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Selection and transcriptomic analysis of coagulase-negative *Staphylococcus* with high proteolytic activity isolated from Chinese Dong fermented pork (Nanx Wudl)

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ABSTRACT

Although coagulase-negative *Staphylococcus* (CNS), along with technological activities, plays a key role in fermented sausage flavour and nutrient production, the molecular mechanism of these activities remains elusive. In this study, 18 CNS strains with high proteolytic activity were isolated from Chinese Dong fermented pork (Nanx Wudl), and their technological and transcriptomic properties were investigated. After biochemical identification and genetic analysis, their technological properties, including nitrate reductase, catalase, antioxidant, and lipolytic activities and their growth under varying temperatures, salt concentrations, and pH levels were evaluated. Their aroma-producing potential was also determined in a model medium resembling fermented sausages. Transcriptomic analysis was performed using the most promising isolates. Biochemical identification and 16S rDNA sequencing revealed that the 18 *Staphylococcus* strains belonged to *Staphylococcus xylosus*, *Staphylococcus saprophyticus*, *Staphylococcus carnosus*, *Staphylococcus sciuri*, and *Staphylococcus equorum*. In terms of technological properties, 16 strains showed a nitrate-reducing ability, while 11 strains had a lipolytic activity. All strains exhibited superoxide dismutase (SOD) and catalase activities; four strains displayed an SOD activity of > 50%. They also tolerated 10% NaCl and 150 mg/kg of nitrite. They showed significant differences in ketone and acid production. The transcriptomic analysis of *S. xylosus* strains Sx3 and Sx6, which were selected because of their excellent enzymatic activities and aroma-producing ability, revealed the remarkable effect of genes related to pyruvate catabolism and amino acid metabolism on aroma generation. Therefore, this study provided valuable insights into the metabolic mechanisms underlying the technological properties of CNS and identified promising candidates as starter cultures in fermented sausage manufacturing.

1. Introduction

Fermented sausages, a popular dish in various countries, have a unique sensory taste and exhibit rich nutritional properties. During ripening, proteolysis is responsible for the sensory and nutritional properties of fermented sausages[1]. Nowadays, the increased awareness of consumers about the health benefits of food has led to the reduction of animal fat and salt in fermented sausages. To enhance the flavour of these reformulated sausages, manufacturers can use coagulase-negative *Staphylococcus* (CNS) strains with proteolytic activity as starter cultures[2-4].

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CNS are among the predominant microorganisms in meat fermentation[5], and their presence is influenced by processing conditions, ripening time, and nature of meat products[6]. The frequently found CNS species in fermented sausages are *Staphylococcus xylosus*, *Staphylococcus carnosus*, *Staphylococcus saprophyticus*, *Staphylococcus equorum*, *Staphylococcus condiment*, and *Staphylococcus sciuri*[7-13]. Studies on the proteolytic activity of CNS in fermented sausages have suggested its contribution to flavour development in low-fat/salt fermented sausages[14-15]. Li et al.[1] revealed that sodium-reduced fermented sausages inoculated with *S. xylosus* exhibit accelerated proteolysis, which enhances the free amino acid content. Other studies have also shown that *S. xylosus* isolated from salami shows proteolytic activity against meat protein and favours the formation of free amino acids, which are converted into aldehydes, ketones, and acids[16-17]. CNS participate in the formation of nitrosomyoglobin through their nitrate-reducing ability and enhance the organoleptic properties of fermented sausages[5]. Sun et al.[15] isolated 8 CNS strains which have been identified with lipolytic activities against pork fat. In addition, CNS can suppress lipid oxidation through catalase and SOD activities, favouring ester formation[18]. Moreover, the aroma-producing potential of CNS has been evaluated in various meat models, which revealed distinct aromas among different *S. xylosus* strains[16,19]. These findings suggest that CNS may enhance and diversify sausage flavours through specific biochemical activities.

Despite the importance of CNS in meat fermentation, the technologically relevant traits are still identified through conventional *in vitro* phenotypical and biochemical approaches. These techniques are inadequate to establish an association between phenotypic traits and specific genes responsible for such traits[20-21]. Consequently, the genetic potential may be underestimated because the applied cultivation conditions may not directly induce gene transcription. Nowadays, the rapid emergence of RNA-seq-based transcriptomic approaches offers promising possibilities for the *in vitro* and *in situ* determination of the transcriptional profiles of specific CNS strains. This advanced method also provides further insights into the functional role of CNS in fermented meats[22-25].

Microorganisms derived from traditional meat products are promising candidates as starter cultures because of their strong adaptability to the ecological niche of fermented meats[26]. Moreover, microorganisms isolated from spontaneous fermentation can produce distinct and attractive aromas based on their unique metabolic activities; as a result, they can enhance and diversify sausage flavour. For instance, Nanx Wudl, a naturally fermented pork consumed by the Dong ethnic minorities in China, is produced without using starter cultures. The CNS species identified in Nanx Wudl include *S. xylosus*, *S. carnosus*, and *S. saprophyticus*[27]. Therefore, these isolates are promising starter cultures in fermented sausage production. CNS strains with proteolytic activity have been isolated from Nanx Wudl[2]. However, their transcriptional profiles have not yet been described.

Therefore, this study was conducted to assess the technological and transcriptomic properties of CNS with high proteolytic activity isolated from Nanx Wudl. The CNS strains with high proteolytic activity were initially screened from Nanx Wudl. The technological properties of the isolated strains as potential meat starter cultures were then investigated. The transcriptional profiles were assessed using a model developed to mimic fermented sausage conditions. The ultimate goal of this study was to obtain new CNS strains with proteolytic activity and provide valuable insights into the functional role of CNS starters.

2. Materials and methods

2.1 Sampling of Nanx Wudl

A total of 12 Nanx Wudl samples from 4 different manufacturers (3 samples from each manufacturer), which are small-scale facilities located in Dong, an ethnic minority region in Hunan (China) and producing Nanx Wudl without using starter cultures, were collected. The main ingredients of Nanx Wudl were pork belly, salt (20–40 g/kg), sticky rice (60–80 g/kg), and additives (sugar and/or capsicum). After collection and during transportation, the samples were kept at 4 ℃ and used within a week.

2.2 Isolation of potential CNS strains

Approximately 25 g of Nanx Wudl from each sample was aseptically homogenised in a sterile pouch containing 225 mL of 0.85% physiological saline (NaCl). The samples were diluted, plated onto mannitol salt agar (MSA, Luqiao, China) in duplicate, and enumerated at 37 ℃ after 48 h. Based on colony morphology, 3–5 colonies were selected from each Nanx Wudl sample and streaked onto MSA plates. The streaking process was repeated 3 times to obtain purified isolates.

2.3 Proteolytic activity assessment using the agar plate method

The proteolytic activity of the pure isolates was evaluated using MSA plates supplemented with 3% skimmed milk powder. The overnight culture of each isolate was centrifuged at 12 000 r/min for 5 min. The resulting pellet was washed with physiological saline and re-suspended in an equal volume of the same buffer. Approximately 40 μL of the cell suspension was pipetted onto the agar plates/into wells bored in the agar plates. After incubation at 37 ℃ for 48 h, the appearance of a clear zone surrounding the colony was observed, indicating the presence of proteolytic activity.

2.4 Proteolytic activity assessment via SDS-PAGE

The proteolytic activity of the isolates was analysed using SDS-PAGE. Sarcoplasmic and myofibrillar proteins were extracted in accordance with a previously described method[28]. After being propagated twice in fresh nutrient broth (Luqiao, China), each isolate was centrifuged at 13 000 × *g* for 5 min. Then, the pellet was washed with physiological saline and re-suspended in an equal volume of the same buffer. The cell suspension of each isolate (1×10^6 CFU/mL) was incubated in 10 mL of sarcoplasmic extract or myofibrillar extract, which was added with 1% glucose and incubated at 37 ℃. For comparison, uninoculated extracts were incubated under similar conditions.

Approximately 1 mL of the culture sample was taken at 0 and 192 h of incubation at 30 ℃. After dilution, each sample was analysed using 12% SDS-PAGE. Electrophoresis was set at 80 V during the stacking gel phase and then increased to 120 V. The gels were stained with Coomassie blue R-250 reagent and destained until the background colour disappeared. Two independent batches were subjected to these experiments.

2.5 Identification of CNS isolates

The isolates with promising proteolytic activities were identified by using the Vitek 2 automated microbial ID/AST testing system (Biomerieux, France) and conducting 16S ribosomal DNA (16S rDNA) sequencing analysis. The biochemical test was performed in accordance with the manufacturer's guidelines. For 16S rDNA sequencing analysis, genomic DNA was extracted using a DNA isolation kit (Tiangen, Beijing, China); then, 16S rDNA was amplified using the universal primer pairs 27F and 1492R[29]. The amplified products were submitted to Thermo Fisher Scientific Co. (Shanghai, China) for sequencing. The obtained sequences were compared with those of similar strains in the GenBank database to identify the isolates at the species level according to a 98%–100% identity criterion.

2.6 Technological properties of CNS isolates

2.6.1 Growth characteristics of CNS isolates under varying temperatures, salt concentrations, and pH levels

Each CNS isolate was propagated twice in fresh nutrient broth and then centrifuged at 13 000 × *g* for 5 min to collect the pellet. Then, the pellet was washed with physiological saline and re-suspended in an equal volume of the same buffer. The cell suspension of each isolate (1×10^6 CFU/mL) was subsequently incubated in nutrient broth. Afterwards, the effect of varying temperatures on the growth of the CNS isolates was assessed at 15 and 25 ℃. The growth characteristics of these isolates were determined at different NaCl concentrations (10% and 15%) at 37 ℃. The influence of different pH values (5.0 and 5.5) on the CNS growth was observed at 37 ℃ in nutrient broth.

The growth of CNS isolates under different conditions was measured based on absorbance ($OD_{600\,nm}$) by using an automatic growth curve analyser Bioscreen C (Oy Growth Curves Ab Ltd., Turku, Finland) after 48 h of incubation. Results were expressed as the growth index (GI) calculated using the following equation: GI = (AbSs/AbSc) × 100, where AbSs refers to the absorbance ($OD_{600\,nm}$) of the sample under different growth conditions, and AbSc represents the absorbance ($OD_{600\,nm}$) of the control sample grown in nutrient broth at 37 ℃[30].

2.6.2 Nitrate reductase activity

The nitrate reductase activity of the CNS isolates was evaluated in accordance with a previously described method[31]. After each CNS isolate was incubated in nitrate broth at 37 and 25 ℃ for 24 h and 15 ℃ for 72 h, a fraction of the overnight culture was collected to determine the dry weight and measure the nitrate reductase activity *via* spectrophotometric assay. The nitrate reductase activity was calculated as the rate of $OD_{540\,nm}$/mg of dry weight.

2.6.3 Measurement of antioxidant (catalase and superoxide dismutase) activity

Catalase activity was measured in accordance with a previously described method[32]. Briefly, approximately 5 mL of each CNS culture ($OD_{600\,nm}$ = 1.0) was centrifuged at 12 000 × *g* for 5 min. After the supernatant was discarded, the pellet was mixed with 1.5 mL of 60 mmol/L H_2O_2 in 20 mmol/L phosphate buffer (pH 7.0). After 5 min of incubation at room temperature, the catalase activity was measured using a spectrophotometer at 240 nm. The activity was quantified and expressed in units of micromoles of H_2O_2 degraded per minute per millilitre of cells.

