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Statistical Optimization of Fermentation Media for Postbiotic Metabolite Production from *Lactobacillus Plantarum* LG138 of Primate Origin

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Abstract

The probiotic metabolites called postbiotics synthesized by microorganisms consist of range of molecules namely organic acids, antimicrobial peptides, short-chain fatty acids, exopolysaccharide (EPS), cofactors, vitamins, immune-modulating compounds, enzymes, neurotransmitters etc. LABs are widely recognized as an efficient EPS producer. Hence, the goal of the current study is to statistically maximize the media components for maximum exopolysaccharide production by *Lactobacillus plantarum* LG138 from primate feces. The de Man, Rogosa and Sharpe (MRS) medium is used for optimization of production process. Batch culture system is used for optimization of exopolysaccharide production in MRS medium using *Lactobacillus plantarum* LG138. The optimization process of EPS production improved its yield by 2.7-folds (from 12.00 mg/ml to 32.88 mg/ml).The enhanced EPS yield was achieved after optimization of different media components such as sucrose (5%), ammonium sulfate (1.2%), temperature (32.5ºC), incubation time (22 h) and pH (6.5) using Response Surface Methodology. The actual experimental value (32.88 mg/ml) was comparable to the predicted maximum EPS

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production by *Lactobacillus plantarum* LG138 (32.87mg/ml) under optimal conditions. The *in vitro* antioxidant assays (free radical scavenging ability and reducing power) revealed antioxidant properties of EPS. These significant activities recommend the possible potential use of EPS in animal food and feed applications.

Keywords: Exopolysaccharide, lactic acid bacteria (LAB), postbiotic, response surface methodology (RSM), optimization

1 Introduction

LAB strains are very well known for possessing probiotic properties and bioactive substances produced by them exhibit similar or correlative health benefits to the consumers [1]. These bioactive substances synthesized by these organisms are called as postbiotics that manifest several bioactive potential[2]. Among microbial postbiotics, exopolysaccharides extracted from lactic acid bacteria are selected and employed for bioactivities under *in-vivo* as well as *in-vitro* conditions [3,4]. EPSs are synthesized as tightly bound capsules or loosely bound slime form on the cell surface by bacteria, microalgae, yeasts and fungi as extracellular polymers in carbon source rich growth medium and under unfavorable environmental conditions [5]. In microorganisms, EPS play prominent functional roles such as biofilm formation, cellular defense mechanisms against osmotic stress, salinity, desiccation, change in temperature, antibiotics, phage attack, antibodies, etc [6,7].These polymers have broad applications in food, cosmetics, pharmaceutical, in medical and petroleum industries as biosurfactants, stabilizers, emulsifiers, bioflocculants, gelling agents, viscosifiers, drug carriers, and ion exchange resins [8].Cellulose, succinoglycan, alginates, curdlan, pullulan, xanthan, dextran, gellan and hyaluronic acid are different commercially available forms of EPS which are extracted from bacteria and fungi. EPSs from LABs have been extensively examined in recent years because of their generally recognized as safe (GRAS) status for human consumption and other distinctive characteristics. [9, 10, 11]. EPSs extracted from different microorganisms have been supplemented in variety of therapeutic applications due to antioxidant, antibacterial, anticancer, anti-inflammatory properties, immuno-modulatory activities, lowering blood cholesterol level, and antidiabetic effects [12, 13]. A major factor constraining the use

of EPS in food and pharma sector is its comparatively low yield [14]. Consequently, this has necessitated to explore possibilities to augment EPS production through microbial strain screening and process optimization approach [15]. The process optimization based upon Design of experiments (DoE) are more recognized as one variable at a time i.e. OVAT approach has several drawbacks [16]. The statistical designs such as Central Composite Design (CCD) and Response Surface Methodology (RSM) having variety of designs has gained attention in modern researches. RSM is an optimization tool having statistical and mathematical methodologies for designing experiments and models, finding most favorable physiochemical conditions for expected responses and assessing the significance of interactions amongst several variables. The media composition and culture conditions significantly affect different variables such as rate of growth, biochemical composition, its type and yield. Hence, optimization of the media elements and culture parameters that augment the yield of polysaccharides is of great significance [17,18]. Recently, RSM has been used for designing different bioprocesses along with EPS yield optimization viz. production media, culture and process conditions [19, 20 ,21], enzyme production [22] and exopolysaccharide production [23,24,25].

In the gastrointestinal tract of primates inhabits variety of special commensal gut microbes which can be a suitable source of novel postbiotics such as exopolysaccharides. Still, investigation of bioactivities of the EPSs purified from lactic acid bacterial isolates from primate feces has never been reported to date. The production of EPS utilizing *Lactobacillus plantarum* LG138, a probiotic microbe with primate origins and the optimizing production parameters for maximal EPS synthesis are the innovative aspects of this work. This study has been aimed on defining the growth parameters having significant effect on maximum EPS production using RSM. This work will provide a base for structural and biochemical property analysis of EPS produced.

