bacterial diversity of different types of traditional Chinese bacon. *Food. Science, 41*(20), 90–96. https://doi.org/10.7506/spkx1002-6630-20200416-210. In Chinese with English abstract.

Zhou, C. Y. (2020). *Study on the mechanism of protein degradation and taste substance formation of Jinhua ham based on modern processing procedure*. Nanjing Agricultural University. PHD thesis.