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NOTES

Isolation of Histamine-Producing *Lactobacillus buchneri* from Swiss Cheese Implicated in a Food Poisoning Outbreak

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A histamine-producing strain of *Lactobacillus buchneri* was isolated from Swiss cheese that had been implicated in an outbreak of histamine poisoning. It produced up to 4,070 nmol of histamine per ml in MRS broth supplemented with 0.1% histidine. The identification of this isolate was based on its biochemical, bacteriological, and DNA characterizations.

Food poisoning incidents involving the consumption of foods containing abnormally high levels of histamine have been recognized for many years. Several outbreaks of histamine poisoning have occurred after the consumption of cheese containing high levels of histamine (4–6, 20). Swiss cheese has been implicated in most of the outbreaks (4, 6, 20).

In 1980, a small outbreak of histamine poisoning associated with the consumption of Swiss cheese occurred in New Hampshire (20). We report the isolation and identification of a unique histamine-producing strain of *Lactobacillus buchneri* from a sample of the Swiss cheese implicated in this outbreak.

A sample of Swiss cheese that had been incriminated in an outbreak of histamine poisoning (20) was obtained from T. J. Keefe, Department of Pathology, Brooke Army Medical Center, Fort Sam Houston, Tex. Bacteriological analysis of the Swiss cheese was carried out by the procedure of Taylor et al. (20). Initially each isolate was Gram stained and its morphology was determined. All of the isolates were identified to the species level with biochemical criteria (3, 17–19).

The ability of the isolates to generate histamine from histidine was assessed in MRS medium supplemented with 0.1% L-histidine hydrochloride and 3.7×10^{-2} M pyridoxal hydrochloride. The conditions for the histamine production experiments were as previously described (2), except 10 ml of MRS medium was used and the incubation temperature was 37°C. Histamine analysis was performed by a modification (8) of the method described by the Association of Official Analytical Chemists (2). Aerobic plate counts were also determined.

The identification of the prolific histamine-producing isolates was confirmed by a DNA hybridization study. The method of DNA extraction and purification was essentially the same as that described by Garvie (7). A competition membrane DNA hybridization method was used (11) to determine the similarity of these isolates to certain lactobacilli.

Thirty-seven isolates were obtained; the tentative identities of these isolates and the frequency of their occurrence are listed in Table 1. The isolates were identified on the basis of their biochemical characteristics only. *Lactobacillus casei* subsp. *casei*, *Streptococcus faecalis*, and *Streptococcus faecium* were the most frequently encountered isolates. Other isolates present in the cheese sample included *L. buchneri*, *Lactobacillus plantarum*, *Lactobacillus cellobiosus*, *Pediococcus cerevisiae*, *Bacillus subtilus*, *Propionibacterium jensenii*, *Propionibacterium freudenreichii* subsp. *globosum*, and *Propionibacterium acidi-propionici*. Representative colonies were chosen for their unique appearances on various types of medium. The goal was to obtain at least one isolate of each colony type. No attempt was made to exhaustively sample colonies of similar appearances from any particular plate. Therefore, these data cannot be used to determine the comparative numbers of the various types of bacteria in the cheese samples. The most commonly encountered isolates were of species able to grow on several different types of medium.

Of the 37 isolates obtained from the Swiss cheese sample, only isolates LBS-1 and LBS-3 were able to produce large amounts of histamine in the supplemented MRS medium. These isolates produced 4,070 and 3,730 nmol of histamine per ml of medium, respectively, within 24 h at 37°C. The other isolates obtained from the Swiss cheese sample were unable to produce more than 10 nmol of histamine per ml. All of the isolates grew well on the supplemented MRS medium.

The histamine-producing isolates LBS-1 and LBS-3 were gram-positive, facultatively anaerobic rods which were catalase negative, produced CO_2 in Gibson semisolid tomato juice medium, grew at 15°C but not 45°C, and produced NH_3 from arginine. These heterofermentative isolates fermented arabinose, melezitose, melibiose, and xylose, but not cellobiose or trehalose. According to the scheme of Sharpe et al. (18), these characteristics most closely fit an identification of *L. buchneri*. Other sugars fermented by these isolates include ribose, galactose, glucose, fructose, maltose, and sucrose. On the basis of these studies, we conclude that LBS-1 and LBS-3 are probably identical.

