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CHARACTERIZATION AND TECHNOLOGICAL EVALUATION OF COAGULASE-NEGATIVE STAPHYLOCOCCI ISOLATED FROM SJENICA SHEEP'S HAM

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ABSTRACT. This study investigates the community of coagulase-negative staphylococci (CNS) in samples of Sjenica sheep's ham from different villages across three seasons over a 120-day maturation period. The CNS group was identified into five distinct species: *Staphylococcus epidermidis, S. saprophyticus, S. carnosus, S. xylosus*, and *S. equorum*. The safety evaluation revealed no hemolytic activity in any tested isolates, while resistance to novobiocin was observed in *S. xylosus* and *S. saprophyticus* isolates. The technological properties of the isolated CNS were assessed, including tolerance to different temperatures, pH values, salt concentrations, as well as proteolytic and lipolytic activity. Results demonstrated the ability of all tested isolates to grow across various pH values and salt concentrations, with isolates of *S. carnosus* and *S. epidermidis* showing tolerance to 45 °C. The best technological properties were shown by *S. carnosus*, followed by *S. equorum*. These findings provide insights into the potential applications of CNS in food processing industries, as putative starters.

Keywords: coagulase-negative staphylococci, *Staphylococcus carnosus*, *Staphylococcus equorum*, safety evaluation, technological properties, starters.

INTRODUCTION

Sheep's ham, a dried meat product derived from a complex production process, requires meticulous attention to raw material quality and stringent sanitary conditions to ensure its production. Various factors, including raw material selection and food technology considerations, influence the quality of sheep litter (AKTAŞ *et al*., 2005). In the production of Sjenica sheep's ham, fattened castrated males and barren sheep, indigenous to the Pešter region, are utilized. The preparation process involves the use of whole sheep carcasses,

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excluding the inner thigh-shoe portion, and the removal of bones, except for distal bone segments measuring up to 5 cm, crucial for suspending the product during drying and fermentation (STAMENKOVIĆ and DEVIĆ, 2006; ŽUGIĆ-PETROVIĆ, 2022). During maturation, water loss occurs, creating an environment conducive to microbial growth, which aids in preserving the meat from spoilage (AKTAŞ *et al*., 2005). The microbial population present during the fermentation process of Sjenica sheep's ham primarily comprises lactic acid bacteria (LAB) and coagulase-negative staphylococci (CNS). This microbial composition originates from microorganisms naturally present in meat or introduced during the production process (ŽUGIĆ-PETROVIĆ, 2022).

The genus *Staphylococcus* (family Staphylococcaceae) comprises Gram-positive cocci, typically spherical bacteria with a diameter of approximately 1 μm, arranged in characteristic grape-like clusters. Staphylococci are characterized by their non-motile nature, facultative anaerobic metabolism, lack of oxidase activity, and positive catalase reaction. These microorganisms exhibit tolerance to elevated salt concentrations, surpassing 10% (GÖTZ *et al*., 2006). Classification of staphylococci into coagulase-positive and coagulase-negative strains is based on their capacity to produce coagulase, a key virulence factor. Coagulase-positive staphylococci (CPS) are known pathogens capable of causing infections and foodborne illnesses, with *Staphylococcus aureus* being a notable representative species (HAIT *et al*., 2014). Conversely, coagulase-negative staphylococci (CNS), while generally considered non-pathogenic, may sporadically trigger opportunistic infections, as observed with species like *S. epidermidis, S. haemolyticus*, and *S. saprophyticus* (HEO *et al*., 2020).

Staphylococci represent a crucial group of microorganisms integral to the process of meat fermentation, exerting notable influence on flavor development and color stability (RATSIMBA *et al*., 2017). CNS strains have been shown to utilize sugars for fermentation processes, particularly in carbohydrate-poor substrates like meat matrices, where alternative energy sources such as nucleosides and arginine are employed (LEROY *et al*., 2017; STAVROPOULOU *et al*., 2018). Variations in metabolic pathways among different CNS species and strains have been noted, presenting opportunities for targeted selection of strains to optimize meat product quality (SÁNCHEZ MAINAR *et al*., 2017).

