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OPTIMIZATION OF TANNASE POSITIVE PROBIOTIC PRODUCTION BY SURFACE RESPONSE METHODOLOGY

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Study in conditions *in vitro* of eight *Lactobacillus* strains procured from culture repositories for their probiotic potential and extracellular tannase activity was the aim of the research. Based upon acid, bile salt tolerance and antibiotic resistance *L. plantarum* MTCC 2621 with high tannase activity was selected for production studies. Optimization of nutrient medium in 3 L bioreactor was optimized by Surface Response Methodology based on the Full Factorial Central Composite Design. A factorial design 2^3 augmented by 6 axial points ($\alpha = 1.68$) and six replicates at the center point was implemented in 20 experiments. The optimized conditions were found to be pH 5.69, contain of lactose 128.58 g/l, peptone 8 g/l. A tenfold increase in the biomass production was observed using the optimized nutrient medium in bioreactor as compared to initial MRS medium.

Key words: *Lactobacillus*, optimization of medium, probiotic, response surface methodology.

Probiotics, according to conclusion of FAO/WHO joint commission, are «live microorganisms which when administered in adequate amount confer a health benefit on the host» [1]. Commonly as probiotics are used the heterogeneous group of lactic acid bacteria, such as lactobacilli, enterococci and bifidobacteria. Also, selected strains must survive in the extreme low pH of stomach and in the presence of bile salt which is to be encountered in the intestine. *Lactobacillus* is generally considered to be safe as they are the normal inhabitants in human and animal intestine and has long been used for its health benefits [2]. Some of the beneficial effects of *Lactobacillus* reported are increase immune response [3, 4], reduce antibiotic associated diarrhea [5, 6] and also decrease in irritable bowel syndrome [7–10].

Catechins (gallotannins and elligitannins) and other simple tannins (tannic acid) present in green, black tea and wine are known to have strong antioxidant properties [11]. Accumulating evidence indicates that they have the ability to suppress carcinogens through detoxification of various oxygen-free radicals generated in the body [12]. Enzyme acylhydrolase (EC. 3.1.1.20) popularly known as tannase, hydrolyses tannic acid completely to gallic acid and glucose [13]. Several reports are available on the production of tannase which hydrolyses gallotannins and elligitannins [14]. The product of enzymatic tannin hydrolysis, gallic acid has antifungal and antiviral properties. Gallic acid acts as an antioxidant and helps to protect human cells against oxidative damage. Several strains of *Lactobacillus* among bacteria are known to produce tannase. The presence of *Lactobacilli* with tannase activity in the human alimentary tract may thus have a significant effect on the medicinal properties of tannins [15]. The aims of recent study were the screening a probiotic *Lactobacillus* strain with high tannase activity and design a nutrient medium for it to achieve higher biomass production.

Materials and Methods

Bacterial strains and culture conditions. *Lactobacillus* strains, such as *L. acidophilus* 2902, *L. acidophilus* 2903, *L. brevis* 2436, *L. casei* 2586, *L. helveticus* 2733, *L. lactis* 2368 and *L. plantarum* 2912 obtained from National Collection of Industrially Important Microorganisms (Pune, India) and *L. plantarum* 2621 from Microbial Type Culture Collection (Chandigarh, India) were used. Frozen stock cultures were transferred to MRS broth (Man-Rogosa-Sharpe, Himedia) and incubated at 35 °C for

24 h and three successive transfers were carried out. The cultures were maintained in MRS agar plates and slants at 4 °C and were subcultured every 30 days. For routine analysis, *Lactobacillus* strains were subcultured in MRS agar every 48 h.

Extracellular tannase activity. Overnight grown *Lactobacillus* culture broth were transferred to MRS broth containing 0.2% gallic acid and incubated at 35 °C in a rotary shaker at 150 rpm for 24 h. 1 ml of the culture broth was centrifuged at 8000 rpm for 15 min. To 0.1 ml of supernatant 1ml of 0.3 mM tannic acid in 0.1 M citrate buffer (pH 3.5) was added. After 30 min incubation at 30 °C the reaction was stopped by adding 0.2 ml HCl (2 N). In the control, 0.2 ml of 2 N HCl was added to 0.1 ml of supernatant. After 30 min incubation 1ml of 0.3 mM tannic acid in 0.1 M citrate buffer was added. In the blank 0.1ml of 0.1 M citrate buffer was added to 1ml of 0.3 mM tannic acid in 0.1 M citrate buffer. After 30 min incubation 0.2 ml of 2 N HCl was added. The quantity of gallic acid released during hydrolysis of tannic acid represents the extracellular tannase activity. Gallic acid was measured with the rhodanine reaction. To 200 µl of standard sample, 300µl of rhodanine solution (0.667% in methanol) was added and the mixture is vortexed. After exactly 5 min, 4.5 ml of aqueous KOH (0.5 M) solution was added and after 20 min incubation, the absorbance was read at 520 nm. ΔA = Absorbance (Test — Blank) — Absorbance (Control — Blank). One unit (U) of tannase activity is defined as 1 µM of gallic acid released per minute in the assay conditions.

