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ANTIBACTERIAL ACTIVITY OF LACTIC ACID BACTERIA*

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INTRODUCTION

The lactic acid bacteria are known for their ability to repress the growth of other microbes with which they are associated. Inhibition and stimulation by lactic acid bacteria have traditionally been of great importance in the dairy industry. Almost all lactic cultures commonly used in this industry (genera *Streptococcus*, *Pediococcus*, *Leuconostoc* and *Lactobacillus)* can inhibit the growth of most organisms causing food spoilage (COLLINS, 1961; MARTH *et al.*, 1962; REDDY *et al.*, 1971; MOSSEL, 1971; HURTS, 1973).

Lactic acid bacteria represent only a small fraction of the microorganisms which develop on uncured meat and bacon stored under aerobic conditions, and do not affect the shelf life of these products. It is for this reason they have received little attention in relation to meat stored in a traditional manner. The situation in complety different in vacuum packed fresh and cured meat. Lactic acid bacteria become the dominat microflora in these foods and the extension of shelf life obtained by vacuum packing is probably a result of inhibition of growth of spoilage organisms *(Brochothrix thermosphacta, Serratia liquefaciens, Pseudomonas* sp., etc.). This inhibition is due in part to the low oxigen and high CO_2 tensión inside the packs. It has been suggested that the spoilage organism are also inhibit by substances produced by the lactic acid bacteria (ROTH and CLARK, 1975; DAINTY *et al.*, 1979).

Lactic inhibition has been also observed in cooked mecanically deboned

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poultry meat (RACCACH *et al.*, 1978), in chicken liver and pig kidney (SMITHER, 1978) and in meat inoculated with milk starter cultures (REDDY *et al.*, 1970) and with meat starter cultures (RACCACH *et al.*, 1978).

The nature of the inhibitory effect of the lactic acid bacteria is not fully understood. The antibacterial activity has been ascribed to undissociated organic acids produced in the fermentation processes (PINHEIRO *et al.*, 1968; SORRELS *et al.*, 1970; DALY *et al.*, 1972; and SMITH *et al.*, 1975) and to a competition for nutrients (HAINES and HAMM, 1973).

Numerous antimicrobial substances have been isolated from lactic acid bacteria. Some are low molecular weight peptides (nisin, diplococcin, acidophilin) active versus Gram positive bacteria (BARIBO and FOSTER, 1951; OXFORD, 1944; VAKIL and SHAHANI, 1965), others are extracellular low molecular weight compounds primarily active against Gram negative organisms (BRANEN *et al.*, 1975).

The production of antibacterial macromolecules has recently been demostrated in many lactobacilli (DE KLERK *et al.*, 1967; WIRAHADIKUSUMAH 1971; UPRETI and HINSDILL, 1975) and in others lactic acid bacteria from fish silage, starter cultures, indonesian foods, etc. (LINDGREN and CLEVSTRON, 1978).

The study described in this paper was iniciated to examine the ability of lactic acid bacteria isolated from fresh and cured vacuun packed meat to inhibit a variety of food spoilage organisms and pathogens, and to try to understand the mechanism by which the lactic acid bacteria exert their inhibitory effects.

MATERIALS AND METHODS

Organisms

The lactic acid bacteria cultures used in this study were obtained from the culture collection of the Microbiology Section of the Meat Research Institute, Langford, grown in cooked meat medium for 48 h at 30°C and then stored at 0°C. All these lactics were isolated from meat and meat products (Table I).

The indicator bacteria included *Serratia liquefaciens* MR 168, a non fluorescens *pseudomonad Ju. 26*, *Pseudomonas fluorescens DC 34*, *Moraxella-like sp.* JER 63, *Moraxella-like sp.* JER 98, *Brochothrix thermosphacta* MR 178, and *Salmonella typhimurium* MR 65, wich were obatined from M. R. I. Microbiology Section culture collection and *Staphylococcus aureus* NCTC 8532 and *Escherichia coli* NCTC 900.