CNS exhibit enzymatic activity, including nitrate reductase production, which contributes to the conversion of nitrate to nitrite and subsequently impacts the color of cured meat products. Catalase produced by staphylococci aids in preventing lipid oxidation, thereby enhancing the taste and color stability of meat products (SÁNCHEZ MAINAR *et al*., 2017). Additionally, CNS secretes proteases and lipases, influencing product texture and flavor through the breakdown of macromolecules into low-molecular-weight compounds (peptides, amino acids, aldehydes, amines, and free fatty acids). These microorganisms demonstrate resilience to high salt concentrations and low temperatures commonly encountered during meat fermentation processes (SEMEDO-LEMSADDEK *et al*., 2016). In meat fermentation, salting, and drying processes, CNS populations typically range from 5 to 7 log CFU/g (LEROY *et al*., 2017).

During the production of traditional fermented meat products such as "Lacón," there is a significant increase in the population of bacteria from the families Micrococcaceae and Staphylococcaceae during the salting and early drying phases (LORENZO *et al*., 2012). Commonly isolated strains from meat and meat products include *S. carnosus, S. equorum, S. succinus,* and *S. xylosus* (SEMEDO-LEMSADDEK *et al*., 2016; STAVROPOULOU *et al*., 2018).

Due to the scarcity of information regarding the potential utilization of wild types of CNS as starter cultures, this study aimed to isolate, identify, and evaluate the safety of indigenous CNS derived from Sjenica sheep's ham. Additionally, the technological properties of these strains were investigated to assess their suitability as potential starter cultures in the food processing industries.

MATERIALS AND METODS

Sampling of Sjenica sheep's ham

For the purposes of the experiment, Sjenica sheep's ham from selected households in the villages of Blato (A), Krajinoviće (B), and Rasno (C) in the Pester region was used over three production seasons (2016/17 – 1; 2017/18 – 2; 2018/19 – 3) during late autumn and early spring periods. The Sjenica sheep's ham was prepared in the same manner in all selected households where the sheep's ham is traditionally prepared. Samples of Sjenica sheep's ham, weighing 300 g, were collected under aseptic conditions for the isolation and identification of autochthonous CNS. For the purposes of the mentioned investigations, the samples were transported in mobile refrigerators to the laboratory of the Department of Agricultural and Food Studies in Prokuplje, where they were stored at a temperature of 4 °C. The research was conducted 24 hours after sampling ("OFFICIAL ROLE OF RS", no. 73/2010; GUIDE TO THE APPLICATION OF MICROBIOLOGICAL CRITERIA FOR FOOD, 2011).

Enumeration and isolation of Staphylococcus spp.

The preparation of samples for isolation of indigenous CNS from the products followed the standardized procedure outlined in SRPS EN ISO 6887-1:2008. Microorganism enumeration in Sjenica sheep's ham commenced on day zero (initial examination of raw sheep meat) and subsequently at intervals of 7, 14, 28, 60, 90, and 120 days following the commencement of indigenous product production. Each sampling event involved three samples collected from households (designated A, B, and C), spanning across three distinct seasons. Sampling procedures involved the use of sterile instruments to collect material from the interior of the product (300 g). Subsequently, 10 g of the sample was isolated and homogenized in 90 ml of sterile physiological solution containing peptone (0.8 g NaCl/mL and 1 g peptone/mL), followed by mixing for 15 minutes on a vortex until complete homogenization was achieved.

A series of decimal dilutions, extending up to 10^{-7}, were prepared from the primary dilution. Subsequently, triplicate plates with nutrient media were inoculated from each dilution. The determination of coagulase-positive staphylococci was conducted in accordance with SRPS EN ISO 6888-1:2009/A2:2018, utilizing Baird-Parker agar. Confirmation of coagulase-positive staphylococci was performed through coagulase testing. Plate incubation occurred at 37 °C for 24 hours, following which colonies on plates containing an average count of more than 20 and less than 300 colonies were enumerated.

Preliminary identification and safety evaluation of coagulase-negative staphylococci

Isolates identified as Gram-positive and catalase-positive cocci were subjected to examination based on identification schemes for coagulase-negative staphylococci (KRISHER *et a*l., 2016). The colony color of tested staphylococci was determined based on the presence of intense yellow pigment (KRISHER *et al*., 2016).

