INNOVARE JOURNAL OF ENGINEERING & TECHNOLOGY

Vol 4, Issue 2, 2016

ISSN - 2347-1573

Research 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]Department of Chemical Engineering, Jadavpur University, Kolkata - 700 032, West Bengal, India. [2]Department of Chemical Engineering, Heritage Institute of Technology, Kolkata - 700 107, West Bengal, India.

Email: ranjana.juchem@gmail.com

Received: 15 October 2015, Revised and Accepted:23 Febuary 2016

ABSTRACT

Objective: This research study is focused on the prebiotic effect of inulin on the antibiotic sensitivity of probiotic *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.

Methods: The sensitivity of *L. casei* toward norfloxacin was determined using well diffusion method. Using the initial values of μ (h^{-1}) of *L. 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 *L. 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 *L. casei* in the presence of inulin suggests that the intake of probiotic *L. casei* and may be done along with prebiotic inulin when a patient is treated with antibiotics such as norfloxacin. Moreover, the model equation correlating the functionality of growth rate of *L. casei* on lactose and inulin will be helpful in fortifying the probiotic milk products and drugs with prebiotics such as 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 biomolecules 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 prebiotics, inulin, a fructan consisting of glycosidic bonds such as fructosyl - fructose with a terminal glucose unit is one of the most popular [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 against broad spectrum antibiotics. Although the genus *Lactobacillus* is inherently resistant to tetracycline, vancomycin, erythromycin, streptomycin, clindamycin, gentamicin, and oxacillin, 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 objectives have been set in the present research by selecting the combination of *L. casei* and inulin as the probiotic bacteria and prebiotic biomolecule, respectively. These are: Determination of the sensitivity of *L. casei* against a broad spectrum antibiotic, namely, Norfloxacin with and without inulin; determination of a statistical growth model; optimization of the growth of *L. casei* against the concentration of inulin and lactose using response surface methodology (RSM). There are no data available in the literature in any of these aspects.

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), di-potassium hydrogen phosphate (2 g/L) (Himedia, India), tri-ammonium citrate (2 g/L) (Himedia, India), magnesium sulfate (0.05/L) (Himedia, India), manganese sulfate (0.05 g/L) (Himedia, India), and lactose (20 g/L) (Merck, India) were used in the present research study.

Microorganisms

L. casei (2651 1951 RPK) culture purchased from NCIM, Pune was used.

Prebiotic

Food grade commercial inulin purchased from Himedia.

Preadaptation of culture

Adaptation of the strain to a medium containing a high concentration of lactose (50 g/dm^3) and inulin (50 g/dm^3) was performed by repetitive subculturing for three times. The preculture process was conducted in an incubator at 37°C using 250 mL Erlenmeyer flasks for 18 hrs, based on sufficient growth (5×10^{10}cfu/mL). The cell from the last adaptation experiment was stored at 4°C for use in further experiments.

Statistical growth model

Batch experiments were conducted to determine the kinetics of growth of *L. casei* using both carbohydrate sources, namely, inulin and lactose simultaneously by varying the initial concentration of lactose, i.e., 10 g/L, 20 g/L, and 30 g/L at each initial inulin concentration of 0.00 g/L, 0.164 g/L, 0.32 g/L, and 0.63 g/L.

Antibiotic sensitivity

The sensitivity of the selected microorganisms, *L. casei* toward a common antibiotic, namely, norfloxacin was also tested using well diffusion method. MRS agar plate containing 20 g/L glucose and MMRS agar plate containing 20 g/L glucose and commercial inulin each was prepared. In each plate, 0.1 g norfloxacin was applied in the well. The *L. casei* cell culture was spread with a glass spreader. The plates were kept in an incubator at 37°C for 24 hrs.

Scanning electron microscopy (SEM)

SEM analysis of 24 hrs batch culture of *L. casei* using MMRS media, one containing 20 g/L lactose and the other containing 20 g/L lactose and 20 g/L commercial inulin each was used to observe the morphological changes of the bacterial cell.

Optimization

The multivariate functionality of μ_{ave} on two independent variables has been determined by conducting 13 batch mode experiments using the central composite design (CCD) technique to set the experimental conditions.

Experimental design and optimization

The face-centered CCD (FCCD) was created by entering the factors *viz.*, the concentration of lactose and concentration of inulin in terms of ±1 levels (Table 1) to perform RSM using Design-Expert 8.1 (Stat-Ease, Inc., Minneapolis, USA). The experiments were conducted randomly to avoid systematic biasness. Accordingly, a design layout was created using 13 experimental runs, with six center points. To investigate the effects of individual parameters as well as their interactive effects on the response variable, a general second order polynomial response surface model was selected and is expressed by Eq. (1):

$$Y_k = b_{k0} + \sum_{i=1} b_{ki} X_i^2 + \sum_{i=1} \sum_{j=1} b_{kij} X_i X_j \quad (1)$$

Where, Y_k is response variable, b_{k0} is a constant intercept; b_{ki}, b_{kii}, and b_{ij} are the linear, quadratic, and interaction regression coefficients, respectively; X_i and X_j represent the coded values of the process variables (factors). The regression Eq. (2) was considered for simultaneous multiple optimizations to maximize Y_k using the numerical optimization program of the same Design Expert software.