2 Materials and Methods

2.1 Isolation of microorganisms

Lactic acid bacteria were isolated from primate feces by plating serially diluted samples on de Man Rogosa Sharpe (MRS) agar containing 1% cysteine and incubated at 37^0C for 48 h anaerobically. Then isolated colonies were further purified by restreaking on respective medium. The cultures thus obtained were stored at 4^0C as slants for future use.

2.2. Screening of EPS producers

For screening, 24h old cultures were streaked on ruthenium red milk agar plates and placed at 37^0C for 24 h. The exopolysaccharide producing isolates producing white colonies will be selected further for quantification.

2.3 Production and quantification of EPS

After preliminary screening, the isolates were inoculated in MRS media for 24 h at 37°C. For EPS extraction the method given by Fashogbon et al [26] was followed. The quantification of EPS was done according to the method of Dubois et al [27] having glucose as a standard and expressed as mg/ml.

2.4 Isolate identification

The 16s rRNA gene sequence analysis of the isolate was performed at Future Biotech (Lucknow, India). The gene sequence was analyzed by sequence alignment and phylogenetic tree was constructed using MEGA v-16 software.

2.5 EPS production Optimization using OVAT

For maximum EPS production the effect of different types of media constituents and culture conditions was investigated. The carbon source in the media was optimized by using sucrose, fructose, glucose, galactose, maltose, xylose, lactose and mannose (2% w/v). The N- source was optimized by using organic nitrogen sources (yeast extract, peptone, tryptone, meat extract and urea) and inorganic nitrogen sources (ammonium chloride, ammonium sulfate, tri-ammonium citrate, potassium nitrate and sodium nitrate at a concentration of 0.5%. The cultivation conditions used for

optimization studies were as follows: inoculum size (1-5% v/v), inoculum age (12 h,14h 16 h, 18 h, 20 h, 22 h, 24 h), incubation temperature (25ºC, 30ºC, 35ºC 40ºC and 42ºC), pH (5, 5.5, 6, 6.5, 7, 7.5 and 8.0) incubation time (12, 24, 36, 48, 60, and 72h). To test the effect of different factors on EPS yield, media components were added into the MRS broth individually and 1% of 24 h old culture is inoculated in each flask.

2.6 Media Optimization Using RSM

After optimization of different parameters for optimum EPS production using OVAT, media optimization using RSM was done employing central composite design (CCD) for maximum EPS production. To study the interaction of different factors the statistical software package i.e. Design-Expert v-13 (Stat Ease Inc., Minneapolis, USA) was used.

2.7 Model Validation

In the course of RSM implementation, the validation of model developed was done by performing check point studies. The software predicted response were contrasted with the experimentally calculated results.

2.6 Free Radical Scavenging Activity

The method described by Yin et al [28] was used to assess the EPS produced for its capacity to scavenge DPPH free radicals [28].

2.8 Reducing Power Assay

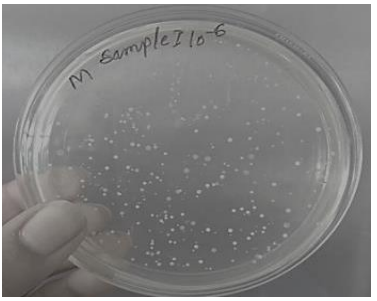
The reducing power of the EPS was calculated according to the method of Vijayalakshmi and Ruckmani [29].

3 Results and Discussion

3.1 Isolation and Identification of Microorganism

Total 50 bacterial isolates were obtained using feces samples collected from different places of Shimla. According to their Gram-positive and catalase-negative nature, they were recognized as presumptive LABs. Twelve of these were discovered to produce EPS and one isolate, LG138 with a maximum production of 12 mg/ml in MRS medium was chosen for further study. The 16s rRNA gene

sequence alignment of selected isolate was done and phylogenetic tree was constructed using the maximum likelihood method and bootstrapped applying 1000 replicates in MEGA v-16.0. The isolate LG138 was similar with *Lactobacillus plantarum* and is named as *Lactobacillus plantarum* LG138 with GenBank accession number of MZ145241.