The identification of these two isolates as *L. buchneri* was confirmed by the DNA hybridization study. The two isolates were 90.6% homologous with *L. buchneri* ATCC 4005 and only 29.3% homologous with *Lactobacillus brevis* ATCC 4006 (Fig. 1).

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TABLE 1. Identification of isolates obtained from Swiss cheese sample[a]

Bacterial species	No. of times isolated (% of total)
Streptococcus faecalis	8 (21.6)
Streptococcus faecium	6 (16.2)
Lactobacillus casei subsp. *casei*	10 (27.0)
Lactobacillus buchneri	3 (8.1)
Lactobacillus plantarum	1 (2.7)
Lactobacillus cellobiosus	1 (2.7)
Pediococcus cerevisiae	2 (5.4)
Bacillus subtilus	2 (5.4)
Propionibacterium jensenii	1 (2.7)
Propionibacterium freudenreichii subsp. *globosum*	1 (2.7)
Propionibacterium acidi-propionici	2 (5.4)

[a] Identification was based on biochemical and bacteriological criteria.

Histamine production by the three *L. buchneri* isolates obtained from the Swiss cheese sample, seven additional *L. buchneri* isolates, seven *L. brevis* isolates, and *Lactobacillus* 30a is depicted in Table 2. Only two of the three *L. buchneri* isolates obtained from Swiss cheese (strains LBS-1 and LBS-3) and *Lactobacillus* 30a were able to produce large amounts of histamine in supplemented MRS medium. The third strain (RC-3) from Swiss cheese produced some histamine but far less than the other two isolates.

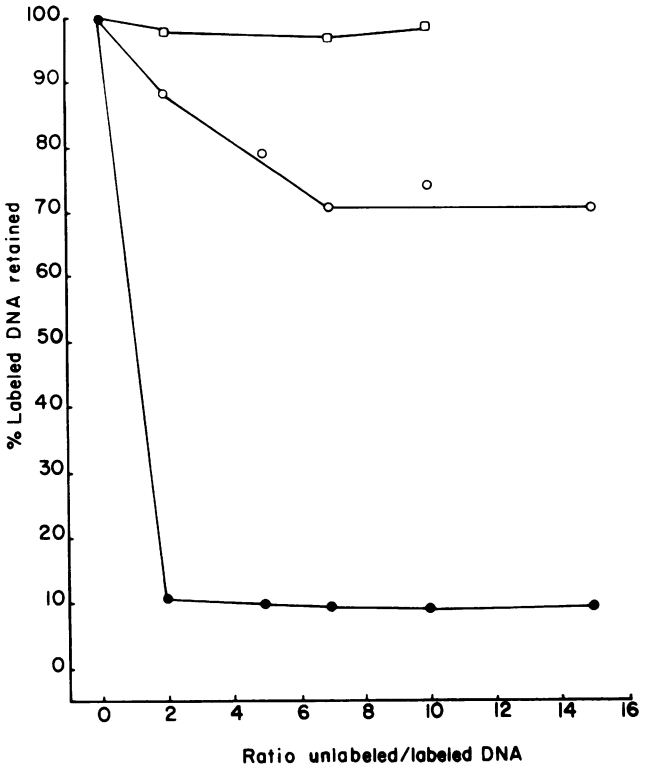

FIG. 1. Competition assay for DNA sequences. Filters containing single-stranded DNA (30 to 40 μg of DNA per cm^2) were incubated with a series of DNA preparations, each of which contained the same amount of [^{3}H]DNA but a different amount of unlabeled competitor. The competitor DNA was from *L. buchneri* LBS-1 (1.5 mg/ml). The [^{3}H]DNAs used were from *L. brevis* (○), *L. buchneri* (●), and *Lactobacillus delbrueckii* (□). The percentage of labeled DNA retained is relative to a control with no unlabeled DNA. The disintegrations per minute of the labeled DNAs ranged from 2,000-8,000/μg of DNA. The concentration of DNA was adjusted so that the disintegrations per minute of the filter quarter in the control vial was between 1,000 and 2,000.

A histamine-producing strain of *L. buchneri* was isolated from Swiss cheese implicated in an outbreak of histamine poisoning. Although this organism was isolated with a lower frequency than were certain other bacteria, it seems quite likely that this *L. buchneri* isolate was responsible for the production of histamine in the implicated Swiss cheese. The isolation procedure was not exhaustive, so no actual indications of the numbers of any particular type of bacteria can be derived from Table 1. In all likelihood, though, *L. casei* subsp. *casei*, *S. faecalis*, and *S. faecium* were present in higher numbers than the other bacteria isolated in the implicated cheese sample. The Swiss cheese implicated in the outbreak, however, was at least 18 months old at the time of consumption (20). The prolonged aging period could allow considerable shifting of the bacterial population. It is possible that *L. buchneri* was present in much higher numbers in this cheese at some earlier stage.

