INVESTIGATIONS ON THE ROLE OF MICROORGANISMS OF THE FAMILY *LACTOBACILLACEAE* IN MEAT PRODUCTS

I. EFFECT OF SOME CHEMICAL COMPOUNDS ON *L. VIRIDESCENS*

The Veterinary Institute, Puławy
Department of Hygiene of Animal Products
Head: Prof. Dr. Zbigniew Gaugusch

EWA OŻDŻYŃSKA

Greening and fading of sausages are among the most important changes appearing in cured meat products. The most frequent agents causing these changes are lactobacilli, especially *L. viridescens* (1, 2, 4, 6, 7, 8, 9). The rather frequent occurence of greening and fading in sausages is theoretically connected with the wide distribution of these microorganisms in the environment. However, there seems to be a lack of information in the literature concerning the sources of contamination of meat products by these microorganisms. Therefore, in the present work we attempted to determine the place of the most frequent appearance of the bacteria mentioned, with the goal of drawing conclusions concerning the route by which *L. viridescens* contaminates the above products.

It is presently known that some lactic-acid bacteria, among them also *L. viridescens*, can multiply at low temperatures (about 4°—6°C), at the relatively low pH found in meat products, and also in the presence of NaCl, KNO_3, and $NaNO_2$ in the amounts occurring in sausages and conserves (10). However, one does not find data in the literature on the subject of experimental studies on the effect of various curing agents on *L. viridescens* multiplication during the storage of meat products. Such data would facilitate development of a curing recipe which would achieve a braking of the growth of the above bacteria and would cause a reduction in the occurrence of *L. viridescens*-related color changes. In connection wih this, the present work attempts to determine the

effect of sodium chloride, potassium nitrate, sodium nitrite, monosodium glutamate, phosphates, sodium ascorbate, sorbic acid, and sucrose on *L. viridescens* during normal storage of the product.

Materials and Methods

The material used for studies to ascertain the occurence of *L. viridescens* in the environment was composed of feces samples: 54 from swine, 92 from cattle, 20 from humans, 40 samples from mixed dung of swine and cattle, 54 samples of fertilized land, 31 from land not fertilized by dung, 43 samples from the air of a slaughterhouse, a processing plant, and the storage room of a processing plant.

Quantitative inestigations were conducted by the plate method of Koch on the medium developed by Kafel (5). From the samples, 1 g of feces was weighed out; next, a row of decimal dilutions in Ringer's solution was made, and 0.2 ml was inoculated onto the aforementioned medium. Plates were incubated in a thermostat at 25°C for 48 hours. After this time the number of black-colored colonies was counted.

In the experiment's second phase, concerning the determination of the effect on *L. viridescens* of various chemical compounds used in meat curing processes, minced-meat sausages were used as experimental material. Sausage meat prepared for this sausage was collected five times in 5.5 kg quantities from the butcher's plant. Before transporting the stuffing to the laboratory, it was divided into 9 portions of 60 g each. To each portion was added one of the following respective substances, dissolved in 20 ml of distilled water in quantities used in the meat industry: NaCl — 18 g (3%), KNO_3 — 1.8 g (0.3%), $NaNO_2$ — 0.12 g (0.02%), monosodium glutamate — 1.2 g (0.2%), phosphates — 3.0 g (0.5%), sodium ascorbate — 0.324 g (0.054%), sorbic acid — 0.6 g (0.1%), sucrose — 6 g (1%). To the portion of stuffing designated to make control sausages, 20 ml of plain distilled water were added. From each portion of stuffing 12 pieces of sausage were made, which were given thermal treatment at 74°—75°C for 45 minutes. Next, the sausages were cooled to 8°C. After cooling, 0.5 ml of *L. viridescens* in suspension was injected to the center of each sausage. The suspension was prepared in the following manner. Liophilized strains of *L. viridescens* producing H_2O_2 were inoculated to liquid APT medium (3) and incubated at 25°C for 24 hours. Then 1 ml of the above culture was dissolved in 100 ml of Ringer's solution, which then comprised the original material for inoculating the experimental sausages. The sausages were stored at 8°C after inoculation. Bacteriological tests were performed on day zero, i.e. the day of production and inoculation of the sausage, and thereafter on the second, fourth, and sixth days after inoculation. The sausages were homogenized in a blender with Ringer's solution in a 1:10 proportion, after weighing them and heat-sterilizing the casing over a flame. Then a row of successive decimal dilutions was made and inoculated in 0,2 ml units on a medium with benzidine (5), incubated at 25°C for 48 hours, and following this period the colonies were counted. The above investigations were repeated three times with regards to three different strains of *L. viridescens*.

