Microorganisms Relating to Color Development in Cured Meats

By Fumisaburo TOKITA, Akiyoshi HOSONO,
Fujio TAKAHASHI and Masayuki MATSUBA
Laboratory of Animal Products Technology, Fac. Agric., Shinshu University

Introduction

In the manufacture of meat products, curing is an important process for the fixation of color in meat. As generally known, the primal reaction for development of a reddish color in meat during curing is the reduction of nitrate to nitrite. This reaction has been traditionally achieved by the action of bacteria, which have the ability to reduced nitrate, found both in meat and in pickle solution. These microorganisms, however, sometimes caused unfavorable situations to meat products such as certain types of spoilage as well as poisoning hazards [6].

For the purpose of breaking off these problems more or less Deibel et al.,[3] Pfützner[16]. and Niinivaara et al.[15] presented new concepts of the use of bacterial pure cultures in the manufacture of meat products and provided, in part, a possible mean of preventing such unfavorable situations.

However, the strains which have been known to be useful as a starter for the development of color in cured meat was relatively small in number. For a better utilization of bacterial pure cultures as a starter for the development of color in cured meat, further extensive isolation of the microorganisms is considered to be necessary.

In the present study, the authors examined to isolate the bacteria which have the ability to reduce nitrate from the cured pork and investigated these microorganisms with ultimate aim of employing them as a starter culture for the color development in meat during curing.

Materials and Methods

1. Isolation and taxonomic study of nitrate reducing bacteria.

1). Media used for the isolation of microorganisms from the cured pork:

Two kinds of media were used for the isolation of the bacteria which have the ability to reduced nitrate (Nitrate reducing bacteria). One (Medium-A) was composed of 1% glucose, 1% peptone, 1% beef extract, 5% NaCl and 2% agar (pH 7.0), the other

Received September 12, 1974

(Medium-B) was the same composition as Medium-A except that Medium-B was composed of 10% NaCl instead of 5% NaCl.

2). Isolation method of microorganisms from the cured pork:

About 20g of pork cured at 4 C for one week in the pickle solution-A in Table 1 was shredded with 60 ml of sterile distilled water in a blender. One ml of the shredded pork sample which was diluted with physiological saline 10 or 100 times was added to 10 ml of each nutrient agar described above. The plates were incubated at 30C for 72 hr. The microorganisms grown on the plates were isolated by streaking methods and pure

Table 1. Composition of the pickle solution

	Pickle solution	
	A	B
	— % (W/V) —	
Potassium nitrate	1.0	1.0
Sodium nitrate	0.015	—
NaCl	17.4	11.7
Sodium ascorbate	0.03	0.03
Nicotinic acid amide	0.03	0.03
Sucrose	0.03	0.03

cultures of the isolated microorganisms were transferred to the agar slants of the same composition.

3). Screening test of nitrate reducing bacteria:

Screening test of nitrate reduding bacteria from the microorganisms isolated from the cured pork was carried out according to Nakanishi's description.[13)]

4). Taxonomic study of the nitrate reducing bacteria:

Diagnostic tests for the morphological and physiological studies of the pure cultures of the nitrate reducing bacteria were carried out according to Bergy's Manual of Determinative Bacteriology.[2)]

2. A test for color development by the isolated nitrate reducing bacteria in various meats.

1). Meat samples and cultural conditions:

Four kinds of meats such as pork, lamb, horseflesh and tuna meat which were purchased from a market were used. Each meat of 10g was cut into small pieces and shredded in a blender with 30 ml of the pickle solution-B listed in Table 1. After shredding, all samples were brought the pH to 5.0 with 5% lactic acid solution and were sterilized on irradiation with ultraviolet rays (2537 Å) for 30 min with continuous stirring. Each of the samples was then asepticaly dispensed, in 25 ml quantity, into a sterilized flask (250 ml capacity) and inoculated 1 ml of 24 hr culture of each strain of the

nitrate reducing bacteria, and inoculated at 30 C for 24 hr.

