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Short communication

Dry sausage fermented by *Lactobacillus rhamnosus* strains

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Abstract

The ability of three probiotic *Lactobacillus rhamnosus* strains GG, E-97800 and LC-705 and one commercial *Pediococcus pentosaceus* starter strain (control) to produce dry sausage was studied. During the fermentation process the numbers of inoculated lactic acid bacteria increased from approx. 7 $\log_{10}$ to 8–9 $\log_{10}$ cfu/g and the pH values decreased from 5.6 to 4.9–5.0. The sensory test indicated that the dry sausages fermented by *L. rhamnosus* LC-705 were inferior to the control sausages. The presence of inoculated experimental strains as predominant organisms in the dry sausages was recognised on the basis of their genetic fingerprints by ribotyping. The concentrations of biogenic amines remained low during the ripening process. These results indicated that the studied *Lactobacillus rhamnosus* strains, especially strains GG and E-97800, are suitable for use as probiotic starter cultures in fermenting dry sausage.

Keywords: Probiotics; Dry sausage; Lactic acid bacteria; *Lactobacillus*

1. Introduction

Meat starter cultures contain living or resting micro-organisms that usually, but not necessarily, develop the desired metabolic activity (Hammes, 1995). The desired properties of meat starter cultures in order to ensure the production of technologically high quality products have been reviewed by Jessen (1995) and Kröckel (1995). In addition to the ability to ensure the safety and the development of consistency in dry sausages the novel starter strain should be culturable on an industrial scale and preserved by freezing or freeze-drying, as well as contribute to high quality sensory properties of the end product (Kalantzopoulos, 1997; Charteris et al., 1998; Holzapfel et al., 1998).

A probiotic is a culture of living micro-organisms — mainly lactic acid bacteria or bifidobacteria — which have a beneficial effect on the health of the host when ingested in certain amounts. Oral consumption of probiotics is suggested to have a positive effect on the maintenance of the intestinal

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microflora and the colonisation resistance against pathogens, as well as to alleviate symptoms of lactose intolerance and promote beneficial immune responses as reviewed by Salminen et al. (1998a). Their safety aspects have recently been reviewed (Salminen et al., 1998b).

Probiotic lactic acid bacteria are widely used in dairy products but not in meat products. Before being consumed most meats are heated, which kills the probiotic bacteria. However, one exception is the various kinds of dry sausages which are processed by fermenting without heating. Arihara et al. (1996, 1998) have shown that the potentially probiotic strain *Lactobacillus gasseri* JCM1131 is applicable for meat fermentation to enhance product safety, and Sameshima et al. (1998) have demonstrated the usefulness of the potential probiotics *L. rhamnosus* FERM P-15120 and *L. paracasei* subsp. *paracasei* FERM P-15121 in meat fermentation. Andersen (1998) fermented dry sausages using a mixture of the traditional starter culture Bactoferm T-SPX (Chr. Hansen) and a potentially probiotic culture of *L. casei* LC-01 or a mixture of the same starter and the probiotic *Bifidobacterium lactis* Bb-12.

Lactobacillus rhamnosus GG is a well known probiotic lactic acid bacterial strain used to ferment dairy products (Saxelin, 1997). *L. rhamnosus* E-97800 is a new potential probiotic strain isolated from human faeces (Kontula et al., 1999). *L. rhamnosus* LC-705 is a bioprotective lactic acid bacterium used to ferment dairy products (Mäyrä-Mäkinen and Suomalainen, 1995) that has recently been studied for its probiotic properties (Lehto and Salminen, 1997; Tuomola and Salminen, 1998). *L. rhamnosus* E-97800, *L. rhamnosus* LC-705 and *L. rhamnosus* GG have been shown to adhere to human intestinal cells (Caco-2) in vitro (Elo et al., 1991; Lehto and Salminen, 1997; Tuomola and Salminen, 1998; Kontula et al., 1999; Kontula et al., 2000a). In addition, *L. rhamnosus* GG and *L. rhamnosus* E-97800 have been studied in a Simulator of the Human Intestinal Microbial Ecosystem (SHIME), which has been used as a model in probiotic studies (Molly et al., 1993, 1994, 1996). Kontula et al. (1998) have demonstrated that after the administration of *L. rhamnosus* GG-fermented oats *L. rhamnosus* GG continued colonising the SHIME model for several days. In vivo studies have shown that *L. rhamnosus* GG continues to colonise the human gut for more than a week after oral dosing (Alander et al., 1999). Feeding the SHIME model with *L. rhamnosus* E-97800 showed that *L. rhamnosus* E-97800 colonised the model. It enhanced the metabolic activity of intestinal microbes and the production of lactic acid and butyric acid increased, whereas production of ammonium and β-glucuronidase decreased (Kontula et al., 2000b).