The pretreatment method of SOD activity described by Mauriello et al.[33] was adopted to obtain the cell pellet of each CNS isolate. The samples were centrifuged at 13 000 × *g* for 5 min, washed in 50 mmol/L K_2HPO_4 (pH 7.8), and re-suspended in the same buffer. After the cells were disrupted using glass beads (0.10–0.11 mm diameter) on a vortex mixer for 5 min, each sample was centrifuged at 13 000 × *g* for 5 min. The SOD activity of the collected supernatant was determined using a superoxide dismutase activity test kit (Beijing Solarbio Science & Technology Co., Ltd., China).

2.6.4 Lipolytic activity

Lipase activity was assessed using a tributyrin agar medium (containing 5 g/L of peptone, 3 g/L of yeast extract, 10 g/L of tributyrin, and 15 g/L of agar, pH 6.0). Approximately 20 μL of overnight CNS culture from each strain was inoculated onto the agar plates and incubated at 25 ℃ for 48 h. Afterwards, the plates were observed to detect the appearance of a clear zone surrounding the inoculated area, which indicated the presence of lipolytic activity.

2.6.5 Detection of pathogenic genes

Pathogenic genes, including enterotoxin (*sea*, *seb*, *sec*, *sed*, *see*) and haemolysin (*hla*, *hlb*), were detected in accordance with established methods[34-35]. Two independent batches were subjected to these experiments.

2.6.6 SPME-GC-MS analysis of volatile compounds in a fermented sausage model medium

A fermented sausage model medium was prepared following the method of Paik et al.[36]. The formulation included 1.2% of meat extract (Sigma-Aldrich, Saint Louis, USA), 1% of glucose, 2% of NaCl, 0.2% of $K_2HPO_4 \cdot 3H_2O$, 0.015% of $MgSO_4 \cdot 7H_2O$, 0.05% of glutamate, and 0.015% of $NaNO_2$. After being propagated twice in fresh nutrient broth, each culture was centrifuged at 10 000 × *g* for 15 min. The pellet was washed twice with sterile physiological saline and re-suspended in the same buffer. Each strain (approximately 1×10^6 CFU/mL) was inoculated into the model medium and incubated at 25 ℃ for 20 days.

The inoculated samples and the non-inoculated control were analysed using solid-phase microextraction coupled with gas chromatography-mass spectrometry (SPME-GC-MS)[37]. The supernatant from each sample was separated by centrifugation at 5 000 × *g* for 10 min. Approximately 2 mL of each supernatant sample was transferred into a 10 mL extraction vial and added with 3-octanol (Sigma Aldrich, Saint Louis, USA) as an internal standard. The vials were then heated at 45 ℃ for 30 min.

Volatile compounds were collected using a 50/30 μm DVB/CAR/PDMS fibre (Supelco, Pittsburgh, USA). The adsorbed molecules were desorbed in a GC-MS 1310/TSQ8000 system (Thermo Fisher Scientific, Waltham, USA) in a splitless mode at 230 ℃ for 5 min; helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The desorbed molecules were then separated using a DB-WAX capillary column (30 m × 0.25 mm, 0.25 μm). The GC oven temperature was programmed as follows: held at 40 ℃ for 3 min, followed by

temperature elevation to 50 °C at 10 °C/min, heated to 120 °C at 4 °C/min, raised to 230 °C at 12 °C/min, and maintained at 230 °C for 8 min. Mass spectral data were obtained in the *m/z* 35–400 by using the scan mode with an electron ionisation (EI) source at 70 eV. The retention index (RI) was calculated using a C5–C20 *n*-alkane standard solution (Sigma-Aldrich, Saint Louis, USA). Eventually, volatile compounds were identified by matching their RI with those listed in the mass spectral database (NIST 11), and were semi-quantified using 3-octanol as the internal standard.

2.7 Transcriptomic sequencing, assembly, and analysis

Each CNS isolate was propagated twice in fresh nutrient broth. The cultures were collected by centrifugation at 10 000 × *g* for 15 min, and the cell pellets were washed twice with sterile physiological saline and re-suspended in the same buffer. Each strain (approximately 1×10^6 CFU/mL) was inoculated onto the fermented sausage model medium and incubated at 25 °C for 24 and 72 h. Subsequently, each culture was collected and flash-frozen in liquid nitrogen.

The transcriptional profile of each strain was analysed by Scale Biomedicine Technology Co., Ltd. (Beijing, China). Briefly, total RNA was extracted using TRIzol (Takara Bio Inc., China) in accordance with the manufacturer's instructions and then tested for integrity by using a Bioanalyser 2100 system (Agilent Technologies, Inc., USA). A total of 12 cDNA libraries were constructed following a previously described method[38] and sequenced *via* Illumina Novaseq high-throughput sequencing. After raw data were obtained, quality control was performed by removing adapter sequences, low-quality reads, and reads containing ploy-*N* stretches to yield clean reads. All differential genes were enriched for Gene Ontology (GO) analysis using the ClusterProfiler R package[39], and GO functional network diagrams were created for these genes. Subsequently, the Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed using the ClusterProfiler R package to assess the functional relevance of differentially expressed genes (DEGs)[40].

2.8 Statistical analysis

All experiments were conducted in triplicate. For the determination of SOD activity, catalase activity and aroma-producing capability of isolated strains, data were presented as mean values and evaluated using SPSS version 19.0 (International Business Machines, USA). Significant differences between mean values ($P < 0.05$) were determined *via* one-way ANOVA with Tukey's test. For transcriptomic analysis, differentially expressed genes were obtained using DESeq2 R package (1.20.0) with an adjusted *P*-value < 0.05.

3. Results and discussion

3.1 Screening of CNS with proteolytic activity

The distinct flavour of fermented sausages is attributed to compounds generated through proteolysis, lipolysis, lipid oxidation, and carbohydrate fermentation; it is also derived from seasonings and curing salts[28]. Proteolysis is caused by endogenous meat enzymes and linked to the activity of the CNS group[1,16,31]. The results showed that the CNS density ranged from 4.26 to 7.39 (lg (CFU/g)) in each Nanx Wudl sample. A total of 50 isolates with representative colony morphotypes were selected and tested for their proteolytic activities. Through the agar plate method, 18 isolates with clear transparent zones were identified on the MSA plates supplemented with skimmed milk powder. Further biochemical identification and 16S rDNA genetic analysis revealed that these isolates belonged to 5 distinct species: *S. xylosus* (9), *S. saprophyticus* (5), *S. sciuri* (2), *S. equorum* (1), and *S. carnosus* (1). The sequencing and NCBI accession data are summarised in Table 1.

Table 1
Identities of the 16S rDNA sequenced genes of isolated strains and the accession numbers of the entries with the highest identity.

Strain number	Species	Identity/%	Accession number
Sx1	*S. xylosus*	99.93	CP031275
Sx2	*S. xylosus*	99.58	MK696228
Sx3	*S. xylosus*	100.00	MH119705
Sx6	*S. xylosus*	100.00	MH119705
Sx7	*S. xylosus*	100.00	MN229554
Sx8	*S. xylosus*	100.00	MK696228
Sx10	*S. xylosus*	100.00	MN229554
Sx11	*S. xylosus*	98.68	MK696228
Sx14	*S. xylosus*	100.00	MH119705
Se16	*S. equorum*	99.91	MN758799
Ssp17	*S. saprophyticus*	98.04	MG719556
Ssp18	*S. saprophyticus*	100.00	MT225743
Ssp20	*S. saprophyticus*	100.00	MT269536
Ssp24	*S. saprophyticus*	100.00	MK026832
Ssp26	*S. saprophyticus*	100.00	FJ380970
Sc27	*S. carnosus*	99.65	FJ795528
Ssc28	*S. sciuri*	99.58	MT214242
Ssc29	*S. sciuri*	99.93	KX344009

Prior to SDS-PAGE analysis, the identified isolates were successfully grown in sarcoplasmic extracts, and their $OD_{600\,nm}$ exceeded 1.0 after 192 h of incubation. The protein profile of the uninoculated control remained the same during this period (Fig. 1). Of the 18 isolates tested, 17 exhibited a decreased intensity of the protein bands at approximately 100, 63, 48, 40, and 30 kDa, implying a high proteolytic activity. However, the isolate Se16 could not hydrolyse sarcoplasmic protein because of its identical electrophoretic profile to that of the uninoculated protein. This result was consistent with previous reports[33,41-42], which demonstrated the proteolytic activity of *S. xylosus*, *S. saprophyticus*, *S. sciuri*, and *S. carnosus*.

In the present study, 34% of the CNS strains isolated from Nanx Wudl could hydrolyse meat protein. By contrast, Sun et al.[15] revealed that more than half of the CNS isolates from fermented dry sausages exhibit a proteolytic activity on meat protein. Palavecino et al.[43] found that only 10% of CNS isolates derived from fermented dry sausages could hydrolyse meat protein. These notable discrepancies could be attributed to different evaluation methods and isolation sources used in each study.

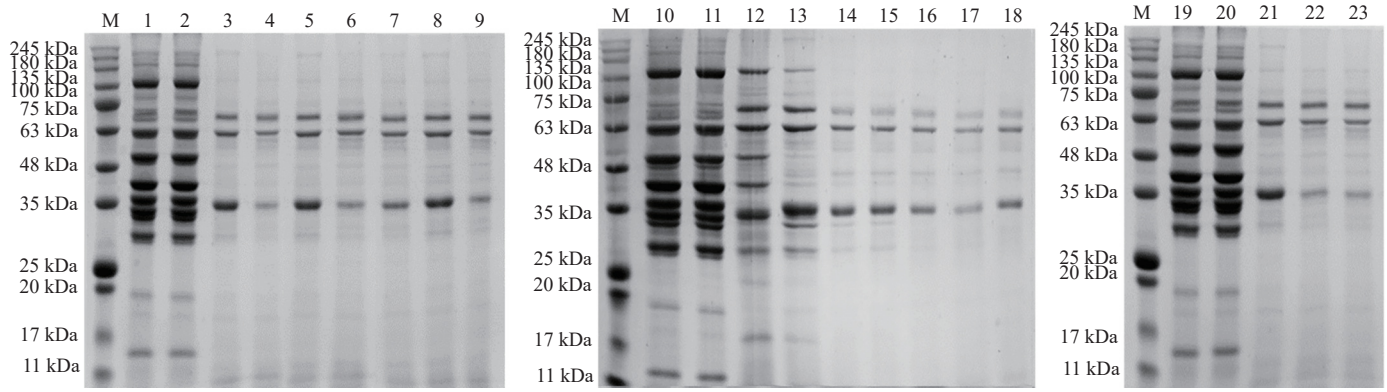

Fig. 1 Electrophoretic profiles of sarcoplasmic proteins hydrolysed by the strains of *Staphylococcus*. Lane 1, 10, 19: uninoculated control at 0 h of incubation; Lane 2, 11, 20: uninoculated control at 192 h of incubation; Lane 3–9, 12–18, 21–23: Sx1, Sx2, Sx3, Sx6, Sx7, Sx8, Sx10, Sx11, Sx14, Ssp17, Ssp18, Ssp20, Ssp24, Ssp26, Sc27, Ssc28, Ssc29.