Experimental procedure

To ascertain reproducibility of the data, each experimental run was conducted in triplicate. FCCD has been used for the optimization study of *L. casei* where lactose concentration was varied from 10 to 30 g/L, and inulin concentration was varied from 0.164 to 0.624 g/L in MMRS medium. In a typical experimental run, all the operating variables were preset at a predetermined design value as per the experimental design layout shown in Table 2.

RESULTS AND DISCUSSION

Growth pattern of *L. casei* in presence of inulin

In Fig. 1, time histories of cell concentration have been plotted with inulin (0-0.624 g/L) as a parameter at initial lactose concentration of 20 g/L. From the analysis of the figure, it is evident that the cell concentration at any reaction time increases with the increase of inulin concentration and passes through the maximum at the inulin concentration of 0.32 g/L. Same tends have also been obtained for other concentrations of lactose (not shown).

Effect of inulin on antibiotic sensitivity

The photographs of petri-plates of *L. casei*, used for the determination of antibiotic (norfloxacin) sensitivity through well diffusion technique with and without inulin are shown in Figs. 2 and 3. By the measurement of the zone of inhibition, it is clear that while it measures 55 mm in the absence of inulin, no inhibition zone is observed in the presence of inulin. Thus, it is revealed that while the strain of *L. casei* used in the present investigation is inherently susceptible toward norfloxacin, resistance to the antibiotic is developed in the presence of inulin. The results signify that norfloxacin act against the *L. casei* strain used under the present investigation. The antibacterial effect of norfloxacin, a fluoroquinolone, may be through the inhibitory action on the enzyme DNA gyrase, essential for replication, repair and transcription of DNA and topoisomerase essential for entangling DNA of the bacteria under attack [9]. However, the exact mechanism may be determined by further investigation. The development of resistance in the presence of inulin may be due to the formation of coating of inulin around the cell wall as observed (Fig. 4b) in the case of growth of the same microorganism on lactose and inulin.

SEM image

The SEM images (Fig. 4a and b) of *L. casei* grown on lactose and lactose-inulin mixture, respectively, reveal that in the presence

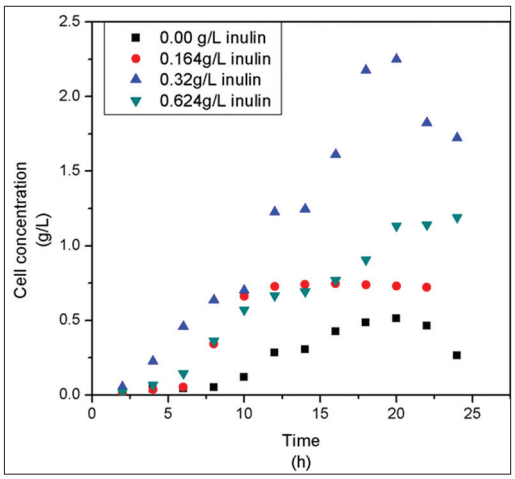

Fig. 1: Growth curves of *Lactobacillus casei* for 20 g/L lactose concentration using concentrations of inulin as a parameter. ■: without inulin, ●:0.164 g/L inulin, ▲: 0.32 g/L inulin ▼: 0.625 g/L inulin

Table 1: Experimental ranges and levels of the factors (process variables) for response surface study

Uncoded factor	Coded factor	unit	Uncoded value	Coded value
Lactose concentrations	A	g/L	10	−1
			20	0
			30	+1
Inulin concentrations	B	g/L	0.164	−1
			0.3225	0
			0.624	1

Table 2: Experimental design matrix

C_L (g/L)	C_I (g/L)	A (g/L)	B (g/L)	Response (μ) (hr^{-1})
2.00	0.32	0	0	0.8285
3.00	0.62	+1	+1	0.092
1.00	0.62	−1	+1	0.1823
2.00	0.75	0	+1	0.151
2.00	0.13	0	−1	0.2173
0.59	0.32	−1	0	0.12
2.00	0.31	0	0	0.8285
3.00	0.00	+1	−1	0.283
2.00	0.32	0	0	0.8285
1.00	0.00	−1	-	0.1787
2.00	0.32	0	1	0.8285
2.00	0.32	0	0	0.8285
3.41	0.32	+1	0	0.4143

of inulin, there is a formation of a distinct layer of inulin over the cell wall. This may be because inulin, being a large molecule cannot be transported inside the cells, and they are decomposed enzymatically to glucose and fructose, which are in turn assimilated by *L. casei* [10].