The aim here was to examine the technological properties of selected probiotic *Lactobacillus rhamnosus* strains, probiotic *L. rhamnosus* GG, and the potential probiotics E-97800 and LC-705, in dry sausage manufacturing. Special emphasis was placed on the growth of added bacterial strains, on tracing them from the sausages, on the pH value and the development of consistency as well as on the formation of biogenic amines in sausages. The ultimate goal is the future industrial production of dry sausage with probiotic lactic acid bacteria.

2. Materials and methods

2.1. Preparation of dry sausages

Dry sausage was manufactured according to the following formulation: beef 200 g/kg, pork 560 g/kg, pork fat 200 g/kg, salt 30 g/kg, glucose 3 g/kg, $NaNO_2$ 0.07 g/kg, KNO_3 0.1 g/kg, bacterial cultures and spices. The experimental strains (Table 1) were provided by Valio Ltd. and VTT Biotechnology as pure cultures. The control strain was isolated from a commercial meat starter culture Condi Rasant (Gewürzmüller, Germany) by separating *Pediococcus pentosaceus* from *Staphylococcus xylosus* using MRS agar (pH 5.6). Selected strains were added into sausage material as pure cultures grown in MRS broth at 30°C for 48 h. The aim for the inoculum was $7 \log_{10}$ cfu/g of raw material (100 ml broth/10 kg sausage material). In addition, a commercial starter culture *Staphylococcus carnosus* (Pökelferment 77, Chr. Hansen, Denmark) was added to the raw sausage material to ensure colour formation. The aim was $6 \log_{10}$ cfu/g of raw material (1 g/10 kg sausage material). The raw sausage material was stuffed into 45-mm diameter artificial casings and fermented for 1 day at 23°C and RH 95% followed by a gradual reduction of temperature (from 22 to 19°C) and relative humidity (from RH 92 to 89%)

Table 1
Bacterial strains used for fermentation

Strain	Code	Supplier
Lactobacillus rhamnosus	GG	Valio Ltd.
Lactobacillus rhamnosus	LC-705	Valio Ltd.
Lactobacillus rhamnosus	E-97800	VTT Biotechnology
Pediococcus pentosaceus	Control	Gewürzmüller GmbH (Condi Rasant)

during the next 5 days. Sausages were also smoked (2 h) twice a day between the second and fifth day of fermentation. After 7 days of fermentation sausages were ripened for 21 days at 17°C (RH 75%). Three different experimental series with each experimental strain were prepared.

2.2. Physical and chemical analysis

2.2.1. pH

pH was measured (Knick Portames 651; electrode Ingold S 2518) directly from the sausage as the mean value of three measurements after 3, 7, 14 and 28 days of fermentation and ripening.

2.2.2. Weight loss

Weight losses were calculated as percentages of raw sausage weight after 3, 7, 14, 21 and 28 days of fermentation and ripening. Two sausages were weighed and the mean value was used.

2.2.3. Consistency

Consistency measurements express the firmness of the product. Consistency (kg) of the sausage was measured from a 5-cm long piece of dry sausage by using an Instron Universal Testing Machine TM-M (Instron Ltd., High Wycombe, England) after 3, 7, 14 and 28 days of fermenting and ripening. The peeled sausages were compressed sideways 1 cm with a 5.5-cm diameter measuring device.