Acid and bile salt tolerance assay. For the acid tolerance, 1ml of overnight grown cultures in sterile MRS broth incubated at 35 °C was inoculated in 9 ml of sterile phosphate-buffered saline (PBS) adjusted to pH 2.5 with 2 N HCl and then incubated at 35 °C for 3 h. Bacterial culture inoculated in PBS adjusted to pH 7 was used as a control. After 0^{th} h and 3^{rd} h cells were serially diluted 7 fold in sterile PBS solution and the number of viable bacteria was determined on an MRS agar plate. The survival rate was calculated as log values of colony forming units per ml (CFU / ml).

For the bile salt tolerance, 1ml of overnight grown cultures incubated at 35 °C were transferred into 9 ml sterile MRS broth containing 1% and 2% bile salt (Himedia, India) and then incubated at 35 °C for 24 h. Bacterial cultures grown in sterile MRS broth without bile salt were used as control. After 24 h incubation cells were serially diluted 7 fold in sterile PBS solution and the number of viable bacteria was determined on an MRS agar plate. The survival rate was calculated as log values of CFU/ml.

Antibiotic resistance. Antibiotic resistance was examined by disk diffusion method [16]. *Lactobacillus* strains were grown in sterile MRS broth at 35 °C for 24 h. The suspensions were swabbed on MRS agar plates. Antibiotic discs (Himedia, India) — ampicillin 25 µg, chloramphenicol 25µg, erythromycin 15 µg, kanamycin 30 µg and tetracycline 30 µg were placed on the MRS agar plates and incubated at 35 °C for 48 h. The inhibition zone around the antibiotic disk was measured.

Optimization of medium for biomass production in bioreactor. The batch fermentations were carried out *in situ* in a 3 L sterilizable stirred bioreactor (Scigenics, India) equipped by dissolved oxygen- (DO) and pH-probes (Mettler Toledo). The DO- and pH-probes were calibrated using standard procedure before launching every batch. The trials were conducted under the following conditions: medium volume 1.6 L, inoculum volume 10%, temperature 35 °C. Aeration and agitation was controlled to keep dissolved oxygen levels above 20% of saturation all the time. pH was maintained by adding appropriate amount of 2 N HCl and 2 N NaOH and temperature was maintained at 35 °C by circulating hot/cold water through the jacket during the fermentation. Sampling was done aseptically for every 1 h interval for analysis. Polypropyleneglycol in concentration of 0.2% was used as the antifoam. 24 h old inoculum developed in MRS broth in shake-flasks was used for the study.

Response surface methodology using central composite design was applied to model the biomass production. The design was based upon the model provided by software Design-Expert (Stat-Ease Inc, Minneapolis, USA). With either a spherical design or a rotatable one, we need number of replicate centre points (points at the origin) to obtain good prediction variance stability. It has been found out that the variance stability will be high when large number of experiments is conducted at centre point. So, actual factorial design 2^3 was augmented by 6 axial points with $\alpha = 1.68$ (α is the distance between the «star points» and the centre of the design space. For a full factorial design (2^3), the formula is $\alpha = (2^3)^{1/4}$ and six replicates at the center point and was implemented in 20 experiments 2^3 (8 trials) plus 6 trials at axial points plus 6 replicate trials at centre points are equal 20 trials. Five levels of variation were selected for each variable shown in Table 1.

