International Journal of Food Microbiology 242 (2017) 141–151

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Predominant processing adaptability of *Staphylococcus xylosus* strains isolated from Chinese traditional low-salt fermented whole fish

Xuefeng Zeng [a,b], Laping He [b], Xu Guo [c], Li Deng [b], Wangen Yang [d], Qiujin Zhu [b,*], Zhenhua Duan [a,*]

[a] Institute of Food Research, Hezhou University, Hezhou, Guangxi, China
[b] School of Liquor and Food Engineering, Guizhou university, Guiyang, Guizhou, China
[c] School of Economics, Guizhou University of Commerce, Guiyang, Guizhou, , China
[d] College of Chemistry and Chemical Engineering, JishouUniversity, Jishou, Hunan, China

ARTICLE INFO

Article history:
Received 28 May 2016
Received in revised form 14 November 2016
Accepted 15 November 2016
Available online 16 November 2016

Keywords:
Suanyu
Staphylococcus xylosus
Starter
Proteolytic activity
Amino acid decarboxylase
Sensory

ABSTRACT

This study aimed to determine the predominant processing adaptability of 27 selected isolates of *Staphylococcus xylosus* in 'Suan yu', a traditional Chinese low-salt fermented whole-fish product. The isolates were screened for proteolytic, lipolytic, and enzymatic profiles; amino-acid decarboxylase content; antimicrobial activities; and tolerance to low temperatures, pH 5.0, and salt. Two *S. xylosus* strains grew at 10 °C in the presence of 10% NaCl and at pH 5.0. Agar-plate assays and sodium dodecyl sulphate–polyacrylamide gel electrophoresis revealed that 21 and 8 of the strains exhibited appropriate proteolytic activities against myofibrillar and sarcoplasmic proteins, respectively. All *S. xylosus* strains also displayed different enzymatic profiles, and most strains showed negative decarboxylase activities. The results of this step were used as input data for a Principal Component Analysis; therefore, the most technologically relevant strain 3 and 8 were combined with *L. plantarum* 120 as MS1 and MS2, respectively, were further selected for the fermented fish surimi, and the fish surimi inoculated with mixed starter cultures (MS1, MS2) scored high for overall acceptability. Free amino acid contents of 1757 and 1765 mg/100 g sample were found in fish surimi inoculated with MS1 and MS2, respectively, after 72 h of fermentation. Therefore, Sx-3 and Sx-8, which presented the best predominant processing adaptability, is an eligible starter culture for fermented fish production.

1. Introduction

Suanyu is a popular low-salt fermented whole fish snack produced in China. The product exhibits a characteristic flavour, is free of fishy odour and taste, and possesses a long shelf-life. Suanyu is prepared by traditional methods without adding starter cultures in small-scale processing units. Autochthonous microbiota in fermented fish products are largely responsible for the organoleptic, nutritional properties and safety of final products after fermentation and ripening (Speranza et al., 2015).

Spontaneous meat fermentation is mainly induced by lactic acid bacteria (LAB), and Gram-positive and Coagulase-negative staphylococci (CNS). CNS, when used as a starter culture, is reportedly an important type of microorganism because of its lipolytic and proteolytic activities (Aida et al., 2013; Jeong et al., 2014). The protease and lipase activities of CNS, which generate considerable aroma compounds, including amino acids, aldehydes, amines, and free fatty acid (FFAs), improve the sensory qualities of fermented food products (Ruaro et al., 2013). CNS can also produce antimicrobial compounds against pathogenic microorganisms, thus stabilizing the product and lengthening the shelf life (Martín et al., 2007). Notably, CNS must be cautiously selected because some of these microbes have decarboxylase activities that adversely affect product safety (Jeong et al., 2014).

