A survey of a factory for the sources of lactobacilli characteristic of vacuum packed, Wiltshire cured bacon

by A.G. Kitchell and G.C. Ingram

Meat Research Institute,

Agricultural Research Council,

Cambridge, England.

Introduction

There are a number of unsolved problems associated with the vacuum packaging of slices of traditionally cured Wiltshire bacon (Kitchell & Ingram, 1963). One is the dominance rapidly achieved by lactic acid bacteria in such packs when stored at 20°or below (Hansen, 1960; Eddy & Gatherum, 1961; and Cavett, 1962). Normally, on Wiltshire sides held under aerobic conditions, the lactobacilli account for less than 1% of the total number of microorganisms and are not known to affect the shelf-life of the bacon. In vacuum packs, their presence in large numbers cannot, however, be ignored as a possible factor in deterioration during storage, especially as souring is reported to occur (Tonge, Baird-Parker & Cavett, 1964).

It is, therefore, of interest to know which kinds of lactic acid bacteria develop on the bacon stored under vacuum and, with a view to their ultimate eradication, to learn something of their origin. Thus, following examination of nearly 600 bacteria (including nearly 140 lactobacilli) isolated from vacuum packed bacon, a survey was made (Kitchell, 1963-64) of the factory in which it was manufactured and packed.

Before presenting an account of the survey and the results it may be helpful briefly to outline a typical sequence of operations in the manufacture of this bacon. Pigs are stunned, bled, passed through a "scalding" tank (60°C for 4-5 minutes) and dehairing machine, singed in a furnace until the skin becomes dark brown, cooled with a cold-water spray, passed through a scraping machine to remove most of the scorched skin, finished by hand, and then passed to the butchers. The entrails are removed and the visceral surfaces sprayed with water. The carcase is split down the back and the back-bone removed. The sides are then

transferred from ambient temperature in the slaughter hall, sometimes via a chiller at -4°C, to chill room at 3-5°C where they remain overnight cooling to 5°C.

Preparation for curing in brine is done in cooled rooms where the sides, passing along roller conveyors, are trimmed and pumped with brine. In the curing cellars (5°C) they are immersed in tanks of brine for 4-5 days. On removal from the tanks they are drained and stacked to mature in the cellar for 10-14 days.

If required, smoking is done by exposing the matured sides in a stove with forced circulation at a temperature of 32-35°C for 4 hours. To facilitate slicing, the rib bones are removed and the sides cooled overnight at -4°C. A cutting machine "shingles" the slices on a conveyor belt from which they are removed by hand, weighed into plastic pouches, and vacuum packed.

Materials and methods

Sampling

A rapid semi-quantitative swabbing technique (Hansen, 1962) was adopted for the purpose of making comparative counts at the sites selected for sampling. Each cotton wool swab, moistened with 6.5% (w/v) NaCl solution, was rubbed over an area of 10 cm^2 delimited by a sterilizable metal template. The exposed side of the swab was used immediately to inoculate a slope of each of two culture media chosen to give respectively a total count and a count of lactic acid bacteria. Ten swabs were used at each site. They were retained and later, in the laboratory, were used in pairs to inoculate 5 plates and a broth culture; these were used for the isolation of strains of lactobacilli required for identification.

Culture technique

The slopes for inoculation with the swabs were prepared from Rogosa acetate agar (Oxoid) or Blood Agar Base (Oxoid) - the total count medium used by Hansen (1962) - containing 6.5% (w/v) NaCl and reinforced with 0.5% (w/v) agar. The plating medium used for isolation was Rogosa acetate agar and the broth was M.R.S. (de Man, Rogosa & Sharpe, 1960).

Incubation of the slopes and plates of Rogosa medium was at

30°C under CO_2 (10% v/v) and H_2 (90% v/v). Slopes of the total count medium and the M.R.S. broth were incubated aerobically at 25°C.

Bacteriological tests

From the plates of Rogosa medium representing each site sampled, about 10 colonies were picked for screening through selected tests (see Rogosa & Sharpe, 1959). These were morphology; Gram stain; catalase; production of CO_2 from glucose; growth in 0.4% Teepol (Sharpe, 1961); growth at 15° and 44°C; production of acid from mannitol and melibiose; hydrolysis of aesculin; and formation of mucoid colonies on 5% (w/v) sucrose agar (Deibel & Niven, 1959).

Site of sampling

Samples were taken from the skin of the pig immediately after slaughter; out of the scalding tank; at the end of the butchery line before going into the chillrooms; on coming out of the chillrooms, during trimming in preparation for curing; and after being cured, matured, and smoked. The visceral surfaces of the dressed carcase were likewise swabbed and samples of faeces taken. Also, certain equipment was examined e.g. scraping machine blades; butchers' knives; roller conveyor on which sides were trimmed; pumping table; blade of the slicing machine; and the conveyor belt and balances used on the packaging line. Finally, samples were taken from slices of freshly packed bacon.

