

# EFFECT OF YEAST INOCULATION ON THE STRUCTURE OF BACTERIAL COMMUNITY IN THE REDUCED-SALT HARBIN DRY SAUSAGES: A PERSPECTIVE OF INTERACTION BETWEEN FUNGI AND BACTERIA

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## I. INTRODUCTION

Salt as an important additive that provides characteristic flavour, ideal texture and inhibits microbial growth in Harbin dry sausages. However, the excessive salt intake has been shown to be associated with a variety of diseases, such as hypertension and cardiovascular. Yeast strains as the promising starter cultures to compensate for the flavour deficiencies of reduced-salt dry sausages have been proven, but their influence on the bacterial community structure has not been clarified. Therefore, this study aimed to investigate the inoculation of two yeast strains, including *Pichia kudriavzevii* and *Debaryomyces hansenii*, on the bacterial community structure in the reduced-salt dry sausages.

## II. MATERIALS AND METHODS

Dry sausages were manufactured based on the methods of Hu *et al.* [1]. Four treatments were manufactured: traditional dry sausage (Ct) and the reduced-salt dry sausages (Cr), containing 2.5% and 1.75% NaCl, respectively, were considered as the controls. The Pk and Dd treatments (containing 1.75% NaCl) were inoculated with *P. kudriavzevii* and *D. hansenii*, respectively. The yeast inoculation level was  $10^6$  CFU/g in meats. The pH and total acid content were determined using the method of Hu *et al.* [1] and Zhang *et al.* [2]. The lactic acid bacteria (LAB) were measured as described by Li *et al.* [3] and bacterial diversity was analysis according to Hu *et al.* [1]. The data were analysed by the General Linear Models procedure of the Statistix 8.1 software package and the analysis of variance (ANOVA) was used in conjunction with Tukey's multiple comparison test to identify significant differences between samples ( $P < 0.05$ ).

## III. RESULTS AND DISCUSSION

As shown in Fig. 1 A, the pH of all dry sausages decreased significantly from 0 d to 8 d ( $P < 0.05$ ). Meanwhile, the trend of total acid content accordingly showed a rapid increase (Fig.1 B). The Ct treatment with the highest salt addition had the highest pH and lowest total acid content throughout the fermentation period. The pH of the inoculated sausages was lower than that of the Cr control at the end of fermentation ( $P < 0.05$ ), which may be attributed to the combined action of yeast and LAB: the yeasts are able to accelerate the metabolism of carbohydrates by LAB. In particular, the dry sausages inoculated with *P. kudriavzevii* showed the lowest pH values and the highest total acid content in the late fermentation stage (8-12 d). The counts of LAB in the inoculated treatments were significantly higher than that in the uninoculated treatment (Cr) ( $P < 0.05$ ), indicating that the inoculation of yeast may promote the growth of LAB to some extent (Fig. 1 C). Twenty-six OTUs were observed to be common in all treatments (Fig. 1 D). *D. hansenii*-inoculated sausages showed the highest level of unique OTUs (36), indicating the low-level similarity of bacterial diversity with the other treatments. From the composition of bacteria in species level, the abundance of *Lactobacillus sakei* was enhanced by inoculating yeast strains (Fig. 1 E). The dissimilarity coefficients based on weighted UniFrac distance between two treatments were reflected in Fig. 1 F, in which the smaller coefficient implied that their similarity on bacterial community structure. Among all treatments, the Ct treatment had the smallest dissimilarity coefficient with the Cr treatment, followed by the treatment on day 0, the Pk and Dh treatments on day 12. In the inoculated dry sausages, the bacterial structure of the Pk treatment was more similar to the traditional dry sausages (Ct treatment), while the Dh treatment was more similar to the reduced-salt dry sausages (Cr treatment). Additionally, principal coordinate analysis showed that the first two principal coordinates (PCo1 and PCo2) explained 96.57% of the variance in bacterial assemblages (Fig. 1 G). The Ct and Cr treatments flocked well with each other located in the third quadrant. While the other treatments were distinctly separated with each other, this result once again illustrated the significant effects of yeast inoculation on the bacterial composition of dry sausages.