2.2.4. Biogenic amines

The formation of biogenic amines (mg/kg) in raw material (0 day) and from sausages was examined after 28 days of fermentation and ripening. Biogenic amines were extracted from 5.0 g of dry sausage with 0.4 M perchloric acid (Merck) and detected as their dansyl derivatives at 254 nm by high-performance liquid chromatography (HPLC-system HP 1090 Series M LC, diode-array detector including gradient module, binary DR5 pumps, autoinjector system with 25 μl loop) (Eerola et al., 1993). The limits of determination in this study for cadaverine, histamine, spermine, spermidine and putrescine were 1 mg/kg, and for tyramine and phenylethylamine 2 mg/kg.

2.3. Microbiological analysis

Ten grams of sausage material was taken aseptically from the core of the sausage and diluted in 90 ml sterile saline (9 g NaCl/l deionized water). Experimental sausages were examined microbiologically after preparation (0 d) and after 3, 7, 14 and 28 days of fermentation and ripening (Table 2). The numbers of inoculated lactic acid bacteria were determined by using both MRS and MRSV agar since all the experimental strains and the control strain are vancomysin (V) resistant (Hamilton-Miller and Shah, 1998; Tynkkynen et al., 1998).

After fermentation and ripening for 28 days, predominating colonies from each sausage were collected from MRS agar plates. A total of 36 predominating colonies isolated from 12 sausages

Table 2
Microbial groups, media and incubation conditions for enumerating representative bacteria from dry sausage

Microbial group	Medium	Incubation conditions
Lactic acid bacteria	MRS, pH 5.6 (Labm 93)	30°C, 3 days
Lactic acid bacteria	MRSV, pH 5.6 (Labm 93 and Abtek IS/LB-A)	30°C, 3 days
Staphylococci	Baird-Parker (Labm 85 and X085X)	37°C, 2 days

(three sausages produced with each experimental strain) were ribotyped by the automated RiboPrinter® Microbial Characterisation System (Qualicon™, USA) according to the manufacturer's instructions (Bruce, 1996). The RiboPrint® patterns of the isolates were compared to patterns from the inoculated strains.

2.4. Sensory evaluation

The sensory evaluation was performed after 28 days of ripening ($n = 24$) from dry sausage slices (h = 3 mm) by an eight-person panel familiar with the sensory evaluation of dry sausages. The panelists were asked to respond to the question 'Do you find the flavour of the experimental sausage better than/as good as/worse than the control sausage?'. Control sausage was fermented by the commonly used commercial starter culture Condi Rasant.

2.5. Statistical analyses

The reported differences between the results of physical, chemical and microbiological analysis of sausages fermented by different strains were tested with one-way analysis of variance (ANOVA, Statgraphics). The results of sensory evaluation were tested with χ^2-test. $P < 0.05$ values were considered to be significant.

3. Results and discussion

The numbers of inoculated lactic acid bacteria were determined on both MRSV and common MRS. The numbers obtained from these media correlated 95% with each other (results not shown). The numbers of lactic acid bacteria increased from 7 $\log_{10}$ cfu/g to 8 $\log_{10}$ cfu/g (LC-705 and GG) and 8.7–8.9 $\log_{10}$ cfu/g (control and E-97800) during the first 7 days of fermentation (results not shown). At the end of ripening the numbers of lactic acid bacteria were slightly lower, but the differences between strains remained the same. The numbers of staphylococci were approx. 6 $\log_{10}$ cfu/g after inoculation (0 d) and remained at that level until the end of ripening (28 d) (results not shown).

The same RiboPrint® patterns (genetic fingerprints) were obtained in from the sausage isolates as from the pure cultures of the relevant experimental strains. The fingerprint patterns of the experimental lactic acid bacterial strains were clearly different. The similarity between GG and LC-705 was 82%, GG and E-97800 91%, and LC-705 and E-97800 71% (results not shown). The ribotyping results indicated that each of the inoculated bacterial strains was the dominating bacterial strain in the final product.

