

Some data concerning the green discoloration of  
meat products caused by Lactobacilli

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Spoilages occurring in fast perishable foods are the main factors impairing the production results of the food industry and a better public supply. A lot of intellectual and material energy is being spent throughout the world in order to prevent these economic losses. The possibility of causal prevention is in our hands if the physical, chemical and biological agents causing spoilage are unveiled.

The green discoloration appearing explosion-like, mainly in summer on the surface of cured meat products /mostly in sausages /, but sometimes on that of raw meat too, is a well-known incidence. This discoloration was suggested to be a consequence of curing defects and a bacterial contamination, however after having excluded the curing defects, the microorganism causing effectively the greening, could not be easily isolated from the mixed flora.

Niven and his co-workers /Allanson, Buettner, Castellani, Evans /1.2.3.4./, then Coretti /5.6.7.8.9./ were dealing with that characteristics of the meat greening lactobacilli in general, while Niven and Evans /1956/ have described the Lb. viridescens nov.spec. and pointed out it's role in greening meat products. We are convinced that curing defects cause

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a greenish discoloration of meat products much less frequently than it has been supposed so far in lack of any better reason. On the other hand, when bacterial greening was suggested, not always could be produced a reliable proof. A mixed flora was found with which some time it was possible experimentally to produce greenish discoloration, the other time not. Until recently it was not always possible to select positively the effective "pathogene organism". Niven et al. have offered us the morphological and biological criteria of Lb. viridescens, in possession of which there has been no difficulty any more in making the differential diagnosis of greenish spoilages. As a matter of fact, since we have known this microorganism, we have always succeeded in isolating it from the greenish discolorations.

This work has followed the objective of detecting the locations, where Lb. viridescens can be found, since no attention has been yet paid to this fact. Furthermore we have tried to study somewhat more minutely the biological characteristics of our isolated strains; finally we examined the microorganisms blamed so far for causing greenish discoloration and to find which of these organisms can be acquitted of this blame in the possession of our present knowledge.

Niven et al. appear to indicate that every salt tolerant, catalase negative microorganism which can grow at low temperatures and oxidate some substances by peroxid-producing, might cause greenish spoilage of meat products.



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The new *Lactobacillus* sp. described by them does respond completely to this postulate. There is no doubt that we have succeeded in all cases to produce greenish discoloration on the meat products by our Lb. viridescens strains.

We have recognized our first Lb. viridescens by selecting systematically from a greenish sausage the mixed cultures grown in usually employed media. The specifically greening colonies have appeared as fine, transparent, dew-drop-like ones among the colonies grown abundantly on glucose-agar, which could be well differentiated as regards their colony characteristics too /Figure 1./ . We have to state that we have failed to produce greenish discoloration by any of the other bacteria isolated from the flora grown on greenish meat products. Accordingly, the only microbe causing always greening has been the Lb. viridescens /Figures 2 and 3 /.

Hereupon we have wished to make clear where Lb. viridescens can be found in the meat plants. We have taken samples from the boning room, from equipments of the mixing and stuffing rooms, from half-finished and finished products, then from the surface of the casings and the aseptically cut surface of sausages available in the trading network, and finally from brines. To our increasing surprise we have been succeeding in isolating practically from every test point and every sample the microorganism which causes green

discoloration and which could now be well recognized on the base of its morphological and biological characteristics.

Consequently the microorganism in question is an ubiquitary one which causes greening depending upon its quantitative presence and the fact whether the local growth conditions turn out to be favorable to produce the oxidation of meat pigments, as a result of the lack of tissue catalase activity and that of sufficient peroxid-production.

In this connection we have to point out that the Lb. viridescens is a delicate, sensible microorganism, which hence, can not be cultivated easely and will be rapidly overgrown in mixed cultures by strains demanding less care. This fact has been pointed out in full details by Niven et al. and also by Coretti. They have suggested an adequately composed medium. According to their view good results can be expected in a medium containing beside the usual components also manganese and citric acid salts. As to us, we could facilitate our earlier uncertain diagnostical work similarly by using a medium containing such compounds.<sup>x</sup>

Beside their specific colouring characteristics /Gram positive/, their carbohydrate fermentation /glucose,

x./ 10 g peptone Witte, 70 g malt extract, 5 g NaCl, 5 g sodium citrate, 0.1 g MnCl<sub>2</sub>, 25 g agar-agar, 400 ml yeast decoction ad 1000 ml tap-water /.



fructose, mannose, maltose, dextrin and potentially sucrose / and salt tolerance / 6.5 % NaCl / , the grown strains have revealed surprising behaviour relating to the heat tolerance. /Table No.1/.

The various strains have different heat tolerance. This is well illustrated by the fact that we have found a strain which could be recovered after a ~~heat-treatment on~~ 50° C. for 170 minutes.