Indigenous starter cultures render fermented fish products hygienic and standardized, with suitable organoleptic properties and short ripening time. Food products prepared with indigenous CNS are generally more adaptable to the local environment than those with commercial starter cultures because of specific metabolic capabilities of the former (Shivanne Gowda et al., 2016). Therefore, characterizing CNS isolated from autochthonous fermented food products is necessary to identify the best strains. Such characteristics include tolerance to acid, NaCl and low temperature, which negatively affect microbial growth and contribute to flavour development throughout maturation. Bonomo et al. (2009) characterizing 37 strains of CNS isolated from traditional fermented sausages of the Basilicata region on the basis of the strains' technological and safety properties. Udomsil et al. (2015) also reported that *Staphylococcus* spp. *CMC5-3-1* and *CMS5-7-5* isolated from fermented fish sauce can serve as a starter culture to improve the umami and aroma of fish sauce. In our previous study, 329 strains of lactobacilli and 156 strains of CNS have been isolated from six whole

* Corresponding authors.
E-mail addresses: ls.qjzhu@gzu.edu.cn (Q. Zhu), 1171983152@qq.com (Z. Duan).

http://dx.doi.org/10.1016/j.ijfoodmicro.2016.11.014
0168-1605/

fish samples subjected to Chinese traditional low-salt fermentation called 'Suanyu' (Zeng et al., 2016). Furthermore, a *Staphylococcus xylosus* strain was isolated from Suanyu. This isolate was combined with two other isolated strains (*Lactobacillus plantarum* and *Saccharomyces cerevisiae*) as mixed starter cultures and used to ferment Suanyu. Results showed that the inoculation with the mixed starter cultures reduced the lag time of fermentation initiation and improved the quality of Suanyu (Zeng et al., 2013). However, the technological properties and predominant processing adaptability of the selected *S. xylosus* strains are relatively less studied. This study aimed to characterize and select *S. xylosus* strains with optimum technological properties, including proteolysis, lipolysis, antimicrobial activity against food borne pathogens, enzymatic activity, decarboxylase activity, and sensory attributes.

2. Materials and methods

2.1. Isolation and identification of CNS

Staphylococci strains were isolated from Chinese traditional low-salt fermented whole fish products (Suanyu). These samples were acquired from the same hand-crafting workshops, in which cooked dried carbohydrate (24% w/w), dried spices (1% w/w), and salt (3% w/w) were combined with the fish samples. The mixtures were then placed in earthenware, ripened at ambient temperature, and stored until desirable taste and aroma were produced under anaerobic conditions. These samples were obtained from products fermented for four different times: SD3, SD7, SD14, and SD28 (fermentation for 3, 7, 14, and 28 days, respectively).

Staphylococcus strains were isolated and purified on mannitol salt agar (MSA; Merck, Darmstadt, Germany) after incubation for 48 h at 30 °C. The resultant colonies were assessed for cell morphology, Gram staining, and catalase production. Oxidation/fermentation test in OF medium was conducted as described by Boulares et al. (2013). Sensitivities to furazolidone, bacitracin, and lysostaphin (Sigma Chemical, St Louis, MO, USA) were examined as described by Boulares et al. (2013). Coagulase production was determined using the tube test with coagulase plasma (Becton, Dickinson & Company, NJ, USA). Pigment production was observed on P-agar (Phillips et al., 1985). The isolates were identified by an API ID 32 STAPH system (BioMérieux, France). Sterile glycerol (10% [v/v]) was added to MSA, and the resulting mixture was frozen at −80 °C.

The *S. xylosus* strains identified above were reconfirmed by species-specific PCR assays as described by Rantsiou et al. (2005). In a typical procedure, DNA was extracted from a single colony by using an InstaGene Matrix (Bio-Rad Laboratories, Hercules, CA). Specific primers geh3 (5′–GTAGAAAAAGCGAAT GAA CAA C–3′) and geh4 (5′–CCT GGT TGC CAATCT TTA TAT AC–3′) designed based on the gehM gene coding for *S. xylosus* lipase (accession number AF208229) were used. After performing PCR, the products were subjected to electrophoresis on a 1% agarose gel in TBE buffer (40 mM Tris-borate, 89 mM boric acid, 2 mM EDTA; pH 8) with a 100 bp DNA ladder (Invitrogen, USA). Finally, the gel was stained with ethidium bromide, and the bands were visualized under UV light.