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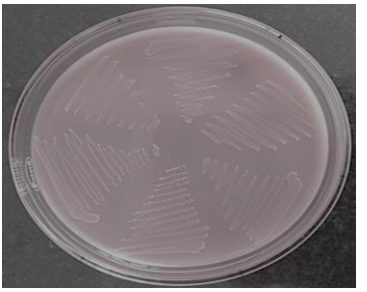


(b)

(c)

Fig.1: (a) Growth on MRS media (b) Screening on ruthenium red agar (c) 16s rRNA gene sequence based phylogenetic tree using MEGA v-16.0 program

3.2 EPS production Optimization Using OVAT

Using OVAT method, the optimized levels of media components and cultivation conditions used for exopolysaccharide production from *Lactobacillus plantarum* LG138 has increased the production yield upto 30 mg/ml from the initial value (12 mg/ml). Various parameters optimized for maximum EPS production, are discussed below:

3.2.1 Inoculum Size

The maximal EPS produced was found to be to 13 mg/ml at 2% inoculum size from *L. plantarum* LG138, whereas with increasing inoculums size, it was decreased to 5 mg/ml at 5%(Fig. 1a). Onilude et al [30] reported similar results where the dextran production from *Leuconostoc* spp. was slowly increased with increase in inoculum size (2% to 4%) and the decrease in EPS production was observed as size of inoculum increased (8% to 10%). A very high as well as very small size of inoculum alters EPS synthesis and it signifies the role of optimal inoculum for fermentation and productivity [31].

3.2.2 Inoculum Age

In the present study, at an inoculum age of 16h the highest EPS level (15 mg/ml) was obtained (Fig. 1b). The optimum inoculum age was determined because this minimizes the lag phase of bacterial growth and increases rate of the fermentation process. Wang et al [31] reported that with 8h old inoculum, the highest quantity (12.64 g/L) of gellan gum were obtained and observed reduction in the gellan gum yields with increase in the age of the inoculum.

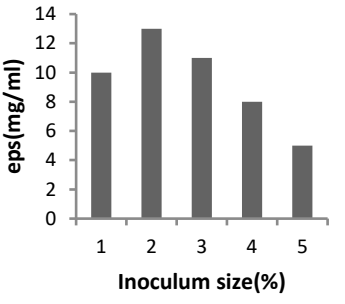
3.2.3 Effect of C-Sources on EPS Production

The effect of addition of different carbon sources (glucose, sucrose, lactose, galactose, xylose, fructose, maltose and mannose) on EPS formation by *Lactobacillus plantarum* LG138 is shown in fig. 2(c-d).The maximum EPS production of 18 mg/ml was achieved using sucrose as exclusive carbon source. This was in good accordance with earlier studies which suggests sucrose as highly suitable C-source for EPS production [33, 34, 25]. The optimum sucrose concentration was also studied using in the range of 0.5-7%w/v in fermentation medium. The highest yield of 22 mg/ml was obtained when the isolate was grown in medium containing 5%w/v sucrose.

For EPS production, different sugars including amino acids can be used as C- source by microbes, among which sucrose and glucose are the most competent C-source [35]. However, EPS production is inhibited by high concentration of sucrose which indicates the feedback inhibition system in LAB cells [36].

3.2.4 Effect of N- Sources on EPS Production

The effects of different nitrogen sources and their different concentrations on EPS production by *Lactobacillus plantarum* LG138 were examined. In comparison to different organic N-sources peptone was the most effective source (Fig. 1e). This might be contributed to the large amount of peptides, free amino acids and growth factors present in peptone. Peptone at the concentration of 4%w/v showed maximum (25 mg/ml) production as shown in the fig. 2f. This is in contrast to the findings of some workers where yeast extracts have been reported as main source of nitrogen for exopolysaccharide production [37,38,39]. Amongst the different inorganic nitrogen source, ammonium sulfate (2%w/v) was most effective one leading to the production of 21 mg/ml of EPS fig. 2g. Previous studies have also reported ammonium sulfate as an efficient N-source for EPS production from LABs [40]). Sirajunnisa et al [41] found that N -sources at low-concentration promote EPS synthesis, which could be due to osmotic stress caused by high concentrations of nitrogen sources in LAB cells.



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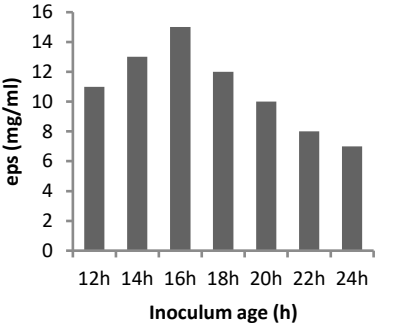