TABLE I
Origin of lactic acid bacteria used in this study

Strain	Isolation* medium	Source
B2 B12, B13 B31	PCA + 1 RSL BQ	Lean area of commercial vacuum packed Danish gammon
B42 B52, B53	PCA + 1 RSL	Lean area of commercial vacuum packed Irish gammon
1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 26, 27 28, 29, 30, 31 40,	RSL MRS BQ » RSL	Vacuum packed beef stored at 1°C for 3 weeks in Cryovac. Lean area: beef from MRI, Langford
VP6, VP9, VP14, VP16, VP41, VP49, VP50, VP52 VP57, VP61, VP70, VP72, VP79 VP80, VP85, VP86, VP89 PK427	MRS RSL » BQ »	Vacuum packed pork from FMC abattoir, Salisbury, stored 11 weeks at 1°C in Cryovac
NZ7, NZ9, NZ10, NZ11, NZ12, NZ15, NZ17, NZ18, NZ19, NZ20, NZ22, NZ50, NZ51, NZ35, NZ36, NZ37, NZ39,	BQ MRS » BQ	Vacuum packed New Zealand lamb stored 5-6 weeks at –2°C in Cryovac
L365, L367, L402, L406	PCA + 1	Vacuum p. English lamb stored 10 weeks at 1°C in Cryovac

Comp 51 = *Lactobacillus plantarum* var. *arabinosus* NCD082

* PCA + 1 = Plate count agar + 1 % NaCl; RSL = Cavettés modification of Acetate agar; MRS = Man, Rogosa, Sharpe broth; BQ = Benzoquinone medium.

They were maintained on Nutrient agar slopes and transferred at two week intervals.

All cultures were checked for purity at monthly intervals by streaking on a appropriate agar medium.

Spoilage organisms were grown at 25°C and the pathogens at 37°C unless stated otherwise.

Demostration of inhibition by lactics grown in liquid media

Media and preparation of test samples:

Cooked meat medium was obtained from Southern Group Laboratory, England. Brain Heart Infusion Broth (BHI, Difco), Nutrient Broth and Skim milk were prepared by conventional procedures, dispensed into Erlenmeyer flasks and sterilized by autoclaving. BHI and Nutrient broth were also prepa-

red containing 0.6 % (w/v) glucose or 0.4 % (w/v) ribose. Fresh acid whey was prepared from non fat dried milk as reported by BRANEN *et al.* (1975).

The media were inoculated with 1 % by volume of stock cultures of lactic acid bacteria and incubated for 24 h and/or 48 h at 30°C.

Supernatants were obtained by centrifuging the culture media (8,000 g, 15 min) to remove the cells and filter sterilized using Millipore membranes (pore size 0.22 µ) before testing for antibacterial activity.

The samples were checked for inhibition by one of the assay procedures outilned below at the naturaly attained pH and after adjustement to pH 7.0 with sterile 2N NaOH.

The supernatants of some lactic cultures were freeze-dried (10 h) and reconstituted with an appropiate volume of sterile water to give a ten fold concentrated material.

Assay of inhibitory activity

1. Agar-well diffusion.

Ten ml of an overnight culture of each indicator organism was added to 100 ml of BHI broth and grown for a few hours. When the optical density (OD) reached *ca* 0.1 at 610 nm, 0.1 ml samples were added to 15 ml of melted DST agar medium (Oxoid), kept at 50°C and the mixture poured in to sterile Petri dishes.

Wells of *ca* 5 mm Ø were cut in each plate using a sterile cork borer. To each well 50 µl of test sample was added.

A modification of this assay was also tried. A standard paper assay disc (Whatman A.A., 6 mm) was dipped into the sample to be tested, touched on the side of the container to remove excess liquid and placed on the assay plate.

After growth for 24 h, the diameter of the zones of inhibition from the edge of the well or paper disc was measured.

2. Turbidometry.

The supernatant from a culture of strain 2 grown in BHI broth + 0.4 % (w/v) glucose for 48 h at 30°C was obtained by centrifugation, divided in two and freeze-dried. One half was reconstituted with the appropiate volume of 0.1 M pH 7.0 potassium phosphate buffer to obtain twice the original concentration. The other half was reconstituted with the same buffer at pH 5.3 (final pH of culture). Both preparations were filter sterilized.

