Biochemical Characteristics and Enzymatic activity of *Micrococcaceae*

Hsiu-Lan Guo, Ming-Tsao Chen and Deng-Cheng Liu
Department of Animal Science, Chung-Hsing University, Taichung, Taiwan, ROC 40227

Introduction

Micrococcaceae was one of the commercial starters utilized in raw sausage production in many countries (Leistner, 1991; Geisen et al., 1994). They are able to produce nitrate reductase and catalase to achieve a stable color and split hydrogen peroxide. Lipolytic activities of Micrococcaceae also play a role in aroma display in meat products (Geisen et al., 1994; Nychas and Arkoudelos, 1990).

Basically, Micrococcaceae were predominant in the Chinese-style sausage (Guo and Chen, 1991). Cultures belonged to Micrococcaceae were selected to achieve a unique product with higher consistency, quality , safety, and longer shelf-life. Accordingly, enzyme activity of *Micrococcus varians*, *Staphylococcus carnosus* and *Staphylococcus xylosus* was the major focus in this study.

Materials and Methods

Starter inoculation and biochemical test: All strains were obtained from Food Industry Research and Development Institute (Taiwan). *M. varians*(CCRC 12272) was inoculated in/on MYP medium (30 ℃, 24hrs) as well as *Staphyl. carnosus* (CCRC 12922) and *Staphyl. xylosus*(CCRC 12930) were inoculated in/on mannitol salt agar (37 ℃, 24hrs). Biochemical test was measured according to the method described by Jean(1976). ***Nitrate reductase activity:*** Prepare 9.5 ml of YEM medium contained 150 ppm $NaNO_2$. A half ml of culture(O. D. 660=1.0) was added and incubated for 24 hr. Absorbance was observed after reacted by Griss solution. ***Catalase activity:*** Catalase was determined by Bergmeyer's (1983) method. ***Metmyoglobin reductase activity:*** Metmyoglobin was prepared and measured according to the method ofArihara et al., (1993). ***NADH-cytochrome b5 reductase activity:*** Specific activity of the cultures was detected by the method of Takesue and Omura (1970). ***Lipolytic Activity:*** Pork fat was emulsified and determined according to methods of Neilsen (1989).

Results and discussion

Characteristics of *M. varians*, *Staphyl. carnosus* and *Staphyl. xylosus* were shown in table 1. Three strains all did not hydrolyze azo casein and sarcoplasma protein. Only *S. xylosus* had a weak gelatin hydrolysis at 20 ℃. The results of carbohydrate oxidation/fermentation test indicated that all test strains got possitive reactions from fructose and mannitol. Additionally, only *Staphyl. xylosus* produced weak acid from glucose and sucrose in aerobical .

Fig. 1 showed that *Staphyl. carnosus* and *Staphyl. xylosus* had higher nitrate reductase activity than that of *M. varians* at 30 ℃. Fig. 2 showed that temperature was an important factor to influence catalase activity. Catalase activity increased with temperature from 20 to 30 ℃. The greatest enzyme activity was obtained from *Staphyl. xylosus*. Metmyoglobin reductase activity was shown as Fig. 3. *Staphyl. xylosus* still reduced metmyoglobin at 20 ℃. It could reduce 5 % metmyoglonin to myoglobin. During 30 ℃ incubation, all strains reduced nearly 4 % of Metmyoglobin to myoglobin in the medium. NADH-cytochrome b5 reductase systems were closely related to Metmyoglobin and nitrate reduction capacity. This relation also evidenced by the result that *Staphyl. xylosus* have highest activity at 30 ℃ in this study(Fig. 4). The results of lipolytic activity were shown in Fig. 5. After 7 day incubation, *Staphyl. xylosus* was stronger activity than that of the other strains. Free fatty acid contents of *Staphyl. Xylosus* at 20 and 30 ℃ were 983 and 1124 mg/kg .

Micrococcaceae is slightly fermentative and grows poorly in anaerobic environment of the interior parts of the sausages(Hammes and Knauf, 1994). The cultures from Micrococcaceas were unable to produce acid from sucrose and glucose. On the other hand, catalase from micrococaceae could split hydrogen peroxide presented and reduce defects in color. It means that Micrococcaceae is useful for Chinese-style sausage because the people can not accept acid flavor. However, Lipolytic activity of *Staphyl. xylosus* was remarkable at 20 and 30 ℃.

conclusions

M. varians, Staphyl. carnosus and *Staphyl. xylosus* incompletely utilized sucrose and fructose very well. *S. xylosus* was the most adaptable culture in 20 and 30 ℃. This strain had the highest reductase activity for nitrate and Metmyoglobin and highest production for catalase and lipase.