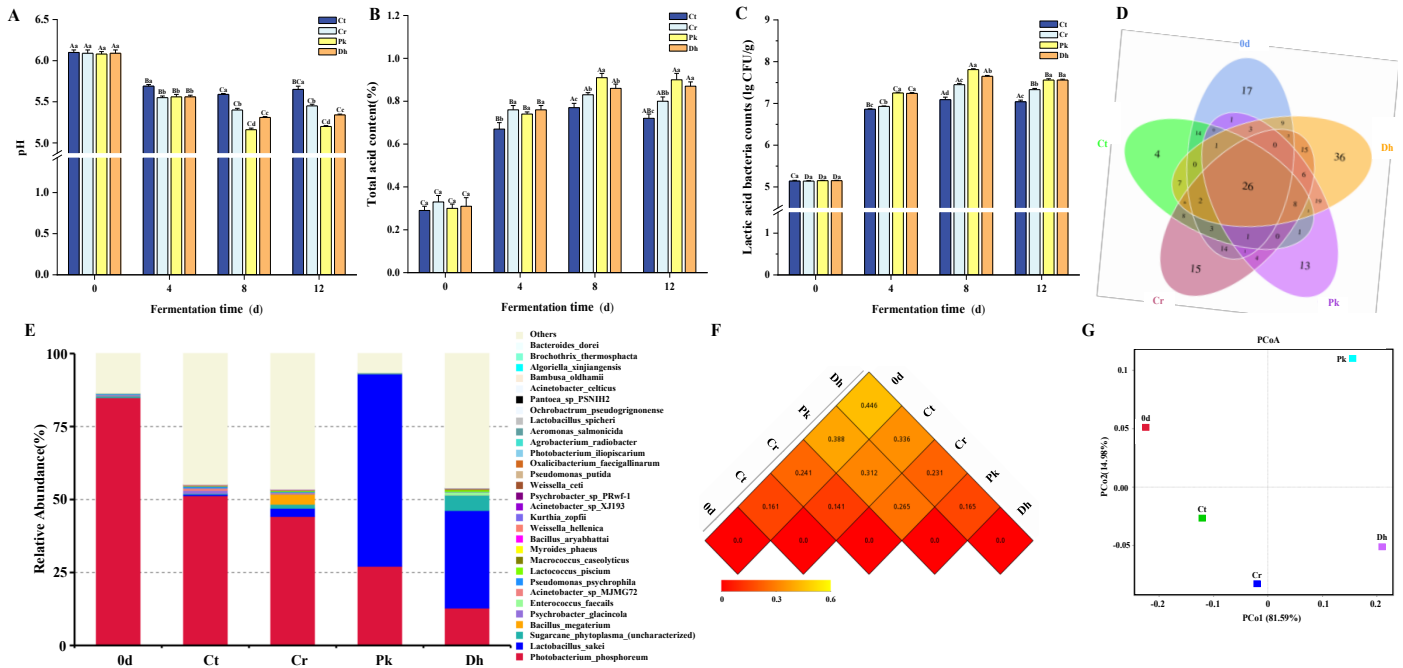


Fig.1 Effect of inoculation with different yeast strains on the physicochemical and microorganism in the reduced-salt dry sausages during fermentation. (A) pH; (B) total acid content; (C) LAB counts; (D) Venn figure diagram based on the OTUs; (E) relative abundance of bacterial compositions at the species level; (F) beta diversity index heatmap and (G) principal coordinate analysis. Means in the same sausage group with different uppercase letters (A to D) differ significantly ( $P < 0.05$ ); means between sausage groups on the same days with different lowercase letters (a to d) differ significantly ( $P < 0.05$ ).

#### IV. CONCLUSION

The inoculation of *P. kudriavzevii* and *D. hansenii* significantly promoted the growth of LAB and increased the relative abundance of *L. sakei* in reduced-salt dry sausages. Accordingly, the reduced pH and increased total acid content were found in the inoculated dry sausages. Beta diversity analysis indicated that the inoculation of yeast strains significantly influenced the bacterial community structure of dry sausages.

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