An actively growing culture of *B. thermosphacta* was obtained by diluting an overnight culture 100 fold with broth and incubating until the OD_{610} reached 0.1. The culture was diluted 1,000 fold to obtain approx. 10^4 cells/ml and 8 ml put into Erlenmeyer flask with 2 ml of the reconstituted freeze-dried material and the mixture incubated aerobically at 20°C. The optical density was read at 4 h intervals and compared with that of control cultures, at the appropiate pH values to which no lactic supernatant had been added.

Demostration of inhibition by lactics grown in solid media

Three techniques were employed:

1. Two layers diffusion method (HAMON, 1956).

Four ml of BHI agar + 0.4 % glucose and two ml of an overnight culture of six different lactics were poured into Petri dishes. After setting 10 ml of peptone water agar was added. The plates were incubated for 48 h at 25°C before inoculating with the indicator organisms and then reincubated for an additional 24 h at 25°C. Inhibition was determined by comparison with plates to which no lactic cultures had been added.

2. Agar surface streak method (MAYR-HARTING *et al.*, 1972).

Streaks, 0.5 cm wide, of selected lactic acid bacteria were made on BHI agar + 0.4 % (w/v) glucose and the plates incubated for 48 h at 25°C. The plates were then exposed to chloroform vapour for 30 min. by inverting over a vessel containing 2 ml of chloroform.

The growth was removed with a glass slide and the agar surface again briefly exposed to chloroform vapour. The plates were then exposed to the air for 20 min. to allow the chloroform to evaporate off. The indicator strains were streaked at right angles to the original streak and the plates incubated for 24 h at 25°C and 37°C (spoilage and pathogens). Inhibition was measured semi-quantitatively.

3. In a modification of method 2, the test strains were streaked on 85 mm diameter, 0.45 µ porosity cellulose acetate membranes (Oxoid) placed on the surface of the agar and the plates incubated for 48 h at 25°C under aerobic and anerobic conditions. The membranes were removed, the indicator bacteria streaked across the original line of growth and the plates incubated for 24 h.

Effect of pH and acetic acid on growth of indicator organisms

In one set of experiments the pH of the medium was adjusted to values between 4.5 and 6.5 by addition of 10 % HCl before autoclaving. The final pH of the solidified medium was checked as described below.

In a second set of experiments, solutions of acetic acid buffered with sodium acetate at pH values of 5.0, 5.5, 6.0 and 6.5 were added to aliquots of BHI agar at the corresponding pH values to gibe final concentrations ranging from 50-4,500 mg/l.

In both cases indicator bacteria were streaked onto the plates which were then incubated for 24 h at 25°C.

Measurements of pH

The pH of solidifed agar was measured a) *in situ* with a surface pH electrode (Russell pH Ltd, Auchtermuchty, Scotland) b) in an aqueous mace-

rate (5 parts dist. water 1 part agar) using a standard combinatum electrode, (Corning-Eel).

Glucose determination

Glucose was determined enzymatically using the glucose oxidase/peroxidase/dye method (Boehringer, Lewes, Sussex).

Fatty acids analysis

Fatty acid were extracted from the agar using the centrifugation method of BUYNITZKY *et al.* (1979). The agar was cut into small pieces and centrifuged (27,000 g, 1 h, 5°C) and a known volume of the supernatant saturated with NaCl and acidified to pH 2 with 2N Hcl. Five volumes of Diethyl ether (at 4°C) were then added, the two layers mixed for 1 min. and then left in ice to separate.

The ether layer was carefully removed and dried over Na_2SO_4 before inyection into the gas chromatograph.

The ether extracts were analysed on a Pye Unican Series 104 Gas Chromatograph (Pye Instruments, Cambridge) fitted with a 2.1 m long × 6.35 mm 0/d glass columm packed with Chromosorb G (AW, DMCS) 80-100 mesh coated with Carbowax 20 M (5 % w/w) and H_3PO_4 (2 %, w/w). Columm packing were obtained from Phase Separations, Deeside Industrial Estate, Queensferry, Clwyd. The flow rates of the carrier gas (O_2-freeN_2) and air supplies to the flame ionization detector were 40, 50 and 400 ml/min. respectivelly. The columm was operated isothermally at 120°C with the inyection port and detector at 200°C. Samples were inyected into the headspace above the packing.

