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THE OCCURRENCE OF VIBRIO IN THE MICROFIONA OF

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THE OCCURRENCE OF <u>VIBRIO</u> IN THE MICROFLORA OF WILTSHIRE CURED BACON SIDES

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SUMMARY. This study was carried out to assess the occurrence of salt requiring Vibrio in the microflora of Wiltshire cured bacon sides. A total of 1,066 sides (1-6 days after tanking) from 15 bacon factories were examined for both total surface counts and a Vibrio count, using the selective medium of Gardner (1973a).

Vibrios were found in 399 (37.4%) sides and data is presented on the relationship between the total and the <u>Vibrio</u> count. Of the 667 sides where <u>Vibrio</u> were not found (i.e. $< 10^3/\text{cm}^2$), 93.2% had total counts of $< 50,000/\text{cm}^2$. Thus the higher the level of microorganisms on the side, the more likely it is that <u>Vibrio</u> will be present.

Both total and <u>Vibrio</u> counts are now carried out as part of the routine bacteriological quality control of bacon production in N. Ireland. <u>Vibrio</u> are rarely isolated from pork or equipmentused prior to curing, but are usually found in cover brines and bacon and associated equipment. They can grow rapidly on bacon sides during maturation, particularly in high temperature (>7°C) and high humidity (>90%) conditions. Thus in interpreting the results of tests on bacon sides, high total counts with high <u>Vibrio</u> counts are usually associated with post-curing production defects, whilst high total counts and low <u>Vibrio</u> counts are associated with pre-curing production defects.

ZUSAMMENFASSUNG. Diese Arbeit hatte zum Ziel, das Vorkommen von kochsalzbedürfenden <u>Vibrio</u> in der Keimflora von Wiltshire gepökelten Baconhälften zu beurteilen. Unter Verwendung des Selektivmediums von Gardner (1973a) wurden die Oberflächengesamtkeimzahl sowie die <u>Vibrio-Zahl</u> von insgesamt 1066 von 15 Betrieben herkommenden Schweinehälften (1-6 Tage nach Entfernung vom Pökelbehälter) ermittelt.

Vibrio wurden bei 399 (37.4%) Hälften nachgewiesen, und es wird besprochen das Verhältnis zwischen der Gesamtzahl und der Vibrio-Zahl.

Die Gesamtzahlen von 93.2% der 667 Hälften, bei welchen keine <u>Vibrio</u> (d.h. < 10³/cm²) erfasst wurden, betrugen < 50 000/cm². Je höher also der Keimgehalt der Hälften, desto grösser ist die Wahrscheinlichkeit, dass <u>Vibrio</u> auch vorkommen werden.

Die Gesamtzahl und die Vibrio-Zahl werden jetzt im Rahmen der üblichen bakteriologischen Qualitätskontrolle der Baconherstellung in Nordirland ermittelt. Vibrio werden nur selten vom Schweinefleisch oder von der vor Pökelung gebrauchten Ausrüstung isoliert; jedoch finden sie sich meistens in Gusslaken, Bacon und der dazu gebrauchten Ausrüstung. Sie können sich auf Baconhälften während der Reifungszeit, besonders bei hoher Temperatur (>7°C) und hoher Luftfeuchtigkeit (>90%) schnell vermehren. Eine Darstellung der Ergebnisse zeigt also, dass hohe Gesamtzahlen mit hohen Vibrio-Zahlen gewöhnlich auf nach der Pökelung eintretenden Herstellungsfehler zurückzuführen sind, während hohe Gesamtzahlen mit geringen Vibrio-Zahlen mit Produktionsfehler vor der Pökelung verbunden sind.

RESUME. Ce travail a été réalisé pour estimer la présence des <u>Vibrio</u> qui veulent du sel dans la microflore des demies de bacon Wiltshire. Un total de 1066 demies (1-6 jours après l'enlèvement de la cuve de salaison) provenant de 15 fabriques ont été examinées pour constater les nombres totaux de bactéries sur la surface ainsi que le nombre de <u>Vibrio</u>, le milieu sélectif de Gardner (1973a) étant employé.

Des vibrios ont été trouvés dans 399 (37.4%) des demies, et des données sont fournies sur la relation entre la teneur totale en germes et le nombre de <u>Vibrio</u>. 93.2% des 667 demies, où <u>Vibrio</u> n'ont pas été isolés (c.-à-d. <103/cm²), avaient des flores totales de <50.000/cm². La présence de <u>Vibrio</u> est ainsi d'autant plus probable que la teneur totale en germes est plus élevée.