3.2 Growth performance under varying temperatures, salt concentrations, and pH levels

Table 2 presents the growth characteristics of the 18 CNS isolates under various conditions typically used for sausage fermentation: temperatures of 15 and 25 ℃, salt concentrations of 10% and 15%, and pH levels of 5.0 and 5.5. The microbial growth of the 18 isolates was measured using the GI and classified into 3 conditions: at 15 ℃, with pH 5.0 or 5.5, and with 10% or 15% NaCl. Only 3 isolates in 15% NaCl did not grow.

Given that NaCl is an essential ingredient in sausage production, microbial halotolerance should be considered as a crucial criterion in starter culture selection[15,41]. In the present study, most *S. xylosus* and *S. carnosus* isolates tolerated 15% NaCl. Similarly, a previous study observed microbial tolerance to 15% NaCl, where 97.5% of *S. carnosus* and 100% *S. xylosus* strains originated from fermented meat[33,41]. Additionally, all isolates in the present study flourished at pH 5.0 and 5.5, likely because of the low pH (< 5.0) of Nanx Wudl samples.

3.3 Nitrate reductase activity

The nitrate reductase activity contributes to the development of the typical red colour of fermented meat products and influences their organoleptic characteristics[44]. Table 2 shows that 89% of the CNS

Table 2
The enzymatic activity and growth characteristics of isolated CNS.

Strains	Species	Enzymatic activities						Growth characteristics[C]					
			Nitrate-reductase[B]			SOD activity (%)	Catalase activity (AU)	25 ℃	15 ℃	pH 5.5	pH 5.0	NaCl (10%)	NaCl (15%)
		Lipase[A]	15 ℃	25 ℃	37 ℃								
Sx1	*S. xylosus*	ND	+	++	++	33.77^{f}	10.17^{ijk}	> 75	> 75	> 75	> 75	25–75	< 25
Sx2	*S. xylosus*	++	++	++	++	24.93^{gh}	13.56^{gh}	> 75	> 75	> 75	> 75	25–75	25-75
Sx3	*S. xylosus*	++	++	++	++	46.69^{d}	16.95^{ef}	> 75	> 75	> 75	> 75	> 75	> 75
Sx6	*S. xylosus*	+	+	ND	ND	38.76^{ef}	5.93^{l}	> 75	> 75	> 75	> 75	> 75	> 75
Sx7	*S. xylosus*	ND	+	+	++	46.92^{d}	22.88^{bc}	> 75	> 75	> 75	> 75	25–75	< 25
Sx8	*S. xylosus*	+++	+	+	++	40.80^{e}	15.26^{fg}	> 75	> 75	> 75	> 75	25–75	< 25
Sx10	*S. xylosus*	++	++	++	+	13.26^{i}	7.46^{kl}	> 75	> 75	> 75	> 75	> 75	> 75
Sx11	*S. xylosus*	+	++	++	++	33.88^{f}	21.36^{cd}	> 75	> 75	> 75	> 75	> 75	> 75
Sx14	*S. xylosus*	ND	+	+	ND	47.05^{d}	14.41^{fg}	> 75	> 75	> 75	> 75	25–75	25–75
Se16	*S. equorum*	+	++	+	+	19.51^{h}	12.54^{ghi}	> 75	> 75	> 75	> 75	> 75	25–75
Ssp17	*S. saprophyticus*	+	++	+	+	53.71^{bc}	21.70^{cd}	> 75	> 75	> 75	> 75	> 75	25–75
Ssp18	*S. saprophyticus*	++	+	++	++	50.67^{cd}	11.02^{hij}	> 75	> 75	> 75	> 75	> 75	25–75
Ssp20	*S. saprophyticus*	ND	ND	ND	ND	63.63^{a}	25.76^{ab}	> 75	> 75	> 75	25–75	> 75	> 75
Ssp24	*S. saprophyticus*	+	+	ND	ND	49.49^{cd}	27.12^{a}	> 75	> 75	> 75	25–75	25–75	25–75
Ssp26	*S. saprophyticus*	++	ND	ND	ND	27.27^{g}	23.05^{bc}	> 75	25–75	> 75	> 75	> 75	25–75
Sc27	*S. carnosus*	ND	ND	+	+	36.72^{ef}	8.47^{ikl}	> 75	> 75	> 75	> 75	> 75	> 75
Ssc28	*S. sciuri*	ND	++	++	++	46.92^{d}	20.68^{cd}	> 75	> 75	> 75	> 75	> 75	> 75
Ssc29	*S. sciuri*	ND	ND	+	ND	58.16^{ab}	18.98^{de}	> 75	> 75	> 75	> 75	25–75	25–75

Note: [A] Lipase activity was determined by measuring the diameter of halo: +, diameter of halo 0.1–2.0 mm; ++, diameter of halo 2–6 mm; ++, diameter of halo > 6 mm; ND: not detected. [B] The nitrate reductase activity was calculated as the rate: $OD_{540\ nm}$/mg dry weight. ND: not detected; +, 1 < $OD_{540\ nm}$/mg dry weight < 6; ++, $OD_{540\ nm}$/mg dry weight > 6. [C] The growth characteristics of CNS isolates under different conditions was expressed as growth index (GI). GI > 75: optimal growth; 75 > GI > 25: partially inhibited; GI < 25: completely inhibited. Different lowercase letters in the same column represent significant difference ($P < 0.05$).

strains could reduce nitrate; however, 2 *S. saprophyticus* isolates did not exhibit nitrate reductase activities at any temperature. Consistent with the present findings, previous reports[45] revealed that *S. saprophyticus* strains do not have nitrate reductase activity.

Interestingly, the nitrate reductase activity of the CNS strains was temperature dependent. In Table 2, 67% of the CNS isolates, except 6 strains (Sx6, Sx14, Ssp20, Ssp24, Ssp26, and Ssc29), exhibited a nitrate-reducing activity at 15, 25, and 37 ℃. Although nitrate reduction is frequently observed in *Staphylococcus* species, the extent of their nitrate reductase activities varies substantially between and within species[46].

3.4 Antioxidant (catalase and SOD) activity

All CNS isolates displayed catalase and SOD activities (Table 2), which participate in suppressing lipid oxidation. Specifically, the catalase activities of *S. sciuri* strains, *S. saprophyticus* strains, and 6 *S. xylosus* strains (Sx2, Sx3, Sx7, Sx8, Sx11, and Sx14) were higher than those of other strains. By comparison, the catalase activity of *S. saprophyticus* strains was higher than that of other *Staphylococcus* species; among them, Ssp24, Ssp20, and Ssp17 achieved the 3 highest catalase activities ($P < 0.05$).

Consistent with the results of the catalase activity, the SOD activities of *S. sciuri* and *S. saprophyticus* strains were higher than those of the other strains. Similarly, Mauriello et al.[33] observed that *S. saprophyticus* strain 68K3 exhibits the highest catalase and SOD activities. Kesmen et al.[47] identified *S. saprophyticus* as the dominant CNS isolated from sucuk, a traditional Turkish fermented meat product.

3.5 Lipolytic activity

Lipase promotes the release of free fatty acids during fermentation, facilitating the production of typical sensory profiles in fermented meats. This study revealed that 61% of the CNS strains, including *S. xylosus* (6), *S. saprophyticus* (4), and *S. equorum* (1), displayed lipolytic activities; among the strains, the isolate Sx8 exhibited the highest lipolytic activity. Mauriello et al.[33] also observed lipolytic activities in *S. xylosus*, *S. saprophyticus*, and *S. equorum* strains isolated from Southern Italy fermented sausages. Likewise, Jeong et al.[48] found lipolytic activities in 90% of 39 isolated *S. equorum* strains. Nevertheless, the lipolytic activity of CNS could be strain dependent, which is consistent with previous findings[49-51].

3.6 Evaluation of relevant safety traits in the isolated CNS strains

Enterotoxin production and haemolytic activity are considered pathogenic traits of CNS. Rosenstein et al.[52] showed that CNS lacks virulence genes encoding enterotoxin and haemolysin. Conversely, 22% of CNS isolated from cheeses produced in Northern Italy demonstrate a β-haemolytic activity[53]. The presence of enterotoxin gene A in CNS (*S. xylosus* and *S. epidermidis* strains) isolated from bakery goods has been reported previously[54]. In the present study, the 18 isolated CNS strains showed no evidence of pathogenic genes, including classical enterotoxin genes (*sea*, *seb*, *sec*, *sed*, and *see*) and haemolysin genes (*hla* and *hlb*). Therefore, CNS isolated from Nanx Wudl could be considered as a safe starter in meat fermentation.

3.7 Aroma-producing ability of CNS strains

The aromatic capabilities of the 18 CNS strains were evaluated using a fermented sausage model medium. Fig. 2 and Tables 3 and 4 list 58 volatile compounds identified in the control and inoculated samples: aldehydes (9), ketones (5), alcohols (12), acids (12), esters (7), phenols (3), and other compounds (10). The volatile compounds produced by the CNS strains and control were notably different. Specifically, the non-inoculated control group comprised 23 volatiles compounds, which were mostly aldehydes and nitrogen compounds. These compounds could be generated through the chemical degradation of amino acids and lipids in the model medium.

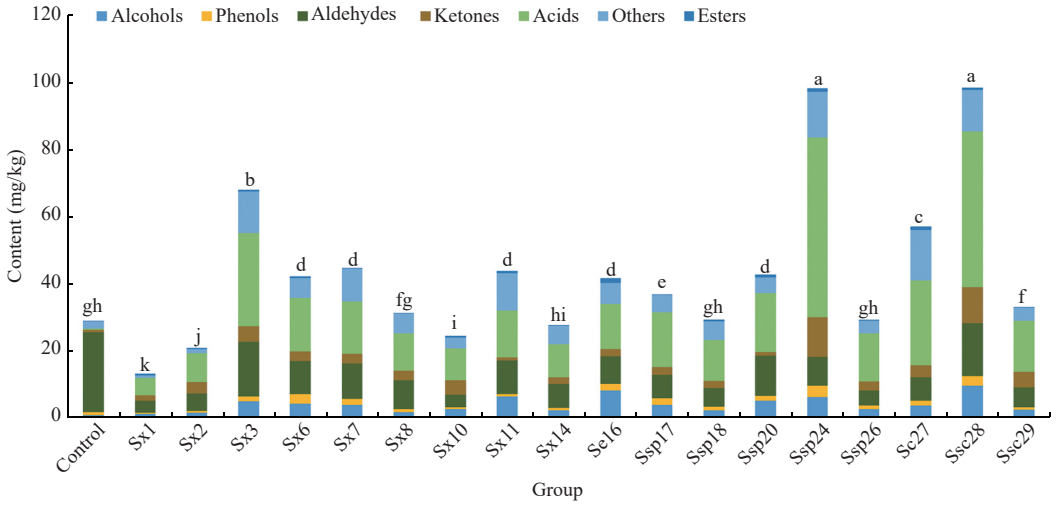

Fig. 2 Content of alcohols, phenols, aldehydes, ketones, acids, esters, and other compounds in non-inoculated model medium (control) and meats model medium inoculated with different CNS strains. Different lowercase letters indicate significant differences of total compounds between groups ($P < 0.05$).

