Biochemical Characteristics of *Micrococcus varians*, *Staphylococcus carnosus* and *Staphylococcus xylosus* and Their Growth on Chinese-Style Beaker Sausage

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ABSTRACT : This study was conducted to investigate protein and carbohydrate utilization of *Micrococcus varians*, *Staphylococcus carnosus* and *Staphylococcus xylosus*. Sensitivity to pH, sodium chloride, potassium sorbate and sodium nitrite of these strains was also determined. In Chinese-style beaker sausage manufacturing, the growth rate of these strains during the curing period (20℃ and 30℃) was evaluated. The results indicated that no strains could hydrolyze azo-casein and sarcoplasmic protein and only *S. xylosus* could hydrolyze gelatin at 30℃. All of these strains could oxidize and ferment fructose and mannitol. *S. carnosus* and *S. xylosus* could slightly oxidize lactose and utilize citrate. Arabinose was oxidized by *S. xylosus* and sorbitol was oxidized by *S. carnosus*. Growth of *M. varians* was restricted at pH 5.0 and *S. carnosus* and *S. xylosus* were restricted at pH 4.5. *S. xylosus* and *S. carnosus* were able to grow with 0.1~0.5% potassium sorbate, 50~200 ppm sodium nitrite or 1~15% sodium chloride. *S. xylosus* had a higher growth rate than the other strains. Staphylococcus species grew well during curing period of Chinese-style beaker sausage then followed by Micrococcaceae. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 3 : 376-380*)

Key Words : Micrococci, Staphylococci, Carbohydrate, Protein, Additives, Sausage

INTRODUCTION

Micrococcaceae such as *Micrococcus varians*, *Staphylococcus carnosus* and *Staphylococcus xylosus* are important starter cultures used in sausage fermentation. The main functions of these cultures are 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. To improve product quality, the original research included isolation and identification of the primary bacteria in Chinese-style sausage and revealed that they were *Micrococcus* and *Staphylococcus* (Guo and Chen, 1991). To investigate the enzymatic activities of these bacteria belong to Micrococcaceae, *M. varians*, *S. carnosus* and *S. xylosus* were selected to determine their NADH cytochrome b5 reductase, nitrate reductase, lipase and catalase activities (Guo et al., 1998).

The high water content (>0.90) and pH (>6.0 after drying), no lactic acid bacterial fermentation and the small diameter of Chinese-style sausages (Chen et al., 1997) may be favourable for the oxidation and growth of Micrococcaceae. Consequently, the pH value remains constant even though more than 8% of sucrose is added to the Chinese-style sausage. This research was conducted to evaluate the feasibility of using a starter culture in Chinese-style sausage, to investigate the utilization by starters of carbohydrate and protein, and the effects of pH, sodium chloride, potassium sorbate and sodium nitrite. 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℃, 24 h), *S. carnosus* (CCRC 12922) and *S. xylosus* (CCRC 12930) were inoculated on mannitol salt agar (37℃, 24 h). Colonies were harvested and resuspended in sterilized distilled water to adjusted absorbance O.D. at 660 nm of 1.0 for biochemical test and 2.0 for beaker sausage inoculation.

Biochemical and physical test

1) Proteolytic ability

Cultures of the different strains were placed in azo-casein solution (Miralles et al., 1996), sarcoplasma protein solution (Zerifi et al., 1992) and gelatin solution (Jean, 1976), individually. Decomposition activities on lysine, phenylalanine, ornithine and urea were determined by using BBL kits (Becton Dickinson

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Microbiology Systems, Becton Dickinson and Company, USA).

2) Oxidation and fermentation of carbohydrate

Phenol red broth plus 1% of glucose, lactose, sucrose, maltose, fructose or mannitol as described by Jean (1976) was used for evaluation. Cultures were then incubated at 37℃ for 24-48 h under aerobic and anaerobic conditions. Results of acid production from oxidation and fermentation were recorded. BBL kits were used to determine utilization of adoritol, arabinose, sorbitol, dulcitol, citrate, and production of indole and diacetyl.

3) Sensitivity to additives and pH

A 100 μl of cultures solution was inoculated on 1% mannitol nutrient broth containing 1-15% of sodium chloride, 50-200 ppm of sodium nitrite, or 0.1-0.5% of potassium sorbate. After incubation at 30℃ for 24 h, absorbance at 660 nm was measured. For pH test, media were adjusted to pH range from 4.5-7.0.

Preparation of beaker sausage

Frozen pork ham was thawed at 4℃ for 24 h, 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. All sausages were made of 80% ground lean pork ham (16 mm plate) and 20% pork back fat. They were diced into 0.8 cm^3 cubes, mixed with NaCl (1.5%), monosodium glutamate (0.5%), sucrose (2%) and 100 ppm $NaNO_2$, and then inoculated separately with *M. varians*, *S. carnosus* and *S. xylosus*. All ingredients of individual treatments were mixed and placed in 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. Ten grams of beaker sausage samples were homogenized, diluted and inoculated on mannitol salt agar plates, and then incubated at 30℃ for 48 h. Colony growth was expressed as cell forming units per gram (CFU/g) of sample.