Table 1. **Experimental design**

Coded values (Z)	Actual values		
	Lactose (g/l)	Peptone (g/l)	pH
−1.68	52.73	5.27	4.39
−1	80	8	4.8
0	120	12	5.4
1	160	16	6
1.68	187.27	18.73	6.41

Preliminary experiments were carried out in 250 ml shaker flasks to determine the individual effects and proper ranges of independent variables using one-factor-at-a-time approach. Lactose concentration, peptone concentration and pH were found to affect the biomass productivity in shaker flasks (in present paper data not shown). Accordingly, these three variables were chosen for bioreactor experiments. Cultivations were carried out with medium containing 0.2 g/l $MgSO_4 \cdot 7\,H_2O$, 0.04 g/l $MnSO_4 \cdot H_2O$; 5 g/l yeast extract with lactose and peptone g/l being the independent variables.

The independent variables will be coded as Z:

$$Z = (X - X_0)/\Delta X \qquad (1),$$

were X — corresponding natural value; X_0 — natural value in the centre of domain; ΔX — increment of X corresponding to one unit of Z.

Second order model of response surface was used to calculate the predicted response (Y):

$$Y = \beta_0 + \Sigma\, \beta_i X_i + \Sigma\, \beta_{ii} X_i^2 + \Sigma\, \beta_{ij} X_i X_j \qquad (2),$$

where Y — response variable; β_0 — interception coefficient; β_i — coefficient of the linear effect; β_{ii} — the coefficient of quadratic effect and β_{ij} — the coefficient of interaction effect. The responses for each run were subjected to multiple non-linear regressions to obtain the coefficients of the polynomial equation. F-test was employed to evaluate the statistical significance of the quadratic polynomial model and the quality of the fit of the model was expressed by the coefficient of determination of correlation R^2. The significance of regression coefficient was tested by t-test. The level of significance was given as values less than 0.05. Three-dimensional surface plots were drawn to illustrate the main and interactive effects of the significant variables on dry cell weight. The global optimum levels of the selected variables were obtained by solving the regression equation and by desirability charts. Experimental validation of the predicted model was also performed.

Biomass measurement. Biomass was determined by drying the pellet obtained by centrifuging 1ml of fermentation broth at 8000 rpm at 4 °C for 12 min. The pellets were dried at 80 °C to constant weight prior weighing. Dry cell weights (DCW) were correlated with optical density measurements at 600 nm in UV-VIS spectrophotometer (LABOMED Inc.) to obtain a calibration of OD_{600} vs DCW. OD readings were subsequently used to determine DCW.

Results and Discussion

Extracellular tannase activity. After 48 h of incubation in enzyme producing medium, extracellular tannase activity of the *Lactobacillus* strains was determined by measuring the amount of gallic acid produced. Gallic acid was measured due to the rhodanine reaction and the quantity of gallic acid released during hydrolysis of tannic acid represents the extracellular tannase activity.

All the eight *Lactobacillus* strains showed extracellular tannase activity (Table 2) but *L. plantarum* 2621 showing highest level. *L. plantarum* 2912, *L. acidohilus* 2903, *L. helviticus* 2733, *L. lactis* 2368, *L. brevis* 2436 and *L. casei* 2586 showed intermediate activity and *L. acidophilus* 2902 showed least activity. All the eight strains were used for further experiment.

Table 2. **Tannase activity of *Lactobacillus* strains**

Strain	Activity (U/ml)
L. plantarum 2912	0.004
L. plantarum 2621	0.009
L. acidophilus 2902	0.0007
L. acidophilus 2903	0.004
L. lactis 2368	0.002
L. brevis 2436	0.001
L. helviticus 2733	0.004
L. casei 2586	0.001

Acid and bile tolerance. Survival of probiotics in the gastrointestinal tract is considered to be one of the most important their properties [1]. Different segment of the gastrointestinal tract have varying levels of acid and bile concentration. Stomach and the segments after stomach have the highest acidity and the pH may fall to pH 2.5 and it takes around 3 h for food to be digested. Thirabunyanon et al. [16], Pennacchia et al. [17], Zoumpopoulou et al. [18] also reported about necessity of probiotic survival at pH 2.5.

According to the guidelines by FAO/WHO the test selection of probiotics *Lactobacillus* strains were carried out *in vitro*. For the acid tolerance assay strains were exposed to a pH of 2.5 for 3 h and their viability was determined by colony counting. All investigated strains retained viability in experiment (Table 3).