Le dénombrement de la flore totale et la numération de <u>Vibrio</u> sont maintenant effectués en Irlande du Nord comme partie du contrôle de qualité bactériologique normal dans la fabrication de bacon. <u>Vibrio</u> ne sont isolés que rarement du porc ou de l'équipement employé avant la salaison, mais ils sont généralement trouvés dans les saumures d'immersion, le bacon et l'équipement y employé. Ils peuvent se multiplier rapidement sur les demies pendant la maturation, surtout dans des conditions de haute température

(>7°C) et de haute humidité relative (>90%). Les résultats des études indiquent ainsi que des nombres totaux élevés de bactéries avec de grands nombres de <u>Vibrio</u> sont attribuables le plus souvent aux défauts de fabrication après la salaison, tandis que de grands nombres totaux avec peu de <u>Vibrio</u> sont occasionnés par des défauts avant la salaison.

Salt requiring <u>Vibrio</u> have been isolated from bacons (Smith, 1938; Ingram, 1952; Gibbons & Rose, 1950; Gardner & Patton, 1969; Gardner, 1971), but their significance as a species capable of producing spoilage characteristics has been less widely recognised (Smith, 1938; Gardner, 1971).

In traditional Wiltshire style curing of bacon as sides, Micrococcaceae have for many years been regarded as the most important bacterial family associated with bacon spoilage. However, we have recently noted that the proportion of Gram negative bacteria in the microflora of bacons, which have spoiled in aerobic conditions, has been significantly higher. In our experience <u>Vibrio</u> is the most frequent type isolated. The reason(s) for this change in the microbiology of bacon spoilage are not fully understood but it may be because of the milder (less NaCl) cures now being used leading to a product with lower salt levels. We do find that in high salt bacons (>8% NaCl in fresh weight) micrococci are the predominant species in spoils

The objective of the work presented in this paper was to assess the occurrence of <u>Vibrio</u> in the microflora of bacon sides produced under normal factory conditions and also examine pork sides and curing brines, to establish their ecological importance in bacon production.

MATERIALS AND METHODS

Bacon sides. Bacon sides were examined at 15 factories in N. Ireland.

3 factories were tested twice, 8 were tested 3 times, 2 were tested 4 times

1 was tested 7 times, and 1 was tested 9 times, thus giving a total of 54

factory visits. In all cases bacon sides were sampled during maturation

(i.e. after removal from the brine, during the draining period). In some
factories sides are matured on pallets and in others the maturation takes
place in stacks. No distinction has been made between these procedures.

Sampling. At each factory visit 20 bacon sides were examined. Each side was sampled using cotton swabs. Five 10 cm² swabs, 3 from the rind and 2 from the inside surface (pleura), were bulked to give a 50 cm² composite sample for the side (Gardner & Kitchell, 1973).

Enumeration of microorganisms. Organisms were removed from the swabs by mechanical shaking (3 min at 1000 oscillations/min) in 50 ml diluent (Gardner & Kitchell, 1973) and plated out by the loop-tile technique

(Kitchell, Ingram & Hudson, 1973) on to a total count medium with 4% NaCl (Gardner, 1968) and CVKA, a selective medium for <u>Vibrio</u> (Gardner, 1973<u>a</u>). Colonies were enumerated after plates of both media had been incubated at 22°C for 3 days. The lower limit of enumeration was $10^3/\text{cm}^2$ in respect of both total and <u>Vibrio</u> counts.

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Brines. Cover brine samples from 16 factories were taken for 10 consecutive weeks and plated out by the "drop and spread" technique on CVKA (Gardner, 1973a). To assess survival, 8 of these samples were stored at 4° in the laboratory and tested at intervals during a period of 14 days.

RESULTS AND DISCUSSION

Relationship between total count and count of Vibrio on bacon sides

In all, 1066 bacon sides were examined. Of these 399 (37.4%) were found to have <u>Vibrio</u> in the flora (i.e. >10³/cm²). The relationship between the total and <u>Vibrio</u> counts is given in Fig.1. In a few sides the <u>Vibrio</u> count exceeded the total, but the majority examined had over 10% <u>Vibrio</u> in the flora and only 15 (3.5%) sides had <1% <u>Vibrio</u> in the flora.

An analysis of the 667 sides, where <u>Vibrio</u> were not found (i.e. $<10^3/\mathrm{cm}^2$) is given in Table 1.

Table 1. Distribution of total viable counts on 667 bacon sides, where Vibrio were not isolated.

Total viable counts (x103/cm	m ²) <1	1-50	51-100	101-200	201–400	401-800	801-1600
No. of sides	52	569	23	11	9	2	1
% of total	7.9	85.3	3.4	1.6	1.3	0.3	0.1

For convenience the counts have been classified into 7 groups. It can be seen that 93.2% of the sides had less than 50,000 organisms/cm² and only 3.3% of sides had a count greater than $100,000/\text{cm}^2$.