Dependence of specific growth rate (μ) on concentrations of lactose and inulin

The values of μ obtained at different values of C_L and C_I have been shown in Table 2.

In accordance with the statistical analysis model fit summary (Table 3), a quadratic model was selected as the best fitted with lower standard deviation (0.060) and lower PRESS value (0.18), predicted residuals sum of squares (1.27), higher adjusted R^2 (0.9666), regression coefficient (0.9108), predicted R^2 regression coefficient (0.8613), and adequate precision in comparison to linear and 2FI, i.e., two-factor interaction model. The data obtained from ANOVA, "analysis of variance" (Table 4), for quadratic model shows insignificant lack of fit (sum of squares = 1.27>0.05), larger F value, Fischer test (70.39), high R^2 value (0.9805), and low "coefficient of variance" value (0.89). The model equation showing the dependence of specific growth rate simultaneously on initial concentration of lactose and inulin is as follows:

Table 3: Model fit summary statistics for final specific growth rate

SD	0.060
Mean	0.44
CV %	13.38
Press	0.18
R^2	0.9805
AdjR^2	0.9666
PredR^2	17.684

SD: Standard deviation, CV: Coefficient of variance

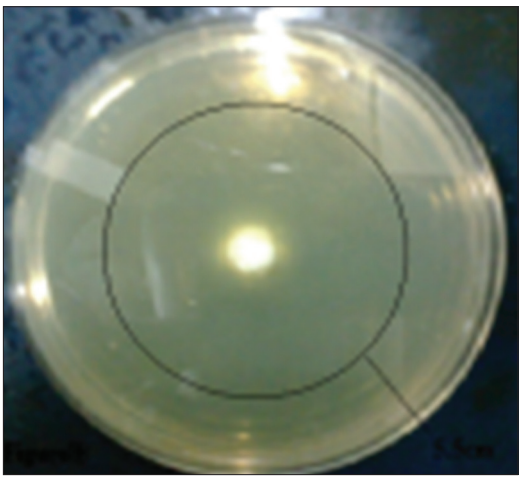

Fig. 2: Effect of norfloxacin on *Lactobacillus casei* in MRS plate. The marked area indicates the zone of inhibition (55 mm)

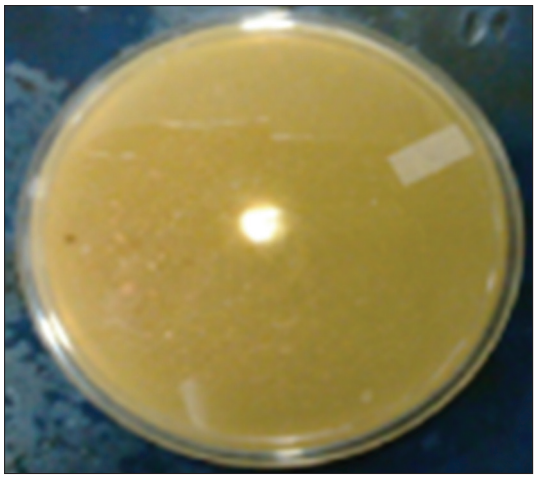

Fig. 3: Effect of norfloxacin on *Lactobacillus casei* in MMRS plate in presence of inulin

$$R1 = +0.83 + 0.054 * A - 0.035 * B - 0.049 * A * B - 0.29 * A^2 - 0.33 * B^2 \quad (1)$$

Where, A and B are the concentrations of lactose and inulin From Eq. 2, it is evident that the factors A, B, and A^2 affected the change in weight of the sample positively.

The ANOVA table is shown in Table 4.

Optimization study

To understand the effect of lactose concentration and inulin concentration on specific cell growth rate, the result obtained through response surface methodology technique have been presented in three dimensional plot shown in Fig. 5a. It is observed that the maximum specific growth rate of *L. casei* is obtained at 20 g/L of lactose concentration and 0.32 g/L inulin concentration. The contour plot shown in Fig. 5b clearly confirms the optimum values of the response variables (Table 5).

CONCLUSION

From the experimental findings, it is ascertained that the presence of inulin imparts resistance in *L. casei* against norfloxacin, a broad spectrum antibiotic. Thus, inulin may be mixed with dairy products using *L. casei* to avoid the destruction of probiotic cells in the human body by the antibiotic. From response surface methodology, it is observed that the specific growth rate is maximum (0.8285 hrs^{-1}) at concentrations of lactose and inulin of 20 g/L and 0.32g/L, respectively.