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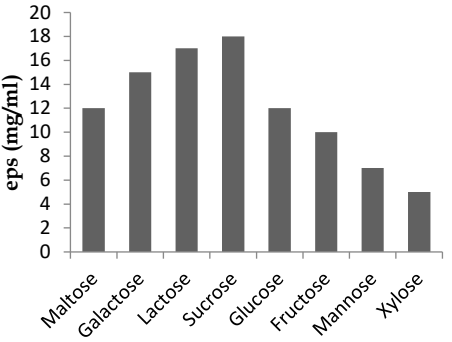
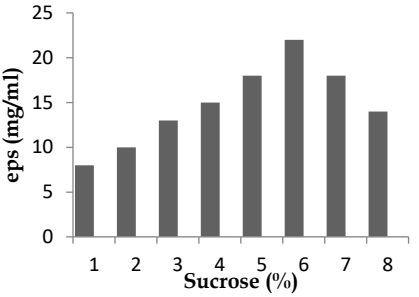
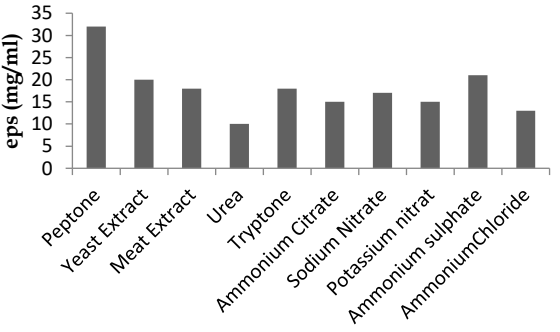

eps (mg/ml)
20
18
16
14
12
10
8
6
4
2
0
Maltose
Galactose
Lactose
Sucrose
Glucose
Fructose
Mannose
Xylose

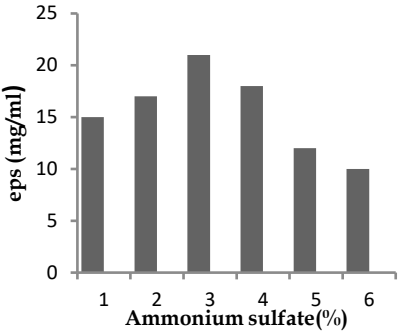
(c)


eps (mg/ml)
25
20
15
10
5
0
1
2
3
4
5
6
7
8
Sucrose (%)

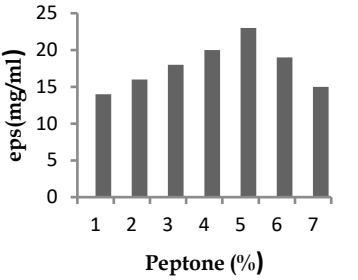
(d)


eps (mg/ml)
35
30
25
20
15
10
5
0
Peptone
Yeast Extract
Meat Extract
Urea
Tryptone
Ammonium Citrate
Sodium Nitrate
Potassium nitrat
Ammonium sulphate
AmmoniumChloride

(e)



(f)



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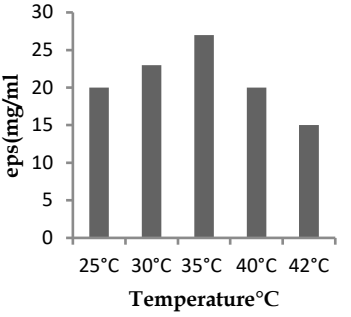
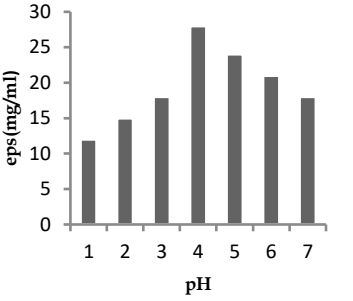
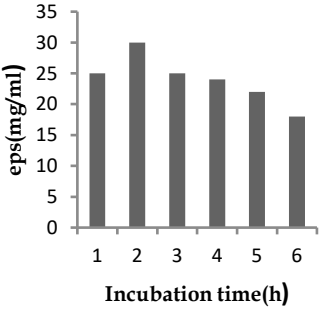


(h)



(i)



(j)

Fig. 2: Effect of different variables on EPS production by *L. plantarum LG138* (a) inoculum size (b) inoculum age (c) c-sources (d) sucrose concentration (e) n- sources (f-g) peptone and ammonium sulfate concentration (h) temperature (i) pH (j) incubation time

3.2.5 Effect of temperature on EPS production

The temperature is a crucial variable for the growth of LAB isolate growth and has to be maintained optimum for metabolite production. The EPS production in the study was found to maximum at 35°C after 24h of incubation period as shown in Fig. 2h. The low or high temperatures inhibit the activity of EPS producing enzymes and any deviation from optimal temperature will have impact on intracellular enzyme activity, subsequently decreasing EPS yield [42, 43]. Between 30-40°C, *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 20081 produced maximum EPS and its production decreased to almost half when grown at 45°C[44].