Coagulase test

In the coagulase test, 50 µl of rabbit plasma was added to sterile microscope slides, into which a colony of the test organism dissolved in a drop of physiological saline solution was introduced. Coagulation was determined by the appearance of clotting (macroscopic aggregation) in the homogenized rabbit plasma and bacteria, defining a positive reaction. *Staphylococcus aureus* ATCC 25923 was used as a positive control in this test (QUINN *et al*., 2011).

Mannitol fermentation

The mannitol fermentation test was conducted by inoculating staphylococcal suspensions onto Chapman agar. After incubation at 37 °C for 24 hours, changes in the color of the medium from red to yellow were observed. Bacteria that caused a change in the indicator color demonstrated the ability to ferment mannitol.

Hemolysis on blood agar

Hemolytic activity was assessed to investigate the pathogenicity of the tested strains (LEDINA *et al*., 2013). Hemolysin induces a hemolytic reaction and is responsible for the breakdown of red blood cells. The hemolysis capability of the tested organisms was analyzed using the method defined by FOULQUIÉ-MORENO *et al*. (2003). Overnight cultures were streaked onto blood agar plates (5% sheep blood) and incubated at 37 °C for 24 hours. After incubation, the presence or absence of clear zones around the colonies was detected. Briefly, α-hemolysis (insufficiently clear zones around colonies), β-hemolysis (clear zones around colonies), and γ-hemolysis (no halo around colonies) indicate positive hemolytic activity, i.e., the potential pathogenicity of the tested strains (MARAGKOUDAKIS *et al*., 2009).

Novobiocin sensitivity

This test is used for the differentiation of CNS and is one of the characteristics of staphylococci that aids in their identification, examined by the disk diffusion method (BAUER *et al*., 1966).

Standardization of bacterial suspension was performed in physiological saline using the McFarland 0.5 standard. The suspension was inoculated onto nutrient agar plates using sterile cotton swabs. Novobiocin disks (5 μg) were aseptically placed on the surface of the inoculated agar. After incubation at 37 ℃ for 24 hours, zones of growth inhibition around novobiocin were measured. A zone diameter less than 12 mm indicates bacterial resistance to the antibiotic, while a zone diameter greater than or equal to 16 mm indicates bacterial susceptibility to novobiocin.

Identification of coagulase-negative staphylococci by MALDI-TOF mass spectrometry

The biochemical identification results were validated using MALDI-TOF mass spectrometry at the Institute of Public Health of Vojvodina. Overnight cultures of CNS grown on blood agar at 37 °C for 24 hours were utilized for identification purposes.

Preliminarily identified CNS samples underwent analysis following the standard procedure (Bruker's direct transfer sample preparation procedure for MALDI-TOF MS). This involved directly applying individual bacterial colonies onto a 96-MALDI plate (Bruker Daltonics, Bremen, Germany) as a thin film using a sterile swab. After application, the MALDI plate was air-dried at room temperature for approximately one minute. Subsequently, the sample was overlaid with 1 μL of matrix solution (Bruker Matrix HCCA; α-cyano-4-hydroxycinnamic acid), as described in GRUJOVIĆ *et al*. (2019).

The mass spectrum results of the identified isolate were compared with those of known microbial isolates from the MALDI BioTyper software database. The similarity measure ranged from 0.000 to 3.000, with values ≥ 2.000 (green color) indicating accurate identification to the species level. Values between 1.700 and 2.000 (yellow color) were considered probable genus identification, while values below 1.700 (red color) indicated similarity between the unknown profile and those in the database (KRZYSZTOF *et al*., 2016).

Technological characteristics of isolated coagulase-negative staphylococci

The technological characteristic of isolated CNS was evaluated through tolerance to different temperatures, pH values and salt tolerance, as well as proteolytic and lipolytic activity.

Temperature tolerance

The growth capacity of CNS isolates at various temperatures was assessed in nutrient broth. Overnight cultures were incubated for 24 hours at temperatures ranging from 4 °C to 50 °C. The presence of turbidity in the seeded isolates after incubation indicated growth (REDA *et al*., 2018).

pH adaptability

The ability of tested CNS strains to grow in media with different pH levels was investigated by inoculating overnight cultures into modified nutrient broths. The pH of the broths was adjusted to values ranging from 4 to 8 using hydrochloric acid. Following incubation under microaerophilic conditions at 37 °C for 24 hours, the growth of seeded isolates was observed (REDA *et al*., 2018).

