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Effect of high intensity ultrasound on the fermentation profile of *Lactobacillus sakei* in a meat model system

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A B S T R A C T

The objective of this study was to investigate the efficacy of high intensity ultrasound on the fermentation profile of *Lactobacillus sakei* in a meat model system. Ultrasound power level (0–68.5 W) and sonication time (0–9 min) at 20 °C were assessed against the growth of *L. sakei* using a Microplate reader over a period of 24 h. The *L. sakei* growth data showed a good fit with the Gompertz model ($R^2 > 0.90$; SE < 0.042). Second order polynomial models demonstrated the effect of ultrasonic power and sonication time on the specific growth rate (SGR, μ, h^{-1}) and lag phase (λ, h). A higher SGR and a shorter lag phase were observed at low power (2.99 W for 5 min) compared to control. Conversely, a decrease ($p < 0.05$) in SGR with an increase in lag phase was observed with an increase in ultrasonic power level. Cell-free extracts obtained after 24 h fermentation of ultrasound treated samples showed antimicrobial activity against *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella typhimurium* at lower concentrations compared to control. No significant difference ($p < 0.05$) among treatments was observed for lactic acid content after a 24 h fermentation period. This study showed that both stimulation and retardation of *L. sakei* is possible, depending on the ultrasonic power and sonication time employed. Hence, fermentation process involving probiotics to develop functional food products can be tailored by selection of ultrasound processing parameters.

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1. Introduction

Ultrasound technology has been employed in various food and bioprocess engineering applications and its use has been directed at attempting to improve process efficiency [1–3]. Application of ultrasound alone, or in combination with other techniques, can be employed to inactivate enzymes and spoilage or pathogenic microorganisms. Inactivation of microorganisms during sonication is due to singular or combination of several chemical and physical effects, including; thinning/disruption of cell membranes, localised heating, intracellular cavitation and sonolysis of water ($H_2O \rightarrow H^+ + OH^-$) leading to the production of free-radicals [4]. The effectiveness of ultrasound on microorganisms is strongly influenced by intrinsic and extrinsic factors, including; microbial ecology (e.g., type of microorganism, medium type and composition), ultrasound parameters (e.g., ultrasound power and frequency), sonication time, pH and temperature [5]. For example, studies have shown that Gram-positive bacteria are more resistant to ultrasound compared to Gram-negative bacteria, probably due to the presence of a thick peptidoglycan layer in the cell membrane of Gram-positive bacteria [6]. In recent years, application of ultrasound technology has expanded beyond simple inactivation of microorganisms. Ultrasound has demonstrated its capacity to enhance the growth of beneficial microorganisms and activity of enzymes, for the production of commercially valuable macromolecules. Beneficial effects of sonication is mainly attributed to the formation of pores on microbial cell membranes, thereby providing a channel for transport of essential nutrients and removal of toxic substances across these membranes [7–9].

Probiotics are live microorganisms primarily derived from *Lactobacillus* and *Bifidobacterium* species which, when ingested in sufficient quantities, can exert beneficial health effects in the host [10]. Lactic acid bacteria (LAB) are Gram-positive bacteria which are widespread in nature and adapted to grow under different environmental conditions. Application of *Lactobacillus* sp. in food products has shown to inhibit the growth of pathogenic and spoilage microorganisms, improve organoleptic properties of food [11,12] and reduce the rate of food spoilage through competitive control of spoilage microorganisms. Additionally, *Lactobacillus* sp. are reported to have beneficial effects on gut health and play a vital role in the alleviation of metabolic diseases [13,14].

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Lactobacillus sakei is generally regarded as a safe strain in food, with its ability to grow at low temperatures and high salt concentrations. Consequently, *L. sakei* strain has been used as a starter culture in the development of various fermented food products. Bacteriocins and other metabolites obtained from probiotics, including *L. sakei*, have been demonstrated to inhibit the growth of various pathogenic microorganisms.

It is evident that ultrasonic treatment can inactivate or activate microorganisms depending on the ultrasound control parameters of ultrasonic power, frequency and sonication time. For example, high intensity sonication treatment of milk containing *Bifidobacterium* sp. has been shown to enhance the production rates of organic acids and the growth of *Bifidobacterium* sp., while concurrently reducing fermentation time [15,16]. Similarly, Yang, Zhang and Wang [17] demonstrated that ultrasound pre-treatment, followed by incubation, enhances the growth of *Brevibacterium* sp. However, the effect of high intensity sonication on the growth behaviour of *L. sakei* has not been reported to date. The objective of this study was to (i) investigate the effect of ultrasound treatment on the growth behaviour of *L. sakei* in MRS broth and meat extract; (ii) characterise the cell-free extract for molecular weight distribution after fermentation and (iii) test the efficacy of cell-free extract against selected pathogenic microorganisms.