3.2.6 Effect of pH on EPS production

The impact of different pH was analyzed in order to enhance the exopolysaccharide production by this isolate. As shown in the fig. 2i, there is a rise in the EPS production with increasing pH of the medium. Highest EPS yield was at a pH 6.5 of the fermentation medium which is in agreement with other findings [45, 46]. The pH effect on EPS production was investigated by various workers and the optimum pH for the production has been found to be strain specific [47, 48].

3.2.7 Effect of Incubation Period on EPS Production

The isolate under study was incubated for various time periods to test the impact of incubation time on EPS production. Data presented in fig. 2j manifest that optimal exopolysaccharide production was attained after 24h incubation which is in unanimity with other workers [49]. Sarwat et al [50] mentioned 20 h as the most favorable incubation time for dextran production by *Leuconostoc* spp. owing to increase in dextransucrase enzyme activity and EPS production with time. Onilude et al [30] reported that with increase in incubation time, the production gradually increased upto the highest production at 20h and decreased afterwards.

3.3 Media Optimization Using RSM

After studying the effect of different variables using OVAT, the RSM with CCD was conducted with five variables viz. sucrose, ammonium sulfate, temperature, pH and incubation time to judge the individual variable effects and the interactions between them. A

statistical model having a set of 26 runs was carried out and every independent variable was evaluated at five different levels (Table1). Every run under study was conducted in triplicate and the EPS yield response values were correlated with the predicted responses derived by quadratic model using the Design Expert v-13 software. Based on the experimental results a polynomial equation of second order was developed to describe the correlation of EPS production response from *L. plantarum* LG138 with other variables: R1= +14.36+3.96A+2.86B+6.75C+0.1016D+4.78E+3.44AB+2.26AC+6.33AD+3.98AE-3.37 BC+3.27BD-2.71BE+3.19CD-2.96CE+1.22DE+3.41A^2 -1.79B^2 +1.21C^2-0.1719D^2-2.77E^2 where R1 is the EPS yield (mg/ml) and the predicted response variable. The A, B , C, D and E are the codes for independent variables, viz., sucrose, ammonium sulfate, temperature, pH and incubation time respectively.

Table 1: The experimental design and results for EPS production using *Lactobacillus plantarum* LG138 from central composite experimental design matrix

Run	**A: Sucrose (%)**	**B: Ammonium Sulfate (%)**	**C: Temperature ºC**	**D: pH**	**E: Incubation time (h)**	**Actual values: EPS yield mg/ml**	**Predicted Values: EPS Yield mg/ml**
1	0.5	2.0	25	8	36	4.69	4.68
2	2.75	1.25	18.8413	6.5	22	6.07	6.06
3	0.5	2.0	40	5	36	4.49	4.49
4	2.75	0	32.5	6.5	22	4.62	4.62
5	2.75	1.25	32.5	6.5	22	15.12	14.36
6	2.75	1.25	32.5	6.5	22	15.19	14.35
7	5.0	0.5	25	8	36	15.14	15.14
8	2.75	1.25	32.5	6.5	0	0	-0.00835
9	2.75	1.25	32.5	6.5	22	13.79	14.36
10	0.5	2.0	40	8	8	14.68	14.68
11	2.75	2.61	32.5	5	22	13.62	13.61
12	0.5	0.5	40	8	36	14.19	14.19
13	0.5	0.5	25	5	8	10.14	10.44

Run	A: Sucrose (%)	B: Ammonium Sulfate (%)	C: Temperature ºC	D: pH	E: Incubation time (h)	Actual values: EPS yield mg/ml	Predicted Values: EPS Yield mg/ml
14	5.0	0.5	40	8	8	20.88	20.88
15	0	1.25	32.5	6.5	22	14.63	14.62
16	5.0	2.0	40	5	8	15.36	15.36
17	2.75	1.25	32.5	3.8	22	13.61	13.60
18	2.75	1.25	32.5	6.5	47.5	13.88	13.87
19	2.75	1.25	46.2	6.5	22	30.66	3.65
20	6.42	1.25	32.5	6.5	22	32.88	32.87
21	2.75	1.25	32.5	6.5	22	13.78	14.36
22	2.75	1.25	32.5	6.5	22	13.88	14.35
23	2.75	1.25	32.5	9.2	22	13.98	13.97
24	5.0	0.5	40	5	36	25.22	25.22
25	5.0	2.0	25	5	36	20.12	20.12
26	5.0	2.0	25	8	8	15.12	15.12