RESULTS

Demostration of inhibition by lactics grown in liquid culture media

Gel diffusion analysis:

A total of 75 strains of lactic acid bacteria were tested for their ability to produce an inhibitor in cooked meat medium. Six strains: 3, 6, VP 85, VP 89, L 406 and L 367 produced very small inhibitory zones, the remainder were negative.

Using ten fold concentrate material, these six lactics did not produce larger zones of inhibition.

Attempts to demostrate inhibitory effects using BHI and Nutrient broth with or without glucose or ribose, skim milk or fresh acid whey for growth of the selected lactic acid bacteria was also negative.

Turbidometric analysis:

Inhibition of growth of *Brochothrix thermosphacta* by concentrated supernatant from lactic acid bacterium strain 2 was tested by a turbidometric method following changes in optical density at 610 mμ. Results are given in Fig. 1. No antibacterial effect could be observed; on the contrary a stimultaion of growth in the test samples was obtained. This was probably due to the concentration of some nutrient (s) or cofactor (s) from the medium during freeze-drying.

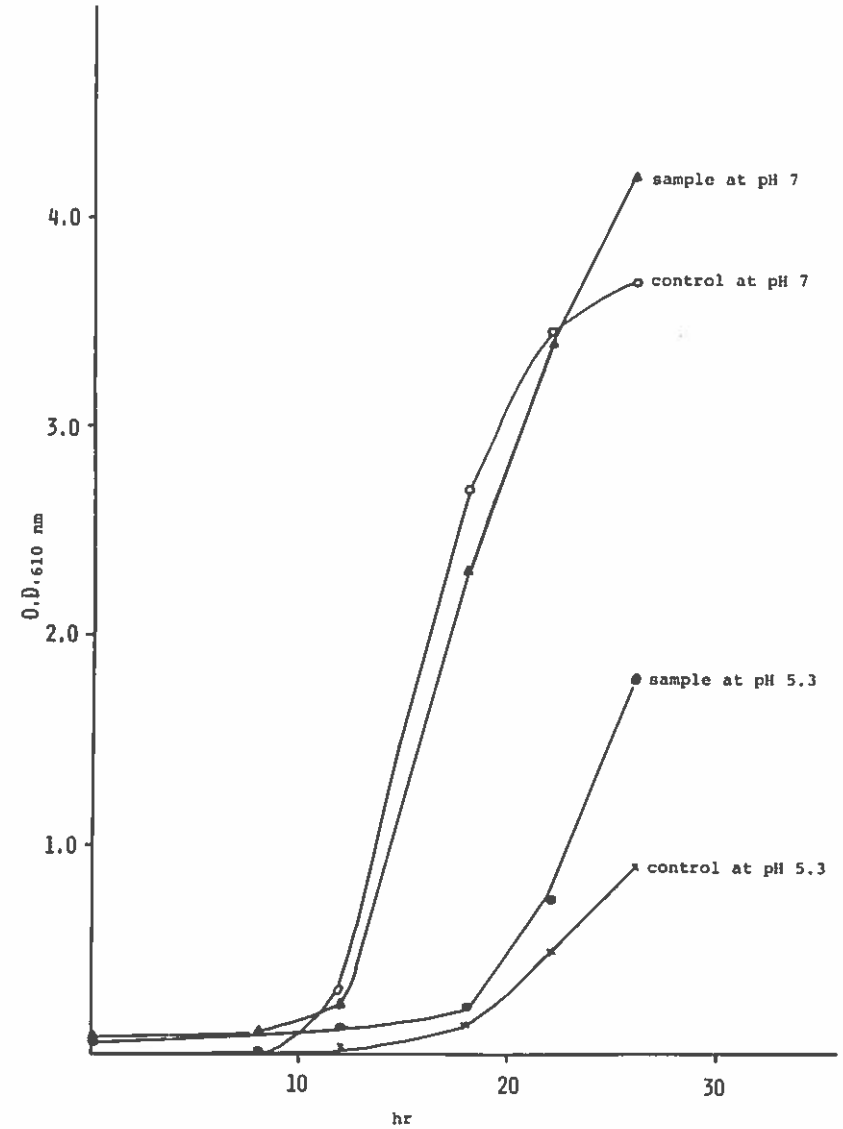

Figura 1.–Inhibition of growth of *Brochothrix thermosphacta* by concentrate supernatant from lactic acid bacterium strain 2.