Results

In the first phase of the investigations (for determining the sources of *L. viridescens*), in swine feces we noted in 1 gram from 2 000 to 42 000 000 of these bacteria, in human feces from 400 000 to 2 450 000, in cattle feces from 4 115 to 18 000 000 and in dung-fertilized earth from 3 000 to 1 250 000. In the air of the butchery, curing plant, and storage room, there were noted from 400 to 2 000 *L. viridescens* on the surface of a plate 10 cm in diameter after exposure of the plates for 10 minutes.

In the second phase of the investigations (concerning the effect of some chemical compounds on the growth of *L. viridescens*), it was shown that the weakest development of these microorganisms occurred in sausages with phosphates added. The remaining chemical compounds

did not produce any noticeable braking effect on the bacteria under discussion. Particular results of the experiments are presented in Figs. 1, 2, and 3.

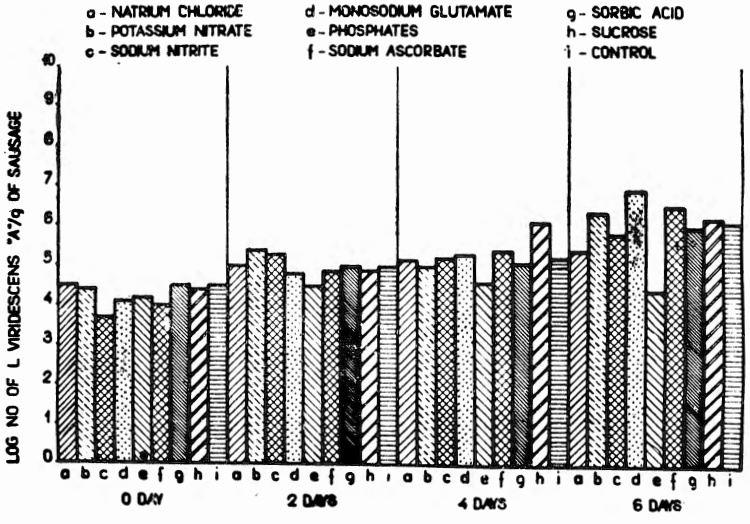

Fig. 1. Influence of curing agents on *L. viridescens* (strain "A") in sausage.

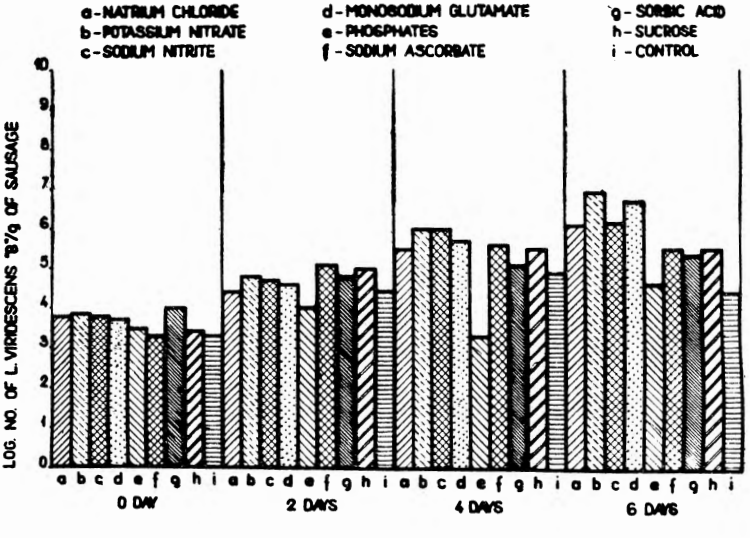

Fig. 2. Influence of curing agents on *L. viridescens* (strain "B") in sausage.