Table 3. **The number of viable cells (lg cfu/ml) of *Lactobacillus strains* incubated at pH 2.5 in 3 h**

Strain	Control lg cfu/ml	pH 2.5 lg cfu/ml	
		0 hour	3 hour
L. plantarum NCIM 2912	9.5	10.1	9.7
L. plantarum MTCC 2621	9.5	10.2	10.1
L. acidophilus NCIM 2903	8	8	8
L. acidophilus NCIM 2902	9.3	9.6	9.4
L. lactis NCIM 2368	9.2	10	9.9
L. brevis NCIM 2436	9.4	8.8	8.6
L. casei NCIM 2586	8.6	9.3	9.3
L. helviticus NCIM 2733	9.3	9.5	9.5

Bile concentrations in the intestine may range between 0.5 to 2.0% during first hour of digestion, the levels may decrease during the second hour. Iyer et al. [19] reported about *Streptococcus* survival at 0.5–2% bile salt concentration. Survival of *Lactobacillus* strains at 0.3% bile salt was also reported by Maragkoudakis et al. [20] and Thirabunyanon et al. [17].

For assay of bile tolerance *Lactobacillus* strains were subjected to 1 and 2% bile salt containing media. The survival of the bacterial strains was determined by plate counting method (Table 4). *L. helviticus* 2733, *L. acidophilus* 2903, *L. brevis* 2436, *L. plantarum* 2621 and 2912, *L. brevis* 2436, *L. helviticus* 2733 survived in bile salt containing media 1% and 2% as well exposed in 24 h. But *L. casei* 2586, *L. lactis* 2368 and *L. acidophilus* 2902 did not show growth both at 1% and 2% bile concentration after 24 hours of incubation.

Antibiotic resistance. Antibiotic resistance of a probiotic strain can be used for development effective therapeutic measures in controlling intestinal infections [21]. Arici et al. [22] reported that probiotics *Lactobacillus* were resistant to kanamycin and sensitive to erythromycin, chloramphenicol and tetracycline. Verdenelli et al. [23] also reported that *L. rhamnosus* and *L. paracasei* to be sensitive to kanamycin and resistant to ampicillin, tetracycline and erythromycin.

Table 4. **The number of viable cells (lg cfu/ml) after 24 h incubation in MRS broth containing bile salt**

Strain	Control, lg cfu/ml	Bile salt concentration (w/v), lg cfu/ml	
		1%	2%
L. plantarum NCIM 2912	9.5	8.3	8.1
L. plantarum MTCC 2621	9.5	8.6	8
L. acidophilus NCIM 2903	8	9.6	8.7
L. acidophilus NCIM 2902	9.3	0	0
L. lactis NCIM 2368	9.2	0	0
L. brevis NCIM 2436	9.4	10.2	10.0
L. casei NCIM 2586	8.6	9.1	9.1
L. helviticus NCIM 2733	9.3	0	0

Original results of antibiotic resistance test of the strains are shown in Table 5. All eight strains were found to be resistant to kanamycin and sensitive to ampicillin, chloramphenicol, erythromycin and tetracycline.

Therefore, our results indicate that *Lactobacillus plantarum* MTCC 2621 and few other strains showed intrinsic antibiotic resistances which is desirable. At the same time, *L. plantarum* 2621 and 2912, *L. acidophilus* 2903, *L. brevis* 2436 and *L. helviticus* 2733 were found to have tannase activity. However, among all the strains tested *Lactobacillus plantarum* 2621 had shown highest tannase activity (0.009 U/ml, Table 2). Further, *L. plantarum* 2621 had exhibited highest acid tolerance (Table 3) and reasonably good bile salt tolerance (Table 4) compared to other strains. Based on these results, *L. plantarum* 2621 was selected for further study.

Optimization of nutrient medium by Response Surface Methodology. The medium was optimized using central composite design for three variables, such as pH, content of lactose and peptone. The experimental and predicted data for the response variable Y (biomass, g/l) are given in Table 6.

