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Modeling of growth and bacteriocin production by Leuconostoc *mesenteroides* E131

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Abstract

Leuconostoc mesenteroides E131, isolated from dry fermented sausages, produces an antimicrobial agent, characterized as bacteriocin. The effect of pH and temperature on growth and bacteriocin production, using MRS broth as growth medium, was studied in a fermentor. The pH value at which the best cell growth was observed (6.5) did not coincided with the value at which the maximum bacteriocin activity was attained (5.5). In contrast, the maximum bacteriocin activity was attained at temperature (25 °C) close to the optimum temperature for cell growth (25–30 °C). Notably, the range of pH and temperature for good bacteriocin production was within the range used for sausage fermentation. An empirical model was developed to describe the growth and bacteriocin production in different pH and temperature conditions. The model was able to describe growth and bacteriocin production and it could be used to predict the kinetic parameters of growth and bacteriocin production within the pH and temperature range examined.
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Keywords: Modeling; Predictive microbiology; *Leuconostoc mesenteroides*; Bacteriocins; Fermentor; Meat products

1. Introduction

Many lactic acid bacteria produce a variety of antimicrobial compounds, such as organic acids, carbon dioxide, hydrogen peroxide, ethanol, diacetyl and bacteriocins (De Vuyst & Vandamme, 1994; Hammes & Hertel, 1996). Bacteriocins are peptides able to inhibit at small concentrations the growth of undesirable bacteria, such as spoilage microorganisms and certain foodborne pathogens (*Listeria monocytogenes* and *Staphylococcus aureus*). Lactic acid bacteria are well adapted to the meat environment and, thus, are able to compete, sufficiently, other undesirable microorganisms (De Vuyst, Callewaert, & Crabbe, 1996; Hugas, Garriga, Aymerich, & Monfort, 1995).

Predictive modeling is applied in order to predict the response of undesirable microorganisms (spoilage and pathogens) to specified environmental conditions (McKellar & Lu, 2004; Skandamis & Nychas, 2003). These models can be used by the meat industry for example in a HACCP plan to predict the shelf-life of the products (Armitage, 1997; McDonald & Sun, 1999). Recently, mathematical equations have been developed, describing the cell growth and bacteriocin production of bacteriocin-producing strains, added to foods as starter or protective cultures (Leroy & De Vuyst, 1999; Leroy & De Vuyst, 2003; Leroy, Verluyten, Messens, & De Vuyst, 2002; Messens, Neysens, Vansieleghem, Vanderhoeven, & De Vuyst, 2002; Messens, Verluyten, Leroy, & De Vuyst, 2003). Modeling contributes to the determination of how environmental conditions influence the growth and bacteriocin production by bacteriocinogenic strains (Leroy et al., 2002; Leroy & De Vuyst, 2003).

The objective of this study was to simulate the kinetic behaviour of *Leuconostoc mesenteroides* E131 in response to commonly encountered environmental conditions (pH and temperature). In this study, the effect of pH and

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temperature that prevail during sausage fermentation, on the growth and bacteriocin production by *L. mesenteroides* E131, was investigated, in order to develop an empirical model capable of simulating the kinetics of cell growth and bacteriocin production.

2. Materials and methods

2.1. Fermentations

The fermentations to study pH and temperature effects on cell growth and bacteriocin production were carried out in a 3-l fermentor equipped with the instrumentation for measurement and control of temperature and pH (MBR BioReactor AG, Switzerland) with 1.5-l culture medium. The fermentor containing the medium (MRS broth, Merck, Darmstadt, Germany) was heat-sterilized at 110 °C for 40 min. The medium was inoculated with 1% v/v (15 ml) inoculum of a 24 h culture of *L. mesenteroides* E131. Agitation was performed at a speed of 100 rpm to keep the fermentation broth homogeneous. The dissolved oxygen during the fermentation was depleted from its initial concentration of 55–60% to 3–7% at the end of exponential growth phase. The pH was controlled by the addition of 5 N NaOH or 5 N HCl. Two additional fermentations for the validation of the model were conducted. All fermentations were done in duplicate. The replicates showed repeatability, thus, the mean value of each parameter (μ_{max}, X_{max}, k_b, k_{inact}, $Y_{X/S}$ and m_S) was taken to be modeled.

