

## BIOPROTECTIVE CULTURES IN ORDER TO PREVENT SLIME IN COOKED MEAT PRODUCTS

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**BACKGROUND**

The formation of ropy slime on vacuum-packed cooked meat products has been a common spoilage problem, in several countries, affecting the marketing of these products with high financial losses. Lactic acid bacteria mainly lactobacilli and *Leuconostoc* are the responsible for this defect (Korkeala et al., 1988). The slime is often formed before the sell-by date and consumers find the appearance of slimy products very offensive, although no off-odours are detected. Several studies have shown that cooked meat products become recontaminated with lactic acid bacteria during handling (i.e. slicing) after cooking. The processing rooms and equipment were considered to be one source of this contamination (Mäkelä et al., 1992).

*Lactobacillus sakei* CTC746 and *Leuconostoc* spp. CTC747 were isolated from slimy cooked ham (sliced and vacuum packed). Both strains were capable to reproduce the defect when they were inoculated in modelized cooked pork stored vacuum packed or in a modified atmosphere at 8°C.

Although lactic acid bacteria are responsible for this problem they have many and well documented applications inside food industry as starter cultures in fermented meat products. Their use as bioprotective cultures in another type of meat products would be interesting because of their ability to produce bacteriocins, compounds of peptidic nature which are active against other lactic acid bacteria and some pathogens like *Listeria monocytogenes* and *Clostridium perfringens*.

**OBJECTIVES**

The aim of this study was to evaluate the effectiveness of the bacteriocinogenic *Lactobacillus sakei* CTC494 (sakacin K) *Enterococcus faecium* CTC492 (enterocin A) and *Pediococcus acidilactici* F (pediocin AcH) as bioprotective cultures against the slime production in sliced packed cooked meat products.

**METHODS****Manufacture of modelized cooked pork****First assay**

Meat was coarsely ground through a 12 mm plate and brined for 48 hours (150 g brine Kg<sup>-1</sup> of meat) at 3-5°C. The brine solution contained (in g Kg<sup>-1</sup>): NaCl, 20; NaNO<sub>2</sub>, 0.15; sodium ascorbate, 0.50; carbohydrate\*, 10; sodium glutamate, 1 and water 115.35. The mixture was stuffed into 7 cm diameter cellulose casings and cooked until the internal temperature reached 67°C. After cooling it was sliced (10g per slice).

\* 5 different lots were prepared each one with a different carbohydrate: lactose, glucose, sucrose, maltodextrines and starch.

For each lot two different types of packaging were used for storage: vacuum and modified atmosphere (80% N<sub>2</sub>: 20% CO<sub>2</sub>) and two storage temperatures 4°C and 8°C. The samples were stored for up to 21 days.

**Inoculum preparation**

*Lactobacillus sakei* CTC746 or *Leuconostoc* spp. CTC747 cultures were grown overnight in MRS at 30°C. Cells were harvested by centrifugation (5000 xg 10 min), resuspended in glycerol and MRS broth (1:1) and stored at -40°C prior to use. Before inoculation, viable counts were determined.

200 µl (containing 6x10<sup>2</sup> cells) were inoculated between two slices. A control lot was done (200 µl water).

**Sampling**

Triplicates from each treatment were sampled at selected times (0, 15 and 21 days) to determine lactobacilli populations (MRS agar incubated in anaerobiosis for 72 h), pH (Ingold electrode 406-M4-S7/35) and slime (visually).

*Lactobacillus sakei* CTC746 and *Leuconostoc* spp. CTC747 were confirmed in its respective lots by their plasmid DNA profile after lysis (Anderson and McKay, 1983), according to a progressive colony sampling plan (Garriga et al., 1996)

**Second assay**

The modelized cooked pork was manufactured as previously described and the following criteria were selected for the assay: sucrose as the carbohydrate of choice, vacuum packaging, and 8°C as storage temperature.

**Strains used**

*Lactobacillus sakei* CTC746, *Leuconostoc* spp. CTC747, *Lactobacillus sakei* CTC494, *Enterococcus faecium* CTC492, *Pediococcus acidilactici* F (kindly provided by B.Ray). Ropy slime strains were inoculated at 6x10<sup>2</sup> cells and the bac<sup>+</sup> strains at 2x10<sup>5</sup> cells both between two slices. Each strain was inoculated both alone and in combination. The inoculum preparation was done as described.

Sampling was done at selected times (0, 7, 15 and 21 days) as described. The strains were confirmed either by plasmid profile, morphology by contrast-phase microscope (for *Pediococcus acidilactici* F, *E. faecium* CTC492).

**RESULTS AND DISCUSSION**

Lactic acid bacteria counts reached 10<sup>8</sup> cfu/g in 15 days of storage in all the samples, but the strain inoculated in each lot was easily verified by its plasmid profile. *Lactobacillus sakei* CTC746 produced ropy slime in all the samples with both packaging methods and temperatures (Table 1) whereas only the samples which contained sucrose as a carbohydrate became slimy when *Leuconostoc* spp. CTC747 was the inoculated strain. For this reason a second assay was carried choosing sucrose, vacuum packaging and 8°C as the storage temperature in order to evaluate several bacteriocinogenic cultures for preventing the default. The results obtained are



reported in table 2. *E. faecium* CTC492 was able to prevent slime formation due to *Lact. sakei* CTC746 up to 7 days of storage and *Lact. sakei* CTC494 until 21 days. However none of the bacteriocinogenic cultures could prevent the defect when *Leuconostoc spp.* CTC747 was the inoculated strain. *Lactobacillus sakei* CTC494 was considered the most effective bioprotective culture to delay the slime production.