The design matrix and the fitness of the each parameter were analyzed by means of Analysis of variance (ANOVA) and a significant second order regression ($P < 0.0001$) was observed (Table 7). Second-order polynomial equation in coded units for the biomass production was found to be,

$$Y = -50.86919 + 18.84911A + 0.091773B + 0.29704C + 9.89583\times10^{-3}AB - 0.077083AC + 9.37500\times10^{-5}\ BA - 1.71342A^2 - 5.78870\times10^{-4}B^2 - 2.86522\times10^{-3}C^2.$$

Table 5. **Antibiotic susceptibility test of the *Lactobacillus strains***
(S — Sensitive, R — Resistant)

Straine	Diameter of inhibition zone (mm)				
	Erythromycin (15 µg)	Ampicillin (25 µg)	Tetracycline (30 µg)	Chloramphenicol (25 µg)	Kanamycin (30 µg)
L. plantarum MTCC 2621	28(S)	34(S)	28(S)	30(S)	8(R)
L. plantarum NCIM 2912	30(S)	39(S)	25(S)	29(S)	6(R)
L. brevis NCIM 2436	23(S)	26(S)	21(S)	28(S)	6(R)
L. acidophilus NCIM 2902	30(S)	40(S)	25(S)	32(S)	8(R)
L. acidophilus NCIM 2903	20(S)	25(S)	28(S)	21(S)	6(R)
L. casei NCIM 2586	31(S)	41(S)	26(S)	31(S)	1(R)
L. helviticus NCIM 2733	27(S)	34(S)	22(S)	26(S)	9(R)
L. lactis NCIM 2368	30(S)	32(S)	26(S)	32(S)	6(R)

Table 6. **Central Composite Design Matrix for observed and predicted response**

Run Order	pH A	Lactose (g/l) B	Peptone (g/l) C	Biomass in 24 h exposition (g/l)		Residual
				Actual	Predicted	
1	4.8	80	8	6.71	6.86	0.15
2	6	80	8	7.6	7.48	−0.12
3	4.8	160	8	7.2	6.95	−0.25
4	6	160	8	8.19	8.52	0.33
5	4.8	80	16	6.1	5.79	−0.31
6	6	80	16	5.4	5.67	0.27
7	4.8	160	16	5.8	5.93	0.13
8	6	160	16	6.9	6.77	−0.13
9	4.39	120	12	5.8	5.97	0.17
10	6.41	120	12	7.4	7.20	−0.20
11	5.4	52.73	12	5.2	5.22	0.02
12	5.4	187.27	12	6.25	6.21	−0.04
13	5.4	120	5.27	9.45	9.39	−0.06
14	5.4	120	18.73	6.98	7.01	0.03
15	5.4	120	12	8	8.33	0.33
16	5.4	120	12	8.5	8.33	−0.17
17	5.4	120	12	8.2	8.33	0.13
18	5.4	120	12	8.5	8.33	−0.17
19	5.4	120	12	8.2	8.33	0.13
20	5.4	120	12	8.6	8.33	−0.27

The Fisher criteria (F-value of 39.25) is indicated the significance of model. Commonly, the determination of coefficient (R^2) is estimated the adequacy of the model. Value R^2 was 0.9725 and if value exceed 0.75, so it indicates aptness of the model. Adjusted determination coefficient Adj R^2 (adjusted for the number of predictors in the model and commonly lower than the R^2) was equal 0. 947. Predicted determination coefficient Pred R^2 indicates how well a regression model predicts responses for new observations and was equal 0.83. If the Adj R^2 and Pred R^2 are less than R^2 and are nearer to 1, we can conclude that the response equation (second order polynomial equation) is robust and can effectively predicts response for new observations as well. Probability plot of the residuals roughly follows a straight line indicating normal distribution of residuals. Consequently, all of mentioned considerations indicate a good adequacy of the regression model.

The significance of each parameter was determined by Student's t-test and p-value (Table 7). In our study, model parameters A, B, C, AB, A^2 and B^2 were found to be significant. Content of lactose and pH of medium have positive effect on biomass production at low concentration as indicated by the positive coefficient but at higher concentrations inhibit the biomass production. The interactive coefficient was found not to be different from zero at a significance level of 0.05. Response surface curves and contour plots for biomass production, keeping one factor fixed while varying the two other, are demonstrated at figs 1–3. The convex curves commonly indicating that well defined optimum conditions lays in the design space.

From the contour plot it was observed that a high concentration of lactose and pH with a lower concentration of peptone is required for higher biomass production. The optimum levels of the tested variables were pH 5.69, lactose 128.58 g/l and peptone 8 g/l, and the biomass production of 9.15 g/l. was predicted from response surface model. To confirm these data, experimental rechecking was done by using a medium representing these optimal points in the bioreactor. The biomass production of 8.97 g/l was obtained as against the predicted 9.15 g/l which is quite satisfactory. A difference of mere 0.18 g/l, which amounts to 2% error, verifies the validity of the response model and the existence of an optimal point. Biomass production was increased 10 times with the optimized medium as compared to the biomass content in the initial MRS broth. The probiotic biomass also showed tannase activity of 0.0085 U/ml, confirming that the desired tannase activity trait is still present in the biomass produced.