Salt tolerance

A tolerance test to varying concentrations of NaCl was performed on nutrient agar supplemented with 4%, 6.5%, and 8% NaCl. Overnight cultures of CNS strains were plated onto a solid medium with the respective salt concentrations and then incubated at 37 °C for 24 hours. The presence of colonies of seeded isolates after incubation indicated growth (REDA *et al*., 2018).

Proteolytic Activity

The proteolytic activity of tested CNS strains was evaluated on a medium composed of nutrient agar and milk (1.6% milk fat) in a 1:1 ratio. Bacteria were aseptically transferred onto the medium and allowed to incubate at 37 °C for 24 hours. The appearance of a transparent zone around bacterial colonies indicated proteolytic activity. *Bacillus subtilis* ATCC 6633 served as a positive control, while *Escherichia coli* ATCC 25922 served as a negative control (ABUBAKR and AL-ADIWISH, 2017).

Lipolytic Activity

Bacterial lipolytic activity was assessed on tributyrin agar. Overnight cultures were inoculated onto the medium and then incubated at 37 °C for 24 hours. The presence of clear zones surrounding bacterial colonies indicated lipolytic activity. *Bacillus subtilis* ATCC 6633 was used as a positive control, and *E. coli* ATCC 25922 was used as a negative control (AKABANDA *et al*., 2014).

Statistical analysis

Where appropriate, data were presented as means ± standard deviations (SD) using Microsoft Excel (Redmond, Washington, DC, USA).

RESULTS AND DISCUSSION

The variation in the number of staphylococci in the product samples from different villages during three different seasons in 120 days of maturation is presented in Table 1. Initially, during the maturation process, a notable divergence in staphylococcal counts is evident among samples, with the highest count observed on day zero in the sample obtained from Blato village (A1). The number of staphylococci escalates until the 28^{th} day, following which it gradually declines.

Table 1. Enumeration of *Staphylococcus* spp.

Day of maturation	Samples								
	A1	**B1**	**C1**	**A2**	**B2**	**C2**	**A3**	**B3**	**C3**
0	3.61 ± 0.01	3.58 ± 0.32	3.05 ± 0.13	3.27 ± 0.02	3.13 ± 0.05	3.05 ± 0.13	3.51 ± 0.01	3.35 ± 0.05	3.33 ± 0.05
7	3.94 ± 0.03	4.03 ± 0.10	3.86 ± 0.27	3.86 ± 0.02	3.82 ± 0.15	3.88 ± 0.07	3.97 ± 0.02	3.98 ± 0.00	3.95 ± 0.04
14	4.03 ± 0.10	4.29 ± 0.04	4.17 ± 0.00	4.24 ± 0.04	4.14 ± 0.09	4.09 ± 0.01	4.17 ± 0.04	4.25 ± 0.05	4.18 ± 0.04
28	4.85 ± 0.05	4.96 ± 0.02	4.81 ± 0.16	4.95 ± 0.03	5.05 ± 0.13	5.01 ± 0.14	4.98 ± 0.02	5.03 ± 0.13	5.02 ± 0.12
60	4.21 ± 0.01	3.94 ± 0.09	4.06 ± 0.07	3.93 ± 0.02	4.00 ± 0.05	3.80 ± 0.08	4.00 ± 0.05	4.21 ± 0.20	4.00 ± 0.00
90	3.61 ± 0.03	3.50 ± 0.01	3.85 ± 0.13	3.50 ± 0.01	3.71 ± 0.02	3.33 ± 0.07	3.68 ± 0.27	3.78 ± 0.05	3.71 ± 0.05
120	3.43 ± 0.01	3.39 ± 0.30	3.65 ± 0.14	3.18 ± 0.20	3.65 ± 0.00	3.08 ± 0.48	3.26 ± 0.02	3.33 ± 0.14	3.51 ± 0.10

Mean values (log CFU/g of sample) ± SD; A – village Blato; B – village Krajinoviće; C – village Rasno; 1 – 2016/17; 2 – 2017/18; 3 – 2018/19

Table 2. Preliminary identification and safety evaluation of species within the genus *Staphylococcus*

Species	***S. xylosus***	***S. carnosus***	***S. saprophyticus***	***S. epidermidis***	***S. equorum***
Morphology	cocci	cocci	cocci	cocci	cocci
Colony color	Orange	Grey	Dark yellow	Grey	White
Coagulase test	–	–	–	–	–
Fermentation of mannitol	+	+	–	–	+
Hemolysis on blood agar	γ	γ	γ	γ	γ
Novobiocin sensitivity	R	S	R	S	S

"+" – positive reaction; "–" – negative reaction; "R" – resistant; "S" – sensitive

Identification and safety evaluation of coagulase-negative staphylococci isolated from Sjenica sheep's ham

A total of 376 Gram-positive and catalase-positive isolates belonging to the CNS group were isolated from the analyzed samples of Sjenica sheep's ham. Characterization and preliminary identification of staphylococci were performed using standard physiological and biochemical tests. The findings are delineated in Table 2.

