THE USE OF NISIN AND LACTOPEROXIDASE IN COMMINUTED MEAT PRODUCTS

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SUMMARY

Pathogens, spoilage organisms and meat starter cultures were characterised for sensitivity to nisin and lactoperoxidase system (LPS). Glucose oxidase (GOD) alone and in conjunction with the LPS was found to have a marked inhibitory effect on *S.monschaui*, *S.aureus*, *P.aeroginosa* and *S.faecalis*. Starter strains were identified that produced sufficient hydrogen peroxide (H_2O_2) to activate the LPS and this may be an important consideration in the preparation of a multicomponent antimicrobial system.

INTRODUCTION

Reducing salt, fat and the chemical load in processed meat products reduces shelf-life due to premature microbial spoilage. To compensate, it may be possible to utilise naturally occurring antimicrobial systems and 'friendly flora' as product ingredients during meat processing. The aim of this study was to measure the antimicrobial effect of Nisin, a commercial antimicrobial used in dairy products and the LPS, an enzyme defence system found in milk, on a range of pathogenic and spoilage bacteria and 'friendly flora' such as starter cultures commonly used in fermented meats.

EXPERIMENTAL METHODS

1. Bacteria

Starter strains (*P.pentosaceus* JC1, *L.plantarum* LP2, *L.sake* LS1, *S.carnosus* SS1 and *M.luteus* JC3) were obtained from Mauri Foods and all other organisms from the FRI Culture Collection. These included (i) gram negative organisms. *Salmonella monschaui* Sm Ref, *P.aeroginosa* Pa Ref, *P.fragi* Pf Ref, *P.fluorescens* Pf1 Ref, *Hafnia alvei* Ha Ref, *E.coli* Ec Ref, *Serratia liquefaciens* S1 Ref, and (ii) gram positive organisms *S.aureus* M6524, *S.faecalis* Sf Ref, *S.faecium* SD1 and *M.luteus* NC1B 8166.

All organisms were maintained by broth culture in Mann Rogosa and Sharpe (MRS) (JC1, LP2, LS1, SS1) Nutrient Broth (JC3, NC1B 8166), M17 (SD1, Sf Ref) and

TABLE 1 NISIN SENSITIVITY OF BACTERIAL STRAINS USING PLATE ASSAY METHOD

Strain	Best High
P. pentosaceus JC1	17.2
L. sake LS1	18.4
S. faecium SD1	20.4
L. plantarum LP2	22.1
M. luteus JC3	26.0
S. carnosus SS1	27.7
M. luteus NCIB 8166	38.5

TABLE 2 THE EFFECT OF GLUCOSE OXIDASE AND LACTOPEROXIDASE SYSTEM ON CELL GROWTH

Concentrations	Treatment	Percentage growth inhibition			
		S. aureus	S. monschaui	P. aeroginosa	S. faecalis
	SCN-	5	0	0	0
GOD 4 IU/mL	H_2O_2	20	15	100	2
LPS 50 U/mL	GOD	42	99	97	100
SCN^- 1mM	GOD + H_2O_2	87	95	99	100
	LPS	0	5	16	4
	LPS + GOD	100	82	100	100
GOD 0.4 IU/mL	SCN-	0	13	0	4
LPS 5 U/mL	H_2O_2	0	0	14	0
SCN^- 100 uM	GOD + H_2O_2	99	100	96	0
H_2O_2 100 uM	LPS	0	0	0	0
	LPS + GOD	99	98	69	0

