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Original Article

THE PREBIOTIC INFLUENCE OF INULIN ON GROWTH RATE AND ANTIBIOTIC SENSITIVITY OF *LACTOBACILLUS CASEI*

DEBOLINA BANERJEE[1], RANJANA CHOWDHURY[1*], PINAKI BHATTACHARYA[2]

[1]Chemical Engineering Department, Jadavpur University, Kolkata 700032, India, [2]Chemical Engineering Department, Heritage Institute of Technology, Kolkata 700107, India
Email: ranjana.juchem@gmail.com

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ABSTRACT

Objective: This research study is focused on the prebiotic effect of inulin on the antibiotic sensitivity of *Lactobacillus casei* and on the determination of functionality of specific growth rate (μ) of the probiotic bacteria on the concentrations of lactose (C_L = 10-30 g/l) and inulin (C_I = 0.164-0.625 g/l) along with the optimization of growth condition through Response Surface Methodology (RSM).

Methods: The sensitivity of *Lactobacillus casei* towards norfloxacin was determined using well diffusion method. Using the initial values of μ (h^{-1}) of *Lactobacillus casei* at different values of C_L (g/l) and C_I (g/l), the functionality of μ on the concentrations of the carbon sources have been derived, and the optimum condition has been identified.

Results: Although *Lactobacillus casei* is sensitive to norfloxacin, resistance is developed in the presence of inulin. Quadratic model equation $\mu = 0.83+0.054*C_L-0.035*C_I-0.049*C_L*C_I-0.29*C_L^2-0.33*C_I^2$ is valid and the optimum value of specific growth rate is 0.8285 h^{-1} at C_L = 20 g/l and C_I = 0.32 g/l.

Conclusion: The interesting observation of the development of antibiotic resistance of *Lactobacillus casei* in the presence of inulin suggests that the intake of probiotic *Lactobacillus casei*, may be done along with prebiotic inulin when a patient is treated with antibiotics like norfloxacin. Moreover, the model equation correlating the functionality of growth rate of *Lactobacillus casei* on lactose and inulin will be helpful in fortifying the probiotic milk products and drugs with prebiotics like inulin.

Keywords: *Lactobacillus casei*, Prebiotic, Inulin, Antibiotic sensitivity, Statistical growth model, Optimization of specific growth rate, Response Surface Methodology

INTRODUCTION

Synergistic combinations of probiotic bacteria and prebiotic carbohydrates are new concepts in food processing [1]. Prebiotic bio-molecules enhance the growth rate of probiotics which in turn act against pathogenic bacteria through the secretion of bacteriocin [2]. Thus, prebiotics may be combined with dairy products namely yoghurt etc. which contain an array of probiotic bacteria [3]. Among different probiotics, inulin, a fructan consisting of glycosidic bonds like fructosyl-fructose with a terminal glucose unit is one of the most popular one [4-7]. Ideally, the prebiotic molecule should not support the growth of pathogens. Although the synbiotic combinations of suitable probiotic-prebiotic pairs are expected to be beneficial for human health, the assessment should be made about the sensitivity of the combinations of broad spectrum antibiotics. The genus *Lactobacillus* (probiotic bacteria) is inherently resistant to tetracycline, vancomycin, erythromycin, streptomycin, clindamycin, gentamicin and oxacillin, but, there may exist some broad spectrum antibiotics against which probiotic strains are susceptible [8].

The combination with prebiotic may also influence the sensitivity of probiotics. No such study elucidating this fact is reported in the literature. Therefore, addressing all these issues regarding the application of synbiotics in food processing, the following novel objectives have been set in the present research by selecting the combination of *Lactobacillus casei (L. casei)* and inulin as the probiotic bacteria and prebiotic biomolecule respectively. These are determination of the sensitivity of *Lactobacillus casei* against a broad spectrum antibiotic, namely, norfloxacin with and without inulin; determination of a statistical growth model; optimization of the growth of *Lactobacillus casei* against the concentration of inulin and lactose using Response Surface Methodology. The functionality of specific growth rate of *Lactobacillus casei* on the concentration of carbohydrate sources, determined for the first time in this report, is very important for the overall production of the microorganism as well as for the metabolic products generated during growth.

MATERIALS AND METHODS

Chemicals

Beef extract (10 g/l) (Merck, India), Yeast extract (5 g/l), Peptone (10 g/l) (Himedia, India), Sodium acetate (5 g/l) (Himedia, India), dipotassium hydrogen phosphate (2 g/l) (Himedia, India), Tri-ammonium citrate (2 g/l) (Himedia, India), Magnesium sulphate (0.05 g/l) (Himedia, India), Manganese sulphate (0.05 g/l) (Himedia, India) and Lactose (20 g/l) (Merck, India) were used in the present research study. These are the composition of Modified de-Man Rogosa Sharp (MMRS) medium. Glucose is used as carbon source for standard de-Man Rogosa Sharp (MRS) medium.

Microorganisms

Lactobacillus casei (2651 1951 RPK) culture purchased from NCIM, Pune were used.

Prebiotic

Food grade commercial inulin purchased from Himedia, India.

Pre-adaptation of culture

Adaptation of the strain to Modified de-Man Rogosa Sharp (MMRS) medium containing a high concentration of lactose (50 g/l) and inulin (50 g/l) was performed by repetitive subculturing for three times. The pre-culture process was conducted in an incubator at 37 °C using 250 mL Erlenmeyer flasks for 18 h, based on sufficient growth ($5x10^{10}$cfu/ml).

The cell from the last adaptation experiment was stored at 4°C for use in further experiments. The pre-adaptation is needed to activate the culture at the high level of concentrations of lactose and inulin exceeding the upper limits of present concentration ranges for the two carbohydrate sources and to maintain the culture in the exponential phase.