At 7 days of fermentation the pH values of the sausages were 5.1 (LC-705), 5.0 (E-97800 and control) and 4.9 (GG). At the end of the ripening time (28 days) the pH of the sausages fermented by GG and E-97800 was 4.9, while the pH of the sausages fermented by LC-705 and Control was 5.0.

Consistency at the end of the fermentation time was lower ($P < 0.05$) in sausages fermented by LC-705 than in control sausages (Table 3.). The consistency of the other experimental sausages was not different ($P > 0.05$) from that of the control sausage. The weight losses were at the level of 40% at the end of ripening (28 days) (results not shown).

The flavour of the sausages fermented by GG and E-97800 was considered to be as good as the flavour of sausages fermented by control, while the flavour

Table 3
Consistency values (kg) during ripening of dry sausages ($n = 9$)

Day	GG		LC-705		E-97800		Control	
	x	S.D.	x	S.D.	x	S.D.	x	S.D.
7	3.3^{a}	0.2	3.2^{a}	0.2	3.6^{a}	0.2	3.5^{a}	0.1
14	5.1^{a}	0.3	$6.1^{a,b}$	0.7	$6.8^{b,c}$	0.5	7.7^{c}	0.5
28	18.6^{b}	2.3	13.5^{a}	0.5	18.1^{b}	0.3	21.2^{b}	2.0

^{a,b,c} Different letters indicate significant difference ($P < 0.05$) between dry sausages fermented and ripened over the same period of time using different strains. x = mean; S.D. = individual standard deviation.

of sausages fermented by LC-705 was inferior ($P < 0.05$) to that of sausages fermented by the control strain (results not shown).

However, more research is needed before final conclusions concerning the impact on sensory properties of these probiotic strains can be reached. The flavour profiles of the dry sausages fermented by these probiotic strains are under study (Erkkilä et al., 2001).

Buckenhüskes (1993) has proposed the absence of biogenic amine formation as a selection criterion for new strains used as meat starter cultures. Biogenic amines are generated by the microbial decarboxylation of amino acids (Askar and Treptow, 1986) and they are generally present in dry sausages, as reviewed by Maijala (1994) and Eerola et al. (1998). The consumption of high amounts of amines may cause toxicological effects (Taylor, 1986; Edwards et al. 1987).

In our study the results indicate that selected *L. rhamnosus* strains do not form biogenic amines. Furthermore, the concentrations of tryptamine, putrescine, cadaverine, histamine, tyramine and spermine in the raw material were low, <5 mg/kg (results not shown). The mean concentration of phenylethylamine was 19.0 mg/kg, and of spermidine 24.0 mg/kg. At 28 days of fermentation and ripening the concentration of tryptamine, putrescine, spermine and spermidine in the sausages remained at the same level as in the raw material. There were only slight increases in the amounts of phenylethylamine, cadaverine and histamine. The levels of tyramine increased approximately ten-fold reaching a level of 25 mg/kg in all sausages (results not shown).

The low amount of biogenic amines in dry sausage was also a sign of the good quality of the raw materials and the favourable hygienic processing conditions as previously discussed by Maijala (1994) and Hernàndez-Jover et al. (1996).

It can be concluded that Probiotic *Lactobacillus rhamnosus* GG, LC-705 and E-97800 seem to be suitable for the production of dry sausages. *L. rhamnosus* E-97800 had the fastest growth rate and acidification. However, the results indicated that *L. rhamnosus* LC-705 was not highly adapted to a the meat environment, resulting in slower growth rates and acidification of the dry sausage.

The inoculated experimental strains were predominant in the sausages. However, as Hammes and Haller (1998) pointed out, health promoting effects caused by probiotics in dairy products should not be generalised. Meats provide differing environments for probiotic bacteria, and more research is required to find the eventual health effects of these probiotic dry sausages. The next steps in this research area will be clinical experiments to determine the dosages of healthy bacteria that are necessary to achieve beneficial effects.

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