Results

Numbers of bacteria

The counts obtained by Hansen's method are presented, in terms of the antilogarithm of the $\log_{10}$ mean value, in Table 1. It should be noted that, as Hansen pointed out, these counts are very much lower than counts made by more accurate sampling and counting procedures, but they are nevertheless suitable for comparative purposes among themselves. Like Hansen, we observed that the standard deviation is normally of the order of 0.5 - 1.0 $\log_{10}$ units.

Distribution of bacon lactobacilli

The characteristics of the lactobacilli isolated from sliced bacon at the end of the present survey and those of strains previously

taken from vacuum packed bacon stored over a wide range of temperatures (unpublished data), together with two named strains, are given in Table 2. The few strains isolated from bacon in this survey share the same reactions in the selected tests as 88% of 119 non-mucoid strains formerly examined. They can, therefore, be taken as typical of the majority of lactobacilli found on sliced bacon packed by this factory.

Though two major sub-groups can be recognized within the bacon strains of lactobacilli neither corresponds with L.casei or L.plantarum, each sharing characters of both named strains. Representatives of each of these sub-groups were found on bacon during the survey in the same proportions (ca. 9:1) as they were found in vacuum packed bacon after storage.

Characterization of 133 cultures made up of about 10 from each of the other sites examined (see Table 1, centre column) established that those sharing the reactions of the bacon strains were few in number up to the point of entry of the sides into the chill rooms i.e. only 8/70 or 11%. On sides leaving the chill rooms, and at sites involved in all later stages of processing, the majority of strains i.e. 58/63 or 92% were of the bacon type.

Heterofermentative lactobacilli constituted 21% of the 70 cultures from the first group of samples but none was found among the 63 isolates from the second group. No lactobacilli of the bacon type were found in faeces but typical L.casei and L.plantarum, together with heterofermentative strains, were isolated.

Discussion and Conclusions

On the slaughter floor and dressing line the total count, still high after the so-called "scalding" tank treatment, falls to a relatively low final value due largely, no doubt, to passage through the furnace. Handling, and contact with the equipment used during the boning procedure lead to re-contamination which remains through curing, maturation, and smoking.

A roughly similar pattern is given by the count of lactobacilli, though numbers of these bacteria appear to be much reduced in the "scalding" tank. Re-infection occurs during boning; and the equipment used for slicing and packing the bacon is a source of contamination

by these bacteria, as samples taken from the final product show.

Characterization of the lactobacilli isolated from the sliced bacon by means of a few selected tests indicates that they do not correspond exactly to either of two broadly similar named strains but share characters of both. They are probably best described, therefore, as casei-plantarum types or, because they fail to ferment mannitol or hydrolyze aesculin, simply as unidentified streptobacteria (c.f. Cavett, 1963).

These organisms are to be found on the skin of the freshly killed pig. It appears, therefore, that they come into the processing area continuously and survive the various heat treatments given to the carcase. At this stage they represent no more than 11% of the lactobacillus types isolated. However, after overnight cooling of the sides of pork at 37°-40°F, they become the dominant type of lactobacillus due, it is presumed, to a selective action of low temperature.

Though it has already been made clear, it should be stressed finally that these results relate to the bacon from one factory and to one survey of that factory. Therefore, the general validity of these observations, with respect to both the lactobacilli of bacon and to their distribution in various parts of the manufacturing process, has yet to be established by surveys made in other factories. Nevertheless, it will be appreciated that further work is greatly facilitated by having, as a result of this initial study, a knowledge of the characteristics of these organisms and of the sites from which they are likely to be isolated.

(The cooperation of Mr. D.P. Gatherum in making the arrangements for and assisting with the factory survey is gratefully acknowledged.)

Summary

The lactobacilli found in large numbers in packs of sliced bacon sealed under vacuum and stored for more than a few days did not resemble any named species but shared some characteristics of both L.casei and L.plantarum. Similar unidentified streptobacteria were found on the pig and throughout the factory, but were only a small proportion of the total number of lactobacilli isolated up to the point where the dressed carcases were chilled overnight. However, they accounted for 93% of the lactobacilli found at all subsequent stages of processing the carcase into bacon. A specific reservoir of these bacteria was not found within the factory. They appear to come in on the pigs and to be selected by the low temperature of the chill rooms.