Table 2: ANOVA (Analysis of Variance) for Quadratic model for EPS production

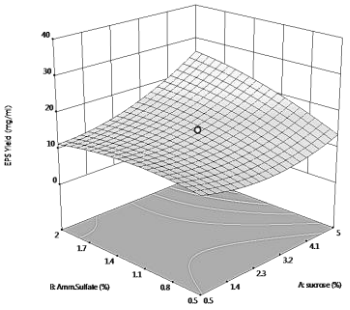
Source	Sum of Squares	df	Mean Square	F-value	P-value	
Model	1381.93	19	72.73	195.93	< 0.0001	Significant
A-Sucrose	58.07	1	58.07	156.42	< 0.0001	
B-Ammonium sulfate	48.98	1	48.98	131.94	< 0.0001	
C-Temperature	126.85	1	126.85	341.71	< 0.0001	
E-Incubation time	126.85	1	126.85	341.71	< 0.0001	
AB	34.48	1	34.48	92.87	< 0.0001	
AC	14.62	1	14.62	39.38	0.0008	

Source	Sum of Squares	df	Mean Square	F-value	P-value	
AD	121.71	1	121.71	327.87	< 0.0001	
AE	46.64	1	46.64	125.63	< 0.0001	
BC	35.73	1	35.73	96.24	< 0.0001	
BD	33.59	1	33.59	90.47	< 0.0001	
BE	22.95	1	22.95	61.81	0.0002	
CD	32.35	1	32.35	87.15	< 0.0001	
CE	27.36	1	27.36	73.71	0.0001	
DE	4.92	1	4.92	13.25	0.0108	
A^2	130.95	1	130.95	352.75	< 0.0001	
B^2	57.79	1	57.79	155.68	< 0.0001	
C^2	30.25	1	30.25	81.47	0.0001	
D^2	0.6147	1	0.6147	1.66	0.2456	
E^2	125.56	1	125.56	338.22	< 0.0001	
Residual	2.23	6	0.3712			
Lack of Fit	0.0695	2	0.0347	0.0644	0.9386	not significant
Pure Error	2.16	4	0.5395			
Cor Total	1384.16	25				
Std. Deviation	0.6571	Mean	14.46, C.V. %	C.V. %	4.54	

Note: predicted R^2= 0.9971, Adjusted R^2 = 0.9922

The F-test and the Analysis of Variance (ANOVA) were used to examine statistical significance of the response surface quadratic model (table 3). A very low probability value (p-value= <0.0001) the model signifies the validity of this model for EPS production from *L. plantarum* LG138. The predicted R^2value (0.9971) was in accordance with the adjusted R^2 value of 0.9922 with a coefficient of variance (CV) (4.54%) which also proved the significance of the model (table 2). The models were adequate, as indicated by the F-value of 195.93 and the Lack of Fit value of 0.0695, and there was only a 0.01% chance that such a value could occur owing to noise. It was noted that all

the parameters except pH were significant in their linear and squared terms. Additionally, the interaction between sucrose (A) and pH (D) was the most significant (p value<0.0001) followed by AE BC, BD, AB, AC, CD, CE. This implies that the cumulative effect of all these interactions was crucial for EPS production by *L. plantarum* LG138. The interaction between DE was not significant (p-value -0.0108).



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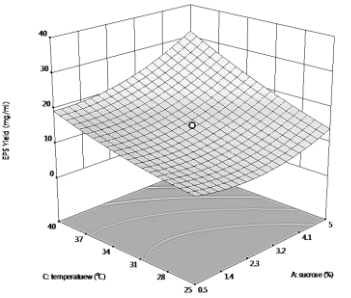

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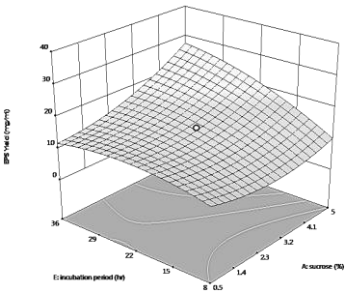

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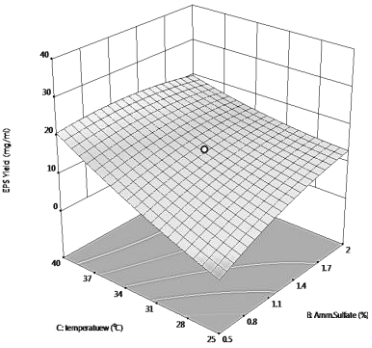
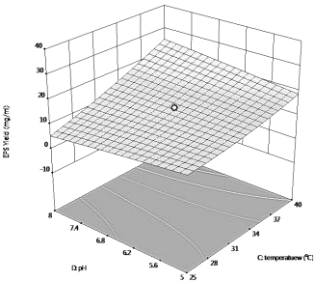
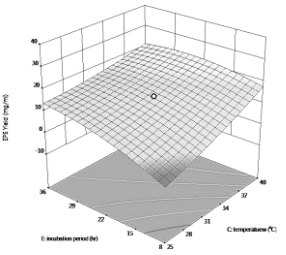
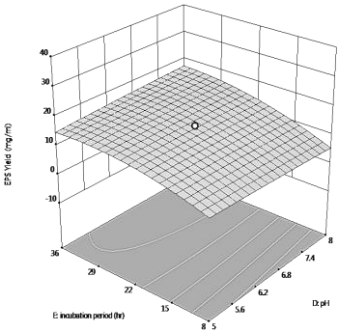