Table 7. **Analysis of Variance for Response Surface Quadratic Model**

Source	Sum of square	Δf	Mean Square	F-value	P-value Prob > F
Model	27.06	9	3.01	39.25	< 0.0001 iignif.
A (pH)	1.81	1	1.81	23.62	0.0007
B (lactose)	1.2	1	1.2	15.65	0.0027
C (peptone)	6.82	1	6.82	89.1	< 0.0001
AB	0.45	1	0.45	5.89	0.0356
AC	0.27	1	0.27	3.57	0.088
BA	1.80×10^{-3}	1	1.80×10^{-3}	0.024	0.8812
A2	5.48	1	5.48	71.59	< 0.0001
B2	12.36	1	12.36	161.4	< 0.0001
C2	0.03	1	0.03	0.4	0.5436
Residual	0.77	10	0.077		
Lack of Fit	0.49	5	0.099	1.8	0.2668 not signif.
Pure Error	0.27	5	0.055		
Cor Total	27.83	19			

$R^2 = 97.25\%$ Adj $R^2 = 94.7\%$

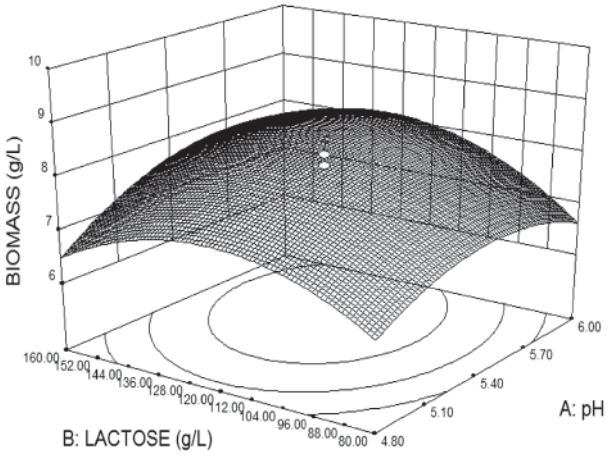

Fig. 1. **Surface Response as a function of lactose and pH (keeping Peptone constant) on biomass production for *L.plantarum* MTCC 2621**

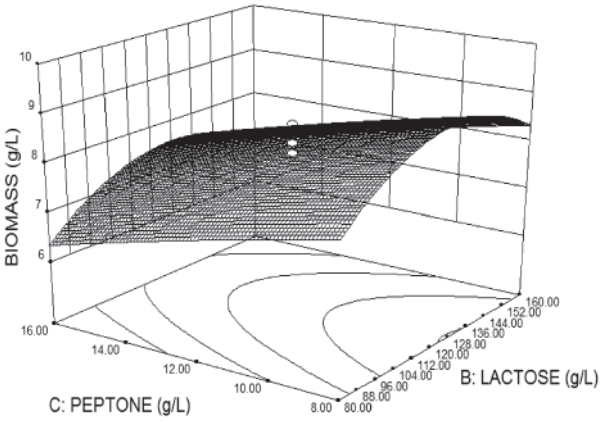

Fig. 2. **Surface Response as a function of peptone and lactose (keeping pH constant) on biomass production for *L.plantarum* MTCC 2621**

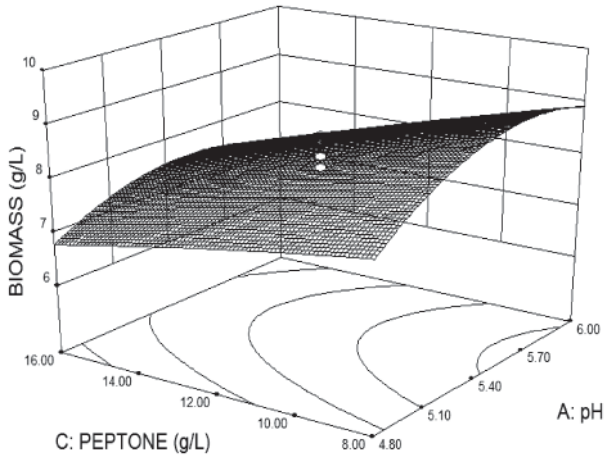

Fig. 3. **Surface Response as a function of peptone and pH (keeping lactose constant) on biomass production for *L.plantarum* MTCC 2621**