The general conclusion which may be drawn is that <u>Vibrio</u> are more frequently found on bacon sides which have relatively high counts and, where found, can constitute relatively large proportions of the flora.

The occurrence of Vibrio on pork carcases and in bacon curing brines

A number of chilled pork carcases were examined before and after butchery along with bone dusts and the surfaces of the butchery belt (Table 2). For the pork carcases and sides only mean results are given. Each of the total of 150 sides from 4 different factories was sampled and tested. Vibrio were not found in any of the samples.

Table 2. Total microbial load and <u>Vibrio</u> of pork carcases and butchery belts.

		Total viable count x10	3/cm ²
Chilled car	cases		
	Mean of 20 sides	1.6	<0.1
	do.	139.0	<0.1
	do.	0.58	< 0.01
	do.	0.99	<0.01
	do.	0.52	<0.01
	Mean of 10 sides	2.0	<0.1
	Mean of 5 sides	5.0	<1.0
Pork sides and brine i	after butchery		
	Mean of 20 sides	2.7	< 0.1
	Mean of 10 sides	17.8	<0.1
	Mean of 5 sides	4.3	<0.1
Bone dust from pork sides*		19.0	<0.1
		180.0	<1.0
Pork butche	ry belts	2.0	<1.0
		2.0	<1.0
		4.0	<1.0
		21.0	<1.0

^{*} Counts/g.

The <u>Vibrio</u> counts in Wiltshire curing cover or immersion brines are given in Table 3. These are the results from 15 different factories over a continuous 10 week period. There are large differences in the levels of contamination between factories, e.g. Vibrios are rarely found in No.8, and No.7 is relatively heavily contaminated. A sudden increase in levels can be seen in Factory 5 between weeks 5 and 6. During this period a number of bacon sides with bacterial slime on the surface (i.e. highly

Table 3. Vibrio counts (x103/ml) in Wiltshire bacon curing brines.

Week No.	1	2	3	4	5	6	7	8	9	10
Factory No.										
1	2.2	1.0		0.5	0.6		0.8	∢0.1 0.8		NT
2					<0.1 1.5				<0.1 <0.1	
4	1.2	9.4	43.0 19.0		15.0		11.1		2.3	7.4
5	<0.1 <0.1		0.2		0.4					
6	0.3	9.6 3.2	16.8 15.2		11.7	11.1				14.2 18.8
7	18.6 26.9	10.0		726.0 93.0	105.0	206.0			13.0	
8			<0.1 0.2		<0.1 <0.1					<0.1
9	0.9	4.3	7.8 7.1	2.4	NT	2.3	NT		<0.1	NT
10	5.1	4.8	9.3		7.3 9.1	0.7			NT	NT
11	<0.1	0.1	0.3	1.3	0.5	0.8	0.5	1.0	< 0.1	1.0
12					1.4					NT
13	0.1	11.1	31.0	24.3	51.0	28.0	22.0	27.0	15.0	8.3
14	0.1	0.6	0.9	0.9	0.4	∠0.1 0.1	0.1	<0.1 <0.1	0.2	<0.1 <0.1
15	0.3				0.2					
16	0.7	10.9	8.5 16.5	4.1	NT		1.7 3.8		<0.1	1.6

NT: Not tested. Single or upper figure in each pair refers to the reconstituted cover brine, and the lower to the spent brine.

contaminated with <u>Vibrio</u>) were retarked and washed with cover brine, Although most of this brine was subsequently discarded, a proportion was left in the bottom of the tanks and in the piping system. These residues were later added to the bulk of the factory brine, resulting in this large increase in the Vibrio count.

Survival of Vibrio in cover brine. These species of <u>Vibrio</u> will not grow in the brine. A number of naturally contaminated brines were examined and the survival was found to be linear with time and thus a D value could be calculated. D = number of days for 90% reduction in numbers at 4°C. The results from 8 brines are given in Table 4. On average it can be seen that the numbers of bacon spoiling <u>Vibrio</u> spp. decrease every week by 90%. This can be seen in the practical situation (Table 3, Factory 5, weeks 6-10).

Table 4. Survival of naturally occurring <u>Vibrio</u> in Wiltshire curing brines at 4°C.