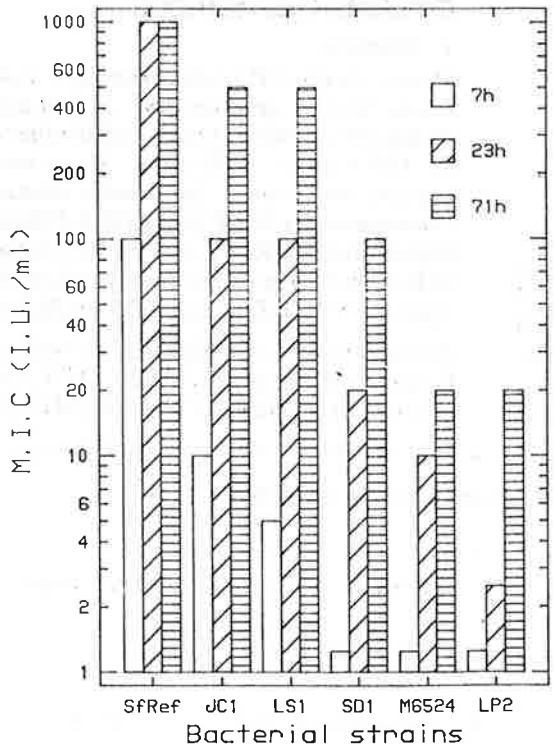

Fig 1 Nisin sensitivity of bacterial strains using the turbidometric method

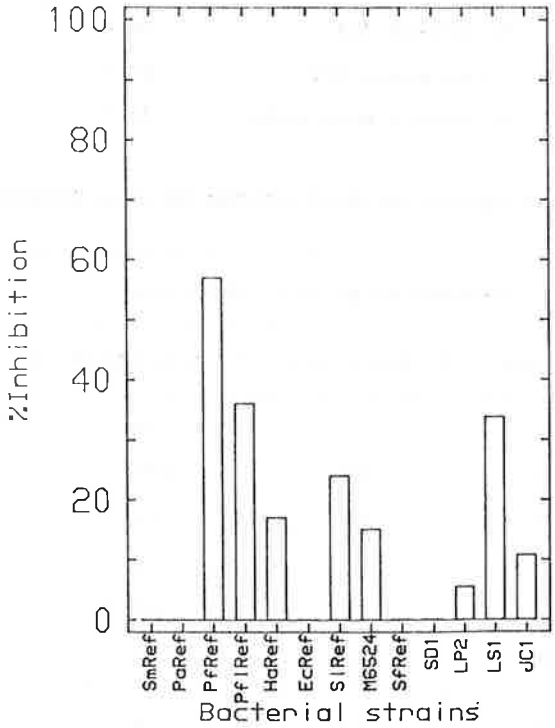

Fig 2 LPS (low level) sensitivity of bacterial strains

Tryptone Soya Broth (TSB) (Sm Ref, Pa Ref, Pf Ref, Pf1 Ref, Ha Ref, Ec Ref, S1 Ref and M6524).

2. Plate Assay Diffusion Method for Nisin Sensitivity

The standard plate diffusion method was used at nisin concentrations of 1.25 IU/mL to 1000 IU/mL and the sensitivity of cultures to nisin expressed as the weighted best high values (Fowler et al 1975). A standard stock solution of Nisaplin (Aplin and Barret Ltd, Trewbridge, England) (10^6 IU/g nisin) was prepared as described by Fowler et al. (1975).

3. Turbidometric Method for Nisin Sensitivity

The sensitivity of organisms to nisin was determined in MRS, TSB, or MI7 broth and nisin added to give 1.25, to 1000 IU/mL. Bacteria were tested at an initial count of 10^8 cfu/mL. Cultures were incubated at 30°C and growth measured by absorbance at 610 nm at 7, 23 and 71 h.

4. Turbidometric Method for LPS and GOD Sensitivity

The LPS was prepared in broth by adding lactoperoxidase (LP) EC 1.11.1.7 (Boehringer Mannheim) at 1 or 10 U/mL together with 100 uM or 1 mM thiocyanate (SNC^-) and 100 mM or 1 mM hydrogen peroxide (H_2O_2). Glucose oxidase (GOD) EC 1.1.3.4 (Boehringer Mannheim) was used at 4 IU/mL or 0.4 IU/mL. Culture tubes containing appropriate enzymes, substrates and controls were inoculated with cells at 10^8 cfu/mL. Cultures were incubated at 30°C for strains JC1, LP2 and LS1 and at 37°C for all other organisms. Growth was determined by absorbance at 610 nm at the times indicated.

RESULTS

1. Effect of Nisin on gram positive bacteria

The plate assay method of determining sensitivity of bacteria to nisin demonstrated a wide variation in sensitivity amongst the strains tested. *P.pentosaceus* JC1 was the most resistant and the decreasing order of resistance was *P.pentosaceus* JC1 *L.sake* LS1 *S.faecium* SD1 *L.plantarum* LP2 *M.luteus* JC3 *S.carnosus* SS1 *M.luteus* NC1B 8166 (Table 1).

The turbidometric method was more convenient than the plate assay method. The organisms tested by both methods had the same relatively with respect to growth in the presence of nisin (*P.pentosaceus* JC1 *L.sake* LS1 *S.faecium* SD1 *L.plantarum* LP2). Decreasing resistance to nisin for all strains tested turbidometrically was (*S.faecalis* Sf Ref *P.pentosaceus* JC1 *L.sake* LS1 *S.faecium* SD1 *S.aureus* M6524 *L.plantarum* LP2 (Fig. 1).

2. The effect of the LPS on gram positive and gram negative bacteria

The LPS is generally regarded as bactericidal toward gram negative bacteria and bacteriostatic toward gram positive bacteria (Reiter and Harnulv, 1984). Figure 2 shows growth inhibition of a range of gram positive and gram negative organisms by the LPS (low level). The organisms, *P.fragi* Pf Ref, *P.fluorescens* Pf Ref, *S.liquefaciens* S1 Ref, *H.alvei* Ha Ref, *S.aureus* M6524 and *L.sake* LS1 showed varying degrees of inhibition to growth after 24 h incubation. Of the starter organisms tested (*P.pentosaceus* JC1, *L.plantarum* LP2 and *L.sake* LS1), *L.plantarum* LP2 was most resistant to the LPS and *L.sake* LS1 was most sensitive (Fig 2). *P.pentosaceus* JC1

