EFFICACY OF ANTIMICROBIAL SUBSTANCES FROM *MONASCUS* METABOLITES ON PRESERVATION OF MEAT

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INTRODUCTION

The molds *Monascus* spp. have been used in the fermentation industry for preparation of wine and native foods such as red rice wine and anka mash port for thousands of years in China and some Asia countries. It produces not only pigments but also enzymes and antimicrobial substances (Lin, 1973; Su and Huang, 1976; Kuei, 1984; Lin, 1986; Wong and Bau, 1977). Some preliminary work on the action of *Monascus anka* and mash on porcine muscle histobiochemical properties also showed that there was some antimicrobial activity from the metabolites of *Monascus* spp. Thus, this study was to screen the strains being capable of producing antimicrobial substances and confirm what antimicrobial substances presented in their metabolites and their efficiency on preservation of pork.

MATERIALS AND METHODS

10 strains of *Monascus* spp. were employed for screening the organisms being capable of producing antimicrobial substances. Yeast extract broth (YEB) containing yeast extract 0.8%, glucose 10% with pH 5.5 which was described by Wong and Bau (1977) was used for antimicrobial substance production. Both static and shaking liquid cultures were kept at 35°C for 15 days. The cultures were taken for testing pH and antimicrobial activity at 9th and 15th days, separately.

The effects of initial pH and incubating temperature on antimicrobial activity were also studied.

Agar diffusion technique method (Booth, 1971; Raccath et al., 1979) and paper disc diffusion method (Casals and Musueus, 1978) were used to study the antimicrobial activity of crude metabolite. Solid cultures with steamed rice and YEB inoculated with 3-5% liquid culture of *Monascus* spp. and kept at 30°C for 20 days. The antimicrobial substance was extracted with ethyl acetate from the solid and liquid cultures and isolated by silica gel adsorption chromatography using eluting solvent of benzene:methanol:chloroform (30:10:9 or 30:20:9, v/v/v) and collected by a fraction collector. Further purification was carried out by silica gel TLC plate and developed with chloroform:methanol (98:2, v/v). *Micrococcus candidus* 11273, *Bacillus cereus* 10250, *Sarcina* spp. *Escherichia coli* 10675, *Clostridium butyricum* 10750, *B. subtilis* 10255, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas* spp. with were obtained from FRDI (Taiwan) were used as testing organisms to investigate antimicrobial activity.

Pork obtained from the local market soaked in 0, 1, and 5% of crude antimicrobial substance by 1:1(v/v), and incubated at 25°C and 2-4°C conditions, and then taken out of the pork samples at different incubation times for determining total bacterial counts, anaerobic bacterial counts and pH value of pork to study the efficacy of *Monascus* antimicrobial substances on the preservation of pork. Ethanol and organic acids presented in the *Monascus* mash were also determined by Boehringer Mannheim GmbH (1986), and HPLC (Shimadzu LC-4A), respectively.

RESULTS

The results showed that *M. pilosus*, *M. purpureus* 31499 and *M. anka* had the highest antimicrobial activity against the growth of the testing organisms (Table 1). Antimicrobial substance as well as pigments production by *M. pilosus* and *M. purpureus* was affected by different strains, medium and culture conditions. After incubation at 30°C for 9 days, the cultural fluid of pH 3.5-4.0 showed inhibiting activity, while the solid culture medium consisting of steamed rice, glucose and yeast extract needed 20 days propagation. The changes of antimicrobial activity for *M. pilosus* and *M.*

purpureus in static and shaking liquid cultures as shown in Table 2. The antimicrobial activity of the strains also varied with the culture conditions such as initial pH and temperature. As initial pH increased up to 6.5 more acid was produced but no antimicrobial activity was detected. No differences among the different incubating temperatures for antimicrobial substance production, but the antimicrobial activity was found in the cultures at 30°C and 35°C earlier than in the culture at 25°C

The crude antimicrobial substance was extracted with ethyl acetate, and the pigments could be precipitated and removed partially from the concentrated crude antimicrobial substance moistened with distilled water. No antimicrobial activity was detected in the pigments. The crude antimicrobial substance was separated and purified by silica gel column chromatography and TLC developing in chloroform: methanol (98:2). The result was found that the substances with Rf values 0.22 and 0.36 showed antimicrobial activities. As methanol level in eluting solvent mixture increased, the elution of the antimicrobial substance was hastened (Fig. 1). The crude antimicrobial substance was acidic and heat-resistant. It did not loss antimicrobial activity when heated at 70°C and 100°C for 30 min. and 121°C for 20 min., but lost antimicrobial activity when the substance diluted ten times with pH 6.0. Partial biochemical color reaction showed acidic reaction and microbial substance was consi-ered as nonpetide structure compound. The minimum inhibition concentration test showed that Staphy. aureus was the highest sensitive to the Monascus antimicrobial substance, the MIC was 2ug/ml. The enzymatic analysis for ethanol test showed the cultural fluid contained 10-25% of methanol. The organic acids determined by HPLS showed that there were fumaric acid, oxalic acid, gluconic acid, succinic acid and citric acid presented in the cultural fluid. The crude antimicrobial substance added to pork and stored at 2-4°C could decrease total bacterial counts and anaerobic bacterial counts significantly, and 5% of antimicrobial substance was more effective to inhibit bacterial growth.