Duration of heat-treat- ment minutes	Temperature /°C/ at which greening and recovery were successful /++/, resp. only the recovery succeeded /+/. <hr/>						
	50	55	60	65	70	75	80
5	++	++	++	++	+	+	-
10	++	++	++	+	+	-	-
15	++	++	+	+	+	-	-
20	++	+	+	+	-	-	-
30	++	+	+	+	-	-	-
40	+	+	+	-	-	-	-
60	+	+	+	-	-	-	-
80	+	+	-	-	-	-	-
100	+	+	-	-	-	-	-
120	+	-	-	-	-	-	-
140	+	-	-	-	-	-	-
145	+	-	-	-	-	-	-
146	-	-	-	-	-	-	-

/+ = the recovery is accidental /

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As it is shown in the above table, the cured meat pulp mixed with 24<sup>h</sup> bouillon culture of Lb. Viridescens and held at a temperature of 65° C for 5 minutes, grew green after an incubation of 24 hours at 20° C. The contaminated meat emulsion held at the aforementioned 65° C for 10 minutes, then incubated at 20° C for 24, later for 48 hours, did not get greening any more; on the other hand the microorganism could be recovered in some cases even when the infected meat pulp was held at a temperature of 65° C for 30 minutes. The inoculated meat emulsion held at 50° C for 30 minutes has taken on a greening, nevertheless as a rule the Lb. viridescens could be successfully recovered from the meat pulp held at this temperature for 145 minutes. Good practical conclusions may be drawn from these tests.

These tests relating to greening and heat tolerance have been effectuated on cured meat pulp packaged in polyethylene pouches. The thickness of the meat paste in the pouches did not exceed 1 mm in order that the heat penetrating conditions should not trouble our tests at the desired temperature. We have chosen the meat pulp as milieu instead of some fluid medium, /e.g. bouillon, Ringer solution, etc/ in order to better approach the natural conditions.

According to our findings up to now, there are fluctuations among the strains also with regard to the salt tolerance. We have succeeded in isolating the microorganism



in question from pickles containing 10-14 % salt, consequently the Lb. viridescens finds the possibility to live also in pumping or cover brines, and its more abundant growth is a function of the local favorable or unfavorable relations.

We have tested different strains from various food inspection laboratories. These bacteria strains have been isolated from sausages showing greenish spoilage. On the base of peroxid-producing and experimental greening we could not find but several *Streptococcus* strains which could be taken into consideration with regard to the aspects examined by us.

We are still continuing our experiments. We suspect that the Lb.viridescens has it's role also in cases, where the activity of other greening species /e.g. *Streptococcus*/ is supposed.

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Figure No.1: Pure culture of *Lb. viridescens* /48 hours, 30° C / on glucose-agar. About 30 x magn.

Figure No.2.: Characteristic shapes of *Lb. viridescens* /see individuals marked by arrows /.

Lumipan, ocular: 7 x , prism: 1.5 x, objective: 90 x, phase-contrast.

Figure No.3: Microphoto of characteristic Lb.viridescens individual, Phase-contrast 6 x linear magn. from negative,

L i t e r a t u r e :

- 1./ Niven C.F. Ir. Castellani A.G. and Allanson V./1949/  
A study of Lacticacid bacteria that cause surface discoloration of sausages. J. Bact. 58. 634-641.
- 2./ Evans I.B. and Niven C.F.Ir. /1951/ Nutrition of the heterofermentative Lactobacilli that cause greening of cured meat products. J. Bact. 62. 599-603.
- 3./ Niven C.F.Ir., Buettner L.G. and Evans I.B. /1953/  
Thermal tolerance of the heterofermentative Lactobacilli that cause greening of cured meat products. Appl. Microbiol. 2. 26-29.
- 4./ Niven C.F.Ir. and Evans I.B. /1956/ Lactobacillus viridescens, nov.spec. a heterofermentative species that produces a green discoloration of cured meat pigments. J.Bact. 73. 758-759.
- 5./ Coretti K /1958/ Die Bakterienflora fehlerhafter Rohwürste. Arch. Lebensmittelhyg. 9. 32.
- 6./ Coretti K. /1958/ Rohwurstfehlfabrikate durch Laktobazillen. Fleischer Bch. 10. 218.
- 7./ Coretti K. /1958/ Die Bakterienflora fehlerhafter Rohwürste. Entgegnung. Arch. Lebensmittelhyg. 9. 126.
- 8./ Coretti K. /1958/ Gehäuftes Auftreten von Kernfärbungen bei Rohwürsten. Die Fleischwirtschaft 800.829.
- 9./ Coretti K. /1958/ Technische Verbesserung des Manganoxyd-Nährbodens zum Nachweis bakterieller Peroxyde. Jahresbericht 1958. Bundesforschungsanstalt für Fleischwirtschaft, Kulmbach, 36-37.