Demostration of inhibition by lactics grown in solid media

In preliminary experiments all three methods used (Methods) were effective as a means of demostrating inhibition by selected lactic acid bacteria. Of these, the cellulose acetate membrane technique, method 3, was chosen to screen a larguer number of lactic strains.

The degree of inhibition of the indicator organisms by the lactic acid bacteria is illustrated semiquantitatively in Table II, together with the pH values and glucose concentrations in the agar media prior to inoculation with the microbial indicator strains. In general there was a clear correlation between low pH and inhibition. Despite this there were some lactics (L 365, PK 427, NZ 39, VP 52 and NZ 37) which gave very low pH values but either no, or much smaller degree of inhibition, than that of other lactics giving a higher pH (L 367, NZ 10, 5, 2, 6, VP 85, etc.).

Effects of pH and acetic acid concentration on growth of indicator organisms

All of the organisms were able to grow down to pH 5.0 (Table III). Only the pathogens and *Serratia liquefaciens* showed significant amounts of growth below this, with *Salmonella typhymurium* and *E. coli* still giving good growth at pH 4.5.

The concentration of acetic acid necessary to inhibit growth of each spoilage organisms increased with increasing pH (Table IV). The two *Moraxella-like* sp and the non fluorescens *Pseudomonas* sp. were the most sensitive organism being inhibited at pH 5.0 by 50-100 µg/ml of acetic acid. At the same pH *Serratia liquefaciens*, *Pseudomonas fluorescens* and *Brochothix thermosphacta* required between 100-400 µg/ml for inhibition. At pH values of 6.0 or greater, concentrations in excess of 1 mg/ml were requered to inhibit growth of any of the bacteria.

Relations between growth, pH, fatty acids production and inhibition in selected lactic acid bacteria

A group of selected lactic acid bacteria were grown on membrane for 48 h on two identical sets of BHI + 0.4 % (w/v) glucose plates. One set was used to detect inhibiton; the other to measure fatty acid concentrations and pH values. The amount of growth was measured by washing the cells from the membrane (5X) with MM (maintenance medium), and measuring the OD at 610 nm after making up to a volume of 10 ml.

All exept two of the orrganisms tested produced in excess of 300 µg/ml of acetic acid, pH values lower than 5.3 and inhibited the growth of the indicator organisms (Table V).

Organisms VP and B73, on the other hand, both produced less than 100 µg/ml of acetic acid, pH values greater than 5.5 and were non-inhibitory.

TABLE II

Inhibition of food spoilage and food poisoning organisms by strains of lactic acid bacteria

Strain	pH	Glucose % (w/v)	Indicator organisms: *Serratia liquefaciens*	Non Fluorescens *Pseudomonas* sp.	*Pseudomonas fluorescens*	*Moraxella-like* 63	*Moraxella-like* 98	*Brochothrix thermosphacta*	*Staphylococcus aureus*	*Salmonella typhimurium*	*E. coli*
8	5.1	0.39	+++	+++	++	++	++	+	+	+	+
NZ11	5.1	»	+	+	+++	+++	+++	+–	–	–	–
NZ12	»	»	+–	+–	+	+	+	–	–	–	–
NZ36	»	»	–	–	+	+	+	–	–	–	–
NZ37	»	»	–	+–	+	+	–	–	–	–	–
NZ39	»	»	–	–	+–	+–	–	–	–	–	–
7	5.2	0.38	+	+–	+	+	+	–	–	–	–
NZ50	»	»	+–	–	+	+	+	–	–	–	–
St. faec	»	»	+++	+++	+++	+++	+	+++	+	+	+
VP89	»	»	+++	+++	+++	+++	+++	+++	+	–	–
NZ9	»	»	+–	+	+	+	++	–	–	–	–
30	5.3	0.38	–	–	+	+	+–	–	–	–	–
40	»	»	+–	+–	+	+	+	+–	–	–	–
NZ7	»	»	+	+	++	++	++	–	–	–	–
L365	»	»	–	–	–	–	–	–	–	–	–
PK427	»	»	–	–	–	–	–	–	–	–	–
26	5.4	0.38	–	+	++	++	+–	–	–	–	–
27	»	»	++	++	++	++	+–	–	–	–	–
31	»	»	–	–	+–	+–	+–	–	–	–	–
VP49	»	»	++	++	+	++	+	+	–	–	–
VP52	»	»	–	–	–	–	–	–	–	–	–
VP86	»	»	–	–	+	+	+–	–	–	–	–
NZ20	»	»	–	–	+	+	+–	–	–	–	–