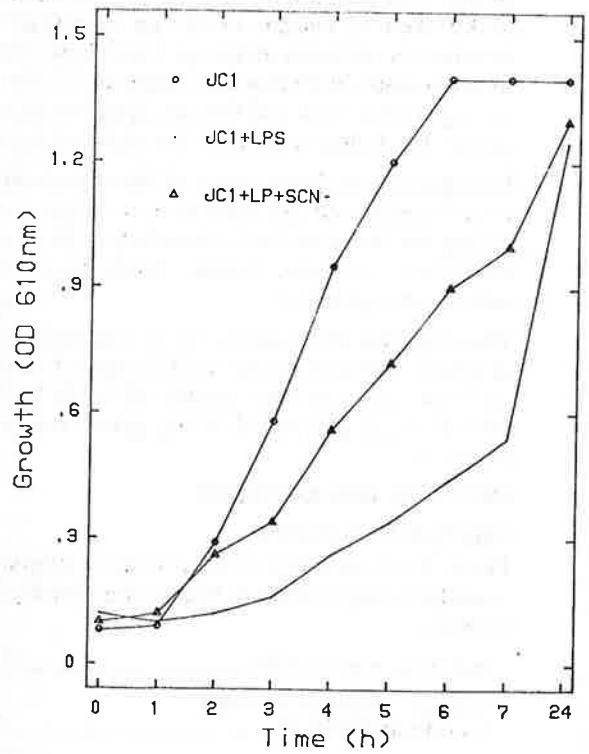

Fig 3a Effect of LPS (low level) on growth of P. pentosaceus JC1

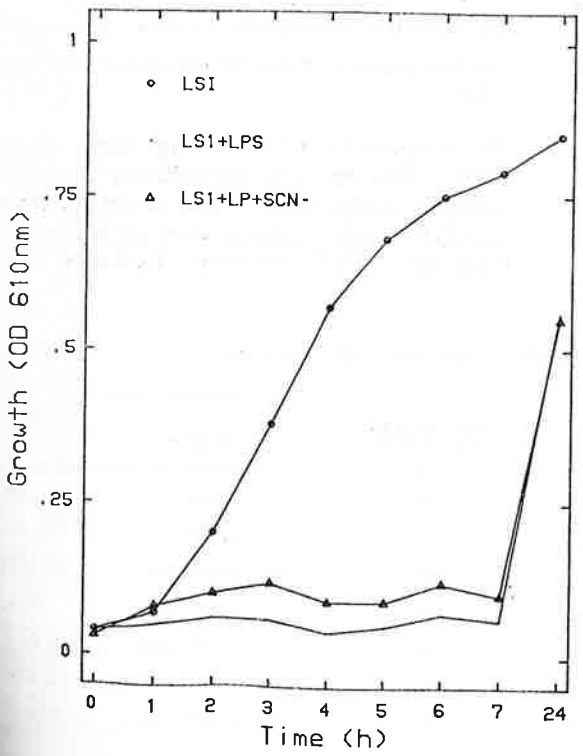

Fig 3b Effect of LPS (low level) on growth of L. sake LS1

and *L.sake* LS1 produced sufficient H_2O_2 to activate the LPS without an exogenous source of H_2O_2 (Fig 3).

3. The effect of GOD on gram negative and gram positive bacteria

GOD would be able to provide a renewable source of H_2O_2 for the LPS. Table 2 shows the effect of LPS and GOD on *S.monschaui*, *S.aureus*, *P.aeroginosa* and *S.faecalis* at LPS (high level) with 4 IU/mL GOD and LPS (low level) with 0.4 IU/mL GOD respectively. At 1 mM H_2O_2, *P.aeroginosa* had complete growth inhibition while *S.aureus* and *S.monschaui* were 20% and 15% inhibited respectively (Table 2). At 100 uM H_2O_2 no growth inhibition occurred except for *P.aeroginosa*, (14% inhibition), an organism known to be sensitive to H_2O_2. Thiocyanate at 1 mM had little effect on any of the organisms tested. The LPS alone had little effect on cell growth at either high or low levels. GOD alone at (4 IU/mL) inhibited growth of all organisms and the addition of 4 IU/mL GOD to the LPS (high level) markedly inhibited growth of all organisms. GOD at (0.4 IU/mL) together with LPS (low level) inhibited the growth of all organisms except *S.faecalis*. These experiments did not determine whether GOD inhibited growth by the production of H_2O_2 or by the removal of glucose.

DISCUSSION

The results of this investigation indicate a wide variation in the sensitivity of different gram positive bacteria to nisin. It may be that nisin does not have a sufficiently broad antibacterial effect against gram positive pathogens and spoilage organisms to be used alone as an effective preservative.

The LPS has not previously been considered for use in meat products. For the LPS to operate optimally, it will be necessary to provide a renewable source of H_2O_2 via the addition of a peroxidase enzyme like GOD or by an H_2O_2 producing meat starter similar to *P.pentosaceus* JC1. The identification of sensitive organisms to antimicrobials (as determined in this study) will be necessary for evaluating antimicrobials in meat products.

CONCLUSIONS

It is unlikely that the development of naturally occurring antimicrobials for use in meat products will result in a single component system. Such a system may involve the use of starter cultures interacting with antimicrobials like the LPS as well as the production of antimicrobials by these organisms in situ, or their addition in pure form (i.e. nisin).

ACKNOWLEDGEMENTS

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