2). Analytical method:

The intensity of the color developed in each meat sample by incubation with each strain of the nitrate reducing bacteria was spectrophotometrically determined by the method of Niinivaara et al.,[14] that is, to each incubated meat sample was added 50 ml of the solvent mixture (ethyl ether: ethyl alcohol =1 : 1) and they were reciprocally agitated for one hour at room temperature. After standing over night, the mixture was centrifuged at 1000 x g for 10 min and the absorbancy of the supernatant was measured on Hitachi spectrophotometer 101 type at 530 mμ.

3. A tese for color development by the extract of the acetone-dried cells prepared from the isolated nitrate reducing bacteria.

1). Preparation method of acetone-dried cells of the isolated nitrate reducing bacteria:

Ten liters of Medium-C or Medium-D, which are the essentially same as Medium-A or Medium-B, respectively, except that Media-C and-D contained no agar was autoclaved in a 20-liter stainless-steel fermentor equipped with a stirring device and an inlet tube for aeration (Marubishi, 202 type). After cooling, 100 ml of 24 hr's culture of each strain of the nitrate reducing bacteria grown in the same medium was inoculated. Sterile air was blown into the medium at a rate if approximately 18 liters per minute and the rate of stirring was adjusted to 2,400 r. p. m. After incubation at 30 C for 80 hr, cells were harvested by centrifugation and washed three times with one liter of sterile distilled water.

Acetone-dried powder of the washed cells was prepared according to the method described by Akabori[1] and preserved in a sealed test tube for 3 months at each temperature, -20C, 4C or 30C. The acetone-dried cells immediately after the preparation was used as a control.

2). Incubation method of horseflesh by the extract of the acetone-dried cells:

One hundred mg of each of the acetone-dried cells preserved under the various temperatures was suspended in 5ml of 1/45M borate buffer (pH4.9). The suspension was incubated at 30 C for 30 min and centrifuged. The sediment was then spun off and the clear supernatant was added to the horseflesh sample prepared by the same method as described above, and incubated at 30 C for 24 hr.

3). Analytical method:

Measurement method of color developed in each cultivated sample was the same as described above.

Results and Discussion

1. Morphological and physiological properties of the nitrate reducing bacteria isolated from the cured pork.

Two (No. 206 and 208) and five (104, 106, 127, 129 and 130) strains which have positive reaction in reduction of nitrate were isolated from the cured pork, using Medium-A and Medium-B, respectively.

These strains were Gram positive, aerobic and coccus (about 1.0 micron in diameter), occurring singly, in pairs or in large clumps. The results of the physiological properties of these strains were summarized in Table 2. From the results, it can be concluded that all of these strains belong to *Micrococcus*, making reference to the Bergy's Manual, 7th

Table 2. Physiological Properties of the nitrate reducing bacteria isolated from the cured pork

Strain No.	104	106	127	129	130	206	208
Gram-reaction	+	+	+	+	+	+	+
Mobility	±	±	±	±	±	±	±
Liquefaction of gelatin	−	−	−	−	−	−	−
Coagulation in skim milk	−	−	−	−	−	−	−
Peptonization in skim milk	−	−	+	+	+	−	−
Hydrolysis of starch	−	−	+	+	−	−	−
Growth on poteto agar	+	+	+	+	+	+	+
Catalase production	+	+	+	+	−	+	+
Nitrate reduction	+	+	+	+	+	+	+
Assimilation							
$NH_4H_2PO_4$	+	+	+	−	+	−	−
Glucose	+	+	+	+	+	+	+
Saccharose	+	+	+	+	+	±	−
Mannitol	±	−	−	−	−	−	−
Glycerin	+	−	−	−	−	−	−

ed. However, no species could be found in the Bergy's Manual, 7th ed. which exactly coincided with these strains. From the comparative studies among the known species in the genus, the strains No. 104, 106 and 127 were similar to *Micrococcus varians*, *Micrococcus conglomeratus* and *Micrococcus caseolyticus*, except for the reactions to glycerin and gelatin, respectively.

The Bergy's Manual states that *M. varians* is negative to assimilation of glycerin and that *M. conglomeratus* and *M. caseolyticus* are positive to liquefaction of gelatin. The strain No. 104 and the strains No. 106 and 127, however, was positive to assimilation of glycerin and were negative to liquefaction of gelatin, respectively.

