Bacteriocinogenic activity of ham microflora

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In recent years there has been an increase in the sale of vacuum packed "ready to eat" meats and in Northern Ireland the most important in this category is sliced cooked ham. The predominant bacterial group in vacuum packed cooked meats has been shown to be lactobacilli (Allen & Foster, 1960; Alm Erichsen & Molin, 1961; Reuter, 1969; Kempton & Bobier, 1970; Mol, Heitbrink, Moller & van Tinteren, 1971). In addition to lactobacilli, Shank & Lundquist (1963) found streptococci in the flora. Miller (1960) isolated non-heat resistant microbacteria and Brooks & Henrickson (1956) micrococci and/or coryneforms as the most important organisms.

In the present paper an investigation was initiated to identify the spoilage microflora of vacuum packed sliced cooked ham as affected by the storage conditions.

MATERIALS AND METHODS

Packs of ham. In each experiment, a number of packs of sliced cooked ham were taken immediately after packing on the production line. These were held at 5° for 24 hrs and then divided into 3 groups and stored at 22° for 4 days, 10° for 7 days and 5° for 11 days. Four such experiments were completed and all the material was obtained from one factory.

Bacteriological and chemical analyses. At the end of each storage treatment duplicate packs were taken for analysis. With aseptic precautions the total contents of each pack were minced and thoroughly mixed by hand in a polythene bag. For bacteriological analysis, 5 g of mince were shaken by hand with 45 ml sterile diluent. The techniques for enumeration and identification of the flora are given earlier (Gardner, 1968), except that all media and the diluent were supplemented to contain a final level of 4% (w/v) NaCl. Identification characteristics of Vibrio spp. and the methods used for determination of pH, NaCl and $NaNO_2$ are given elsewhere (Gardner, 1971).

Detection of bacteriocins. One loop of a broth culture of the producer strain (Vibrio) was streaked in a line across a plate of the total count medium. After incubation for 24 hr at 22° the cells were removed and the plate exposed to chloroform vapour for 30 min. The plates were then held at 44° for 2-3 hr to remove the chloroform and cross streaked with APT broth (Evans & Niven, 1951) cultures of lactic acid bacteria (indicator strains) grown for 24 hr at 30°. The plates were incubated for 5 days at 30°. Only zones greater than 5 mm denoting inhibition of growth of the indicator strain were regarded as "sensitive". Four Vibrio strains were tested against a total of 34 strains of lactic acid bacteria including both meat and dairy cultures.

RESULTS

The results of both chemical and microbiological analyses of the stored ham are summarized in Table 1. There were no significant differences between treatments in relation to pH, NaCl and $NaNO_2$. Similarly the mean total viable counts were of the same order (60.9-141.1 x 10^6/g). The main types of bacteria isolated from the spoilage microflora were Microbacterium thermosphactum, Vibrio and Leuconostoc. Storage treatment did not markedly affect the qualitative aspect of the microflora, except perhaps that leuconostocs were much less important at 5°.

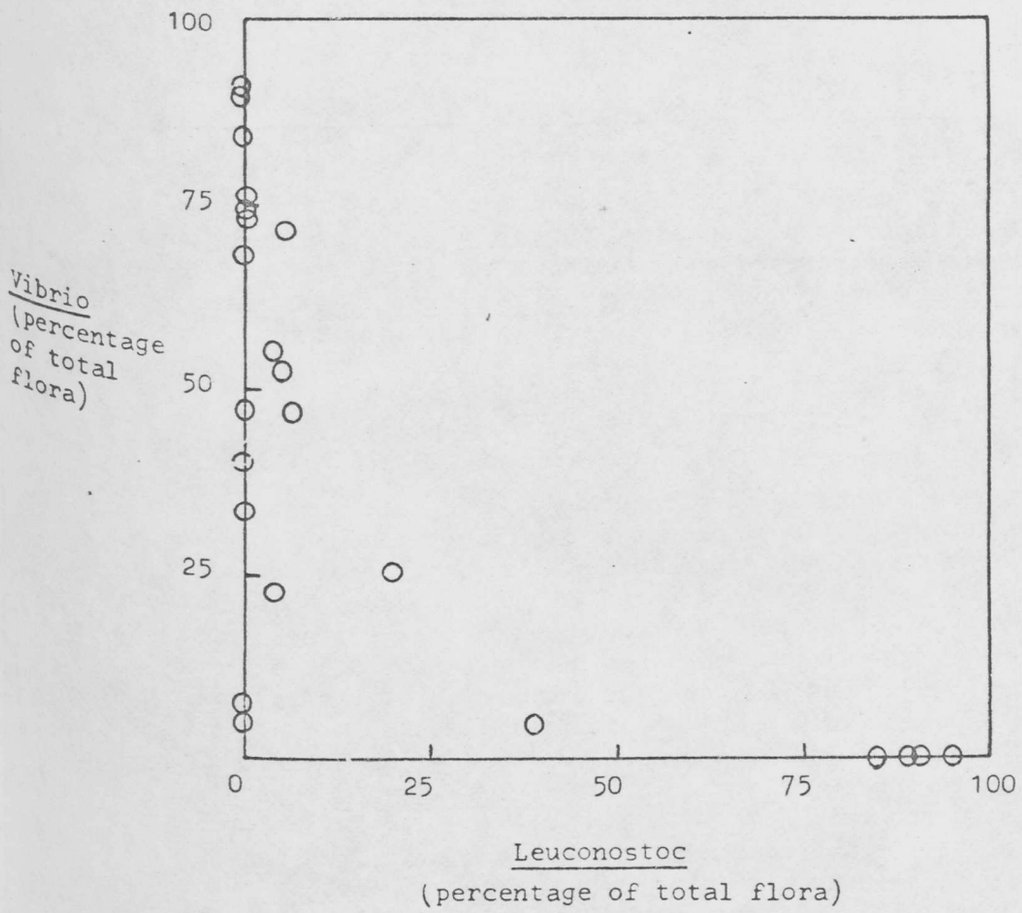
TABLE 1. Chemical and microbiological observations* of stored vacuum packed cooked ham.