Based on preliminary identification (Table 2), five distinct species of CNS were provisionally identified within the samples of Sjenica sheep's ham. *S. epidermidis* isolates exhibited characteristics including grey-colored colonies and the absence of mannitol fermentation. Conversely, *S. saprophyticus* isolates displayed round colonies with a dark yellow hue and no mannitol fermentation. Isolates of *S. carnosus* featured shiny gray colonies and demonstrated robust mannitol fermentation. Meanwhile, *S. xylosus* isolates presented with yellow-orange colonies and exhibited proficient mannitol fermentation. Finally, *S. equorum* isolates were characterized by white, shiny colonies, showcasing efficient mannitol fermentation.

The results of the safety evaluation demonstrated that none of the tested isolates showed hemolytic activity on blood plates, while resistance to novobiocin was observed for *S. xylosus* and *S. saprophyticus* isolates (Table 2).

The preliminary identification of CNS isolates was subsequently affirmed using MALDI-TOF mass spectrophotometry. Scores equal to or greater than 2,000 (indicated in green) were interpreted as precise identifications down to the species level).

The distribution of isolates through the seasons and place of sampling are delineated in Table 3. The species *S. epidermidis* (11 isolates, 2.92%) was recovered from all producers during the initial production year. However, it was not detected in the second year of the study. In the third year, isolates of *S. epidermidis* were identified in samples collected from households in the villages of Blato (A) and Krajinoviće (B). *S. saprophyticus* (46 isolates, 12.23%) was isolated from all producers during the first production year. In the second year, *S. saprophyticus* was only isolated from producers in the villages of Krajinoviće (B) and Rasno (C). During the third year, this species was detected in samples from households in the villages of Blato (A) and Rasno (C). *S. carnosus* (64 isolates, 17.2%). *S. xylosus* (103 isolates, 27.39%), and *S. equorum* (151 isolates, 40.42%) were consistently identified across all producers and throughout all research periods from the Sjenica sheep's ham samples.

Table 3. The distribution of *Staphylococcus* spp. isolates in Sjenica sheep's ham

Producers/ seasons	**A1**	**B1**	**C1**	**A2**	**B2**	**C2**	**A3**	**B3**	**C3**	**Total**	**(%)**
S. equorum	17	20	20	19	11	13	15	18	19	152	40.42
S. xylosus	13	5	12	14	12	15	10	10	12	103	27.39
S. carnosus	8	9	10	2	2	5	7	11	10	64	17.02
S. saprophyticus	7	9	2	0	10	5	5	0	8	46	12.23
S. epidermidis	2	1	4	0	0	0	2	2	0	11	2.92
Total	47	44	48	35	35	38	39	41	49	376	10.00

A – village Blato; B – village Krajinoviće; C – village Rasno; 1 – 2016/17; 2 – 2017/18; 3 – 2018/19

Investigation of staphylococcal biodiversity in Pastrima highlighted *S. vitulinus* as dominant, along with *S. equorum*, *S. saprophyticus*, and *S. xylosus* (FETTAHOĞLU *et al*., 2019). In CNS isolated from various meat products, *S. xylosus* prevailed (ESSID *et al*., 2007; KABAN and KAYA, 2009, while *S. equorum* dominated in Spanish dry-cured products and *S. carnosus* in German and Belgian fermented meats (RECKEM *et al*., 2019). CNS isolated from the traditional product "Sujuk" consisted of species such as *S. xylosus*, *S. saprophyticus*, *S. equorum*, and *S. carnosus* (KABAN and KAYA, 2009). Identification of CNS isolated from

Sjenica sheep's ham samples revealed that *S. xylosus*, *S. carnosus*, and *S. epidermidis* were predominant across all research periods and producers. *S. saprophyticus* was exclusively isolated in the first production year, while in subsequent years, it was found in specific households.