2.2. Technological characterization

2.2.1. Proteolytic activity

The proteolytic activities of the carps' (*Cyprinuscarpio* L.) myofibrillar and sarcoplasmic proteins were initially estimated using the method of Landeta et al. (2013). Meanwhile, the sarcoplasmic and myofibrillar proteins were extracted in accordance with the modified method of Landeta et al. (2013). The proteins were then added to sterile medium (0.25% yeast extract, 0.1% glucose and 1.5% agar), hereafter denoted as PA medium, at 1 mg/mL concentration. The medium was poured into Petri dishes. After solidification, the wells were bored and filled with 30 μL of cell suspension, and the mixtures were inoculated at 30 °C for 48 h. The agar disc was removed from each dish and stained with 0.05% (w/v) Coomassie blue R-250 in methanol: acetic acid: water (50:10:40). After 30 min, the samples were destained by 20% (v/v) methanol: ethanol: aceticacid: water (20:10:5:65). Proteolytic activity was determined by the clear zones around the wells.

Strains that exhibited proteolytic activity were further tested using sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis (PAGE). The overnight culture was cryo-centrifuged (Sigma Laborzentrifugen, Model 4K15, Osterode, Germany) at 5000*g* for 15 min at 4 °C. The resulting cell pellets were washed three times with PBS buffer. Afterwards, 10 μL of cell suspension was inoculated in 1 mL of PA medium containing sarcoplasmic and myofibrillar proteins without agar supplementation and incubated at 30 °C for 7 days. The culture biomass was then separated by refrigerated centrifugation as described above, and the supernatant was mixed (1:1 [v/v]) with the SDS–PAGE sample buffer (0.125 M Tris–HCl, pH 6.8; 2% SDS; 10% glycerol; 2% β-mercaptoethanol; and 0.005% bromophenol blue). The mixture was maintained in boiling water for 3–5 min prior to electrophoresis. In accordance with the method of Paramithiotis et al. (2000), we conducted SDS–PAGE in a vertical gel electrophoresis unit (Mini-Protean-3 Cell. Bio-Rad, Richmond, CA, USA) using a 12% separating gel with 4% stacking gel. After electrophoresis, staining and destaining were carried out as described by Landeta et al. (2013). The molecular weights of the proteolysis products were estimated by comparing with those of standard proteins. PA media lacking strains and containing sarcoplasmic or myofibrillar proteins incubated for seven days at 30 °C were designated as control samples. Proteolytic activity was analyzed by comparing the protein profile of the control with those obtained after incubation with proteolytic strains.

2.2.2. Lipolytic activity

Lipolytic activity was assessed as described by Vignolo et al. (1988). Cell suspensions (1 mL) of each strain was inoculated into 10 mL of sterile broth containing 1% tryptone (Merck), 0.5% yeast extract (Merck), and 3% NaCl at pH 7, supplemented with 4% (w/v) of pork fat. After incubation for seven days at 30 °C, lipolytic activity was determined through titration. Lipid extracts were obtained by adding 10 mL of petrol ether (Merck) and shaking for 1 min. Free fatty acids in the upper phase (lipid extract) were titrated with NaOH (0.1 M) in ethanol, with two drops of 1% phenolphthalein ethanol solution as the indicator. Lipolytic activity calculated as Lipolytic activity $= (\alpha \times N \times 28.2)/g$, where α is the volume (mL) of NaOH used for titration, N is the normality of NaOH, 28.2 is the percent equivalent weight of oleic acid, and g is the weight of pork fat.

Strains that exhibited lipolytic activity could be further analyzed on the ability for FFA release from triglycerides utilizing the medium as described above with fish fat instead of pork fat. After incubating at 37 °C for 72 h, the lipids of each culture were extracted as described by Bonomo et al. (2009), and the FFAs were separated from the triglycerides using the columns of NH_2-aminopropyl in accordance with the method proposed by Kaluzny et al. (1985).

As described by Shehata and Alexander (1970), the methyl esters of FFAs were prepared and quantified by gas chromatography by calculating the area at each peak using an internal standard (C13:0) at 4000 ppm (Franco et al., 2006). FFA content was expressed as mg fatty acid per 100 g of fat.

2.2.3. Effects of pH, temperature, and NaCl on microbial growth

The *S. xylosus* strains were evaluated for their growth abilities under different temperatures, pH, and NaCl concentrations as described by Landeta et al. (2013). In a typical procedure, each strain was separately subcultured in tryptone soya broth with 0.6% (w/v) yeast extract by adding 1% (v/v) inoculum and incubating overnight at 30 °C. This subculture was maintained until standardization to a final optical density at 600 nm (OD_{600}) of 0.2. Subsequently, the meat extract medium was