(d)





(e) (f)



(g)

Fig. 3: Response surface 3-D curves for EPS production (a) sucrose vs. ammonium sulfate (b) sucrose vs.temperature (c) sucrose vs. incubation period (d ammonium sulfate vs. temperature (e) ammonium sulfate vs. pH (f) ammonium sulfate vs. incubation temperature (g) temperature vs. pH (h) temperature vs. incubation time (i) pH vs. incubation time

All the results have shown a consensus between the predicted and experimental values. Hence, it was elucidated that the present statistical models were best suited for the replication of EPS production in this study. The interactions between the variables is depicted in 3D response surface plots of *L. plantarum* LG138 (Fig. 3). The 3-D response surface plots depicting the interaction between different variables (sucrose, ammonium sulfate, temperature, pH and incubation period) on EPS synthesis from *Lactobacillus plantarum* LG138 has been shown in Fig.3. The interactive effect of sucrose and ammonium sulfate (AB) Temperature and sucrose (AC), incubation period and sucrose (AE) is shown in Fig. 3a-c. At low N-source (ammonium sulfate) concentration, and elevated sucrose concentration, produced EPS amount was the highest (Fig. 3a). With increase in temperature and sucrose concentration, EPS production increases (Fig. 3b). A decrease in EPS production was seen as incubation time increased. (Fig.3c). Interactive effect between temperature and ammonium sulfate is significant, high temperature and low ammonium sulfate concentration increases EPS yield (Fig. 3d). The interaction of temperature with ammonium sulfate and incubation time (CD and CB) was also statistically significant. Fig.

3d and 3f depicts that increasing the level of one factor and decreasing other factor culminated in increased EPS production. The interaction of pH with temperature and incubation period (DC and DE) shows increase in EPS yield with increase in temperature and incubation time (Fig. 3e and Fig. 3g).

By using the Design Expert v-13, the optimum EPS production using *L. plantarum* LG138, the optimum culture conditions were sucrose, (6.4%) and ammonium sulfate, (1.25 %), temperature of 32.5°C, pH of 6.5 and incubation time of 22h. The EPS yield was around three times (2.7 folds) greater under these optimized conditions than the EPS generation from MRS media. Fig. 4a explains the variation in response for EPS production from *Lactobacillus plantarum* LG138 using perturbation curve. The variables did not diverged much in relation to the reference point. *Lactobacillus plantarum* LG138 has shown an increase in a EPS production using RSM which agrees with the data reported previously. A study on *Scenedesmus* sp. SB1, a freshwater microalga, media optimization using the Plackett-Burman Design (PBD) and RSM showed a 1.8-fold higher EPS production (48 mg/L) than for unoptimized media) [17]. Chen et al [19] reported that using optimized conditions EPS yield increased upto 496.64 mg/L which is 76.70% more than that of unoptimized conditions i.e. 281.07 mg/L. While studying eps synthesis by the yeast *Lipomyces starkeyi* VIT-MN03, significant effect of culture conditions viz., sucrose and NaCl concentration, temperature, pH, and incubation time was observed. Under optimal conditions (NaCl 3%, sucrose 2%, pH-4, incubation period 30 days and temperature 25 °C), the maximum EPS recovery was 4.87 g L^{-1} that revealed sixfold increase in contrast to EPS produced in minimal media (0.79 g/ L) [51].