Table 3
Volatile compounds (mg/kg) identified in meat model medium inoculated with different CNS strains.

Compounds	RI	Control	Sx1	Sx2	Sx3	Sx6	Sx7	Sx8	Sx10	Sx11	Sx14
Alcohols											
1-Butanol	1 129	n.d.	0.28^{eg}	0.40^{defg}	0.96^{bc}	1.06^{b}	0.67^{bcde}	0.18^{e}	0.52^{cde}	1.00^{bc}	0.52^{cde}
3-Methyl-1-butanol	1 192	n.d.	0.11^{g}	0.30^{fg}	n.d.	0.44^{defg}	0.59^{cdef}	0.39^{efg}	0.42^{defg}	1.03^{bc}	n.d.
2-Ehyl-1-hexanol	1 470	0.37^{cd}	0.20^{d}	n.d.	1.14^{a}	0.62^{b}	n.d.	n.d.	n.d.	0.67^{b}	0.40^{cd}
1-Nonanol	1 663	n.d.	0.24^{h}	0.36^{fg}	n.d.	0.47^{cde}	0.73^{b}	0.43^{defg}	0.32^{gh}	0.55^{c}	0.44^{cdef}
1-Pentanol	1 192	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1-Undecanol	1 871	n.d.	n.d.	n.d.	1.74^{a}	0.14^{c}	n.d.	n.d.	n.d.	0.12^{c}	n.d.
2-Furanmethanol	1 674	n.d.	n.d.	n.d.	n.d.	n.d.	0.20^{a}	n.d.	n.d.	0.22^{a}	n.d.
3-Furanmethanol	1 678	0.20^{b}	n.d.	n.d.	n.d.	0.18^{bcd}	n.d.	n.d.	0.1^{g}	n.d.	0.13^{fg}
Benzyl alcohol	1 854	0.13^{gh}	0.11^{h}	0.16^{efgh}	0.72^{a}	0.26^{cde}	0.27^{cd}	0.22^{cdefg}	0.15^{fgh}	0.53^{b}	0.21^{cdefgh}
Phenylethyl alcohol	1 915	0.20^{c}	0.29^{c}	0.45^{c}	0.53^{c}	1.12^{c}	0.88^{c}	0.63^{c}	0.80^{c}	0.28^{c}	0.55^{c}
Geraniol	1 784	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.98^{a}	n.d.
2-Propyl-1-pentanol	1 471	n.d.	n.d.	n.d.	n.d.	n.d.	0.54^{a}	n.d.	0.35^{c}	n.d.	n.d.
Phenols											
Phenol	> 2 000	n.d.	0.07^{b}	n.d.	n.d.	n.d.	0.16^{b}	0.83^{a}	n.d.	n.d.	n.d.
2,4-Bis(1,1-dimethylethyl)-phenol	> 2 000	0.97^{defg}	0.32^{g}	0.39^{fg}	1.33^{cdefg}	2.71^{ab}	1.51^{cdef}	n.d.	0.40^{fg}	0.59^{efg}	0.66^{efg}
P-Tert-butyl-phenol	> 2 000	n.d.	0.05^{e}	0.08^{de}	n.d.	0.14^{b}	0.11^{bcd}	n.d.	0.08^{de}	0.14^{b}	0.09^{cde}
Aldehydes											
3-Methyl-2-butenal	1 207	n.d.	0.07^{f}	0.09^{def}	n.d.	n.d.	0.15^{bc}	n.d.	0.11^{cdef}	n.d.	0.08^{ef}
2-Undecenal	1 753	0.19^{b}	n.d.	n.d.	n.d.	0.18^{b}	n.d.	n.d.	n.d.	n.d.	n.d.
3-Furaldehyde	1 466	0.33^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Benzaldehyde	1 500	22.20^{a}	2.35^{k}	3.84^{ihjk}	13.42^{b}	6.36^{defg}	7.56^{de}	5.91^{efg}	2.52^{k}	7.72^{d}	5.31^{ghi}
3,4-Dimethyl-benzaldehyde	1 786	0.82^{ef}	0.50^{f}	0.93^{ef}	n.d.	2.19^{ab}	2.16^{abc}	1.41^{cde}	0.87^{ef}	2.06^{abcd}	n.d.
Benzeneacetaldehyde	1 620	n.d.	0.18^{de}	0.11^{def}	n.d.	0.70^{a}	0.37^{b}	0.44^{b}	0.07^{ef}	n.d.	0.36^{b}
Furfural	1 448	n.d.	0.17^{f}	0.30^{cde}	0.66^{a}	0.54^{b}	0.49^{b}	0.38^{c}	0.27^{def}	0.20^{f}	0.36^{cd}
Octanal	1 280	0.29^{abc}	0.29^{abc}	n.d.	n.d.	n.d.	n.d.	0.41^{ab}	n.d.	0.15^{bc}	n.d.
2,4-Dimethyl-benzaldehyde	1 784	n.d.	n.d.	n.d.	2.28^{b}	n.d.	n.d.	n.d.	n.d.	n.d.	1.16^{d}
Ketones											
2,3-Butanedione	984	n.d.	0.55^{i}	1.11^{e}	0.46^{ij}	0.76^{gh}	0.82^{fg}	1.00^{ef}	1.36^{d}	n.d.	0.31^{j}
3-Hydroxy-2-butanone	1 267	n.d.	0.85^{h}	1.91^{cdef}	4.13^{b}	1.29^{efgh}	1.21^{fgh}	1.33^{efgh}	2.67^{c}	n.d.	1.12^{fgh}
Acetophenone	1 626	0.60^{gg}	0.26^{j}	0.39^{i}	n.d.	0.67^{ef}	0.78^{c}	0.59^{g}	0.35^{i}	0.74^{cd}	0.50^{h}
4'-Hydroxy-acetophenone	1 803	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.18^{a}	n.d.
3-Octanone	1 235	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Esters											
5-Hexyldihydro-2(3*H*)-furanone	> 2 000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.16^{b}	n.d.
4-Dodecanolide	> 2 000	n.d.	n.d.	n.d.	n.d.	0.12^{c}	n.d.	n.d.	n.d.	0.19^{b}	n.d.
Phenethyl acetate	1 818	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.14^{c}	n.d.
Dibutyl phthalate	> 2 000	0.15^{g}	0.22^{efg}	0.35^{de}	0.60^{c}	0.15^{g}	0.23^{efg}	0.22^{efg}	0.46^{cd}	0.18^{fg}	0.20^{efg}
Isopropyl myristate	> 2 000	0.11^{a}	n.d.	n.d.	n.d.	0.12^{a}	n.d.	n.d.	n.d.	0.11^{a}	n.d.
Ethyl octanoate	1 436	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Methyl hexanoate	1 189	n.d.	0.22^{a}	n.d.	n.d.	0.13^{b}	n.d.	n.d.	n.d.	n.d.	n.d.
Acids											
Acetic acid	1 442	0.11^{j}	2.76^{i}	4.24^{h}	11.16^{c}	6.18^{fgh}	8.03^{de}	5.22^{ghi}	5.08^{ghi}	4.08^{i}	4.78^{hi}
Butanoic acid	1 630	n.d.	0.67^{j}	1.15^{h}	3.05^{c}	2.04^{d}	1.86^{de}	1.46^{h}	1.17^{h}	1.97^{de}	1.32^{gh}
3-Methyl-butanoic acid	1 192	n.d.	0.22^{h}	0.43^{g}	0.85^{cd}	0.82^{cd}	0.72^{cde}	0.52^{fg}	0.44^{g}	1.45^{b}	0.46^{fg}
Hexanoic acid	1 844	n.d.	0.49^{j}	0.89^{hi}	2.18^{c}	1.54^{de}	1.61^{d}	1.17^{fgh}	0.85^{i}	1.49^{de}	0.98^{ghi}
Nonanoic acid	> 2 000	0.06^{c}	0.14^{b}	0.22^{b}	0.83^{b}	2.03^{b}	0.46^{b}	0.62^{b}	0.24^{b}	2.26^{b}	0.29^{b}
Octanoic Acid	> 2 000	0.13^{g}	0.63^{fg}	1.15^{fgh}	3.22^{bcd}	2.25^{de}	1.98^{ef}	1.60^{efg}	1.10^{fgh}	1.82^{ef}	1.45^{efg}
2-Methyl-propanoic acid	1 551	n.d.	0.16^{g}	0.30^{fgh}	0.80^{c}	0.48^{d}	0.48^{d}	0.37^{defg}	0.31^{fgh}	0.51^{d}	0.32^{efg}
n-Decanoic acid	> 2 000	0.11^{f}	0.10^{f}	0.16^{ef}	0.40^{bcd}	0.34^{cde}	0.35^{cde}	0.17^{def}	0.20^{cdef}	n.d.	0.20^{cdef}
trans-Cinnamic acid	1 607	n.d.	n.d.	n.d.	5.40^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Benzoic acid	> 2 000	n.d.	n.d.	0.07e	n.d.	0.12c	n.d.	n.d.	n.d.	0.14b	n.d.
Propanoic acid	1 525	n.d.	n.d.	n.d.	n.d.	0.17a	n.d.	n.d.	0.12b	0.14b	n.d.
Pentanoic acid	1 734	n.d.	0.03^{c}	n.d.	n.d.	0.11^{ab}	0.11^{ab}	n.d.	n.d.	n.d.	0.06^{bc}

Table 3 (*Continued*)

Compounds	RI	Control	Sx1	Sx2	Sx3	Sx6	Sx7	Sx8	Sx10	Sx11	Sx14
Other nitrogen/Sulphur compounds											
2-Acetylthiazole	1 621	0.54^{ijk}	0.40^{k}	0.45^{jk}	n.d.	1.22^{c}	1.00^{cde}	0.76^{fghi}	0.41^{k}	0.57^{ghijk}	0.78^{efgh}
1-Isocyano-2-methyl-benzene	1 897	0.26^{ef}	0.09^{i}	0.19^{gh}	0.58^{b}	0.27^{ef}	0.30^{e}	0.22^{fgh}	0.16^{h}	0.37^{d}	0.20^{g}
Benzonitrile	1 580	n.d.	n.d.	n.d.	n.d.	3.81^{de}	6.23^{c}	4.25^{d}	1.86^{i}	8.71^{a}	3.53^{ef}
O-Decyl-hydroxylamine	1 399	0.42^{bcde}	n.d.	0.16^{f}	n.d.	n.d.	0.63^{bc}	0.30^{def}	n.d.	n.d.	n.d.
3-(Methylthio)-propanal	1 436	0.47^{b}	0.05^{g}	0.09^{efg}	n.d.	0.23^{c}	0.14^{d}	0.12^{de}	0.07^{fg}	0.24^{c}	0.13^{de}
2,5-Dimethyl-pyrazine	1 302	n.d.	n.d.	n.d.	n.d.	n.d.	0.76^{ab}	n.d.	0.51^{e}	0.82^{a}	0.54^{de}
3-Ethyl-2,5-dimethyl-pyrazine	1 441	0.45^{b}	0.16^{e}	0.24^{ef}	n.d.	0.40^{bc}	0.45^{b}	0.30^{de}	0.20^{gf}	0.45^{b}	0.29^{de}
Others											
2,6,11-Trimethyl-dodecane	1 446	n.d.	n.d.	n.d.	5.92^{b}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2-Methyl-eicosane	1 641	n.d.	n.d.	n.d.	3.64^{a}	n.d.	0.27^{e}	n.d.	n.d.	n.d.	n.d.
2,6,10-Trimethyl-	1 452	n.d.	n.d.	n.d.	2.23^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Note: Results are expressed as means of 3 replicates of each strains. Different lowercase letters in the same row indicate significant differences ($P < 0.05$). n.d.: not detected.