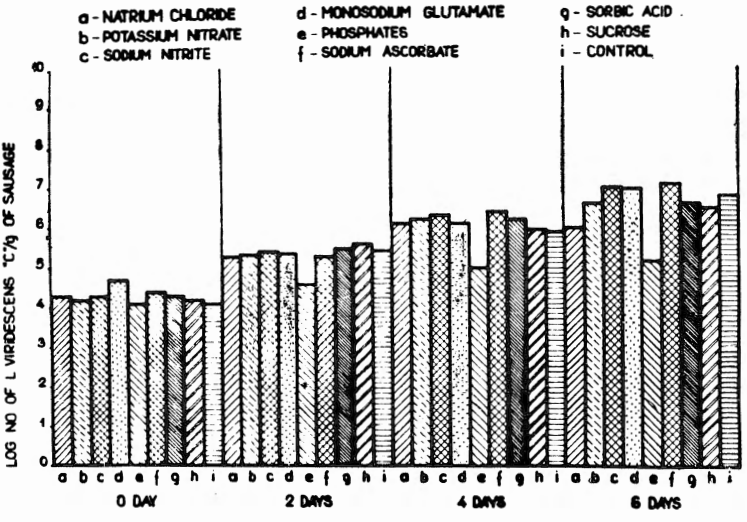

Fig. 3. Influence of curing agents on *L. viridescens* (strain "C") in sausage.

Discussion

The first phase of the investigations showed that *L. viridescens* occurs most often and in rather large numbers in human and animal feces. Probably it is passed from there to meat production rooms, to curing brine, and to raw meat, especially under unsuitable sanitary conditions during the whole production cycle.

In the second phase investigations conducted with pieces of minced-meat sausage, it was shown that the addition of NaCl, KNO_3, $NaNO_2$, monosodium glutamate, sodium ascorbate, sorbic acid, and sucrose did not affect in any noticeable manner the growth of *L. viridescens*. However, a braking influence on these bacteria was seen with the addition of phosphates to sausage.

Conclusions

1. The main source of *L. viridescens* contamination in meat production departments and in sausage is the intestinal tract of humans and animals.

2. No visible effect on the growth of *L. viridescens* in minced-meat sausage was produced by these chemical compounds: NaCl, KNO_2, $NaNO_2$, monosodium glutamate, sodium ascorbate, sorbic acid, and sucrose.

3. Phosphates added to sausages made from cured meat and contaminated by *L. viridescens* inhibited the development of these microorganisms when the above products were stored at 8°C.

REFERENCES

1. *Bartels H., Coretti K.*: Fleischwirtschaft 8, 639, 1961. — 2. *Bartels H., Coretti K., Schadeck M.*: Fleischwirtschaft 12, 991, 1961. — 3. *Evans J. B., Niven C. F., Jr.*: J. Bact. 62, 599, 1951. — 4. *Grever A. B. G., Schuddeboom L. J.*: Fleischwirtschaft 4, 234, 1958. — 5. *Kafel S.*: Biuletyn IIIzj. PTNW ,Lublin 1966. — 6. *Lörinch F., Inche K.*: Fleischwirtschaft 5, 406, 1961. — 7. *Niven C. F., Jr., Castellani A. G., Allanson V.*: J. Bact. 58, 633, 1949. — 8. *Niven C. F., Jr.*: American Meat Institute Foundation Bulletin No. 13, 1951. — 9. *Niven C. F., Jr., Evans J. B.*: J. Bact. 73, 758, 1957. — 10. *Niven C. F., Jr.*: Reprint from Proceedings of the Fourth Research Conference. Sponsored by the Council on Research, American Meat Institute, at the University of Chicago, March 20 and 21, 1952.