Storage treatment	pH	NaCl** (%)	$NaNO_2$** ug/g	Total viable count x 10^6/g	Total no. of isolates examined	Incidence (%) in the flora of								
						Microbacterium thermosphactum	Vibrio	Leuconostoc	Lactic streptococci	Faecal streptococci	Atypical streptobacteria	Unclassified lactobacilli	Yeasts	Acinetobacter
22° for 4 days	5.92	3.93	10	141.1	157	40.8	29.9	24.2	1.3	0.6	1.9	1.3	0	0
10° for 7 days	6.14	4.06	14	60.9	136	17.6	44.8	33.1	2.2	0	0	2.2	0	0
5° for 11 days	6.12	4.24	17	89.1	155	38.1	52.9	3.2	0	0	0	0	1.3	4.5

* Each result is the mean of 8 packs. ** Levels are expressed in the aqueous phase of the ham.

However, analyses of the microflora for the individual packs showed that in general when Vibrio was present in the flora, the proportion of Leuconostoc was very low or absent. The reverse was also found. When the proportion of Vibrio was very low or absent, leuconostocs were predominant. The relationship between these two organisms in the flora of all packs examined is given in Fig.1.

Fig.1. Relationship between the proportions of Vibrio and Leuconostoc in the flora of vacuum packed cooked ham stored at 22°, 10° and 5°.



In only 7 packs out of 23 were both organisms isolated together and in 6 of these only small proportions (< 7%) of Vibrio were found in a flora dominated by Leuconostoc and vice versa.

Examination of Vibrio spp. for the production of a bacteriocin(s) effective against lactic acid bacteria was carried out and the results are summarized in Table 2.

TABLE 2. Sensitivity of some lactic acid bacteria to bacteriocins produced by Vibrio spp. isolated from vacuum packed cooked ham.

Indicator strain	Number of strains	
	Sensitive	Insensitive
Leuconostoc	2	1
Lactobacillus viridescens	8	0
Lactobacillus casei-plantarum	1	2
Lactobacillus brevis	0	1
Enterococci	0	19

From a collection of isolates from ham, bacon and dairy sources the existence of such a bacteriocinogenic property in four Vibrio isolated from the vacuum packed ham was demonstrated and sensitive strains were found of Leuconostoc, Lactobacillus viridescens and Lactobacillus casei-plantarum types. 19 isolates of faecal streptococci were insensitive to the vibriocin.

DISCUSSION

Although it has been generally reported that lactic acid bacteria are the most important bacteria which grow and cause spoilage of vacuum packed cooked cured meats, there have also been a number of exceptions, e.g. Alm et al. (1961) reported that Achromobacter predominated in vacuum packed Bologna type German sausage, and Kempton & Bobier (1970) found that after 15 weeks storage at 5°, 12% of the flora of vacuum packed cooked ham were catalase positive. For packs of pickle and pimento and Bologna the proportion of catalase positive bacteria was 17 and 8% respectively. Mol et al. (1971) found in their storage trials a number of atypical spoilage flora, i.e. not predominantly Lactobacillus. In these cases Enterobacteriaceae, Microbacterium thermosphactum, faecal streptococci, lactic streptococci and Aerococcus were found. In the present work M. thermosphactum, Vibrio and leuconostocs were the most important organisms, and in some earlier unpublished work we have found large proportions of Enterobacteriaceae in the flora of vacuum packed cooked ham. These Vibrio spp. are similar to those isolated from Wiltshire cured bacon (Gardner & Patton, 1969; Gardner, 1971).