Table 4
Volatile compounds (mg/kg) identified in meat model medium inoculated with different CNS strains.

Compounds	RI	Control	Se16	Ssp17	Ssp18	Ssp20	Ssp24	Ssp26	Sc27	Ssc28	Ssc29
Alcohols											
1-Butanol	1 129	n.d.	0.67^{bcde}	0.83^{bcd}	0.73^{bcde}	0.67^{bcde}	0.71^{bcde}	0.85^{bc}	n.d.	2.18^{a}	0.65^{bcde}
3-Methyl-1-butanol	1 192	n.d.	0.46^{defg}	0.75^{cde}	n.d.	0.53^{defg}	3.10^{a}	0.82^{cd}	n.d.	1.48^{b}	n.d.
2-Ehyl-1-hexanol	1 470	0.37^{cd}	0.57^{bc}	0.54^{bc}	n.d.	n.d.	n.d.	n.d.	1.16^{a}	1.01^{a}	n.d.
1-Nonanol	1 663	n.d.	0.50^{cd}	0.41^{defg}	0.38^{efg}	n.d.	n.d.	0.26^{h}	0.95^{a}	n.d.	0.33^{fgh}
1-Pentanol	1 192	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.27^{a}	n.d.
1-Undecanol	1 871	n.d.	0.24^{b}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2-Furanmethanol	1 674	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3-Furanmethanol	1 678	0.20^{b}	0.18^{bcd}	0.19^{bc}	0.15^{cde}	n.d.	n.d.	0.14^{efg}	n.d.	0.49^{a}	n.d.
Benzyl alcohol	1 854	0.13^{gh}	0.25^{cdef}	0.31^{c}	0.24^{cdef}	0.47^{b}	0.70^{a}	0.23	0.53^{b}	0.52^{b}	0.20^{defgh}
Phenylethyl alcohol	1 915	0.20^{c}	5.45^{a}	0.84^{c}	0.82^{c}	3.46^{ab}	1.82^{bc}	0.39^{c}	1.07^{c}	1.79^{bc}	0.90^{c}
Geraniol	1 784	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2-Propyl-1-pentanol	1 471	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.48^{b}
Phenols											
Phenol	> 2 000	n.d.	0.14^{b}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.27^{b}	n.d.
2,4-Bis(1,1-dimethylethyl)-phenol	> 2 000	0.97^{defg}	1.61^{bcde}	1.89^{bcd}	1.01^{defg}	1.45^{cdefg}	3.30^{a}	0.99^{defg}	1.46^{cdefg}	2.24^{abc}	0.63^{efg}
P-Tert-butyl-phenol	> 2 000	n.d.	0.14^{b}	0.10^{cde}	0.12^{bcd}	n.d.	n.d.	0.10^{cde}	n.d.	0.23^{a}	n.d.
Aldehydes											
3-Methyl-2-butenal	1 207	n.d.	0.13^{cde}	0.19^{b}	0.14^{bcd}	n.d.	n.d.	0.14^{bcd}	n.d.	0.59^{a}	0.19^{b}
2-Undecenal	1 753	0.19^{b}	0.44^{ab}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.74^{a}	0.10^{b}
3-Furaldehyde	1 466	0.33^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Benzaldehyde	1 500	22.20^{a}	5.26^{ghi}	4.19^{hij}	3.57^{ijk}	9.79^{c}	5.43^{fgh}	2.95^{jk}	7.03^{def}	10.48^{c}	3.70^{ijk}
3,4-Dimethyl-benzaldehyde	1 786	0.82^{ef}	1.43^{bcde}	1.99^{abcd}	1.17^{ef}	n.d.	n.d.	1.33^{de}	n.d.	2.63^{a}	1.47^{bcde}
Benzeneacetaldehyde	1 620	n.d.	n.d.	0.42^{b}	0.23^{cd}	0.42^{b}	n.d.	n.d.	n.d.	0.35^{bc}	n.d.
Furfural	1 448	n.d.	0.48^{b}	0.18^{f}	0.33^{cd}	n.d.	n.d.	n.d.	n.d.	0.52^{b}	0.21^{ef}
Octanal	1 280	0.29^{abc}	0.45^{ab}	0.14^{bc}	0.08^{c}	n.d.	n.d.	n.d.	n.d.	0.52^{a}	0.28^{abc}
2,4-Dimethyl-benzaldehyde	1 784	n.d.	n.d.	n.d.	n.d.	1.79^{c}	3.30^{a}	n.d.	n.d.	n.d.	n.d.
Ketones											
2,3-Butanedione	984	n.d.	0.48^{ij}	0.59^{hi}	0.62^{ghi}	1.18^{de}	3.85^{b}	0.56^{hi}	n.d.	4.45^{a}	2.01^{c}
3-Hydroxy-2-butanone	1 267	n.d.	1.01^{gh}	1.14^{fgh}	0.97^{gh}	n.d.	7.94^{a}	1.76^{defg}	2.40^{cd}	4.12^{b}	2.19^{cde}
Acetophenone	1 626	0.60^{gg}	0.70^{de}	0.61^{fg}	0.51^{h}	n.d.	n.d.	0.50^{h}	1.12^{b}	1.19^{a}	0.41^{i}
4'-Hydroxy-acetophenone	1 803	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3-Octanone	1 235	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.86^{a}	n.d.
Esters											
5-Hexyldihydro-2(3*H*)-furanone	> 2 000	n.d.	0.39^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4-Dodecanolide	> 2 000	n.d.	0.33^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Phenethyl acetate	1 818	n.d.	0.33^{b}	n.d.	n.d.	0.52^{a}	n.d.	n.d.	n.d.	n.d.	n.d.
Dibutyl phthalate	> 2 000	0.15^{g}	0.15^{g}	0.19^{efg}	0.33^{def}	0.42^{d}	1.15^{a}	0.41^{d}	0.96^{b}	0.34^{def}	0.15^{g}
Isopropyl myristate	> 2 000	0.11^{a}	0.11^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table 4 (*Continued*)

Compounds	RI	Control	Se16	Ssp17	Ssp18	Ssp20	Ssp24	Ssp26	Sc27	Ssc28	Ssc29
Ethyl octanoate	1 436	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.44^{a}	n.d.
Methyl hexanoate	1 189	n.d.	n.d.	0.11^{b}	0.27^{a}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Acids											
Acetic acid	1 442	0.11^{j}	4.83^{hi}	8.78^{d}	6.64^{efg}	7.99^{de}	19.21^{b}	7.34^{def}	11.55^{c}	30.44^{a}	8.80^{d}
Butanoic acid	1 630	n.d.	1.61^{efg}	1.81^{de}	1.41^{fgh}	2.84^{c}	3.97^{b}	1.71^{def}	3.18^{c}	4.56^{a}	1.63^{efg}
3-Methyl-butanoic acid	1 192	n.d.	0.57^{efg}	0.74^{cde}	0.51^{fg}	0.77^{cde}	1.37^{b}	0.95^{c}	1.66^{b}	1.84^{a}	0.67^{def}
Hexanoic acid	1 844	n.d.	1.26^{efg}	1.51^{de}	1.18^{fgh}	2.06^{c}	3.35^{a}	1.38^{def}	2.81^{b}	3.04^{b}	1.31^{efg}
Nonanoic acid	> 2 000	0.06^{c}	2.31^{b}	0.45^{b}	0.28^{b}	0.30^{b}	11.03^{a}	0.46^{b}	0.96^{b}	1.17^{b}	0.41^{b}
Octanoic Acid	> 2 000	0.13^{g}	2.09^{ef}	1.96^{ef}	1.66^{efg}	2.45^{cde}	7.14^{a}	1.68^{ef}	3.89^{b}	3.38^{bc}	1.76^{ef}
2-Methyl-propanoic acid	1 551	n.d.	0.34^{fgh}	0.45^{de}	0.32^{efg}	0.72^{c}	0.92^{b}	0.23^{fg}	0.83^{bc}	1.17^{a}	0.40^{def}
n-Decanoic acid	> 2 000	0.11^{f}	0.36^{bcde}	0.31^{cdef}	0.21^{cdef}	0.39^{bcde}	1.43^{a}	0.26^{cdef}	0.43^{bc}	0.57^{b}	0.25^{cdef}
trans-Cinnamic acid	1 607	n.d.	n.d.	n.d.	n.d.	n.d.	5.22^{b}	n.d.	n.d.	n.d.	n.d.
Benzoic acid	> 2 000	n.d.	0.11^{d}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.32^{a}	n.d.
Propanoic acid	1 525	n.d.	n.d.	0.13^{b}	0.11^{b}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pentanoic acid	1 734	n.d.	n.d.	0.10^{ab}	n.d.	n.d.	n.d.	0.2^{a}	n.d.	n.d.	0.08^{bc}
Other nitrogen/Sulphur compounds											
2-Acetylthiazole	1 621	0.54^{ijk}	0.99^{cde}	0.93^{def}	0.80^{efg}	n.d.	2.22^{a}	0.65^{ghij}	1.17^{cd}	1.47^{b}	0.55^{hijk}
1-Isocyano-2-methyl-benzene	1 897	0.26^{ef}	0.24^{fg}	0.24^{fg}	0.22^{fgh}	0.54^{bc}	0.66^{a}	0.19^{gh}	0.48^{c}	0.50^{c}	0.21^{fgh}
Benzonitrile	1 580	n.d.	3.84^{de}	2.85^{gh}	3.03^{fg}	n.d.	n.d.	2.45^{hi}	8.25^{b}	6.43^{c}	2.12^{i}
O-Decyl-hydroxylamine	1 399	0.42^{bcde}	n.d.	0.51^{bcd}	0.34^{cde}	0.71^{b}	n.d.	0.32^{cde}	1.36^{a}	1.11^{a}	n.d.
3-(Methylthio)-propanal	1 436	0.47^{b}	0.23^{c}	n.d.	0.10^{ef}	n.d.	n.d.	n.d.	n.d.	1.55^{a}	n.d.
2,5-Dimethyl-pyrazine	1 302	n.d.	0.69^{bc}	n.d.	0.67^{bc}	n.d.	n.d.	n.d.	n.d.	0.45^{e}	0.63^{cd}
3-Ethyl-2,5-dimethyl-pyrazine	1 441	0.45^{b}	0.37^{cd}	0.39^{bc}	0.33^{cd}	n.d.	n.d.	0.30^{de}	n.d.	0.85^{a}	0.31^{de}
Others											
2,6,11-Trimethyl-dodecane	1 446	n.d.	n.d.	n.d.	n.d.	n.d.	7.75^{a}	n.d.	3.24^{c}	n.d.	0.19^{d}
2-Methyl-eicosane	1 641	n.d.	n.d.	0.24^{e}	n.d.	2.68^{b}	2.24^{c}	n.d.	0.66^{d}	n.d.	n.d.
2,6,10-Trimethyl-pentadecane	1 452	n.d.	n.d.	n.d.	n.d.	0.78^{b}	0.65^{c}	n.d.	n.d.	n.d.	n.d.

