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Exopolysaccharide production from *Bacillus safensis* using statistical experimental design

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Abstract

Microbial polysaccharide is an important biopolymer secreted either by bacteria, fungi, or yeast as natural non-toxic, biodegradable and renewable sugar monomers. It has a wide range of industrial application such as in pharmaceuticals, cosmetics, food and Microbial enhanced oil recovery. This study is aimed at a cost-effective EPS production from renewable raw material feedstock using statistical design. Six isolates WN3, WN5, WN7, WN9, WZI and WZ2 showing mucoid morphology isolated from Bonny River in Rivers State, Nigeria were examined for the Exopolysaccharide production using renewable feedstock of Sweet Potato peel and Bean Bran as carbon and nitrogen sources respectively. WN7, WZ1 and WZ2 had higher EPS yield where WN7 had EPS yield of 0.362g/l, WZ1 had EPS yield of 1.02g/l while WZ2 had EPS yield of 0.615g/l. isolate WZ1 that had the highest EPS yield was identified using the 16srRNA sequence as Bacillus safensis. Two statistical designs Plackett-Burman and Box-Behnken were used for the optimization studies where Plackett-Burman design indicated that K2HPO4, MgSO4H2O, CaSO4H2O, NaCl, Potato Peel, Bean Bran are significant ($p<0.05$) model terms. Box-Behnken design revealed that optimum conditions of pH 4.5, Potato Peel 15g/l, Bean Bran 3.13g/l and Incubation Time of 5 days results in maximum EPS production of 10.2g/l. After production, EPS was extracted and quantified by phenol sulphuric acid method using glucose as standard, Identified using Fourier Transform Infrared (FT-IR) and Gas Chromatography–Mass Spectroscopy (GC–MS). The presence of glycosidic linkage (C-O-C) stretching vibration, C=O, C-H and O-H stretching vibrations as revealed by the FT-IR clearly elucidated the production of EPS. This was also confirmed by the presence of Glucose, Mannose and Galactose in the GC-MS characterization of the EPS.

Keywords: Exopolysaccharide; Plackett-Burman; Box-Behnken; FT-IR; GC-MS

1. Introduction

Microbial exopolysaccharides (EPS) are the polymers that consists principally carbohydrates and are excreted by some bacteria and fungi outside of their cell wall. EPS is occur in two forms depending on their location, i.e. capsular polysaccharide (capsule) where the polymer is closely associated with the cell surface and as the slime polysaccharides that are loosely associated with the cell surface (1). Extracellular polysaccharides (EPS) are produced by a great variety of bacteria, including environmental bacteria, pathogens, and food bacteria. These polymers may be assembled as capsular polysaccharides (CPS) tightly associated with the cell surface, or they may be liberated into the growth medium (2). Bacterial exopolysaccharides (EPSs) represent a large group of carbohydrate polymers which can be either covalently associated with the cell surface, forming a capsule, or loosely attached or even as homo- or heteropolysaccharides, depending on whether they are composed, respectively, of one or more than one type of sugar. EPSs are widely distributed among bacteria. They can have a protective function in the natural environment against phagocytosis and predation by protozoa, phage attack, antibiotics or toxic compounds, and osmotic stress (3). Moreover, EPSs also have a role in cell recognition, adhesion to surfaces, and biofilm formation (4). Over the past few decades, the number of known exopolysaccharide (EPS) produced by microbial fermentation has gradually increased.