## CONCLUSIONS

*Lactobacillus sakei* CTC494 and *Enterococcus faecium* CTC492 (to a lesser degree) had been proved to be effective enhancing the shelf life of sliced cooked meat products in vacuum packages. Studies are being carried in order to evaluate their bacteriocins as biopreservatives in the same type of products.

**Table 1. Effect of different parameters on ropiness of modelized cooked pork inoculated with ropy slime-producing bacteria.**

BATCH	SUGAR	T=0 days		T=15 days								T=21 days									
		LAB*	pH	VACUUM-PACKED				MAP (80% N <sub>2</sub> : 20% CO <sub>2</sub> )				VACUUM-PACKED				MAP (80% N <sub>2</sub> : 20% CO <sub>2</sub> )					
				LAB*	pH	SLIME		LAB*	pH	SLIME		pH	SLIME		pH	SLIME					
						4°C	8°C			4°C	8°C		4°C	8°C		4°C	8°C	4°C	8°C		
Control	Lactose	1.60	6.36	6.45	6.43	---	---	---	---	6.35	6.29	---	---	6.17	6.10	---	---	6.10	6.04	---	---
	Glucose	2.15	6.35	7.90	6.19	---	---	---	---	8.27	6.14	---	---	5.97	5.53	---	---	5.97	5.53	---	---
	Starch	2.25	6.36	6.32	6.36	---	---	---	---	5.98	6.33	---	---	6.20	6.08	---	---	6.12	6.10	---	---
	Maltodextrine	1.74	6.34	5.85	6.24	---	---	---	---	6.62	6.23	---	---	5.95	5.75	---	---	6.04	5.59	---	±--
	Sucrose	1.54	6.34	6.07	6.25	---	---	---	---	5.96	6.25	---	---	6.06	5.59	++±	±±±	6.03	5.57	--+	++±
<i>Lact. sakei</i> CTC 746	Lactose	1.95	6.35	8.73	6.15	+++	±±±	8.72	6.16	+++	++±	6.15	6.10	+++	++±	6.07	6.09	+++	+++		
	Glucose	3.15	6.36	8.93	6.01	+++	+++	8.55	5.97	+++	+++	5.79	5.39	+++	+++	5.75	5.25	+++	+++		
	Starch	2.08	6.33	8.72	6.13	+++	++±	8.60	6.15	+++	+++	6.12	6.11	+++	+++	6.08	6.06	+++	+++		
	Maltodextrine	2.18	6.34	8.64	6.06	+++	+++	8.64	5.97	+++	+++	5.74	5.42	+++	+++	5.67	5.28	+++	+++		
	Sucrose	1.81	6.33	8.89	6.15	+++	+++	8.50	6.15	+++	+++	6.09	5.69	+++	+++	5.87	5.62	+++	+++		
<i>Leuconostoc spp</i> CTC 747	Lactose	2.64	6.35	8.71	6.33	---	---	7.72	6.22	---	---	6.19	5.98	---	---	6.15	6.09	---	---		
	Glucose	2.94	6.36	8.96	5.93	---	---	8.44	6.08	---	---	5.79	5.44	---	---	5.77	5.56	---	---		
	Starch	2.15	6.34	8.63	6.25	---	---	8.63	6.21	---	---	6.17	6.16	---	---	6.18	6.14	---	---		
	Maltodextrine	2.59	6.34	8.84	5.87	---	---	8.79	5.70	---	---	5.87	5.56	---	---	5.70	5.52	---	---		
	Sucrose	2.67	6.34	9.09	6.14	+++	+++	8.74	5.91	+++	+++	5.79	5.71	+++	+++	5.81	5.71	+++	+++		

**Table 2. Effect of bacteriocinogenic lactic acid bacteria against ropy-slime producing bacteria inoculated in modelized cooked pork.**

BATCH	T=7 days			T=15 days			T=21 days		
	LAB*	pH	SLIME	LAB*	pH	SLIME	LAB*	pH	SLIME
Control	nd	6,35	---	8,08	5,79	---	8,06	5,79	±±-
<i>Lactobacillus sakei</i> CTC 746	7,51	6,29	++±	nd	5,81	+++	8,54	5,36	+++
<i>Leuconostoc spp</i> CTC 747	8,11	5,80	+++	nd	5,63	+++	7,09	5,58	+++
<i>Enterococcus faecium</i> CTC 492	nd	6,18	---	nd	5,48	---	8,71	5,29	---
<i>Lactobacillus sakei</i> CTC 494	nd	6,13	---	nd	5,86	---	8,61	5,61	---
<i>Pediococcus acidilactici</i> F	nd	6,36	---	nd	5,98	---	8,63	5,62	---
<i>Lact. sakei</i> CTC 746 + <i>E. faecium</i> CTC 492	8,68	6,07	---	8,90	5,40	±±±	8,77	5,41	±±±
<i>Lact. sakei</i> CTC 746 + <i>Lact. sakei</i> CTC 494	8,03	6,13	--±	8,73	5,82	---	8,76	5,68	±±±
<i>Lact. sakei</i> CTC 746 + <i>P. acidilactici</i> F	6,24	6,30	--±	8,58	5,72	+++	8,56	5,57	±±±
<i>Leuconostoc spp</i> CTC 747 + <i>E. faecium</i> CTC 492	8,79	5,72	+++	nd	5,58	+++	7,89	5,56	+++
<i>Leuconostoc spp</i> CTC 747 + <i>Lact. sakei</i> CTC 494	8,86	5,78	+++	nd	5,58	+++	8,70	5,56	+++
<i>Leuconostoc spp</i> CTC 747 + <i>Pediococcus acidilactici</i> F	8,77	5,86	+++	nd	5,62	+++	7,52	5,59	+++

Values of LAB (\* log cfu/g) and pH are the average of three samples.  
nd: not determined.

Slime: + (slime production), - (no slime production), ± (weak slime production).

## LITERATURE

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