Note: Results are expressed as means of 3 replicates of each strains. Different lowercase letters in the same row indicate significant differences ($P < 0.05$). n.d.: not detected.

Conversely, most volatile compounds produced by the CNS strains were mainly acids, such as acetic acid and butanoic acid. These acidic compounds are regarded as key aroma contributors in fermented sausages. The concentration of benzaldehyde detected in the non-inoculated control was higher than that found in the CNS-inoculated samples. This result indicated the considerable role of catalase activity in benzaldehyde degradation by these strains.

The CNS strains showed significant differences ($P < 0.05$) in ketone and acid production. Ketones, primarily produced through amino acid catabolism or carbohydrate metabolism, are considered key aroma contributors in fermented sausages (Tables 3 and 4). In addition, 3-hydroxy-2-butanone (acetoin) was produced by all inoculated samples except *S. xylosus* Sx11 and *S. saprophyticus* Ssp20. Acetoin is widely used in the food industry as a flavour enhancer, giving a buttery taste[55]. The highest acetoin concentration was produced by *S. xylosus* Sx3. Diacetyl (2,3-butanedione) was also generated by all inoculated samples except *S. xylosus* Sx11 and *S. carnosus* Sc27. Conversely, the acetophenone content of the samples inoculated with *S. carnosus* Sc27 and *S. sciuri* Ssc28 was higher. Acetophenone, which is derived from aromatic amino acid catabolism, contributes to the almond odour of the model medium.

Carboxylic acids not only function as aroma compounds but also serve as precursors of other aromatic compounds, methyl ketones, alcohols, lactones, and esters[56]. In the present study, the largest proportion of acids produced by the CNS strains was acetic acid, followed by butanoic acid and octanoic acid. *S. sciuri* Ssc28 produced the highest levels of acetic acid and butanoic acid ($P < 0.05$), and *S. saprophyticus* Ssp24 generated the highest concentration of octanoic acid ($P < 0.05$). Among the *S. xylosus* strains, *S. xylosus* Sx3 successfully generated the highest levels of acetic acid, butanoic acid, and octanoic acid ($P < 0.05$). Given that *S. xylosus* is a common CNS species in commercial starter cultures, it is a promising candidate that can enhance the aroma of fermented sausages because of its ability to produce considerable levels of acetoin and acids.

3.8 Transcriptomic analysis of CNS strains

RNA-seq was performed to assess the functioning of *S. xylosus* in fermented meats. On the basis of preliminary screening results, Sx3 and Sx6 were selected for RNA-seq analysis because they exhibit satisfactory enzymatic activities and aroma-producing abilities. After the quality control process, 57 066 483 clean reads were obtained from 12 CNS samples. The transcriptome data showed a mean GC content of 34.82%. The clean data were assembled and compared against GO and KEGG; thus, the expressed genes with relevant functional information were annotated.

In comparison with the transcriptional profiles of Sx3 and Sx6 at 24 h, their transcriptomic profiles at 72 h displayed 634 and 620 DEGs, respectively. After incubation in the fermented sausage model medium for 72 h, Sx3 had 297 upregulated and 337 downregulated genes, while Sx6 had 295 upregulated and 325 downregulated genes (Fig. 3). Vermassen et al.[57] reported that *S. xylosus* strain C2a showed 993 DEGs, accounting for 38%–48% of the total genes at 72 h possibly because of varying culture conditions.

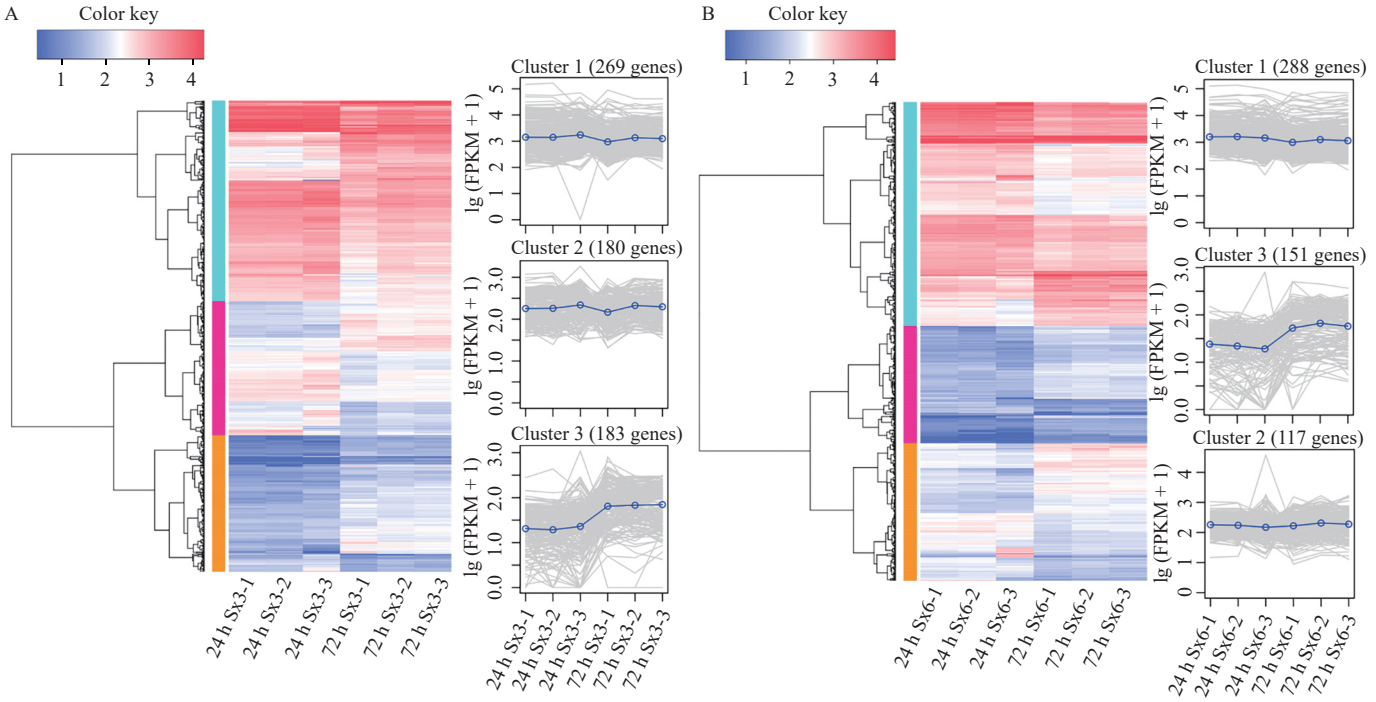

Fig. 3 DEGs cluster heat map of Sx3 and Sx6 at 24 h *vs*. 72 h. Heat map obtained from hierarchical clustering analysis of all DEGs, each row represents a DEG, and each column represents different samples. Blue represents low expression, and red represents high expression. The gray lines show how each gene's expression changes across samples within a cluster. The blue line is the average expression change of all genes in that cluster. (A) Sx3-1, Sx3-2, Sx3-3; (B) Sx6-1, Sx6-2, Sx6-3.

Fig. 3 illustrates the hierarchical clustering of DEGs in Sx3 and Sx6 into 3 primary clusters based on their expression patterns. The expression patterns in Cluster 1 (269 genes in Sx3; 288 genes in Sx6) and Cluster 2 (180 genes in Sx3; 177 genes in Sx6) were relatively stable, but the expression levels were significantly different. Cluster 1 comprised highly expressed genes, indicating their vital roles during fermentation. Conversely, Cluster 2 contained genes with consistently low expression levels, suggesting their contribution to non-essential or auxiliary cellular functions. Cluster 3 (183 genes in Sx3; 151 genes in Sx6) showed increasing expression levels at 72 h, indicating their potential involvement in processes or pathways activated during fermentation.

DEGs were assigned to GO function annotation. A total of 1 279 genes in Sx3 and 1 272 genes in Sx6 were significantly enriched across 26 GO terms. The 3 most upregulated DEG clusters were cellular processes, metabolic processes, and catalytic activity. Conversely, the three most downregulated DEG clusters were catalytic activity, cellular anatomical entity, and cellular processes. These findings indicated the dynamic functional regulation in both strains under the given experimental conditions.

In Fig. 4, 350 DEGs in Sx3 and 351 DEGs in Sx6 were grouped into 5 primary categories consisting of 24 pathways. Both strains shared 22 pathways. Among them, the top 3 enriched pathways were carbohydrate metabolism, amino acid metabolism, and metabolism of cofactors and vitamins. In comparison with Sx3, Sx6 contained 22 additional DEGs, suggesting a more pronounced regulation of these metabolic processes in Sx6. Conversely, Sx3 showed greater enrichment in nucleotide metabolism and energy metabolism pathways. These results indicated distinct metabolic profiles between the two strains.

Sx3 and Sx6 (Q-value ≤ 0.05) shared the following pathways: 'valine, leucine, and isoleucine biosynthesis' and 'cysteine and methionine metabolism' in amino acid metabolism, and 'glycolysis/gluconeogenesis' in carbohydrate metabolism. Notably, the glycolysis/gluconeogenesis pathway is involved in ATP synthesis, which supports cellular growth and metabolic activities. Additionally, the breakdown of branched-chain amino acids, such as leucine, isoleucine, and valine, during meat fermentation contributes to microbial protein synthesis and improves fermentation efficiency; consequently, the organoleptic attributes of fermented meat products are enhanced[58].

Other important pathways in Sx6 were linked to amino acid metabolism, including 'tyrosine metabolism', 'alanine, aspartate, and glutamate metabolism', 'histidine metabolism', and 'aminoacyl-tRNA biosynthesis'. This observation suggested that the utilisation and processing of amino acids were remarkably altered. Conversely, Sx3 exhibited more diverse pathways, including 'C5-branched dibasic acid metabolism' and 'butanoate metabolism' in carbohydrate metabolism and the 'HIF-1 signalling' pathway in signal transduction. These differences indicated a link between the phenotypes of *S. xylosus* and specific genetic pathways.

The metabolic pathways related to volatile compound production in *S. xylosus* primarily depend on pyruvate catabolism, amino acid catabolism, lipolysis, and fatty acid oxidation[59]. In pyruvate catabolism, Sx3 and Sx6 synthesise acetoin and diacetyl from pyruvate. The diacetyl reductase gene was significantly upregulated by 1.7-fold (P < 0.05; Fig. 5), facilitating the conversion of diacetyl into acetoin. The gene encoding acetoin reductase was significantly downregulated by 1.3-fold (P < 0.05). This finding might account for the 2.2-fold increase in acetoin levels in Sx3 compared with that in Sx6; therefore, acetoin production in *S. xylosus* exhibited intraspecies variability, as previously reported[17].

