15 Discoloration of cured meat pigment by bacteria

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Introduction

Niven, Castellani and Allanson /1949/ described first the organism /L. viridescens/ frequently responsible for greening of cured meat products. This organism was also described as causative agent of discoloration of cured sausages by Lőrincz and Incze /1961/. Other workers have found other lactobacilli with the ability of causing greenish discoloration, namely unclassified streptobacteria from hams /Skovgaard, 1959/ and from frankfurter /Drake et al. 1960/, as well as heterofermentative lactobacilli, like L. pastorianus / Coretti, 1958/.

Recent cases of almost regular greening in summer months in one of our plants made our studies timely.

Materials and methods

Meat Product — Discoloured Bologna sausage samples were taken, homogenized and inoculated onto media below.

Culture media — MRS agar was used for isolation of lactic acid bacteria, APT agar for growth /Evans and Niven, 1951/.

Discolouring ability — Isolated strains were tested for catalase activity, negative strains were inoculated onto MnO_2-plate. /Kneteman, 1946/. The bacteria forming a halo around colonies were H_2O_2-producers and capable of causing greening. Discolouring ability has also been tested on surface of meat product.

Temperature requirement — APT-broth was inoculated with the H_2O_2-positive strain and incubated at 6, 10, 15, 20, 30, 37, 40 and 43°C. Growth was checked after 1, 2, 4 and 10 days.

Salt tolerance — APT-agar plates containing 3, 5, 6,5 , 7 and 10% NaCl were inoculated with the isolated H_2O_2-positive strain /10^5/ml/ and incubated at 20 °C. Growth was checked after 48, 96 and 168 hrs.

Biochemical tests — In addition to carbohydrate fermentation ability other special tests /e.g. nitrate reduction, NH_3 from arginin, haemolysis etc./ have also been carried out.

Heat resistance — Heating menstruum /APT-broth/ was preheated in ultrathermostate to 65 °C and inoculated with pure culture of H_2O_2-positive strain to a final density of approx. 10^8/ml. Since the volume of APT-broth was 50 times higher than that of the inoculum, this latter reached the desired, temperature without delay. Samples were taken at regular intervals and total viable count of survivors determined by serial dilution technique and plating method.

Results — From the direct isolations of discoloured Bologna sausage the vast majority of bacteria proved to be lactobacilli on MRS and APT plates. When inoculated onto plates without glucose, very slight or no growth could be detected. Selecting catalase negative bacteria and inoculating onto MnO_2-agar a strain showed intensive H_2O_2-production manifested by definite halo around colonies. A streak with liquid culture of this strain on a slice of Bologna sausage confirmed that the isolate is capable of causing greenish discolouration. Temperature requirement studies proved that no growth occurs below 3°C and above 40 °C. Although above 40°C /at 43°C/ a slight growth could be detected after longer incubation time, nevertheless degenerated, filamentous forms are resulted. At 45°C no growth occurred.

Table 1. Temperature requirement of H_2O_2-positive strain

Incubation time /days/	Incubation temperature °C									
	3	6	10	15	20	30	37	40	43	45
	G R O W T H									
1	-	-	-	+	++	+	+	-	-	-
2	-	-	+	++	+++	++	+	-	-	-
4	-	+	+	+++	0	+++	++	+	-	-
10	+	+	++	0	0	0	0	+	+	-

0 = not tested further

The greening strain grew well in presence of 2 and 5 % NaCl, slower at a concentration of 6,5 % and no growth could be detected at 7 % even after 168 hrs.
Results of biochemical tests are shown in table 2. and 3.

Table 2. Fermenting activity of H_2O_2-positive strain

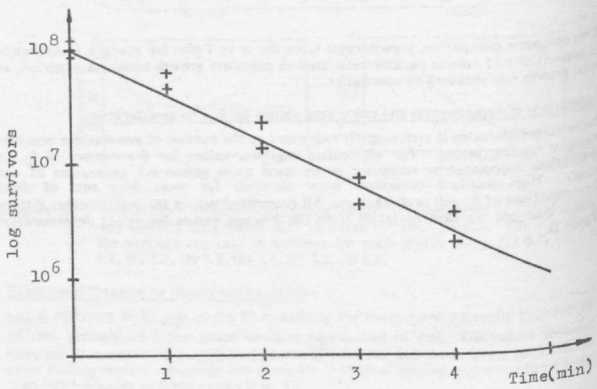
Gas from glucose	-
acid from glucose	+
lactose	-
arabinose	-
maltose	+
mannitol	-
melezitose	-
melibiose	-
raffinose	-
salicin	+
sorbitol	-
trehalose	+
aesculin	+
laevulin	+
sucrose	+

Table 3. Other biochemical characteristics

Ammonia from arginin	-
Nitrate reduction	-
Catalase	-
Oxidase	-
Blood agar	α - haemol
Gelatin liquefaction	-
Mucoid colonies on 5 % sucrose agar	-

In our heat resistance studies the strain gave a D_{10}-value of 2,5 mins at 65 °C, when APT-broth was the heating menstuum/Fig.1./

Fig. 1 Heat resistance of H_2O_2-positive strain. /heating menstruum: APT-broth; temp.: 65°C/



Discussion

On the basis of the biochemical tests and temperature requirement, the isolated strain, responsible for greening of cured meat pigment, can be classified as Streptobacterium, being homofermentative and showing growth at 15°C, no growth at 45°C. Our strain resembles in many respects the 140 strains isolated from bacon by Kitchell and Shaw /1975/. Most of the biochemical test results are common with the exception of melibiose and melezitose fermentation, where 128 out of 140 strains showed positive, our strain negative results. It is of interest to mention, that most of the strains isolated by them proved to cause greening.
Our isolate was a non-motile Gram positive catalase negative rod with no tendency of forming cocooid shape, which was closely related to atypical streptobacteria, more precisely to Lactobacillus leichmannii with a deviation in lactose fermentation. Since these bacteria have a relatively high salt resistance and grow well at lower temperatures, there is a possibility for their growth on the meat during chilling, which means high initial number. Although its heat resistance is smaller than that of the L. viridescens, nevertheless this can be significantly higher in meat emulsion by the protective effect of fat and protein.
Considering the high initial count and the relatively high heat resistance it is easily comprehensible that under favourable conditions these lactobacilli survive regular heat treatment of meat products and may thus cause discolouration. Effect of higher temperatures also in chilling rooms during summer is evident: higher initial count before heat treatment gives higher number of survivors.

References

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