It is generally known that bacteria belonging to the genus, *Micrococcus* were frequently detected in raw and cured meats and play important roles in the development of flavor and color in meat during curing.[8~12] Although detailed information has also been obtained concerning spoilage effects of these bacteria on meat products, it could not be

discussed within the limits of this experiment whether the seven strains isolited in the present study have pathogenic properties or not pathogenic.

2. Development of color in various meat samples incubated with the isolated nitrate reducing bacteria.

Intensities of the color developed in pork, tuna meat, lamb and horseflesh which were incubated by each of the isolated nitrate reducing bacteria at 30 C for 24 hr were shown in Fig. 1. As may readily be seen from this Figure, strengh of the color developed

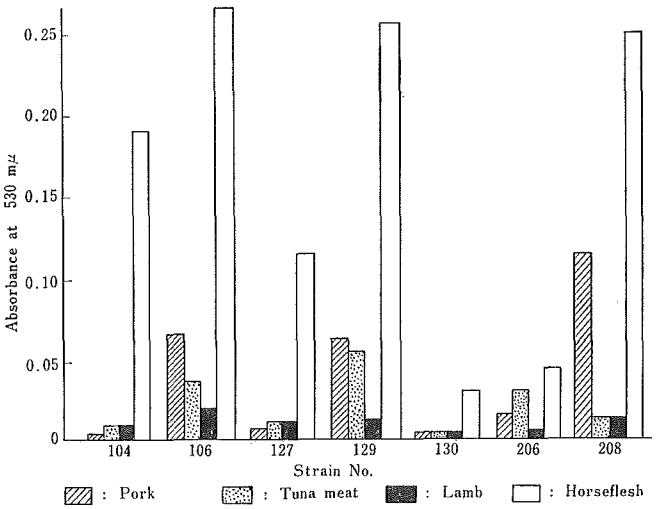

Fig. 1. Intensities of color development in the various meats incubated by the isolated nitrate reducing bacteria.

was observed to be most significant in all cultivated horseflesh samples. Relatively strong color also developed in the pork incubated by the strains No. 208, 129 or 106, comparing with the lamb and the tuna meat incubated by the same strains.

As might be easily expected,[7] these significant differences in the intensity of developed color among the tested meat samples were considered to be mainly effected by the myoglobin and haemoglobin contents of the meats examined.

It is also well known that development of color in meat during curing is greatly influenced by pH of meat and of the pickle solution.[6] Therefore, effect of pH on the development of color in cured meats was investigated using the isolated strains No. 106, 129 and 208.

In the present study, the shredded horseflesh sample prepared by the same method described above was dispensed, in 25 ml quantity, into twenty sterilized flasks (250ml capacity) and they were adjusted to various pH values, 4.7 to 5.5 with 5% solution of either phosphoric, lactic, citric or acetic acids, and the strains No. 106, 129 or 208 was

inoculated to all of the samples and incubated in the same manner as described above.

The intensity of color developed in each meat sample was shown in Fig. 2. As seen in this Figure, development of color in these cultivated meat samples is noted to greatly

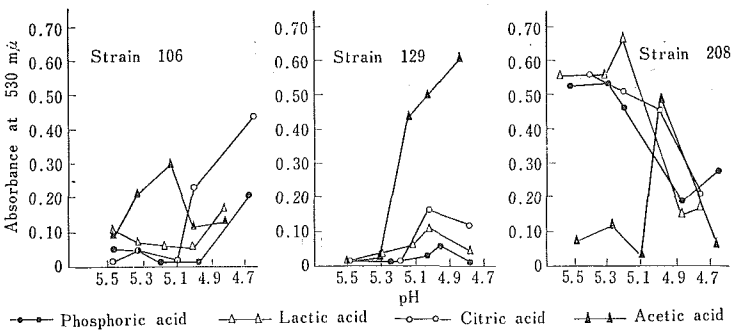

Fig. 2. Effect of acids on the development of color in the horseflesh samples.

influenced by acids which were used for adjusting pH of the meat samples as well as kind of the nitrate reducing bacteria. These findings may be taken to indicate that an optimum pH alone will enhance cured meat color development without regard to microbial activity.

3. Development of color in the horseflesh samples incubated with the extracts of the acetone-dried cells prepared from the isolated nitrate reducing bacteria.