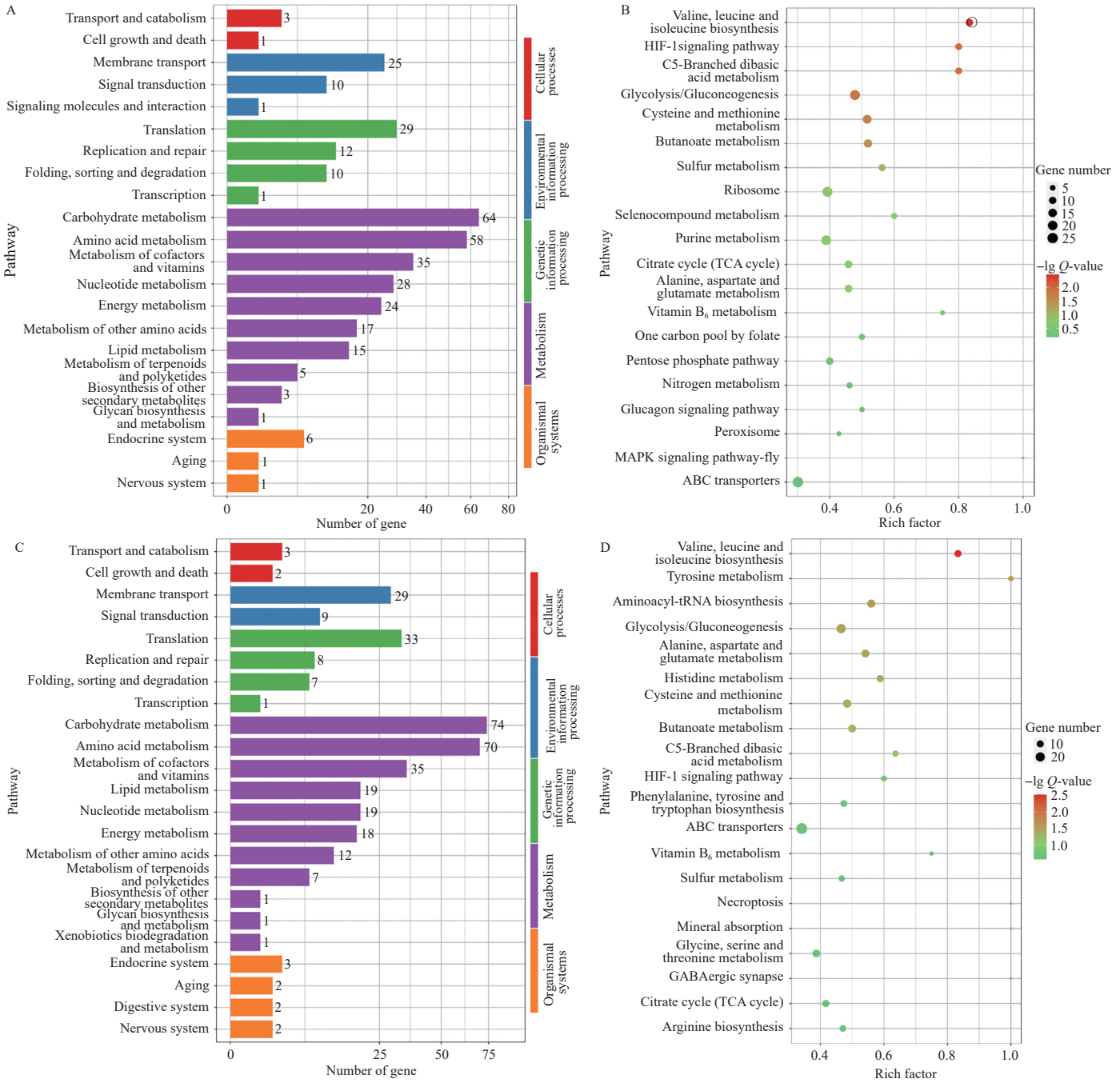

Fig. 4 The DEGs were analyzed for KEGG enrichment and KEGG enrichment bubble map of Sx3 and Sx6 at the 24 h *vs.* 72 h. KEGG enrichment analysis (Q-value < 0.05) of (A) Sx3, (C) Sx6. Bubble map of (B) Sx3, (D) Sx6 was used to show KEGG enriched top 20 pathways. The intensity of the color indicates the significance (Q-value < 0.05) of the pathway.

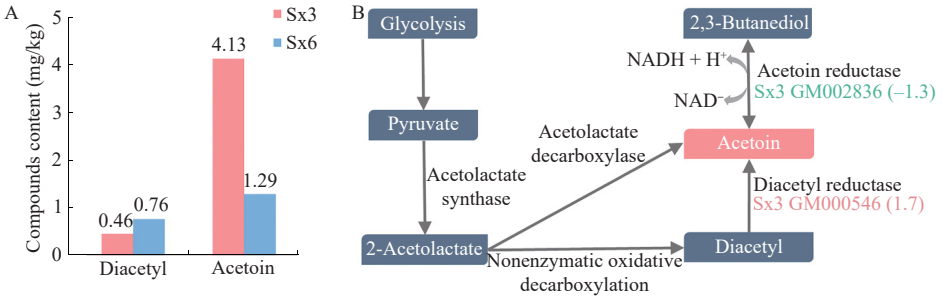

Fig. 5 (A) Compounds content of diacetyl and acetoin of Sx3 and Sx6; (B) Butanoate metabolism pathway for acetoin and diacetyl production. Red: up-regulated gene, Green: down-regulated gene, and DEGs fold changes are shown in parentheses.

4. Conclusion

This study demonstrated the technological properties of 18 CNS strains isolated from Nanx Wudl. Of these strains, 16 showed a nitrate-reducing ability, 11 displayed a lipolytic activity, and 15 tolerated 15% NaCl and 150 mg/kg of nitrite. Furthermore, all strains exhibited catalase and SOD activities, and the highest activity was observed in *S. saprophyticus*. The CNS strains produced acids and ketones as the predominant volatile compounds in the fermented sausage model medium. Among them, Sx3 generated the highest levels of acetoin. Comprehensive transcriptomic analysis revealed the considerable effect of genes related to pyruvate catabolism and amino acid metabolism on aroma production, especially acetoin production, in *S. xylosus*. Therefore, these findings helped elucidate the metabolic mechanisms underlying the technological properties of CNS strains and their potential use as starter cultures in fermented sausage manufacturing.

Conflict of interest

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

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Reference

[1] H.Y. Li, J.Q. Zhu, X. Chen, et al., Isolation and characterization of coagulase negative staphylococci with high proteolytic activity from dry fermented sausages as a potential starter culture, Food Res. Int. 162 (2022) 111957. https://doi.org/10.1016/j.foodres.2022.111957.

[2] X. Chen, R.F Mi, B. Qi, et al., Effect of proteolytic starter culture isolated from Chinese Dong fermented pork (Nanx Wudl) on microbiological, biochemical and organoleptic attributes in dry fermented sausages, Food Sci. Hum. Wellness 10 (2021) 13-22. https://doi.org/10.1016/j.fshw.2020.05.012.

[3] S. Heo, J.H. Lee, D.W. Jeong, Food-derived coagulase-negative *Staphylococcus* as starter cultures for fermented foods, Food Sci. Biotechnol. 29 (2020) 1023-1035. https://doi.org/10.1007/s10068-020-00789-5.

[4] N. Sirini, P.E.S. Munekata, J.M. Lorenzo, et al., Development of healthier and functional dry fermented sausages: present and future, Foods 11 (2022) 1128. https://doi.org/10.3390/foods11081128.

[5] A. Khusro, C. Aarti, Metabolic heterogeneity and techno-functional attributes of fermented foods-associated coagulase-negative staphylococci, Food Microbiol. 105 (2022) 105. https://doi.org/10.1016/j.fm.2022.104028.

[6] G. Blaiotta, C. Pennacchia, F. Villani, et al., Diversity and dynamics of communities of coagulase-negative staphylococci in traditional fermented sausages, J. Appl. Microbiol. 97 (2004) 271-284. https://doi.org/10.1111/j.1365-2672.2004.02298.x.

[7] R. Talon, S. Leroy, Diversity and safety hazards of bacteria involved in meat fermentations, Meat Sci. 89 (2011) 303-309. https://doi.org/10.1016/j.meatsci.2011.04.029.

[8] N.P. Mangia, M. Cottu, M. Aponte, et al., Technological and safety characterization of coagulase-negative staphylococci isolated from Sardinian fermented sausage made by ovine meat, Foods 13 (2024) 633. https://doi.org/10.3390/foods13040633.

[9] J. Lofblom, R. Rosenstein, M.T. Nguyen, et al., *Staphylococcus carnosus*: from starter culture to protein engineering platform, Appl. Microbiol. Biotechnol. 101 (2017) 8293-8307. https://doi.org/10.1007/s00253-017-8528-6.

[10] A. Połka, V. Rebecchi, L. Pisacane, et al., Bacterial diversity in typical Italian salami at different ripening stages as revealed by high-throughput sequencing of 16S rRNA amplicons, Food Microbiol. 46 (2015) 342-356. https://doi.org/10.1016/j.fm.2014.08.023.

[11] S. Leroy, P. Giammarinaro, J.P. Chacornac, et al., Biodiversity of indigenous staphylococci of naturally fermented dry sausages and manufacturing environments of small-scale processing units, Food Microbiol. 27 (2010) 294-301. https://doi.org/10.1016/j.fm.2009.11.005.

[12] M.C.G. Fontán, J.M. Lorenzo, S. Martínez, et al., Microbiological characteristics of "androlla", a Spanish traditional pork sausage, Food Microbiol. 24 (2007) 52-58. https://doi.org/10.1016/j.fm.2006.03.007.

[13] B. Geniş, Y. Tuncer, Determination of antibiotic susceptibility and decarboxylase activity of coagulase-negative *Staphylococcus* and *Macrococcus caseolyticus* strains isolated from fermented Turkish sausage (sucuk), J. Food Process Preserv. 42 (2018) e13329. https://doi.org/10.1111/jfpp.13329.

[14] K. Candogan, F.B. Wardlaw, J.C. Acton, et al., Effect of starter culture on proteolytic changes during processing of fermented beef sausages, Food Chem. 116 (2009) 731-737. https://doi.org/10.1016/j.foodchem.2009.03.065.

[15] J. Sun, C. Cao, M. Feng, et al., Technological and safety characterization of coagulase-negative staphylococci with high protease activity isolated from traditional Chinese fermented sausages, LWT-Food Sci. Technol. 114 (2019) 108371. https://doi.org/10.1016/j.lwt.2019.108371.

[16] A.K. Søndergaard, L.H. Stahnke, Growth and aroma production by *Staphylococcus xylosus*, *S. carnosus* and *S. equorum*-a comparative study in model systems, Int. J. Food Microbiol. 75 (2002) 99-109. https://doi.org/10.1016/s0168-1605 (01)00729-2.

[17] F. Ravyts, L. Steen, O. Goemaere, et al., The application of staphylococci with flavour-generating potential is affected by acidification in fermented dry sausages, Food Microbiol. 27 (2010) 945-954. https://doi.org/10.1016/j.fm.2010.05.030.

[18] C. Barrière, D. Centeno, A. Lebert, et al., Roles of superoxide dismutase and catalase of *Staphylococcus xylosus* in the inhibition of linoleic acid oxidation, FEMS Microbiol. Lett. 201 (2001) 181-185. https://doi.org/10.1111/j.1574-6968.2001.tb10754.x.