DISCUSSION

Growth rate, pigments and antimicrobial substance production varied with strains, media, initial pH and cultural conditions. Alcohols or organic acids or antimicrobial substance were detected in the metabolites. It is needed more work on which is responsible for the antimicrobial action and how to improve the production of the antimicrobial substance from these three selected strains. The optimal conditions for the antimicrobial substance production from these strains are also needed more studied. Since the production of antimicrobial substance is usually accompanied by increase pigment production, both target products can be used to replace the nitrite used in meat products passibly.

CONCLUSION

M. pilosus and M. purpureus 31499 and M. anka which could produce the metabolite with antimicrobial activity were detected. The optimum cultural conditions for producing antimicrobial substance were pH 5.5 and 30°C. The antimicrobial substance was acid stable and heat-resistant. 5% of crude solution combined with low temperature to cure pork could inhibit microbial growth. This antimicrobial substance had a broad spectrum of antimicrobial activity. It may be a valuable natural preservative and seems to be needed further work.

ACKNOWLEDGEMENT

We would like to acknowledge the financial support for this research from the National Science Council of Republic of China.

REFERENCES

Booth, C. (1971): Introduction to general method in broth. Method in Microbial. Vol. 4. Academic Press. New York, USA.

Casals, J. B., and Musaeus, J. (1978): Standardized antimicrobial sensitivity

testing in veterinary practice. Nord. Ved-Med. 30: 97-106.
Kuei, C. H. (1984): Effects of anka mash treatment on the electrophoretic characteristics of porcine muscle during storage. Master thesis, National Chung-Hsing University, Taiwan.
Lin, C. F. (1973): Isolation and cultural conditions of Monascus sp. for production of pigment in a submerged culture. J. Ferment. Technol. 51: 407.
Lin, K. P. (1986): Studies on changes in chemical and histological properties of porcine muscle soaked in liquid anka mash. Master thesis, National Chung-Hsing University, Taiwan.
Raccach, M. et al. (1979): Potential application of microbial antagonism to extended storage stability of a flesh type food. J. Food Sci. 44: 43-46.
Su, Y. C., and Huang, J. H. (1976): Studies on the production of anka pigment of the Chinese Agric. Chem. Soc. 14: 59-67.
Wong, H. C., and Bau, Y. S. (1977): Pigmentation and antibacterial activity of fast neutron- and X-ray-induced strains of Monascus purpureus Went. Plant Physiol. 60: 578.

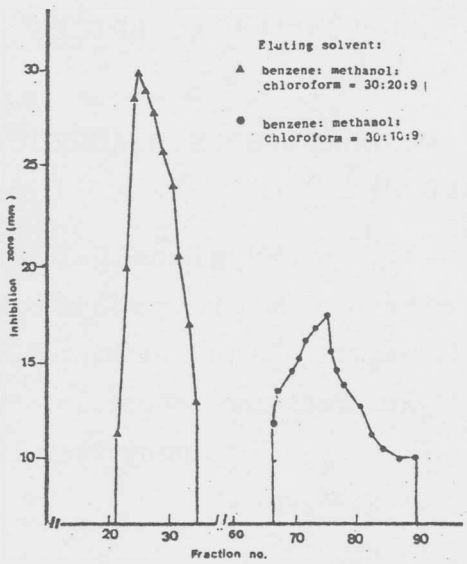

Fig. 1. Effect of different eluting solvent on the antibacterial activity of *Monascus* by column chromatograph.

Table 1. The effect of culture fluid on the growth of microorganisms of Monascus sp.

strains	Test organisms ※ B. subtitis	M. candidus	Ps. sp.	B. cereus	Scar. sp.	E. coli	Sal. typhi	Sta. aureus
M. kaoliang	−	−	△	±	±	△	±	±
M. pilosus	+	+	+	+	*	*	*	*
M. purpureus 31499	±	±	+	+	*	*	+	+
31501	−	△	−	−	−	−	−	−
31504	−	△	△	−	−	−	−	−
31540	−	−	△	±	△	±	−	±
31536	±	+	±	+	+	+	±	+
M. sp.31746	±	+	±	+	+	+	±	*
M. ruber van Tieghem	−	−	△	±	±	±	±	±
M. anka	+	±	+	+	*	*	+	+

※: Antimicrobial activity on the diameters of zone of inhibition :
△: stimulate growth, −: no effect on growth, ±: 10 - 12 mm,
+: 12 -15 mm, *: 15 -20 mm,

Table 2. Effects of shaking culture and static culture on pH and antibaciterial activity of Monascus sp.

Item	pH value				Diameter of inhibition zone (mm)			
treatment	static culture		shaking culture ※		static culture		shaking culture ※	
time (day)	9	15	9	15	9	15	9	15
M. pilosus	4.19 ±0.82	3.67 a ±0.52	3.87 ±0.42	7.24 c ±1.23	13.4 ±0.28	14.25 ±0.32	14.63 ±1.30	_※
M. purpureus	4.25 ±0.68	3.97 a ±0.45	4.09 ±0.42	6.48 b ±1.07	13.3 ±1.20	13.8 ±0.89	14.38 ±0.69	_

※:" _ " : no inhibition zone , incubation temp. 35℃
※: shaking ,100-120 rpm.
a,b,c, Means within each column with different superscript letters are significantly different ($p<0.05$)