TABLA II
(Continuación)

Strain	pH	Glucose % (w/v)	Indicator organisms								
			Serratia liquefaciens	Non Fluorescens *Pseudomonas* sp.	*Pseudomonas fluorescens*	*Moraxella*-like 63	*Moraxella*-like 98	*Brochothrix thermosphacta*	*Staphylococcus aureus*	*Salmonella typhimurium*	*E. coli*
NZ22	»	»	−	−	+−	+−	−	−	−	−	−
NZ35	»	»	−	−	+	+	+	−	−	−	−
L406	»	»	+++	+++	+++	++	+++	+	+	−	−
6	5.5	0.4	++	++	+	++	+	+	−	−	−
12	»	»	−	−	+	+−	+	+−	−	−	−
NZ51	»	»	+	+	++	++	+	−	−	−	−
VP85	»	»	++	+	+	+	+	+−	−	−	−
NZ15	»	»	−	−	+	+	+−	−	−	−	−
NZ17	»	»	−	−	−	−	−	−	−	−	−
NZ18	»	»	−	−	+	+	+	−	−	−	−
NZ19	»	»	−	−	+	+	+	−	−	−	−
10	5.6	0.4	++	+	++	+	+	−	−	−	−
L367	»	»	++	++	+	+	+	−	−	−	−
B31	5.8	0.4	−	−	−	−	−	−	−	−	−
B42	»	»	−	−	−	−	−	−	−	−	−
5	»	»	++	+	++	++	++	+−	−	−	−
9	»	»	+	+−	+	+	+	+−	−	−	−
11	»	»	−	−	+−	+−	+−	+−	−	−	−
15	»	»	+	+−	+	+	+	−	−	−	−
16	»	»	−	−	−	−	−	−	−	−	−
NZ10	»	»	+	+	+	+	+	−	−	−	−
3	5.9	0.42	−	−	−	−	−	−	−	−	−
VP9	»	»	−	−	−	−	−	−	−	−	−
VP84	»	»	−	−	+	+	−	−	−	−	−
B2	»	»	−	−	−	−	−	−	−	−	−
1	6.0	»	−	−	−	−	−	−	−	−	−
4	»	»	+	+	++	+	+	+−	−	−	−
Comp51	»	»	−	−	−	−	−	−	−	−	−
2	6.1	»	+	+−	++	++	+	−	−	−	−
B12	6.3	N.D.	−	−	−	−	−	−	−	−	−
B13	»	»	−	−	−	−	−	−	−	−	−
B52	»	»	−	−	−	−	−	−	−	−	−
17	6.5	»	−	−	−	−	−	−	−	−	−
VP15	»	»	−	−	−	−	−	−	−	−	−
VP61	»	»	−	−	−	−	−	−	−	−	−
VP16	»	»	−	−	−	−	−	−	−	−	−
VP57	»	»	−	−	−	−	−	−	−	−	−
VP14	6.7	»	−	−	−	−	−	−	−	−	−
VP70	»	»	−	−	−	−	−	−	−	−	−
B73	»	»	−	−	−	−	−	−	−	−	−
29	»	»	−	−	−	−	−	−	−	−	−
VP50	6.8	»	−	−	−	−	−	−	−	−	−
VP72	»	»	−	−	−	−	−	−	−	−	−

(−) = no inhibition; (+−) = slight inhibition; (+) = inhibition only in zone of lactic growth; (++) = zone of inhibition betwee
(+++) = more than 4 mm of inhibition zone.
N. D. = not determined.

