IDENTIFICATION OF ATYPICAL STREPTOBACTERIA ISOLATED FROM AUSTRALIAN VACUUM-PACKAGED BEEF

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SUMMARY

Using DNA/DNA homology, the atypical streptobacteria isolated from Australian vacuum-packaged beef by Hitchener et al. (1982) have been identified. Organisms of group 1 are strains of *L.curvatus* and those of groups 2 and 3, including the hydrogen sulphide-producing strain L13, are strains of *L.sake*. The occurrence of these organisms on meat is discussed.

INTRODUCTION

Lactic acid bacteria are the major component of the microbial flora which develops on vacuum-packaged fresh meats stored at 0°C (Kitchell and Shaw 1975; Gill and Newton 1978; Dainty et al. 1979; Egan 1983). Until recently it has proved difficult to precisely identify strains of lactic acid bacteria isolated from meats, presumably because the methods of identification are based upon the properties of organisms from quite different environments (Kitchell and Shaw 1975; Reuter 1975).

The lactic acid bacteria of meats, especially processed meats, were studied extensively by Reuter, and these studies are summarised in papers published in 1975 and 1981. He found that atypical streptobacteria were commonly isolated.

In 1982 we studied the properties of 177 psychrotrophic strains of lactic acid bacteria isolated from vacuum-packaged beef produced in Australia. Atypical streptobacteria formed a significant component of the flora of this product. These strains, which could not be assigned to a particular species, appeared to fall into three distinct groups (Hitchener et al. 1982).

The work of Kandler and Kagermeier using DNA hybridisation has allowed more precise classification of the atypical strains of lactobacilli. They used this technique to study strains isolated by Reuter and these appeared to be strains of *Lactobacillus sake* and *Lactobacillus curvatus* (Kagemeier 1980; Reuter 1981). Recently, several studies have reported that *L.sake* and *L.curvatus* commonly occur on meats (see Discussion). As a result of these studies, we have now re-examined the atypical streptobacteria groups 1-3 isolated in our previous study. The results reported here show them to be strains of *L.sake* and *L.curvatus*.

EXPERIMENTAL METHODS

The isolation and properties of the organisms studied have been described by Hitchener et al. (1982). Determination of the G+C% content of DNA and DNA/DNA homology have been described in previous publications (Lauer et al. 1980; Kandler and Kunath 1983). The type cultures of *L.sake* and *L.curvatus* were included as reference strains in each experiment.

RESULTS

Table 1 lists some phenotypic properties of representative strains of the three groups of atypical streptobacteria. The properties of the type cultures of *L.sake* and *L.curvatus* are included for comparison.

DISCUSSION

On the basis of the properties of their DNA, the isolates of the atypical streptobacteria group 1 of Hitchener et al. (1982), are strains of *L.curvatus*. The organisms of groups 2 and 3 are strains of *L.sake*. Table 2 shows the

TABLE 1: Relationship between strains from meat and Lactobacillus curvatus and Lactobacillus sake

Strain	% Homology with		$R_{iso\ I}$*	Mole % G+C	Fermentation of														
	L.curvatus	L.sake			ribose	arabinose	xylose	sorbitol	mannitol	raffinose	melibiose	rhamnose	sucrose	maltose	trehalose	lactose	cellobiose	salicin	inulin
L.curvatus																			
DSM 20010	100	41	1.60	43.9	+	-	-	-	-	-	-	-	-	+	-	+	-	-	-
group 1:																			
P40	104	37	1.58	o	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-
P28	89	46	1.61	42.2	+	-	-	-	-	-	-	-	-	-	+	-	+	+	-
L.sake																			
DDM 20017	51	100	1.60	42.2	+	+	-	-	-	-	+	+	+	+	+	+	+	+	-
group 2:																			
L13	53	79	1.57	43.7	+	-	-	-	-	-	+	-	+	-	+	-	+	-	-
L75	o	o	1.59	o	+	-	-	-	-	-	+	-	+	-	+	-	+	-	-
group 3:																			
L70	48	88	1.58	42.7	+	+	-	-	-	+	+	-	+	-	+	+	-	+	-
L24	45	99	1.55	41.0	+	+	-	-	-	-	+	-	+	-	-	-	+	+	-

* relative electrophoretic mobility of the Mn^{2+} and FDP-activated L-LDH
o = not determined

TABLE 2: DNA/DNA homology among strains from meat (groups 1-3) and type strains of the subgenus Streptobacterium

^{3}H-DNA$_s$ / DNA$_f$	L.sake 20017	L.spec. L24	L.curvatus 20010	L.spec. P28	L.xylosus 20175	L.casei 334	L.farciminis 20184	L.alimentarius 20249
L.sake 20017*	100	99	51	46	7	7	10	12
L.bavaricus 20269	82	82	31	30	o	8	o	11
L.spec. L70	88	100	48	48	6	7	10	11
L.spec. L13	79	o	53	o	7	10	13	12
L.curvatus 20010	41	45	100	89	6	6	8	8
L.spec. P40	37	46	104	100	7	11	10	11
L.xylosus 20175	6	9	14	17	100	5	6	6
L.casei 20011	12	8	9	7	o	32	o	o
L.farciminis 20184	8	8	8	6	5	5	100	27
L.alimentarius 20249	15	14	13	17	o	o	o	100

* DSM number

DNA homology among the strains of group 1-3 and type strains of homofermentative lactobacilli.

Subsequent to the work of Kagemeier (1980) there have been several studies which have reported the isolation of strains of *L.sake* and *L.curvatus* from meats. A numerical taxonomic study of lactic acid bacteria from vacuum-packaged beef, pork, lamb and bacon by Shaw and Harding (1984) classified the streptobacteria into two clearly defined clusters. The strains of one were identified as *Lactobacillus sake*.

Niemand and Holzapfel (1984) found that streptobacteria dominated the lactic acid bacteria isolated from radurised minced beef, and *L.sake* and *L.curvatus* were the most common strains. A later study confirmed *L.sake* as the predominant strain on radurised minced beef following a radiation dose of 5 kGy (Hastings and Holzapfel 1987 a, b). Morishita and Shiromizu (1986) reported that *L.sake* and *L.curvatus* were the predominant types of streptobacteria isolated from fresh meats and meat products in Japan.

Based on information now available it seems likely that many of the streptobacteria isolated from meats in earlier studies were strains of *L.sake* or *L.curvatus* (eg. studies of Thornley and Sharpe, 1959; Cavett 1963; Mol et al. 1971). In addition some of the isolates studied by Keddie (1959) may be *L.sake* strains. These were isolated from silage and this suggests a likely origin for the organisms which now appear to be so common on meats.

Strain L13, now shown to be *L.sake* (Table 1), is capable of producing hydrogen sulphide during growth on vacuum-packaged meat or from cysteine during growth in culture media (Shay and Egan 1981). The presence of large numbers of *L.sake* on meats has significant implications in spoilage.

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