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Predictive modeling of the growth of *Lactobacillus viridescens* under non-isothermal conditions

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Abstract

Food spoilage by microorganisms is a major problem that can generate large economic losses to industries, making critical the application of technologies for predicting shelf life, aiming to obtain products with higher quality. The Lactic Acid Bacteria (LAB), including *Lactobacillus viridescens*, are among the main groups of microorganisms responsible for spoilage of refrigerated meat products, vacuum packed and under modified atmosphere. The growth of the LAB can be predicted by mathematical models, which describe the influence of various environmental factors (such as non-isothermal conditions) on microbial growth. The objective of this study was to obtain a mathematical model able to predict the growth of *L. viridescens* in non-isothermal conditions in culture medium (MRS broth). Six isothermal growth curves (at 4, 8, 12, 16, 20 and 30 ºC) were described by Baranyi and Roberts model and the dependence of maximum specific growth rate (μ_{max}) parameter on the temperature was described by square root secondary model. The model was validated using *L. viridescens* experimental data in the temperature ranging from 6 to 10 °C and 5 to 11 °C, changing every 12 and 24 h, respectively. The results showed that it was possible to predict safely (bias factor greater than 1) the growth of *L. viridescens* in MRS broth under non-isothermal conditions. The observed prediction deviations may have been caused by abrupt temperature changes, generating intermediate adaptation phases.

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Keywords: non-isothermal conditions; predictive modeling; *Lactobacillus viridescens*

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1. Introduction

The lactic acid bacteria (LAB) are Gram positive, non-spore-forming, strictly fermentative producing lactic acid as a major end product[1]. These bacteria are present in the natural microflora of meat products under vacuum and modified atmosphere, and it is considered among the main bacterial species that causes the spoilage. *Lactobacillus viridescens* (*Weissella viridescens*) is considered one of the most common species in the deterioration of meat products[2].

The spoilage of meat and meat products during processing, distribution, storage and sale is a subject of great interest in the literature[3]. Meat is considered a highly perishable food and requires a very detailed storage control. The temperature is one of the main factors associated with the spoilage of meat products[4,5]. Thus, the control of temperature throughout the meat processing and storage is of paramount importance to the safety and quality of food[3].

Monitoring food storage temperature helps to control the proliferation of spoilage microorganisms, such as LAB. According to the Brazilian Sanitary Surveillance[6], meat products must be stored in a temperature range between 0 °C and 10 °C, but that is not the real situation in cold chain of refrigerated products in Brazil. Temperature abuses impact shelf life of these products, and the inadequate refrigeration of food is still a factor that causes food spoilage, even with many recommendations on the handling of chilled foods and their ideal storage temperatures[7,8,9].

The knowledge and control of the microbiota present in the raw material during meat processing are essential for ensuring the quality and sensorial characteristics of the final product. During the storage and distribution of food, the temperature of these foods may vary dramatically. Therefore, it is necessary to develop a non-isothermal model able to describe the influence of temperature fluctuations on microbial growth. These models can be validated and used to predict the shelf life of foods, as they can simulate the microbial growth in a certain temperature range[4,10].

The study of the growth kinetics of *L. viridescens* in culture medium, considering the temperature changes, allows knowledge of the microbial growth, and can be used to compare with the actual conditions of contamination in meat products. Modeling LAB growth in culture medium is easier than in food matrices and results can be extrapolated and they are useful for estimating the growth of these bacteria in foods. This subject have great interest to the meat industries because the investigation allow different possibilities to control the development of undesirable microorganisms in food. The objective of this study was to obtain a mathematical model able to predict the growth of *L. viridescens* under different non-isothermal conditions in MRS broth.

2. Material and methods

2.1. Microorganism and inoculum preparation

L. viridescens (CCT 5843 ATCC 12706, Lot 22.07) used in this study was purchased in lyophilized form from André Tosello Foundation of Tropical Culture collection (Campinas, Brazil). The strains were rehydrated, grown in MRS broth (Difco, Le Pont de Claix, France). Then it was stored in Eppendorf tubes with MRS medium containing 20 % glycerol at – 24 °C until its use. The reactivation to prepare seed culture was carried out in MRS medium at 30 °C in incubators (Dist, Florianopolis, Brazil) for 18 h[11].

2.2. Growth conditions and growth curves

The experiments in non-isothermal conditions were conducted in 500 mL Erlenmeyer flasks with 320 mL of MRS broth and 1 % (v/v) of seed culture. The initial concentration of the experiments was approximately 10^3 CFU.mL^{-1} and the initial pH was 6.0 (pH meter V620, Analion, Ribeirao Preto, Brazil). The flasks were inserted in incubators (Dist, Florianopolis, Brazil) in two temperature profiles ranging from 6-10 °C and 5-11 °C, changing every 12 and 24 h, respectively. All experiments were conducted until the stationary growth phase, and the temperature was recorded by mini data loggers (Testo 174, Lenzkirch, Germany).

For growth curves construction, the classic method of counting viable cells to determine the growth of *L. viridescens* over time was applied. MRS agar (Difco, Le Pont de Claix, France) with a double layer was used and inserted in incubators (Dist, Florianopolis, Brazil) at 30 °C for 48 h. The count was expressed in CFU.mL^{-1}.