There may be a number of reasons for the discrepancies in the observations of the spoilage microflora of vacuum packed cooked cured meats. Firstly, the chemical and physical characteristics of the product, i.e. NaCl, $NaNO_3$, $NaNO_2$, pH, degree of vacuum etc. Different conditions may result in different growth

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rates, thus selectively encouraging some bacterial species in the product. Secondly, the observations may be reflected in the bacteriological techniques employed, e.g. some workers used both total count and selective media and based their findings on comparison of these. Others, including the present work, isolated and identified the spoilage flora from total count plates. Even the media used for this differed between workers and so could contribute to variation in results. Unfortunately there is no universal acceptance of a total count medium. Thirdly, we have observed that one species in the flora (Vibrio) can inhibit the growth of other organisms, particularly leuconostocs, Lactobacillus viridescens and some Lactobacillus casei-plantarum species on total count plates. Thus although the organisms may be present in the flora of the ham, because of the bacteriocinogenic properties of the Vibrio they are not isolated. It is known that some pathogenic Vibrio spp. can produce a vibriocin which prevents the growth of other Vibrio spp. (Jayawardene & Farkas-Himsely, 1970) and Enterobacteriaceae (Datta & Prescott, 1969). An analogous case was noted by Kafel & Ayres (1969), who found that enterococci can act antagonistically on species of Clostridium, Bacillus and Lactobacillus in canned hams.

Although we have demonstrated antagonistic activity between Vibrio and Leuconostoc and Lactobacillus on plates, it is not yet known whether the same antagonism exists within the vacuum packed cooked ham. The nature of this property and an investigation into a method of estimating "total" viable counts is at present in progress.

REFERENCES

ALLEN, J.R. & FOSTER, E.M. (1960). Spoilage of vacuum packed sliced processed meats during refrigerated spoilage. Fd. Res. 25, 19.

ALM, F., ERICHSEN, I. & MOLIN, N. (1961). The effect of vacuum packaging on some sliced processed meat products as judged by organoleptic and bacteriological analysis. Fd. Technol., Champaign 15, 199.

BROOKS, R.F. & HENRICKSON, R.L. (1956). Microflora of prepackaged luncheon meats. Bull. Mo. Agric. Exp. Sta. No. 611.

DATTA, A. & PRESCOTT, L.M. (1969). Effect of vibriocins on members of the Enterobacteriaceae. J. Bact. 98, 849.

EVANS, J.B. & NIVEN, C.F. (Jr.) (1951). Nutrition of heterofermentative lactobacilli that cause greening of cured meat products. J. Bact. 62, 599.

GARDNER, G.A. (1968). Effects of pasteurization or added sulphite on the microbiology of stored vacuum packed baconburgers. J. appl. Bact. 31, 462.

GARDNER, G.A. (1971). Microbiological and chemical changes in Wiltshire bacon lean during aerobic storage. J. appl. Bact. 34, in press.

GARDNER, G.A. & PATTON, J. (1969). Variations in the composition of the flora on aWiltshire cured bacon side. J. Fd. Technol. 4, 125.

JAYAWARDENE, A. & FARKAS-HIMSLEY, H. (1970). Mode of action of vibriocin. J. Bact. 102, 382.

KAFEL, S. & AYRES, J.C. (1969). The antagonism of enterococci on other bacteria in canned hams. J. appl. Bact. 32, 217.

KEMPTON, A.G. & BOBIER, S.R. (1970). Bacterial growth in refrigerated vacuum packed luncheon meats. Can. J. Microbiol. 16, 287.

MILLER, W.A. (1960). Microbiology of self-service packaged slices of cooked ham. J. Milk Tech. 23, 311.

MOL, J.H.H., HIETBRINK, J.E.A., MOLLEN, H.W.M. & van TINTEREN, J. (1971). Observations on the microflora of vacuum packed sliced cooked meat products. J. appl. Bact. 34, in press.

REUTER, G. (1969). Untersuchungen zur Mikroflora von vorverpackten, aufgeschnittenen Brüh- und Kochwürsten. Proc. 15th European Meeting of Meat Research Workers, Helsinki.

SHANK, J.L. & LUNDQUIST, B.R. (1963). The effect of packaging conditions on the bacteriology, colour and flavour of table-ready meats. Fd. Technol. Champaign 17, 1163.