Reference

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Table 1. Biochemical test of three different strains

	Micrococcus varians	*Staphylococcus carnosus*	*Staphylococcus xylosus*
protein hydrolysis			
gelatin	—	—	V
azo-casein	—	—	—
sarcoplasma	—	—	—
oxidative/fermentation test at 37 ℃			
fructose	O/F	O/F	O/F
lactose	—	—	—
glucose	—	—	—
mannitol	O/F	O/F	O/F
sucrose	—	—	—
maltose	—	—	—

O/F : positive of oxidative and fermentation ;
+: : positive ; — : negative ; V:weak hydrolysis

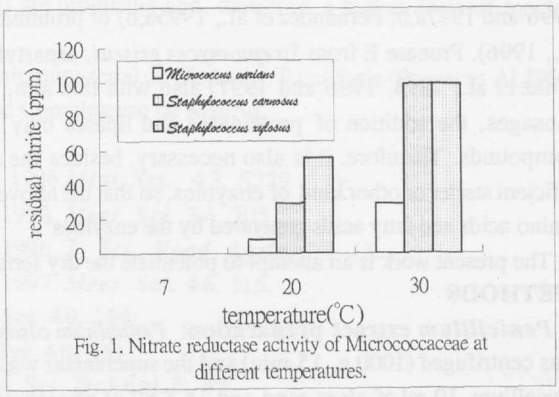

Fig. 1. Nitrate reductase activity of Micrococcaceae at different temperatures.

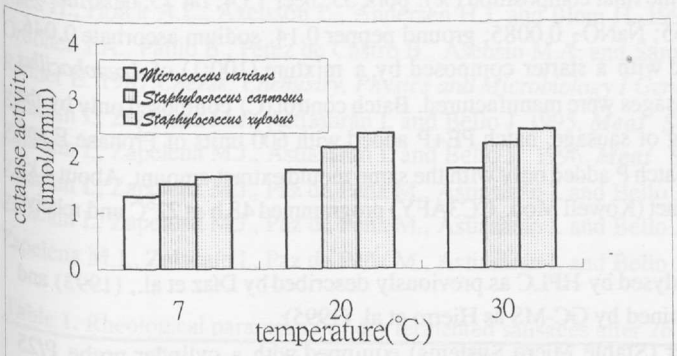

Fig. 2. Catalase activity of Micrococcaceae at different temperatures.

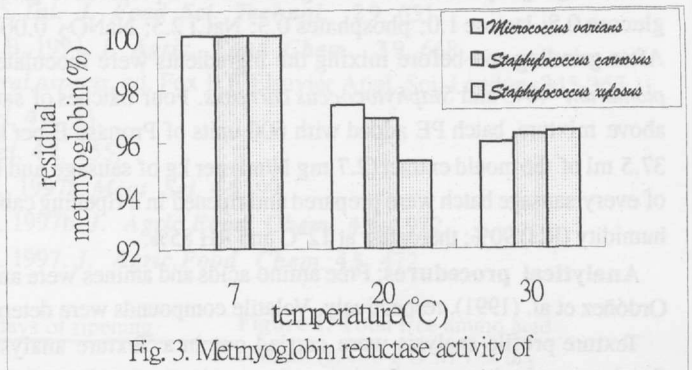

Fig. 3. Metmyoglobin reductase activity of Micrococcaceae at different temperatures.

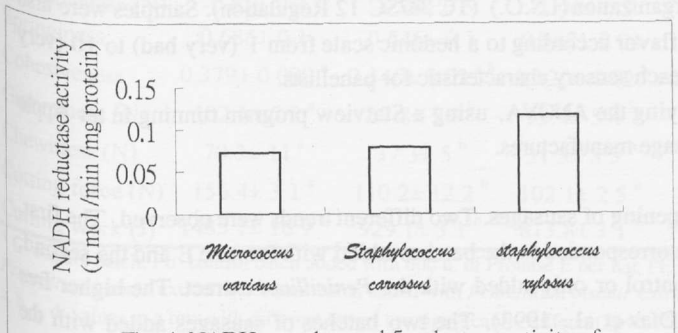

Fig. 4. NADH-cytochrome b5 reductase activity of Micrococcaceae at 30 ℃.

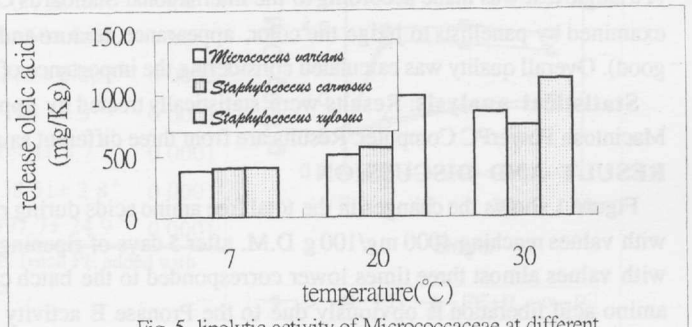

Fig. 5. lipolytic activity of Micrococcaceae at different temperatures.