This strain of *L. buchneri* produces histamine very efficiently in supplemented MRS medium. The average 24-h production of histamine at 37°C in MRS broth supplemented with 0.1% histidine was 3,900 nmol/ml, a level approximately equivalent to 42 mg/100 g of medium. The hazard action level set by the U.S. Food and Drug Administration for histamine in tunafish is 50 mg/100 g, so this 24-h accumulation of histamine could be toxicologically important. Since Swiss cheese is intentionally held in a warm room, the potential for histamine production by *L. buchneri* would be

TABLE 2. Histamine production in supplemented MRS medium by selected lactobacilli

Species and strain	Net histamine production (nmol/ml) at:		Log aerobic plate count at:	
	6 h	24 h	0 h	24 h
L. buchneri				
LBS-1	330	4,070	6.9	8.7
LBS-3	568	3,730	6.8	8.5
RC-3	23	97	6.8	8.5
ATCC-1916	<1	2	7.0	9.0
ATCC-4005	2	<1	7.4	8.7
ATCC-1838	<1	<1	5.9	6.9
ATCC-12935	<1	<1	4.6	5.9
ATCC-12936	<1	<1	5.4	6.8
RA[a]	8	28	7.1	9.5
NCDO-110[b]	13	78	6.8	9.3
L brevis				
ATCC-367	<1	<1	7.1	8.6
ATCC-4006	<1	6	6.9	9.0
ATCC-8287	<1	<1	7.7	9.4
ATCC-11577	<1	<1	7.1	9.0
ATCC-13648	<1	<1	7.6	9.4
ATCC-14434	<1	<1	7.2	8.8
ATCC-14869	<1	7	6.3	8.2
Lactobacillus 30a[c]	976	4,700	7.1	8.7

[a] Obtained from G. Oliver, Centro de Referencia para Lactobacilos, Tucuman, Argentina.

[b] Obtained from the National Collection of Dairy Organisms, National Institute for Research in Dairying, Reading, England.

[c] Obtained from E. E. Snell, University of Texas, Austin.

substantial if it were to contaminate the cheese at an early stage of production.

The limitation of histamine formation in Swiss cheese might be the amount of free histidine available in milk. The Swiss cheese implicated in the outbreak of histamine poisoning contained 187 mg of histamine per 100 g (20). Fresh milk has very little free histidine, but milk protein can contain as much as 9.6 g of histidine per 100 ml (10). Aged Swiss cheese may have as much as 370 mg of histidine per 100 g (9), which undoubtedly arises via proteolysis.

Histamine production or histidine decarboxylase activity or both have been noted previously in lactobacilli (1, 13, 16). Surveys of *Lactobacillus* starter cultures have revealed that these strains do not produce histamine or possess histidine decarboxylase (15, 21). The only previous report of a histamine-producing *Lactobacillus* isolate from foods was a *L. brevis* isolate from delicatessen salads (12). Histamine production does not appear to be a common trait among *L. brevis* or *L. buchneri* (Table 2).

The histamine-producing strain tentatively identified as *L. buchneri* by biochemical characterization was confirmed to be *L. buchneri* by a DNA characterization study. Few studies of nucleic acid homology have been reported with lactobacilli. Although only one strain of each species was tested by DNA-RNA hybridization, *L. brevis* and *L. buchneri* appear to be dissimilar in base sequences, despite other similarities (14). Miller et al. (14) reported a 6.7% homology between *L. buchneri* BC1 and *L. brevis* XI.

Edwards and Sandine (6) attempted to isolate histamine-producing bacteria from Swiss cheese after an outbreak of histamine poisoning in which Swiss cheese was implicated (4). Numerous histamine-producing strains were obtained. Although none of their isolates appear to be capable of the same magnitude of histamine generation as the *L. buchneri* strain, it is still possible that other histamine-producing bacteria will ultimately be identified in Swiss cheese. The histamine-producing enteric organisms commonly associated with the spoilage of tunafish would not be expected to proliferate in cheese, but additional histamine-producing lactobacilli and other dairy-related bacteria might exist, especially since very few searches for such bacteria have been performed.

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