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Micrococcus varians, *Staphylococcus carnosus* AND *Staphylococcus xylosus* GROWTH ON CHINESE-STYLE BEAKER SAUSAGE

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Background:

Micrococcaceae such as *Micrococcus varians*, *Staphylococcus carnosus* and *Staphylococcus xylosus* are important starter cultures used in sausage fermentation. The main performance of these cultures was to break down peroxide to improve color and flavor (Geisen *et al.*, 1992). They are important starter cultures used in Western-style sausages for many years (Metz, 1993). However, most Chinese-style sausages do not utilize starter culture and are non-cured products. Basically, the higher water activity (>0.90) and pH (>6.0 after drying), no lactic acid bacterial fermentation and the smaller diameter of sausages characteristics (Guo and Chen, 1997) may be compatible to the oxidation and growth of Micrococcaceae. This research was conducted to evaluate the feasibility of using a starter culture in Chinese-style sausage.

Objectives:

This work was to investigate the effects of pH, sodium chloride, potassium sorbate and sodium nitrite on these starters. Growth rate of the starter in the Chinese-style sausage environment was also evaluated.

Materials and methods

Starter inoculation: All strains of microorganisms were obtained from the Food Industry Research and Development Institute (Taiwan). *M. varians* (CCRC 12272) was inoculated on MYP medium (30℃, 24hrs), *S. carnosus* (CCRC 12922) and *S. xylosus* (CCRC 12930) were inoculated on mannitol salt agar (37℃, 24hrs). Colonies were harvested and resuspended in sterilized distilled water to adjusted absorbance (O. D. 660 nm $=1.0$) for sensitivity test and 2.0 for beaker sausage inoculation.

Sensitivity on additives: Cultures were streaked on 1 % mannitol nutrient agar plates containing 1-15% of sodium chloride, 50-200ppm of sodium nitrite, or 0.1-0.6 % of potassium sorbate. After incubated at 30℃ for 24 hrs, colony formation was examined as a positive reaction.

Sensitivity on pH: Adjusted pH range from 4.5-7.0 of nutrient agar (containing 1% mannitol) and then streaked cultures on agar plates were utilized. Colony formation was examined as a positive reaction.

Growth rate: Ten ml of nutrient broth contained 1 % mannitol per tube was prepared. After sterilized, 0.5 ml of the three strains of cultures were added and incubated at 20℃ and 30℃ for 1, 2 and 3 days. The cells were centrifuges at 3000 rpm for 10 min and then discharge permeabilized in distilled water and made a volume to 5 ml. Absorbance at 660nm was measured against a blank.

Preparation of beaker sausage: Frozen pork ham was thawed at 4 ℃for 24 hr, cooked in water at 100℃ for 3 min and then surface sterilized with 95% alcohol flame. Before grinding the burned, pork ham surface was trimmed with an aseptic knife. Treatments were designated by the ingredients as A (blank), B (control), C (inoculated with *M. varians*), D (inoculated with *S. carnosus*), and E (inoculated with *S. xylosus*) as shown in Table 1. All ingredients of individual treatments were mixed and stuffed into sterilized beakers (70 mm ×25 mm i.d.), and designated as beaker sausage samples. The samples were incubated at 20℃and 30℃for 1, 2, and 3 days.

Growth on Mannitol salt agar*:* Tens gram of beaker sausage samples were homogenized, diluted and inoculated on Mannitol salt agar plates, and then incubated at 30 ℃ for 48 hrs. Colony was calculated as cell forming units per gram (CFU/g) of sample.

Results and discussions

Table 2. showed that the growth of Micrococcus was limited by lower pH than was Staphylococcus. *S. xylosus* could adapt to pH 4.5 and *S. carnosus* to 5.0, *M. varians* did not grow very well until the pH value rose to 5.0. However, lower pH value also inhibits enzymatic activity of Micrococcaceae. To illustrate, Faustman and Cassens (1990) has found that the metmyoglobin reduction ability was slower when the pH value was lower than 5.8. During the time that pH values were lower than 5.5, the nitrate reduction was also slower (Brankoua et al., 1987). Lipolytic activity was inhibited at pH value of 5.0. Sorensen et al., (1993) also indicated that the lower the pH value, the lower the NADH related enzymatic activity.

As showed in Table 3, The growth of *M. varians* was restricted by 0.4% of potassium sorbate but *S. carnosus* and *S. xylosus* could adapt to 0.6 % of potassium sorbate. These three strains could also adapt to 50-200 ppm of sodium nitrite and 1-5% of sodium chloride. In Taiwan, potassium sorbate is a regulated additive used in sausage products (2g/ Kg). From these results, it suggests that these three strains may grow very well in this concentration. Toth (1983) has reported that 200 ppm of nitrite in appropriate culture media will inhibit the growth of *Cl. botulinum* and other microorganisms. In Chinese National Standards, the limited contents of residual nitrite is 70 ppm, in these manufactured product and this allows, 100-150 ppm of sodium nitrite or potassium nitrite to be added to these products. From this study, these three strains may grow very well under typical procedures and established use of additives. These three strains can also adapt to 15% sodium chloride content but *M. varians* indicates only weak colony formations when the concentration of sodium chloride rose to 10%. Sorensen and Jakobsen (1996) indicated that the higher sodium chloride content the lower lipase activity. Normally, the content of sodium chloride of Chinese-style sausage in Taiwan was no further than 3 %. The normal sodium content will not inhibit these three strains.

Fig. 1 and 2 show that *S. xylosus* has the highest growth rate at 20℃ and 30℃. *S. carnosus* had the next and *M. varians* was the last. After 24 hrs of incubation, absorbance of these three strains increase 2-3 times and increased with incubation time.