TABLE III
Effect of pH on growth of indicator organisms

pH of agar	*Serratia liquefaciens*	Non fluorescens *Pseudomonas* sp.	*Pseudomonas fluorescens*	*Moraxella* like 63	*Moraxella* like 98	*Brochothrix thermosphacta*	*Staphylococcus aureus*	*Salmonella typhimurium*	*E. coli*
	Indicator organisms								
6.5	+	+	+	+	+	+	+	+	+
6.0	+	+	+	+	+	+	+	+	+
5.5	+	+	+	+	+	+	+	+	+
5.0	+	+	+	+	+	+	+	+	+
4.85	+	–	+–	–	–	+–	+	+	+
4.75	+	–	+–	–	–	–	+	+	+
4.65	+	–	–	–	–	–	+	+	+
4.5	+–	–	–	–	–	–	–	+	+

(+) = growth; (–) = no growth; (+–) = sligth growth.

TABLE IV
Effects of pH and acetic acid concentration on growth of indicator organisms

Organisms	Acetic acid (ppm)															
	0				50				100				225			
	5	5.5	6	6.5	5	5.5	6	6.5	5	5.5	6	6.5	5	5.5	6	6.5
Serratia liquefaciens	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+
Pseudomonas fluorescens	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Non fluorescens *Pseudomonas* sp.	+	+	+	+	+	+	+	+	–	+	+	+	–	+	+	+
Moraxella like 63	+	+	+	+	+	+	+	+	–	+	+	+	–	+	+	+
Moraxella like 98	+	+	+	+	–	+	+	+	–	+	+	+	–	+	+	+
Brochothrix thermosphacta	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+

	450				900				2,225				4,500			
	5	5.5	6	6.5	5	5.5	6	6.5	5	5.5	6	6.5	5	5.5	6	6.5
Serratia liquefaciens	–	–	+	+	–	–	+	+	–	–	–	+	–	–	–	+
Pseudomonas fluorescens	–	–	+	+	–	–	+	+	–	–	–	+	–	–	–	+
Non fluorescens *Pseudomonas* sp	–	–	+	+	–	–	+	+	–	–	+	+	–	–	–	+
Moraxella like 63	–	–	+	+	–	–	+	+	–	–	–	+	–	–	–	–
Moraxella like 98	–	–	+	+	–	–	+	+	–	–	–	+	–	–	–	–
Brochothrix thermosphacta	–	–	+	+	–	–	+	+	–	–	–	+	–	–	–	+

(+) = growth; (–) = no growth.

TABLE V
Antibacterial activity and growth, pH and acetic acid concentration produced by lactics grown in solid media

Strain	$O.D._{610nm}$*	pH	Acetic acid (ppm)	n-butiric acid (ppm)	inhibition**
VP52	1.38	5.9	60	0	Negative
B73	1.86	5.5	86	0	Negative
2	2.04	5.3	300	8	Positive
19	2.64	5.2	340	12	Positive
NZ11	3.0	5.1	480	0	Positive
15	2.7	4.7	680	0	Positive
NZ51	2.22	4.7	734	0	Positive
6	2.04	4.7	725	0	Positive
4	1.98	4.8	730	0	Positive
VP79	1.62	4.7	730	0	Positive
5	1.44	4.7	680	0	Positive

* Inhibition tested against food spoilage organisms cited in Materials and Methods section.
** Amount of growth expressed as optical density at 610 nm.

There was no obvious link between the concentrations of acetic acid produced and the amount of cell growth.