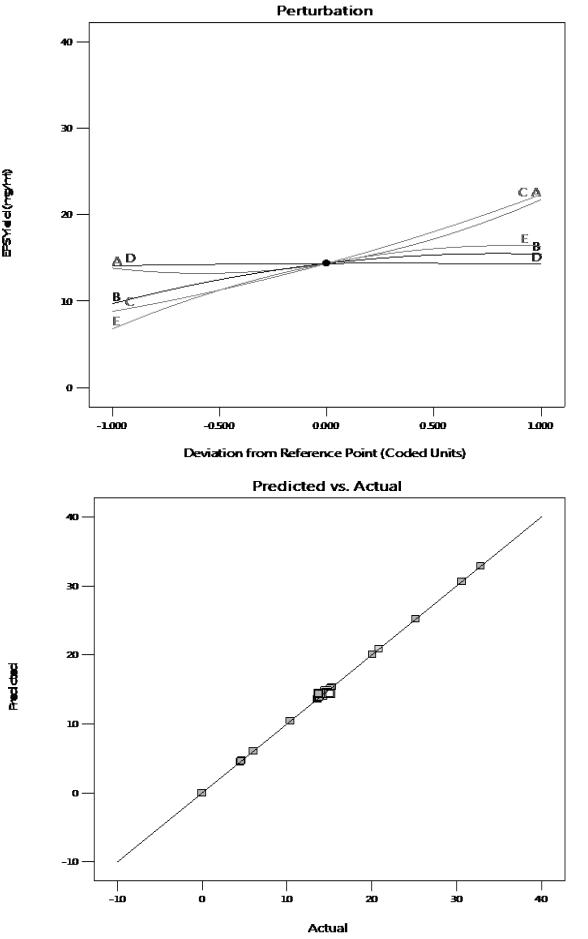


Fig. 4: a) Predicted vs Actual response of EPS production b) The response changes shown in perturbation plot the as each variable varies from the chosen reference point while maintaining other variables constant

3.4 Free radical scavenging activity

The scavenging activity of EPS using DPPH found to be increased with the increasing concentration of exopolysaccharide as is shown in Fig. 5a. It was found that EPS's scavenging activity was less than the control value (ascorbic acid). The DPPH activity of EPS increased upto 66% at of 5 mg/ml concentration. A study by Zhang et al [52] reported DPPH scavenging activity of 50.41% (8 mg/ml) by EPS of the *Lactobacillus kimchi* SR8. The EPS isolated from *Lactobacillus plantarum* showed free radical scavenging activity of 52.23% at

concentration of 4.0 mg/ml [53]. These findings shown that the EPS obtained from the isolate under study exhibits considerable DPPH scavenging capacity.

3.5 Reducing power assay

An increase in reducing power activity of EPS and standard (Ascorbic acid) was observed with increasing EPS concentration (5mg/ml to 100mg/ml) (Fig. 5b). In a study using *Lactobacillus lactis* subsp. *lactis* 12 EPS exhibited reducing power activity similar to the standard antioxidant used [54].

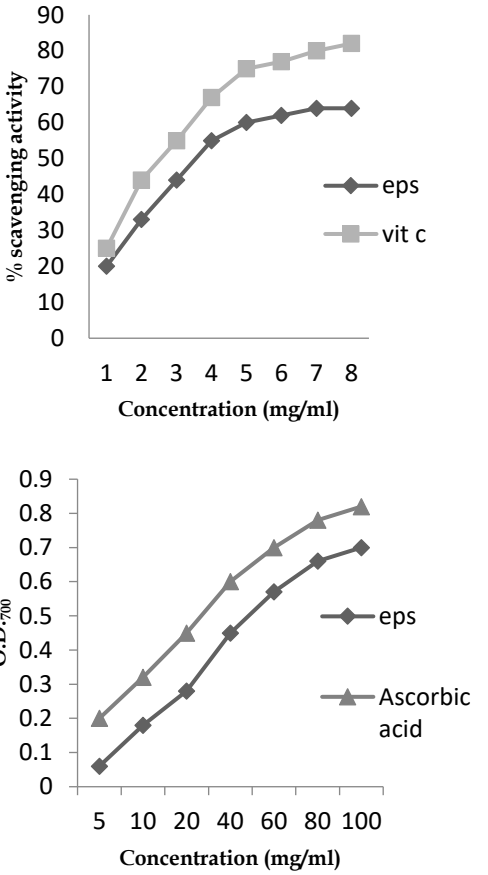


Fig. 5: (a) Free radical scavenging activity (b) Reducing power assay

4. Conclusion

As far as we know, this is the first reported study on EPS production using lactic acid bacterial isolate from primate feces of Shimla region. In this work, the impact of different physiochemical parameters on EPS production was examined and by using RSM, the optimized conditions were determined for EPS production The maximum EPS yield of 32.88 mg/ml was obtained under the optimal conditions with a 2.7-fold elevation in EPS yield as compared with the EPS production before optimization (12.00 mg/ml) in MRS medium. Besides, the significant antioxidant activity of EPS derived from *lactobacillus plantarum* LG138 suggests its potential application as an antioxidant agent in food or feed industry. For industrial production of EPS, cost effective and optimum culture medium is required. So further investigation of optimized culture parameters is suggested.

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Conflict of Interest: The authors declare no conflict of interest.

Authors Contribution:

Concept and design of the work: Dr. SS and RK, Data collection: PL, Data analysis and Interpretation: KBS and SR, Drafting the article: RK, Critical review of the article: Dr. SS

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