After the three different strains were added to Chinese-style beaker sausage, the growth rate was observed as shown in Fig. 3 and Fig. 4. These results suggested that the same trends as observed for these strains inoculated in the media environment also occurred in the sausage. Greater growth rate of *S. xylosus* indicated that this strain might be more appropriate in the environment of Chinese-style sausage. The same growth curve of *S. carnosus* and *M. varians* during incubated at 20℃ was observed. The *M. varians* will have a lower growth rate than the other two strains. Perhaps anaerobic condition in the beaker sausage was a limiting factor for *M. varians* germination.

Conclusions:

The growth of Micrococcus was limited by lower pH than was Staphylococcus. These three strains may grow very well in regular concentration of potassium sorbate, sodium nitrite and also sodium nitrite. Staphylococcus may be the most appropriate to use in the procedure of Chinese-style sausage with the current use of additives.

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Table 1. Sausage formulation*.

Ingredients	Treatment				
	A	B	C	D	E
Pork (Kg)	1	1	1	1	1
Sodium chloride(g)	15	15	15	15	15
Sucrose(g)	20	20	20	20	20
Sodium nitrite(PPM)	100	100	100	100	100
Sodium nitrate(PPM)	-	150	150	150	150
M. varians(ml)	-	-	50	-	-
S. carnosus(ml)	-	-	-	50	-
S. xylosus(ml)	-	-	-	-	50
Sterilized distilled water (ml)	50	50	-	-	-

*: Based on meat weight.

Table 2. Effects of pH values on *Micrococcus varians*, *Staphylococcus carnosus* and *Staphylococcus xylosus*

Strains / pH value	*Micrococcus varians*	*Staphylococcus carnosus*	*Staphylococcus xylosus*
4.5	−	+	−
5.0	−	+	+
5.5	+	+	+
6.0	+	+	+
6.5	+	+	+
7.0	+	+	+

+ : positive − : negative

Table 3. Effects of additives on *Micrococcus varians*, *Staphylococcus carnosus* and *Staphylococcus xylosus*

Additives / Strains	*Micrococcus varians*	*Staphylococcus carnosus*	*Staphylococcus xylosus*
Potassium sorbate (g/kg) 0.1	+	+	+
0.2	+	+	+
0.4	−	+	+
0.6	−	+	+
Sodium nitrite (ppm) 50-200	+	+	+
Sodium cholide (%) 1-15	+	+	+

+ : positive − : negative

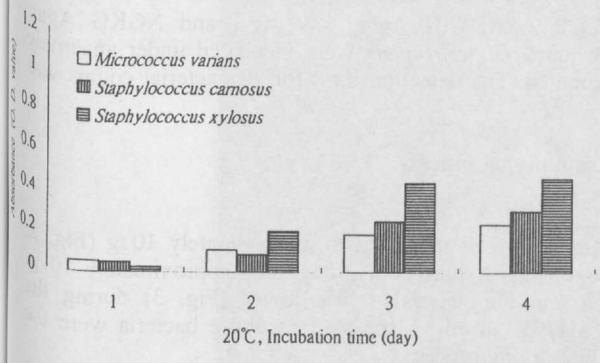

Fig. 1. Growth rate of different strains on agar plate during 20℃ incubation.

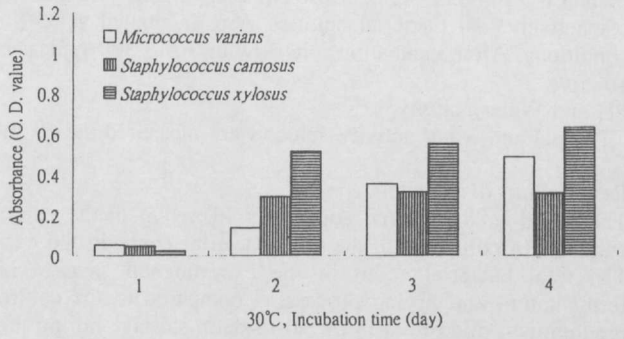

Fig. 2. Growth rate of different strains on agar plate during 30℃ incubation.

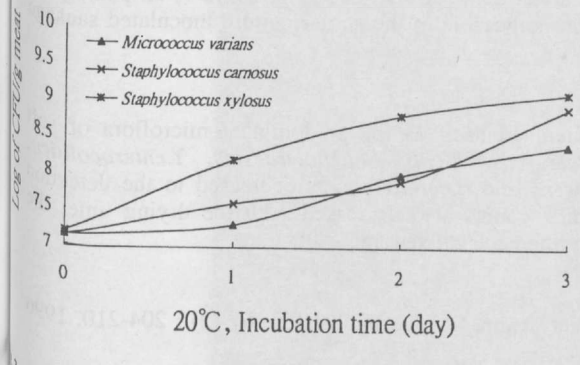

Fig. 3. Growth rate of three strains on beaker sausage during 20℃ incubation.

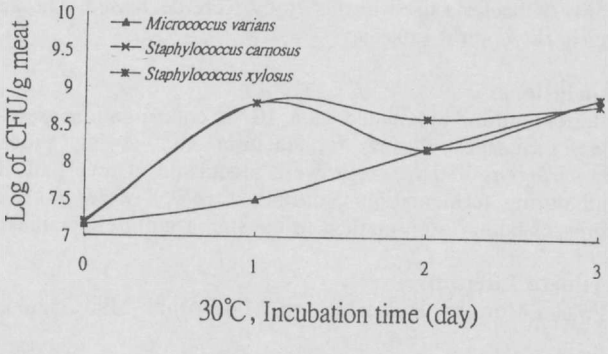

Fig. 4. Growth rate of three strains on beaker sausage during 30℃ incubation.