Brine No.	D* value (days)	Curi NaCl (%)	ing salts in NaNO3 (%)	brine NaNO2 (ppm)	рН
499	5	27.8	0.24	275	6.2
503	7	27.3	0.11	485	6.3
523	8	24.8	0.11	500	6.3
526	7	25.2	0.11	1080	6.2
529	6	23.6	0.05	1160	6.2
532	7	27.0	0.10	915	6.2
535	8	29.3	0.23	525	6.2
541	6	25.4	0.07	1220	6.1

^{*}D value: number of days at 4°C for a 90% reduction in the numbers of Vibrio.

Thus the level of <u>Vibrio</u> in any brine sample is the balance between the numbers which die and those which the brine acquires. Counts of <u>Vibrio</u> are therefore important in the routine microbiological examination of bacon curing brines (Gardner, 1973b).

It would appear that in Wiltshire bacon production the pork carcases are contaminated by <u>Vibrio</u> during the tanking process (i.e. from the cover brine) and subsequently these can grow on the bacon side during maturation and in most instances can form a significant proportion of the

spoilage microflora.

Use of the Vibrio count in routine bacteriological quality control of Wiltshire style bacon production

Since the beginning of 1972 this laboratory has used as part of the centralised quality control of bacon from 19 factories the distribution of total counts of 20 bacon sides (Gardner, 1973c). This scheme has proved valuable in assessing the bacteriological quality of the final product. High total counts of mature bacon can arise from microbial contamination and growth at all stages of production.

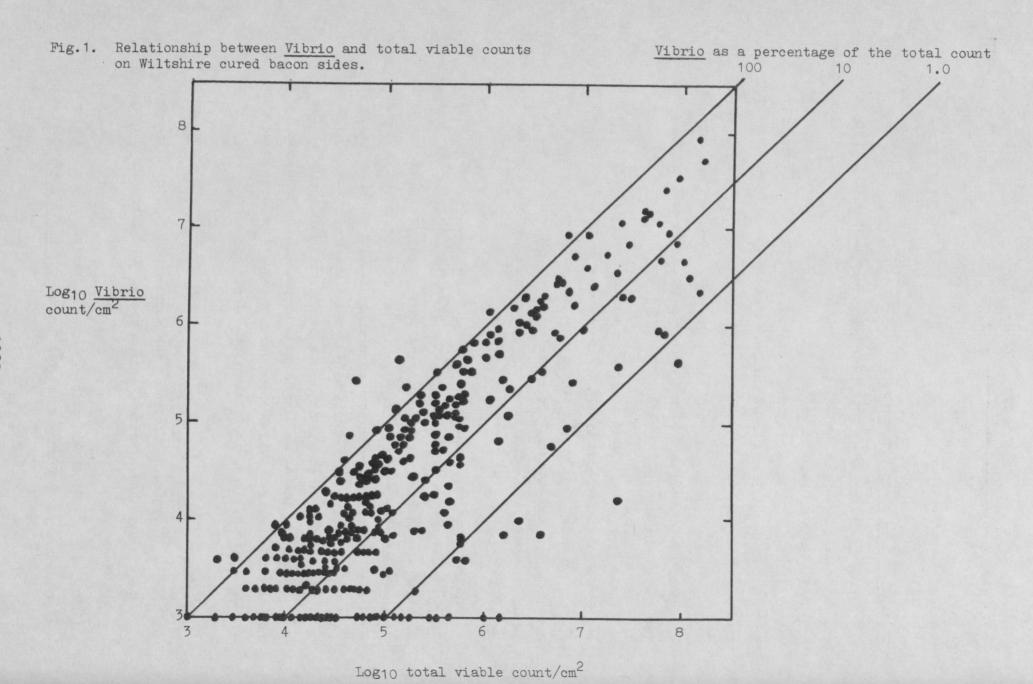
<u>Vibrio</u> are rarely isolated from pork and associated equipment, but can be found in cover brines and on cured sides, as well as equipment used for their handling.

These <u>Vibrio</u> are unable to grow in bacon curing brines, but can survive in them for some time. Their presence in cover brines, we have shown, can arise from direct or indirect contamination of the brine by cured meats. Subsequently the cover brine can contaminate all pork sides being immersed, thus completing the recontamination cycle of bacon to brine to bacon.

These <u>Vibrio</u> spp. require salt for growth, and the inclusion of a <u>Vibrio</u> count with a total count in the microbiological analysis of bacon sides can be useful in distinguishing post-curing growth on the product. As a general rule we find that high total counts on bacon with high <u>Vibrio</u> counts are associated with poor maturation conditions (i.e. temperature >7°C and/or relative humidity >90%). In these conditions <u>Vibrio</u> can grow rapidly on bacon sides and achieve a predominant proportion of the microflora. On the other hand high total counts on bacon with low <u>Vibrio</u> counts can usually result from production defects before curing, e.g. poor slaughterline hygiene, inefficient chilling.

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