Intensity of the color developed in the horseflesh sample incubated with each extract of the acetone-dried cells preserved at −20C, 4C and 30C for three months was shown in Fig. 3. Meanwhile, the absorption value at 530 mμ of the control dried cells of the strains No. 106, 129 or 208 was 0.08, 0.20 or 0.15, respectively. Accordingly, judging from these results, nitrate reducing activity in each acetonedried cells can be recognized to be relatively stable, when it was preserved at −20C.

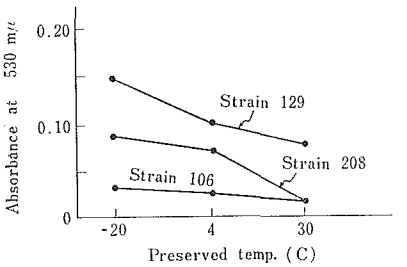

Fig. 3. Intensity of color developed in the horseflesh samples incubated by the aceton-powdered cells of the nitrate reducing bacteria preserved under various temperatures.

On the other hand, certain difficulties are reported concerning to the utilization of bacterial pure cultures. For instances, Deibel et al.[4] reported that salt tolerance of the bacteria decreased, when they were lyophilized. Niinivaara et al.[15] and Gyllenberg et al.[5] reported that some strains could not be used a very long time as a starter because, in time, they lost part of their desired characteristics.

Furthermore, several difficulties still remain in the utilization of bacterial pure cultures in the manufacture of meat products, e. g. the number of undesired bacteria in meat can be usually several millions per g, and their in influence on chemical changes is expected to be more significant than the influence of inoculated bacteria.

Although many efforts have been made to resolve these problems by many workers, more extensive studies are considered to be necessary for the better utilization of the bacterial pure cultures as a starter for the development of color in cured meat.

Summary

For the fixation of color in cured meat, the use of bacterial pure cultures is advantageous especially in both shortening of curing period and providing against certain types of spoilage as well as poisoning hazard.

The authors examined to isolate nitrate-reducing bacteria from the cured pork and investigated these microorganisms with ultimate aim of employing them as a starter culture for the color development in meat during curing.

The results obtained were summarized as follows:

(1) Seven strains (Strains No. 104, 106, 127, 129, 130, 206, and 208) which are significantly capable of reducing nitrate were isolated from the cured pork. From the taxonomic studies concerning to these strains, it was concluded that all of these strains belong to the genus, *Micrococcus*.

(2) Nitrate reducing activities of these strains in various kind of meats from such species as horse, swine, lamb and tuna were examined. As results, it was found that the strains No. 106, 129 and 208 had relatively strong nitrate reducing activities in these meats, and that the development of reddish color in the horseflesh was the most significant among the meats examined.

(3) Addition of either lactic, citric, phosphoric or acetic acids to the shredded horseflesh sample greatly affected on the nitrate reducing activities of these strains, and the modes of these affections were considerably differed among the acids used.

(4) Nitrate reducing activities of the acetone-dreid cells prepared from the strains No. 106, 129 and 208 were found to be relatively stable when after three months of the preparation, when they were preserved at −20C. Therefore, it may be concluded that preparation of the acetone-dried cells from the isolated nitrate reducing bacteria is a promising method in the utilization of these bacteria as a starter for the development of

color in cured meat.