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Microbial biopolymers have many applications in biotechnology fields including pharmaceutical, tissue engineering, cosmetics, food, textile, oil recovery, metal mining and metal recovery (5). Several researchers have carried on the EPS production by the bacteria and their biotechnological applications. However, it is important to study the optimal culture conditions for a good production of exopolysaccharides, because factors such as the culture medium, pH, temperature and agitation influence the production of EPS and their composition. The influence of the initial pH of the culture medium on the EPS production by several bacteria has been clearly demonstrated (6). Bacillus safensis is a Gram positive,spore forming rod, aerobic and chemoautotrophic bacterium(7). It is a mesophilic and halotolerant microorganism with optimal temperature of 10-50 ^{0}C and 0-10% salt range (7). The production of microbial EPS are highly affected with nutritional and environmental conditions (8) Compared with conventional methods, RSM can be used to design experiments, build models, search optimum factors for desirable responses, and evaluate the relative significance of several influence factors even in the presence of complex interactions (9). The new era of microbial biopolymer production on industrial scale is directed for the production from inexpensive sources like agro-industrial wastes. A co-mixture of sweet potato peel and bean bran can serve as carbon and nitrogen sources for the bacteria while exploiting its potential to produce exopolysaccharide. Large scale production of microbial polysaccharide requires intensive research activities for the application of innovative ideas on a large scale. This research aimed to enhance EPS production from most potent bacterial strain using powerful statistical experimental design as one of the most important steps toward production at the industrial level.

2. Material and methods

2.1. Sample Collection and Screening for EPS Production

Water sample were collected from Bonny River in Rivers state Nigeria. To isolate EPS producing bacteria, the seawater sample was serially diluted in normal saline and plated on nutrient agar (NA) as well as Zubell Marine Agar (ZMA) and incubated for 48hrs. Plate counts of bacterial isolates were done while identification of likely EPS producing microorganisms were initially done to physical identification of isolated that had mucoid morphology. Mucoid colonies were then examined for their ability to produce exopolysaccharide was by growing the isolates on a synthetic media containing g/l: KH_2PO_4 0.2, K_2HPO_4 1.5, $MgSO_4.7H_2O$ 0.2, $CaSO_4.2H_2O$ 0.1, $FeCl_3$ 0.002, yeast extract 0.5 and sucrose 20 (15). The cell-free broths after centrifuging at 10,000 rpm for 10 minutes were mixed with two volumes of cold ethanol and stored at 4 ^{o}C overnight. The precipitates were collected centrifuging at 10,000 rpm for 20 minutes, dried, weighed and dissolved in distilled water (10). They were quantified by phenol sulphuric acid method using glucose as standard (11). The feed raw material feedstock comprising of sweet potato peel and bean bran were sourced locally in Oyigbo L.G.A, in Rivers state.

2.2. Biochemical analysis and Identification of bacterial isolate by 16S rRNA gene and sequencing techniques

The bacterial isolates were biochemically analyzed with the oxidase, catalase, methyl red Voges Proskauer (MR-VP) test, motility, indole production and citrate utilization according to guidelines outlined by Bergey's Manual of Systematic Bacteriology (12). Zymo Quick DNA Fungal/Bacterial Kit was used for the DNA extraction. Bacterial 16S rRNA gene was amplified from the extracted genomic DNA using universal primers, forward 27F (5' AGAGTTTGATCMTGGCTCAG 3') and reverse primer 1525R (5'AAGGAGGTGWTCCARCCGCA 3'). PCR was performed with 2.5µl of 10x PCR buffer,1µl of 25mM MgCl2, 1µl each of forward primer and reverse primer, 1µl of DMSO, 2µl of 2.5mMDNTPs, 0.1µl of 5µ/µl Taq DNA polymerase, and 3µl of 10ng/µl DNA. The total reaction volume was made up to 25µl using 13.4µl Nuclease free water. The PCR program has initial denaturation at 94 °C for 5mins, followed by 36 cycles of denaturation at 94˚C for 30sec, annealing at 56˚C for 30secs and elongation at 72˚C for 45sec. This is then followed by a final elongation step at 72˚C for 7 minutes and hold temperature at 10˚C forever. Amplified fragments were visualized on Safe view-stained 1.5% agarose electrophoresis gels. The size of the amplicon is about 1500bp and the DNA ladder used is Hyperladder from Bioline.