RESULTS AND DISCUSSION

Table 1 shows that the three strains of bacteria were unable to hydrolyze azo-casein or sarcoplasmic protein, and only *S. xylosus* could hydrolyze gelatin; *M. varians* and *S. carnosus* could utilize urea as a nitrogen source. These three strains were unable to utilize lysine, phenylalanine and ornithine during incubation at 37℃. A number of researchers have reported that Micrococcaceae have no proteolytic activity. Bernell et al. (1992) and Cornejo and Carrascosa (1991) indicated that *S. xylosus* or other strains belonging to Micrococcaceae isolated from Spanish ham had no proteolytic activity. Comi et al. (1992) and Miralles et al. (1996) also indicated that Micrococcaceae had deficiency in proteolytic activity. Reports from Campbell-Platt and Cook (1995) indicated that *M. varians* may hydrolyze gelatin but *S. carnosus* or *S. xylosus* could not. This study also indicated that only *S. xylosus* could hydrolyze gelatin. Proteolytic activity of starter cultures was shown to be related to sausage flavor, but the results indicated that the proteolytic enzyme activity from Micrococcaceae was weaker than that of the other enzymes.

Table 1. Utilization of proteins and amino acids by *Micrococcus varians*, *Staphylococcus carnosus* and *Staphylococcus xylosus*

Protein \ Strains	*Micrococcus varians*	*Staphylococcus carnosus*	*Staphylococcus xylosus*
Protein			
Gelatin	-	-	+
Azo-casein	-	-	-
Sarcoplasma	-	-	-
Amino acid			
Lysine	-	-	-
Phenylalanine	-	-	-
Ornithine	-	-	-
Urea	+	+	-

+=positive reaction; - =negative reaction.

Table 2 shows the utilization of carbohydrates by the three bacteria. All of them could oxidize and ferment fructose and mannitol, but only *S. carnosus* and *S. xylosus* could oxidize lactose, slightly. In contrast, all of these three strains could not use glucose, sucrose and maltose as a carbon source. *S. xylosus* could oxidize arabinose and *S. carnosus* oxidize sorbitol; citrate could not be utilized by *M. varians*. Indole and diacetyl production by these three strains was not detectable. These results indicated that these three strains could not utilize sucrose or glucose which is commercially used in Chinese-style sausage. Therefore, during Chinese-style sausage manufacture, acid production from the Micrococcaceae, starter culture was not detectable.

Nychas and Arkoudelos (1990) indicated two metabolic pathways for Micrococcaceae, namely the Embden-Meyerhof and hexose monophosphate pathways. However, some of Micrococcaceae isolated from a variety of fermentation products vary in their utilization of different carbohydrate sources. Molina et al. (1989) reported that *S. xylosus* could utilize various carbohydrates including glucose, fructose, mannose, maltose, lactose, mannitol, xylose and sucrose. Rosa et al. (1990) indicated that glucose had variable

utilization by different species of Micrococcaceae for oxidation or fermentation or both. Seager et al. (1986) indicated that under aerobic environments *S. carnosus* could not produce acid from sucrose degradation, but *S. xylosus* could.

Table 2. Utilization of carbohydrates by *Micrococcus varians*, *Staphylococcus carnosus* and *Staphylococcus xylosus*

Carbohydrate \ Strains	*Micro-coccus varians*	*Staphylo-coccus carnosus*	*Staphylo-coccus xylosus*
Oxidative/Fermentation at 37℃			
Fructose	O/F	O/F	O/F
Lactose	-	O (W)	O (W)
Glucose	-	-	-
Mannitol	O/F	O/F	O/F
Sucrose	-	-	-
Maltose	-	-	-
Oxidative at 37℃			
Adonitol	-	-	-
Arabinose	-	-	O
Sorbitol	-	O	-
Ductiol	-	-	-
Citrate	O	-	O
Indole production	-	-	-
Diacetyl production	-	-	-

O/F=acid production from oxidation and fermentation; - = no acid production; O=acid production from oxidation; W=weak reaction.

Table 3 shows that the growth of Micrococcus was limited by low pH more than was Staphylococcus. *S. xylosus* and *S. carnosus* could adapt to pH 5.5. *M. varians* did not grow very well until the pH value rose to 6.0. 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 (Brankova et al., 1987). Lipolytic activity was inhibited at pH value of 5.0. Sorensen et al. (1993) and Selgas et al. (1988) also indicated that the lower the pH value, the lower the NADH-related enzymatic activity.