[19] J. Ye, X. Zhang, S. Yuan, et al., An Investigation of volatile flavor compounds and lipolysis-oxidation in coppa as affected by the inoculation of coagulase-negative *Staphylococcus* during the air-drying stage, Foods 13 (2024) 2723. https://doi.org/10.3390/foods13172723.

[20] D. Van der Veken, F. Leroy, Prospects for the applicability of coagulase-negative cocci in fermented meat products using omics approaches, Curr. Opin. Food. Sci. 48 (2022) 100918. https://doi.org/10.1016/j.cofs.2022.100918.

[21] J.H. Lee, S. Heo, D.W. Jeong, Genomic insights into *Staphylococcus equorum* KS1039 as a potential starter culture for the fermentation of high-salt foods, BMC Genom. 19 (2018) 136. https://doi.org/10.1186/s12864-018-4532-1.

[22] S. Choi, J. Jung, C.O. Jeon, et al., Comparative genomic and transcriptomic analyses of NaCl-tolerant *Staphylococcus* sp. OJ82 isolated from fermented seafood, Appl. Microbiol. Biot. 98 (2014) 807-822. https://doi.org/10.1007/s00253-013-5436-2.

[23] S. Heo, J. Park, E. Lee, et al., Transcriptomic analysis of *Staphylococcus equorum* KM1031, isolated from the high-salt fermented seafood jeotgal, under salt stress, Fermentation 8 (2022) 403. https://doi.org/10.3390/fermentation8080403.

[24] S. Heo, T. Kim, H.E. Na, et al., Transcriptomic analysis of *Staphylococcus equorum* KM1031 from the high-salt fermented seafood jeotgal under chloramphenicol, erythromycin and lincomycin stresses, Sci. Rep. 12 (2022) 15541. https://doi.org/10.1038/s41598-022-19897-9.

[25] M. Chung, V.M. Bruno, D.A. Rasko, et al., Best practices on the differential expression analysis of multi-species RNA-seq, Genome Biol. 22 (2021) 121. https://doi.org/10.1186/s13059-021-02337-8.

[26] I. Essid, and M. Hassouna, Effect of inoculation of selected *Staphylococcus xylosus* and *Lactobacillus plantarum* strains on biochemical, microbiological and textural characteristics of a Tunisian dry fermented sausage, Food Control 32 (2013) 707-714. https://doi.org/10.1016/j.foodcont.2013.02.003.

[27] Z.J. Li, Isolation and identification of staphylococci in Chinese traditional cured meat and its application, Biol. Bull. 3 (2006) 5. https://doi.org/10.3969/j.issn.1002-5464.2006.03.018.

[28] G. Mauriello, A. Casaburi, F. Villani, Proteolytic activity of *Staphylococcus xylosus* strains on pork myofibrillar and sarcoplasmic proteins and use of selected strains in the production of "Naples type" salami, J. Appl. Microbiol. 92 (2002) 482-490. https://doi.org/10.1046/j.1365-2672.2002.01551.x.

[29] J.S. Johnson, D.J. Spakowicz, B.Y. Hong, et al., Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis, Nat. Commun. 10 (2019) 5029. https://doi.org/10.1038/s41467-019-13036-1

[30] X. Zeng, J. Fan, L. He, et al., Technological properties and probiotic potential of yeasts isolated from traditional low-salt fermented Chinese fish Suan yu, J. Food Biochem. 43 (2019) e12865. https://doi.org/10.1111/jfbc.12865.

[31] A.G. Casaburi, G. Blaiotta, O. Mauriello, Technological activities of *Staphylococcus carnosus* and *Staphylococcus simulans* strains isolated from fermented sausages, Meat Sci. 71 (2005) 643-650. https://doi.org/10.1016/j.meatsci.2005.05.008.

[32] H. Aebi, Methods of Enzymatic Analysis, 2nd ed., New York&London, Academic Press, 1974.

[33] G. Mauriello, A. Casaburi, G. Blaiotta, et al., Isolation and technological properties of coagulase negative staphylococci from fermented sausages of Southern Italy, Meat Sci. 67 (2004) 149-158. https://doi.org/10.1016/j.meatsci.2003.10.003.

[34] J.P. Rosec, O. Gigaud, Staphylococcal enterotoxin genes of classical and new types detected by PCR in France, Int. J. Food Microbiol. 77 (2002) 61-70. https://doi.org/10.1016/S0168-1605(02)00044-2

[35] S. Jarraud, C. Mougel, J. Thioulouse, et al., Relationships between Staphylococcus aureus genetic background, virulence factors, agr groups (alleles), and human disease, Infect Immun. 70 (2002) 631-641. https://doi.org/10.1128/IAI.70.2.631-641.2002.

[36] H.D. Paik, J.Y. Lee, Investigation of reduction and tolerance capability of lactic acid bacteria isolated from kimchi against nitrate and nitrite in fermented sausage condition, Meat Sci. 97 (2014) 609-614. https://doi.org/10.1016/j.meatsci.2014.03.013.

[37] K.A. Gutsche, T.B. Tran, R.F. Vogel, Production of volatile compounds by *Lactobacillus sakei* from branched chain alpha-keto acids, Food Microbiol. 29 (2012) 224-228. https://doi.org/10.1016/j.meatsci.2014.03.013.

[38] S.F. Chen, Ultrafast one-pass FASTQ data preprocessing, quality control, and deduplication using fastp, iMeta. 2 (2023) e107. https://doi.org/10.1002/imt2.107.

[39] M.D. Young, M.J. Wakefield, G.K. Smyth, et al., Gene ontology analysis for RNA-seq: accounting for selection bias, Genome Biol. 11 (2010) R14. https://doi.org/10.1186/gb-2010-11-2-r14.

[40] C. Xie, X.Z Mao, J.J. Huang, et al., KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases, Nucleic Acids Res. 39 (2011) W316-22. https://doi.org/10.1093/nar/gkr483.

[41] A. Müller, G. Fogarassy, A. Bajac, et al., Selection of *Staphylococcus carnosus* strains based on *in vitro* analysis of technologically relevant physiological activities, Ann Microbiol. 66 (2016) 479-487. https://doi.org/10.1007/s13213-015-1133-y.

[42] R.K. Majumdar, S. Gupta, Isolation, identification and characterization of *Staphylococcus* sp. from Indian ethnic fermented fish product, Lett. Appl. Microbiol. 71 (2020) 359-368. https://doi.org/10.1111/lam.13362.

[43] N.Z. Palavecino Prpich, M.P. Castro, M.E. Cayré, et al., Autochthonous starter culture selection to keep traditions in the manufacture of dry sausages alive, Ann. Microbiol. 65 (2015) 1709-1719. https://doi.org/10.1007/s13213-014-1010-0.

[44] R. Bosse, M. Gibis, H. Schmidt, et al., Nitrate reductase activity of *Staphylococcus carnosus* affecting the color formation in cured raw ham, Food Res Int. 85 (2016) 113-120. https://doi.org/10.1016/j.foodres.2016.04.021.

[45] M.S. Mainar, F. Leroy, Process-driven bacterial community dynamics are key to cured meat colour formation by coagulase-negative staphylococci *via* nitrate reductase or nitric oxide synthase activities, Int. J. Food Microbiol. 212 (2015) 60-66. https://doi.org/10.1016/j.ijfoodmicro.2015.03.009.

[46] M.S. Mainar, D.A. Stavropoulou, F. Leroy, Exploring the metabolic heterogeneity of coagulase-negative staphylococci to improve the quality and safety of fermented meats: a review, Int. J. Food Microbiol. 247 (2017) 24-37. https://doi.org/10.1016/j.ijfoodmicro.2016.05.021.

[47] Z. Kesmen, B. Yarimcam, H. Aslan, et al., Application of different molecular techniques for characterization of catalase-positive cocci isolated from sucuk, J. Food Sci. 79 (2014) M222-M229. https://doi.org/10.1111/1750-3841.12328.

[48] D.W. Jeong, H. Seulhwa, J.H. Lee, Safety and technological characterization of *Staphylococcus equorum* isolates from jeotgal, a Korean high-salt-fermented seafood, for starter development, Int. J. Food Microbiol. 188 (2014) 108-115. https://doi.org/10.1016/j.ijfoodmicro.2014.07.022.

[49] A.M. Fiorentini, M.C. Sawitzki, T.M. Bertol, et al., Influence of a native strain of *Staphylococcus xylosus* on the microbiological, physicochemical and sensorial characteristics on milano salami type, Braz. Arch. Biol. Techn. 53 (2010) 961-974. https://doi.org/10.1590/S1516-89132010000400027.

[50] A. Rebecchi, F. Miragoli, C. Lopez, et al., Exploring coagulase-negative staphylococci diversity from artisanal llama sausages: assessment of technological and safety traits, Microorganisms 8 (2020) 629. https://doi.org/10.3390/microorganisms8050629.

[51] G. Landeta, J. A. Curiel, A. V. Carrascosa, et al., Characterization of coagulase-negative staphylococci isolated from Spanish dry cured meat products, Meat Sci. 93 (2013) 387-396. https://doi.org/10.1016/j.meatsci.2012.09.019.

[52] R. Rosenstein, C. Nerz, L. Biswaset al., Genome analysis of the meat starter culture bacterium *Staphylococcus carnosus* TM300, Appl. Environ. Microbiol. 75 (2009) 811-822. https://doi.org/10.1128/AEM.01982-08.

[53] A. Ruaro, C. Andrighetto, S. Torriani, et al., Biodiversity and characterization of indigenous coagulase-negative staphylococci isolated from raw milk and cheese of North Italy, Food Microbiol. 34 (2013) 106-111. https://doi.org/10.1016/j.fm.2012.11.013.

[54] M.D. Cunha, E. Peresi, R.A. Calsolari, et al., Detection of enterotoxins genes in coagulase-negative staphylococci isolated from foods, Braz J. Microbiol. 37 (2006) 70-74. https://doi.org/10.1590/S1517-83822006000100013.

[55] S.J. Bae, S. Kim, and J.S. Hahn, Efficient production of acetoin in *Saccharomyces cerevisiae* by disruption of 2,3-butanediol dehydrogenase and expression of NADH oxidase, Sci Rep. 6 (2016) 27667. https://doi.org/10.1038/srep27667.

[56] S. Zhang, T. Wang, Y. Zhang, et al., Effects of *Monascus* on proteolysis, lipolysis, and volatile compounds of camembert-type cheese during ripening, Foods 11(2022) 1662. https://doi.org/10.3390/foods11111662.

[57] A. Vermassen, S. Leroy, A. de La Foye, et al., Adaptation of *Staphylococcus xylosus* to pork meat batter during the fermentation step, ICoMST 61 (2015) hal-02743715f. Clermont-Ferrand, France.

[58] M. Flores, A. Olivares, Flavor, Handbook of Fermented Meat and Poultry, 2nd ed., Chichester, Wiley, 2015.

[59] S. Leroy, A. Vermassen, G. Ras, et al., Insight into the genome of *Staphylococcus xylosus*, a ubiquitous species well adapted to meat products, Microorganisms 5 (2017) 52. https://doi.org/10.3390/microorganisms5030052.