Résumé

Les lactobacilles présents en grand nombre dans les paquets de lard en tranches, scellés au vide, et emmagasinés pendant une période dépassant plusieurs jours, ne ressemblent à aucune espèce nommée mais assument quelques caractéristiques et du L.casei et du L.plantarum. Des streptobactéries semblables, non identifiées, furent trouvées sur les cochons et partout dans l'usine mais ne représentèrent qu'une menue proportion du nombre total des lactobacilles isolés jusqu'au moment où les carcasses furent frigorifiées pendant la nuit. Or, ils s'élèvent à 93% des lactobacilles trouvés dans toutes les étapes ultérieures que la carcasse subit pour en faire du lard. Un reservoir spécifique de ces bactéries ne fut pas trouvé dans l'usine. Il paraît qu'elles entrent l'usine avec les cochons et qu'elles sont ensuite selectionnées par la basse température de la chambre frigorifique.

Zusammenfassung

Lactobazillen, die in grossen Mengen in in vacuo versiegelten, länger als ein paar Tage aufgespeicherten Paketen von Schinkenspeckscheiben aufgefunden wurden, glichen nicht irgendeiner bekannten Art, sondern teilten die Charakteristiken von L.casei und L.plantarum. Gleichartige, nicht identifizierte Streptobakterien wurden auf den Schweinen und in der Anlage aufgefunden, aber sie stellten nur einen

kleinen Anteil der Gesamtheit aller Laktobazillen dar, die bis zur Arbeitsstufe der übernächtlichen Kühlung der Kadaver isoliert wurden. Es stellte sich jedoch heraus, dass diese Art 93% aller Laktobazillen ausmachte, die in allen späteren Arbeitsstufen bei der Verarbeitung bis zum Schinkenspeck vorgefunden wurden. Ein spezifisches Reservoir für diese Bakterien wurde in der Anlage nicht entdeckt. Es scheint dass sie zusammen mit den Tieren in die Fabrik hereingebracht, und dort durch die niedrige Temperatur in den Kältelagerräumen ausgewählt werden.

TABLE 1. Total counts and the numbers of lactic acid bacteria, together with the percentage of bacon types, found by swabbing carcases and equipment in the operating sequence.

Samples taken from carcases	Total count	Lacto-bacilli	% bacon -type lactobacilli	Lacto-bacilli	Total count	Samples taken from equipment
	No. bacteria* / 10 cm^2		%+	No. bacteria* / 10 cm^2		
Skin after slaughter	-‡	70	20			
" ex scalding tank	2800	< 10	10			
			20	< 10	2800	Scraping machine blades
Visceral surfaces	300	< 10	20			
			0	30	1400	Butchers' knives
Faeces	-	-	0			
Skin into chiller	200	< 10	10			
" out of chiller	200	< 10	100			
			33	< 10	3200	Boning table
" during boning	3200	30	100			
			90	20	2800	Pumping table
" of smoked side of bacon	2800	100	100			
			100	20	400	Blade of slicing machine
Slices of bacon	1100	70	100			
			100	250	2000	Slice conveyor, trimming boards and balances

* Anti log of the logarithmic mean value

\+ Results based on identification of about 10 bacterial isolates from each site (total = 133, see text).

‡ No result

(B2)

TABLE 2. Characters of the non-mucoid streptobacteria isolated from sliced bacon compared with those of typical dairy strains.

Sample	No. isolates	CO_2 from glucose	Growth in 0.4% Teepol	Growth at 15°	Growth at 44°	Acid from mannitol	" " melibiose	Hydrolysis of aesculin	Mucoid colonies on 5% sucrose
Sliced Bacon (this survey)	9/10	-	+	+	-	-	+	-?	-
	1/10	-	-	+	-	-	+	-?	-
Vacuum Packed Bacon (Kitchell, unpub.)	97/119	-	+	+	-	-	+	-?	-
	8/119	-	-	+	-	-	-	-?	-
L.plantarum		-	+	+	-	+	-	+	-
L.casei		-	-	+	-	+	+	+	-

* only the named strains gave an unequivocal result in this test.

References (B2)

Cavett, J.J. (1962). J. appl. Bact. 25, 282.

idem (1963). ibid. 26, 453.

Deibel, R.H. & Niven, C.F. (1959). Appl. Microbiol. 7, 138

Eddy, B.P. & Gatherum, D.P. (1961) 7th European Meeting Meat Res. Workers. (Warsaw)

Hansen, N-H. (1960). Danish Meat Res. Inst., Roskilde, publication No. 28

Kitchell, A.G. (1963-64). Ann. Rep. Low Temp. Res. Sta., Cambridge, H.M.S.O. London. p.26

Kitchell, A.G. & Ingram, M. (1963). Fd. Proc. & Pack. 32, 3.

de Man, Rogosa & Sharpe (1960). J. appl. Bact. 23, 130.

Rogosa, M. & Sharpe M.E. (1959). J. appl. Bact. 22, 329.

Sharpe, M.E. (1961). Ann. Inst. Pasteur (Lille) 12, 133.

Tonge, R.J., Baird-Parker, A.C. & Cavett, J.J. (1964). J. appl. Bact. 27, 252

* Hansen, N-H. (1962). J. appl. Bact. 25, 46.