DISCUSSION

Growth of many strains of the lactic acid bacteria on membranes placed on BHI + Glucose agar inhibited growth of one or more of the indicator organisms. This was not due to substrate limitation since there was still adequate glucose in the agar at the time of inoculation of the indicator organisms. However, cell free culture supernatants of the same isolated grown in BHI glucose broth, or in other liquid media chosen to give a range of nitrogen and carbon sources and concentrations, were not inhibitory. This cannot be explained by assuming bacteria *per se* are required for inhibition in view of the finding with the solid media. Nor does it appear to be a concentration effect since 10-fold concentrated supernatant from a cooked meat culture was still non inhibitory, though it is possible that an inhibitor was present but inactivated during concentration by freeze drying. It is also unlikely that an inhibitor unable to diffuse from the wells into the agar due to its molecular size was produced (Lindgren and Clevstrom, 1978), since growth of the lactic acid bacteria on dialysis membrane still produced inhibition in the underlying agar. It therefore appears that, as in the case of colicin B production by *S. typhi* (Nicholle and Prunet, 1964), the inhibitor (s) are not produced in liquid media. The failure to demostrate inhibition of *B. thermosphacta* (in fact there was a slight stimulation of growth) by lactic acid bacterium strain 2, using a turbidometric method is consistent with this suggestion.

The inhibitory activity in solid medium was most pronounced in bacteria which lowered the pH of the medium below pH 5.5 (Table II), but could not be

atributed to an hydrogen ion effect alone because the indicator organisms grew well on BHI agar adjusted to pH 5.0 with HCl. The pathogens grew at even lower pH values (Table III). Moreover, several lactics produced pH values below 5.5 but did not inhibit any of the indicator organisms while a few others produced pH values close to 6.0, yet still inhibited a range of indicator organisms (Table II).

Table IV and V indicate that the inhibition is due to a combination of pH and acetic acid concentration. Thus, at pH 5.5 the addition of 225 to 450 ppm acetic acid to BHI agar inhibited growth of the indicator organisms and all of the lactics which produced this level of acetic acid inhibited, while those producing less did not.

This well established relationship between pH, acetic acid concentration and bacterial inhibition, has been interpreted as showing that the undissociated form of the acid is the active inhibitory species (MEYNELL, 1963; HENTGES, 1967).

Whether these conclusions can be used to explain the inhibitory properties of lactic acid bacteria against other organisms growing on meat is not clear. The laboratory medium was relatively rich in carbohydrates (0,2-0,6 % w/v), which were clearly the source of the acetic acid detected. In normal pH meat carbohydrates are not so plentiful (< 0,1 %). And in high pH meat they are even less so. There must therefore be some doubt whether inhibitore concentrations of acetic acid are formed in meat.

SUMMARY

The inhibitory effects of some lactic acid bacteria isolated from meat and meat products on selected spoilage and pathogen bacteria were investigated. The demostration of the inhibitory properties were studied in liquid and solid media. A perpendicular streak technique was used as a screening procedure to determine relative degrees of inhibition by lactics grown in solid media. Agar gel diffusion and turbidometric analysis was also used to measure the antibacterial activity produced by supernatants from selected lactic acid bacteria against indicator organisms.

The existence of antibacterial activity was only demostrated in solid media. The inhibitory properties of lactic acid organisms studied can be explained on the basis of their ability to produce substancial concentration of acetic acid.

ACTIVIDAD ANTIMICROBIANA DE CIERTAS BACTERIAS ACIDOLACTICAS

RESUMEN

Se ha estudiado en este trabajo el efecto inhibidor de un número elevado de cepas de bacterias ácido lácticas aisladas de carne y productos cárnicos, sobre un grupo de bacterias que intervienen en la alteración de los alimentos y en la producción de intoxicaciones y toxiinfecciones alimentarias.

La producción de sustancias inhibidoras por las bacterias lácticas fue estudiada en diferente medios de cultivo líquidos y sólidos.

Para determinar el grado de inhibición producido por las bacterias lácticas cultivadas en medios sólidos se utilizó una técnica de siembra perpendicular en superficie.

Se utilizaron también los métodos de difusión en gel de agar y turbidimétrico para medir la actividad antibacteriana de las cepas lácticas cultivadas en medios líquidos.

Unicamente las cepas cultivadas en medios sólidos son capaces de producir inhibición frente a los organismos indicadores.

Las propiedades antibacterianas que exhiben las bacterias lácticas pueden explicarse en función de su capacidad de producir concentración suficiente de ácido acético en forma no disociada.

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