In the current study, eight tannase positive *Lactobacillus* strains were taken and *in vitro* probiotic properties were determined. Among eight strains, L. plantarum 2621 and 2912, *L. acidophilus* 2903, *L. brevis* 2436 and *L. helviticus* 2733 were found to possess significant acid tolerance, bile salt tolerance and desirable antibiotic resistance. As *Lactobacillus plantarum* 2621 had shown highest tannase activity and significant acid and bile tolerance, it was chosen for further study.

A nutrient medium was designed comprising lactose, peptone and salts making use of "one-factor-at-a-time" approach in shake flasks. Based upon the results, bioreactor trails were conducted to optimize the medium by Response surface modeling technique. Altogether 20 batch trails were conducted in a 3 L bioreactor as per the central composite design. A polynomial response model was generated using software, Design-Expert (Stat-Ease Inc., Minneapolis, USA). The adequacy of the model equation was tested by ANOVA, and response surface and contour plots were generated. Using point prediction tool, optimum points of the variable were predicted and verified by conducting two more bioreactor trials.

Thus a specific nutrient medium for biomass production for *L. plantarum* 2621 has been designed successfully using response surface methodology. A tenfold increment in biomass production was achieved in the new optimized medium as compared to initial culture medium. The biomass produced had shown significant tannase activity. As the optimization trails were conducted in 3 L bioreactor, the process is ready for scale-up.

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ОПТИМІЗАЦІЯ ПРОДУКУВАННЯ ТАНАЗОАКТИВНОГО ПРОБІОТИКА НА ОСНОВІ МЕТОДИКИ ВІДГУКУ ПОВЕРХНІ

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Метою роботи було дослідження за умов *in vitro* пробіотичних властивостей та оптимізації продукування позаклітинного ензиму танази у восьми штамів *Lactobacillus*, одержаних із музейних культур. Для вивчення промислової продуктивності обрали штам *L. plantarum* МТСС 2621, толерантний до кислот та жовчі, стійкий до дії антибіотиків, з підвищеною таназною активністю. Оптимізацію живильного середовища в 3-літровому біореакторі було проведено з використанням методики відгуку поверхні на основі повного факторного аналізу головних компонент. Факторний аналіз 2^3, розширений шістьма додатковими точками (α = 1,68) і шістьма повторними навколо центральної точки, відтворено в 20 експериментах. Встановлено оптимальні параметри живильного середовища: pH 5,69, вміст лактози — 128,58 г/л, пептону — 8 г/л. За використання оптимізованого живильного середовища одержано 10-кратне підвищення продукції біомаси порівняно із застосуванням стандартного середовища MRS.

Ключові слова: *Lactobacillus*, пробіотик, оптимізація складу середовища, методика відгуку поверхні.

ОПТИМИЗАЦИЯ ПРОДУЦИРОВАНИЯ ТАННАЗОАКТИВНОГО ПРОБИОТИКА НА ОСНОВЕ МЕТОДИКИ ОТКЛИКА ПОВЕРХНОСТИ

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Целью работы было исследование в условиях *in vitro* пробиотических свойств и оптимизации продуцирования внеклеточного энзима танназы у восьми штаммов *Lactobacillus*, полученных из музейных культур. Для изучения промышленной продуктивности избрали штамм *L. plantarum* МТСС 2621, толерантный к кислотам и желчи, устойчивый к действию антибиотиков, с повышенной танназной активностью. Оптимизация питательной среды в 3-литровом биореакторе проведена с использованием методики отклика поверхности на основе полного факторного анализа главных компонент. Факторный анализ 2^3, расширенный шестью дополнительными точками (α = 1,68) и шестью повторными вокруг центральной точки, был реализован в 20 экспериментах. Установлены оптимальные параметры питательной среды: pH 5,69, содержание лактозы — 128,58 г/л, пептона — 8 г/л. При использовании оптимизированной питательной среды получено 10-кратное увеличение продукции биомассы по сравнению с применением стандартной среды MRS.

Ключевые слова: *Lactobacillus*, пробиотик, оптимизация питательной среды, методика отклика поверхности.