2. Material and methods

2.1. Culture and sample preparation

L. sakei DSM 15831 was obtained from DSM, Germany. The bacteria were cultured in Man Rogosa Sharpe (MRS; Oxoid Ltd., Cambridge, UK) medium at 30 °C in an microaerophilic chamber for 48 h. Microbial cells were harvested at 5000×*g* for 10 min and subsequently added to meat model system (MRS broth). MRS broth is considered as a best meat model system for fermentation studies [18]. MRS broth (Oxoid Ltd., Cambridge, UK) was prepared as per the manufacturer's instruction. Additionally, fermentation studies were also carried out in the meat extract. Meat extract was prepared by cooking minced beef meat (500 g) in 2 L of water at 90 °C for 30 min, followed by centrifugation at 4000×*g*. Fat collected from the upper surface of samples was removed manually and the supernatant autoclaved at 121 °C for 15 min, and subsequently cooled to room temperature.

2.2. Ultrasound treatment

MRS broth and meat extract solution (100 mL) containing *L. sakei* (1×10^6 cfu/mL) culture was sonicated in sterilised containers. The ultrasonic treatment of samples was carried out using a 550 W ultrasonic probe (XL2020 Heat Systems, Misonix Inc., Farmingdale, NY, USA) operating at 20 kHz with a 13 mm diameter ultrasonic probe operating in an amplitude range of 24–120 μm. The energy input was controlled by setting the amplitude of the ultrasound probe and ultrasonic power (P) power dissipated to the sample was calculated by using the method outlined by Tiwari, Muthukumarappan, O'Donnell and Cullen [19] using Eq. (1). Samples were subjected to sonication treatment at varying power level (0–68.5 W) and sonication time (0–9 min) operating at a frequency of 20 kHz. The temperature of the samples during sonication was monitored using thermocouples (Radionics, Ireland). Samples were sonicated at a temperature of ca. 20 °C and the final temperature of samples ranged from 30 to 40 °C depending on the applied ultrasonic power. These conditions were selected based on preliminary studies carried out prior to this study. Samples without ultrasound treatment were considered as the control. Samples were incubated at 30 ± 0.5 °C for 24 h in a temperature controlled incubation chamber (Model HCP 246, Memmert, Germany).

$$P = mC_p\left(\frac{dT}{dt}\right)_{t=0} \tag{1}$$

where (dT/dt) is the change in temperature over time (°C min^{-1}), C_p is the specific heat of water (4.18 kJ kg^{-1} °C^{-1}), and m is the mass (kg).

2.3. *L. sakei* growth measurement

The growth of control and ultrasound treated *L. sakei* was monitored by measuring the optical density change at a regular interval of 30 min using 96 well plates in a temperature controlled microbial growth analyser (Multiskan™ Microplate Spectrophotometer, Thermo Scientific) at a wavelength of 595 nm.

2.4. Cell-free extract preparation

Cell-free extract samples were obtained by centrifuging the samples at 4000×*g* for 15 min at 20 °C. The supernatant obtained was filtered using a sterile 0.22 μm pore size filter unit (Millex GS Millipore) and subsequently, freeze-dried. Freeze-dried samples were stored at 4 °C for molecular weight analysis, SDS–PAGE and antimicrobial activity.

2.5. Lactic acid estimation

Lactic acid estimation was carried out using the method outlined by Nguyen, Lee and Zhou [16] with slight modifications. HPLC (Waters, e2695 Separation module) equipped with an autosampler and Rezex ROA-Organic acid H^+ (8%) (350 mm × 7.8 mm) column (Phenomenex, UK) was utilised. Fermented MRS broth and meat extract solution (1 g) was mixed with distilled water (25 mL) and centrifuged at 10,000×*g* for 10 min. samples were filtered using a 0.45 μm syringe filter.

2.6. Molecular weight analysis and SDS–PAGE

High Performance Size Exclusion Chromatography (HP-SEC) for each sample was carried out using a Waters Alliance 2795 Chromatography Separations Module (Waters Corp., Milford, USA) coupled to a Waters 2996 PDA detector at a wavelength of 254 nm. Separation was achieved using Tris–HCl 0.1 M as a mobile phase (0.85 mL/min) and serial connected Zorbax GF-450 (9.4 × 250 mm, 6 μm) and Zorbax GF-250 (4.6 × 250 mm, 4 μm) columns. A calibration curve was developed using albumin (66 kDa), carbonic anhydrase (29 kDa), cytochrome c (12.4 kDa), aprotinin (6.5 kDa), angiotensin II acetate (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe, 1.046 kDa) and leucine enkephalin (Tyr-Gly-Gly-Phe-Leu, 0.555 kDa).

SDS–PAGE was carried out using a Compact PAGE AE-7350 (Atto, Tokyo, Japan) and ready-made 5–20% polyacrylamide gels (c-PAGEL compact gel C520L; Atto), as recommended by the manufacturer. Each extract solution (100 μg/mL) was mixed with an equal volume of 80 mM Tris–HCl buffer (pH 6.8) containing 140 mM SDS, 8 M urea, 20% glycerol, 0.01% bromophenol blue and 200 mM dithiothreitol and heated to 100 °C for 5 min. Following denaturation, an 8 μL aliquot was subjected to electrophoresis. BLUE Star Pre-stained Protein-Ladder (Nippon Genetics, Tokyo, Japan) was used as a reference. On completion of the analysis, proteins were visualised by staining with Quick-CBB Plus (Wako, Osaka, Japan).

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