At constant time intervals, 50 ml samples were aseptically withdrawn from the fermentor to measure optical density (OD) at 600 nm, bacteriocin activity, glucose, lactic and acetic acid concentration. Optical density was transformed into cell dry mass (CDM l^{-1}). Cell dry mass was determined by linear regression from plots of OD versus cell dry weight (g l^{-1}) at points during the exponential growth phase. The residual glucose concentration was measured using the Glucose PAP kit (EliTech, Labarthe Inard, France). Cell free samples, needed for the measurement of lactic and acetic acid with high-performance liquid chromatography (SpectraSystem P2000, Spectra-Physics Analytical, USA), were prepared as follows: in 1 ml of supernatant fluid, after centrifugation (10,844*g* for 15 min at 4 °C) and sterilization through microbiological filter (Acrodisc, 0.22 μm) (Gelman, Michigan, USA), 10 μl of Trifluoroacetic acid (TFA, Merck) was added and incubated at 4 °C for 18 h (overnight), for the precipitation of peptides. After centrifugation (10,844*g* for 15 min at 4 °C) the supernatant fluids were filtrated through 0.22 μm microbiological filter (Millex-GV, Millipore, Bedford, USA) and analyzed with HPLC. High-performance liquid chromatography analysis was performed under the following conditions: HPLC Organic Acid Analysis Column, Aminex HPX-87 H Ion Exclusion Column, 300 × 7.8 mm (Bio-Rad Laboratories, California, USA); sample, fermented MRS broth, 20 μl; eluent, 0.005 M sulfuric acid (Merck); flow rate, 0.7 ml min^{-1}; temperature, 65 °C; Detection, UV at 210 nm (Spectra FOCUS, Spectra-Physics Analytical).

The influence of pH on growth and bacteriocin production was studied at values of 5.0, 5.5, 6.0 and 6.5 ± 0.1 at the optimum temperature for growth (30 ± 0.1 °C). The influence of temperature was studied at 10, 14, 18, 25 and 30 ± 0.1 °C at the optimum pH for bacteriocin production. The conditions (pH and temperature) for the two additional fermentations were: 6.5, 25 °C; and 5.5, 20 °C, respectively.

2.2. Bacteriocin activity assay

The cells were harvested by centrifugation at 10,844*g* for 15 min at 4 °C and the supernatant fluid was adjusted to pH 6.2–6.5 with 5 N NaOH, then it was treated with catalase and sterilized through a 0.22 μm microbiological filter (Acrodisc, Gelman). To determine the bacteriocin activity, two-fold dilutions of the cell free extracts were made in sterile 1/4-strength Ringer's solution. Then 50 μl of the diluted samples were spotted on MRS agar plates containing the indicator strain, *Lactobacillus curvatus* E33. The lawns were prepared by adding a 24 h culture, grown in MRS broth and incubated at 30 °C, of the indicator strain in MRS agar. After incubation at 30 °C for 24 h the arbitrary units of activity (AU ml^{-1}) of the bacteriocin were determined as the reciprocal of the highest dilution showing inhibition of the indicator strain (Barefoot & Klaenhammer, 1983). The bacteriocin-producing strain (*L. mesenteroides* E131) and the indicator strain (*Lb. curvatus* E33) were isolated from naturally fermented Greek sausages.

2.3. Calculation of the various parameters

The mathematical estimation of the parameters was performed using the following equations (Lejeune, Callewaert, Crabbe, & De Vuyst, 1998; Leroy & De Vuyst, 1999):

Cell growth:

$$\frac{dX}{dt} = \mu_{max} \times (1 - X/X_{max}) \times X \tag{1}$$

Bacteriocin production:

$$\frac{dB}{dt} = k_b \times (dX/dt) - k_{inact} \times X \times B \tag{2}$$

Glucose consumption:

$$\frac{dS}{dt} = -\left(\frac{1}{Y_{X/S}}\right) \times \left(\frac{dX}{dt}\right) - m_S \times X \tag{3}$$

Lactic or acetic acid production:

$$\frac{dL}{dt} = -Y_{L/S} \times \left(\frac{dS}{dt}\right) \tag{4}$$

$$\frac{dA}{dt} = -Y_{A/S} \times \left(\frac{dS}{dt}\right) \tag{5}$$

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