FONTÁN *et al*. (2007) suggested that novobiocin-sensitive staphylococci isolated from meat products originates from human and animal skin during raw material handling and throughout production. The presence of resistance in staphylococci isolated from animal-derived food may be attributed to frequent antibiotic administration to animals for therapeutic or growth-promoting purposes (DE MESQUITA SOUZA SARAIVA *et al*., 2022). Our research indicates that *S. xylosus* and *S. saprophyticus* isolates showed resistance to novobiocin. Other tested isolates were novobiocin-sensitive.

Technological properties of isolated coagulase-negative staphylococci

The technological properties of isolated CNS were evaluated through tolerance to different temperatures, pH values, salt tolerance as well as proteolytic and lipolytic activity. The results are presented in Table 4.

The results indicated that all tested isolates belonging to the CNS group showed the ability to grow in different pH values of the medium and in all tested salt concentrations. All isolates demonstrated the ability to grow at temperatures up to 15 °C, while isolates of *S. carnosus* and *S. epidermidis* grew at 45 °C. Only isolates of *S. saprophyticus* did not exhibit proteolytic and lipolytic activity, while isolates of *S. epidermidis* did not show proteolytic activity.

Table 4. Technological properties of isolated coagulase-negative staphylococci

Technological properties	**Species**	***S. xylosus***	***S. carnosus***	***S. saprophyticus***	***S. epidermidis***	***S. equorum***
	№	103	64	46	11	152
Temperature	4 ºC	+	+	+	+	+
	15 ºC	+	+	+	+	+
	45 ºC	–	+	–	+	–
	50 ºC	–	–	–	–	–
pH value	4.0	+	+	+	+	+
	5.0	+	+	+	+	+
	6.0	+	+	+	+	+
	8.0	+	+	+	+	+
% of NaCl	4.0	+	+	+	+	+
	6.5	+	+	+	+	+
	8.0	+	+	+	+	–
Lipolytic activity		+	+	–	+	+
Proteolytic activity		+	+	–	–	+

"+" – positive reaction; "–" – negative reaction; № (tested isolates).

The assessment of technological properties in isolated CNS sheds light on their potential applications in food processing industries. Results indicate versatile growth capabilities across pH and salt ranges, suggesting adaptability to various production conditions. Notably, their ability to thrive at low temperatures, such as 15 °C, is promising for refrigerated environments. CARVALHO et al. (2012) similarly observed significant growth of CNS within the 15 °C to 20 °C range, indicating the potential for fermentation processes. Remarkably, *S. carnosus* and *S. epidermidis* showed heat tolerance up to 45 °C, suggesting suitability for heat-resistant starter cultures.

Conversely, *S. saprophyticus* lacked proteolytic and lipolytic activity, while *S. epidermidis* lacked proteolytic activity, highlighting species-specific enzymatic variations. Despite limited data on CNS from dry sheep ham, LANDETA et al. (2013) found proteolytic activity in *S. xylosus* and *S. equorum* from Spanish dried meat, aligning with our findings.

Furthermore, out of 104 *Staphylococcus* spp. isolates, 30% exhibited proteolytic and 42% lipolytic activity, with *S. xylosus* and *S. equorum* showing optimal growth at 15 °C, pH 5.5, and NaCl concentrations of 10% to 15% (CARVALHO *et al*., 2012). In our study, *S. xylosus* and *S. carnosus* isolates exhibited the best technological properties and great potential for use as starter cultures. *S. equorum* isolates also demonstrated good technological properties, except for heat tolerance and growth at 8% NaCl. However, *S. xylosus* showed phenotypic resistance to antibiotics, therefore, the molecular analysis should be performed.

These findings contribute to our understanding of the technological capabilities of CNS and provide a basis for further exploration of their utility as functional ingredients or starter cultures in food manufacturing. However, further research is warranted to elucidate the specific mechanisms underlying the observed variations in enzymatic activity and their potential impact on food quality and safety. Finally, the selection of CNS as starter cultures depends on their specific technological properties, safety considerations, and desired characteristics in the final product. Proper strain selection, along with stringent quality control measures, is essential to ensure the effectiveness and safety of CNS-based starter cultures in food production.

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