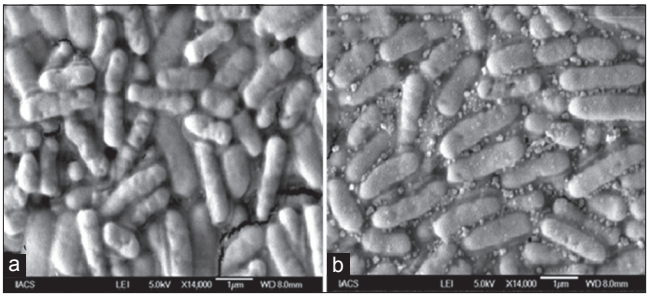

Fig. 4: Scanning electron microscopy (SEM) image of *Lactobacillus casei* cell without inulin (a), SEM image of *L. casei* cell in presence of inulin (b), Scale bar 1 μm

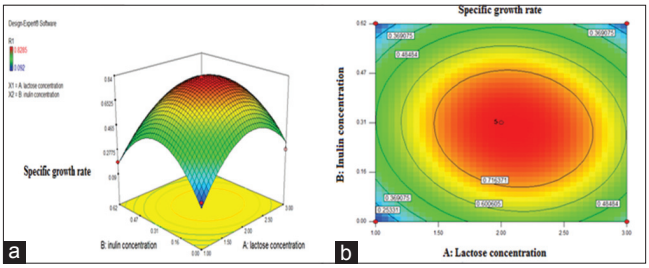

Fig. 5: (a) Three dimensional plot for optimization of specific growth rate of *Lactobacillus casei,* (b) Contour plot for optimization of specific growth rate of *L. casei*

Table 4: ANOVA for response surface quadratic model

Source	Sum of squares	df	Mean square	F value	P value P>F
Model	1.25	5	0.25	70.39	<0.001 (significant)
A - lactose concentration	0.023	1	0.023	6.53	0.0378
B - inulin concentration	9.882E-003	1	9.882E-003	2.79	0.1387
AB9.467E-003	1	9.467E-003	2.67	0.1460	
A^2 0.59	1	0.59	166.47	<0.0001	
B^2 0.77	1	0.77	217.31	<0.0001	
Residual	0.025	7	3.541E-003		
Lack of fit	0.025	3	8.262E-003		
Pure error	0.000	4	0.000		
Cor total	1.27	12			

Table 5: Solutions for optimal conditions of specific growth rate

Number	Lactose concentration	Inulin concentration	Desirability
1	2.00	0.32	1.0 (selected)
2	1.00	0.62	0.909
3	1.00	0.00	0.909

ACKNOWLEDGMENT

The first author (Debolina Banerjee) acknowledges the financial support offered by Council of Scientific and Industrial Research (CSIR), India by providing Senior Research Fellowship (File number: 9/96(0725)2K12-EMRI).

REFERENCES

1. Gaggìa F, Mattarelli P, Biavati B. Probiotics and prebiotics in animal feeding for safe food production. Int J Food Microbiol 2010;141 Suppl 1:S15-28.
2. Saad N, Delattre C, Urdaci M, Schmitter JM, Bressollier P. An overview of the last advances in probiotic and prebiotic field. LWT - Food Sci Technol 2013;50:1-16.
3. Kosin B, Rakshit SK. Microbial and processing criteria for production of probiotics: A review. Food Technol Biotechnol 2006;44(3):371-9.
4. Manso J, Luz MM, Yanez - Sedeno P, Jose Pingarron M. Bienzyme amperometric biosensor using gold nanoparticle-modified electrodes for the determination of inulin in foods. Anal Biochem 2008;375(2):345-53.
5. de Souza O, Perego P, de Oliveira MN, Converti A. Effect of inulin as prebiotic and synbiotic interactions between probiotics to improve fermented milk firmness. J Food Eng 2011;107:36-40.
6. Azza A, Abou-Arab Hala AT, Ferial M, Salem A. Physico-chemical properties of inulin produced from Jerusalem artichoke tubers on bench and pilot plant scale. Aust J Basic Appl Sci 2011;5(5):1297-309.
7. Toneli JT, Park KJ, Ramalho JR, Murr FE, Fabbro IM. Rheological characterization of chicory root (*Cichorium intybus* L.) inulin solution. Braz J Chem Eng 2008;25(3):461-71.
8. Ashraf R, Shah NP. Antibiotic resistance of probiotic organisms and safety of probiotic dairy products. Int Food Res J 2011;18(3):837-53.
9. Nelson DL, Cox MM. Lehninger Principles of Biochemistry. New York: W.H. Freeman; 2004. p. 778-80.
10. Ricca E, Calabrò V, Curcio S, Iorio G. Fructose production by chicory inulin enzyme hydrolysis: Kinetic study and reaction mechanism. Process Biochem 2009;44:466-71.