As shown in table 4, the three strains could adapt to 0.5 % of potassium sorbate. They 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 (2 g/kg). Results reveal that these three strains may grow very well in this concentration. To destroy *Clostridium botulinum*, nitrite is an important additive used in sausage products. Toth (1982) reported that 200 ppm of nitrite

Table 3. Effect of pH on growth of *Micrococcus varians*, *Staphylococcus carnosus* and *Staphylococcus xylosus* (30℃, 24 h)

pH value \ Log_{10} CFU/ml	*Micro-coccus varians*	*Staphylo-coccus carnosus*	*Staphylo-coccus xylosus*
Initial counts	5.21	5.16	5.33
4.5	0	4.45	3.30
5.0	0	4.66	2.79
5.5	5.49	7.38	7.38
6.0	7.56	7.16	7.09
6.5	7.56	7.72	7.56
7.0	7.10	7.52	7.30

Table 4. Effects of additives on the growth of *Micrococcus varians*, *Staphylococcus carnosus* and *Staphylococcus xylosus*

Additives \ Log_{10} CFU/ml	*Micro-coccus varians*	*Staphylo-coccus carnosus*	*Staphylo-coccus xylosus*
Initial counts	5.21	5.16	5.33
Potassium sorbate (g/kg)			
0.1	7.59	7.59	7.59
0.2	7.72	7.44	7.84
0.4	7.79	7.35	7.65
0.5	7.50	7.32	7.72
Sodium nitrite (ppm)			
50	7.05	6.56	7.45
100	7.56	6.38	7.67
150	7.80	7.90	7.56
200	7.92	7.57	7.69
Sodium chloride (%)			
1	7.76	7.53	6.37
2	7.33	7.44	6.68
3	6.57	7.22	7.30
5	6.27	7.05	6.53
10	4.77	4.83	4.85
15	4.21	3.65	3.65

Log_{10} number of three strains without any additives were 7.68, 7.64, 7.73 after incubation for *Micrococcus varians*, *Staphylococcus carnosus* and *Staphylococcus xylosus*.

in appropriate culture media could inhibit the growth of *C. botulinum* and other microorganisms. However, other factors such as pH, water activity and additives may also play an important role. It was suggested that 80-100 ppm of sodium nitrite was a possible danger threshold. In Chinese National Standards, the limit for residual nitrite in the processed product is 70 ppm, and this allows 100-150 ppm of sodium nitrite or potassium nitrite to be added to these products during manufacture. From this study, the three strains may grow very well under typical procedures and established use of additives. These three strains could

adapt to 15% sodium chloride content but reduced growth was observed when the concentration of sodium chloride increased to 10%. Sorensen and Jakobsen (1996) indicated that the higher the sodium chloride content the lower the lipase activity. Normally, the content of sodium chloride in Chinese-style sausage in Taiwan is no more than 3%. The normal sodium chloride content will not inhibit these three strains.

After the three different strains were added to Chinese-style beaker sausage, growth rates observed were shown in figures 1 and 2.

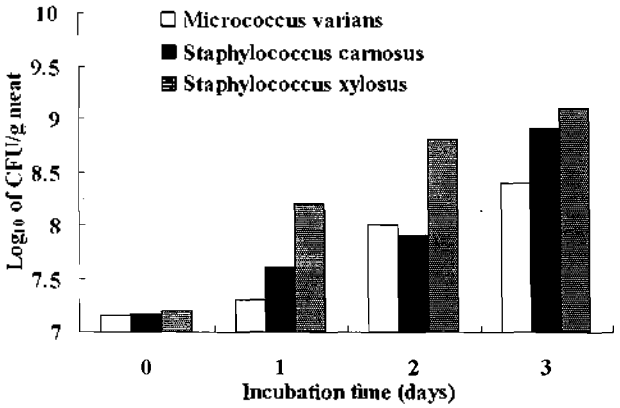

Figure 1. Log_{10} number of three strains growing on beaker sausage during incubation at 20℃

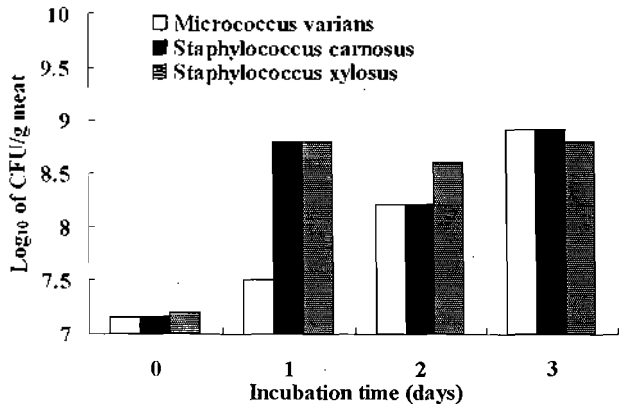

Figure 2. Log_{10} number of three strains growing on beaker sausage during incubation at 30℃

These results suggest that the trends seem when these strains were inoculated in the media environment were also observed in the sausage. Greater growth rate of *S. xylosus* indicated that this strain might be more appropriate to grow in the environment of Chinese-style sausage. *M. varians* had a lower growth rate than the other two strains. Perhaps anaerobic condition in the beaker sausage was a limiting factor for *M. varians* germination.

In conclusion, these three strains appeared to have little or no proteolytic activity. They had no acid production from sucrose and glucose, which are commercially used in Chinese-style sausage. 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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