Literatures

1) Akabori, S. ed. 1956. *Kosokenkyuho I.* p. 15–17. Asakura-shoten. Tokyo.
2) Breed, R. S., Murray, E. G. D. and Smith, N. R. ed. 1957. *Bergey's Manual of Determinative Bacteriology. 7th Ed.* Williams & Wilkins Co. Baltimore.
3) Deibel, R. H., Niven Jr, C. F., Keyahian, Th. and Doty, D. M. 1955. Role of microorganisms in curing hams. *American Meat Institute Foundation, Anual Report.* No. 22, S. 22.
4) Deibel, R. H., Wilson, G. D. and Niven Jr, C. F. 1961. Microbiology of meat curing IV. A lyophilized *Pediococcus cerevisiae* starter culture for fermented sausage. *Appl. Microbiol.* **9**:239–243.
5) Gyllenberg, H. G. and Hackman, C. R. 1961. A bacteriophage-lying strain of Staphylococcus employed in the manufacture of dry sausage. *Appl. Microbiol.* **9**:480–483.
6) Hashimoto, Y. 1966. *Chikuniku no Kagaku to Seizo.* p. 133–144. Yokendo. Tokyo.
7) Hashimoto, Y. 1963. *Shokuniku Nikuseihin Handbook.* p. 323–352. Asakura-Shoten. Tokyo.
8) Kaneko, S. 1971. Namaniku ni sonzai suru Biseibutsu I. *New Food Ind.* **13**(2):81–86.
9) Kaneko, S. 1971. Namaniku ni sonzai suru Biseibutsu II. *New Food Ind.* **13**(3):76–82.
10) Leistner, S. 1958. Bakterielle Vorgänge bei der Pökelung von Fleisch. I. Der Keimgehalt von Pökellaken. *Fleischwirtschaft.* **10**:74–79.
11) Leistner, S. 1958. Bakteielle Vorgänge bei der Pökelung von Fleisch. II. Gunstige Beeinflussung von Farbe, Aroma und Koservierung der Pökelfleisches durch Mikroorganismen. *Fleischwirtschaft.* **10**:226–234.
12) Leistner, S. 1958. Bakterielle Vorgänge bei der Pökelunk von Fleisch. III. Die Verderbnis von Pökellaken. *Fleischwirtschaft.* **10**:530–536.
13) Nakanishi, T. 1967. *Gyunyu to Nyuseihin no Biseibutsu.* p. 282–301. Chikyu-Syuppan. Tokyo.
14) Niinivaara, F. P. und Pohja, M. S. 1957. Über die Reifung der Rohwurst. II. Mitteilung beim Reifungsprozeβ. *Z. Lebensm. -Untersuch. u. -Forsch.* **106**:187–196.
15) Niinivaara, F. P., Pohja, M. S. and Komulainen. 1964. Some aspects about using bacterial pure cultures in the manufacture of fermented susages. *Food Tech.* **18**:147–153.
16) Pfützner, W. 1959. Die Kontrollierte Fermentierung von Fleischwaren, insbesondere Rohwurst. *Fleischwirtschaft* **6**:464–467.

肉色発現に関与する食肉内微生物

鵜田文三郎・細野明義・高橋富士雄・松葉　正幸
信州大学農学部　畜産製造学教室

要　　約

塩漬肉における硝酸塩の亜硝酸塩への還元は通常，肉に自然棲息する硝酸還元菌の作用により行なわれているが，塩漬の際に硝酸還元菌をスターターとして添加することにより，塩漬期間の短縮，塩漬肉の熟成促進さらには他の有害菌の増殖を防止するなど，望ましい多くの効果が期待される。

本報はそのスターターを実用化するための基礎研究であり，塩漬肉より分離した硝酸還元菌の各種塩漬肉における硝酸塩の還元性を調べ，硝酸還元菌の利用上における諸要因について検討した。

1)　塩漬豚肉より7株（No. 104, 106, 127, 129, 130, 206, 208）の硝酸還元菌を分離した。それら菌株についての形態的，生理的試験から，いずれも *Micrococcus* に属する菌種であることが認められた。

2)　馬肉，豚肉，羊肉ならびに鮪肉の発色に対するこれら分離菌株の作用性を調べると，No. 106, 129, 208の3株がそれぞれの肉に対し強い発色作用を示し，特に馬肉における発色が最も著しいことが認められた。

3)　ピックル液とともに破砕した馬肉試料を乳酸，クエン酸，酢酸により種々のpHに調整し，上記3菌株を作用させると，菌種の違いによる発色度の異なりの他に，使用した酸の種類により肉の発色が顕著に影響されることが認められた。

4)　No. 106, 129, 208の3菌株よりアセトン粉末菌体を調製し，それらのスターターとしての有用性を馬肉を用い試験した。その結果，いずれの粉末菌体とも肉の発色に対する作用性を有し，かつ−20°Cで保存すると，3ヶ月経過後においても硝酸塩に対する還元力があまり低下しないことが認められ，アセトン粉末菌体のスターターとしての有用性を認めた。