Probiotic potential of yeasts isolated from Xuanwei ham

Jiaming Cai, Wangang Zhang

College of Food Science and Technology, Nanjing Agricultural University, China

Objectives: Nowadays, the focus on yeast is gradually shifting from fermentation starter cultures to probiotic strains, while *Saccha- romyces cerevisiae* is the only one recognized as probiotic yeast (Hatoum, Labrie, & Fliss, 2012). Dry-cured ham, a fermented meat product, contains a rich variety of yeast. Thus, this work was designed to explore the probiotic properties of yeasts from Xuanwei ham, and select novel probiotic yeasts which have abilities of resistance to the intestinal environment, auto-aggregation, antimicrobial, and antioxidant.

Materials and Methods:

1. Ham sampling and yeast isolation Xuanwei ham was fermented for 24 months (Laopujia Ham Co., Ltd., Yunnan, China). The sur- face of Xuanwei ham was wiped with 75% ethanol, and 25 g of *biceps femoris muscle* at 2 cm under the surface of the ham was cut up and placed into 225 mL sterile normal saline. Yeast was selected in Rose Bengal medium and purified in YPD medium at 25 ℃.
2. Growth at pH 2.5 and in bile salts All isolates were incubated in YPD broth for 24 h at 28 ℃. YPD broth was acidified with 2 M HCl to reach pH 2.5 and added 2% (w/v) bile salts, respectively. Then, 10^6 cells/mL of strain was incubated in modified YPD broth for 48 h at 28 ℃. The strain cultured in the YPD broth without bile salts and acidification was set as control.
3. Auto-aggregation capacity The auto-aggregation capacity of isolates was tested followed by the method described by Gil-Rodri- guez, Carrascosa, and Requena (2015).
4. Antimicrobial activity The yeast cultures (200 μL) were added to the indicator bacteria (*Escherichia coli*, *Staphylococcus aureus*, and *salmonella sp.*) LB medium. The antimicrobial capacity of yeasts was measured by the size of the inhibition zone.
5. Antioxidant activity of yeast The DPPH radical scavenging activity of fermentation broth was assessed as described by Gil-Rodriguez et al. (2015)
6. Yeast identification Yeast DNA was extracted by fungus DNA Extraction Kit and the ITS1-5.8S rRNA-ITS2 region was amplified by PCR using primer pair ITS1 and ITS4. Yeast was identified by the alignment of sequences to similar fungal genes in NCBI and by comparison of the phylogenetic tree. 3.

Results and Discussion:

1. Ability to grow at pH 2.5 and in bile salts In this study, 108 yeast strains isolated from Xuanwei ham were evaluated for their ability to grow at pH 2.5 and 1% bile salts. The number of isolates that could grow at pH 2.5 was 41 and isolates that could grow at 1% bile salt was 50. A total of 27 isolates were able to grow both at pH 2.5 and in 1% bile salt which presented a higher resistance to the intestinal environment and were selected for the following parts.
2. The auto-aggregation ability of yeasts The auto-aggregation values gradually increased with extended incubation time and reached the maximum at 24 h of incubation. It was worth noting that 13 strains showed outstanding auto-aggregation capacity (more than 90% at 24 h) which had the potential to prevent pathogenic microorganisms from disrupting the intestinal balance.
3. The antibacterial properties of yeasts Results showed that most isolates displayed an obvious antibacterial activity on *salmonella sp.* and half of the isolates could effectively against *S. aureus*. However, fewer isolates presented the inhibition capacity against *E. coli.*
4. The antioxidant activity of yeasts The DPPH radical scavenging values of 27 isolates were all above 74%, among which 12 isolates were over 84%. Combining all probiotic indexes, two strains (XHY69 and XHY79) with potential probiotic properties were select- ed for strain identification.
5. Strain identification XHY69 and XHY79 were identified as *Yamadazyma triangularis* which was also identified in Spain Iberian ham and Danish cheese(Gallardo et al., 2014). However, the probiotic and antioxidant properties of *Yamadazyma triangularis* have not been reported.

Conclusions: In this study, it was first shown that *Yamadazyma triangularis* (XHY69 and XHY79) isolated from Xuanwei ham presented excellent probiotic properties. Further research is worth exploring the effect of XHY69 and XHY79 on improving gut microbiota and disease.

References:

Gallardo, G., Ruiz-Moyano, S., Hernández, A., Benito, M. J., Córdoba, M. G., Pérez-Nevado, F., & Martín, A. (2014). Application of ISSR-PCR for rapid strain typing of Debaryomyces hansenii isolated from dry-cured Iberian ham. Food Microbiology, 42, 205- 211.

Gil-Rodriguez, A. M., Carrascosa, A. V., & Requena, T. (2015). Yeasts in foods and beverages: In vitro characterisation of probiotic traits. Lwt-Food Science and Technology, 64(2), 1156-1162.

Hatoum, R., Labrie, S., & Fliss, I. (2012). Antimicrobial and probiotic properties of yeasts: from fundamental to novel applications. Frontiers in Microbiology, 3, 421.

Key words: Probiotic, Yeast, Xuanwei ham