2.3. Preparation of raw material feedstock

Preparation of the feedstock of sweet potato and bean bran was done by washing, drying, milling and sieving of the various feedstock to obtain mesh which are diluted in distilled water and sterilized at 121ºC for 40 minutes (13).

2.4. Optimization of EPS using Plakett–Burman (PB) design

The PB experimental design was applied to screen the significant variables that influence EPS production(14). Ten variables of medium composition and culture conditions were tested at low (−1) and high (+1) levels as shown in Table 1. Based on PB matrix design, two-level factorial design, it allows the investigation of 12 variables (n + 1)(15). This design consisted of three replicated center points to avoid error.

$$Y = ß_0 + \sum ß_{ixi} \quad \ldots\ldots\ldots\ldots (1)$$

where Y is the predicted response, $ß_0$ is the model intercept; $ß_i$ is the linear coefficient and X_i is the level of the independent variable.

EPS production was measured in triplicate and the aver- age value was taken as the response. The variables significant at 95% level ($p < 0.05$) were considered to have significant effect on EPS production and thus used for further optimization.

2.5. Response surface methodology

Box–Behnken design (BBD) with three factors and three levels, including three replicates at the center point, were used for fitting a second-order response surface to provide a measure of process stability and inherent variability (16) The center points and parameters were selected according to Plackett–Burman design. Fifteen trials for three differ- ent variables including medium consisted of sugarcane molasses, yeast extract and incubation temperature were designed. Three levels, "high (+1)", "middle (0)" and "low (-1)" as shown in Table 2. Regression analysis was per- formed on the data obtained using software package 'Design Expert' software (Version 7.0) and confirmed by Microsoft Excel 2010(17) The accuracy of polynomial model equation was expressed by coefficient of determination R^2. All experiments were performed in triplicates. The design is rep- resented by a second-order polynomial regression model as follows:

$$Y = ß_0 + \sum ß_{ixi} + \sum ß2_{ixi} + \sum ß_{ijxij} + \sum \quad \ldots\ldots\ldots\ldots (2)$$

where Y is the predicted response, $ß_0$ is the intercept, $ß_i$ is the linear coefficient, $ß_{ij}$ is the interactive coefficients, $ß_{ii}$ is the quadratic coefficients, and $\sum$ is the error, and x_i and x_j are the coded independent variables.

2.6. Characterization of the exopolysaccharide

The exopolysaccharide produced was characterized using the FT-IR and GC-MS techniques. Buck scientific M530 USA FTIR was used for the analysis which was equipped with a detector of deuterated triglycine sulphate and beam splitter of potassium bromide. BUCK M910 Gas chromatography equipped with HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization system which utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 mL/min.

3. Results

3.1. Sample Collection and Screening for EPS Production

A total of thirteen marine bacterial isolates were isolated from Bonny River, River state, Nigeria. Only three of these isolates WN7, WZ1 and WZ2 on phenotypic examination were found to be EPS producer based on their mucoid colonies and Ropy strands. While screening for EPS production, these colonies produced different productivity rates where WN7 had EPS yield of 0.362g/l, WZ1 had EPS yield of 1.02g/l while WZ2 had EPS yield of 0.615g/l.

3.2. Biochemical analysis and Identification of bacterial isolate by 16S rRNA gene and sequencing techniques

The ESP-producing isolates with the highest yield was differentiated and identified by a 16S rRNA PCR technique to belong to the genus Bacillus. Identification of the isolate was achieved by studying its morphological characters and biochemical features, and confirmed genetically by using 16S rRNA PCR sequence techniques. Molecular characterization of the selected isolate was achieved by performing PCR for the 16S ribosomal RNA gene, followed by alignment of the sequence produced by the PCR with the GenBank database, which also showed high similarity Bacillus. Sequence alignment results revealed that; the isolate belongs to bacillus species with maximum relation to *Bacillus safensis* and assigned with accession number OPO49917. The phylogenetic tree was constructed using the neighbor-joining tree making algorithm (Fig.1).

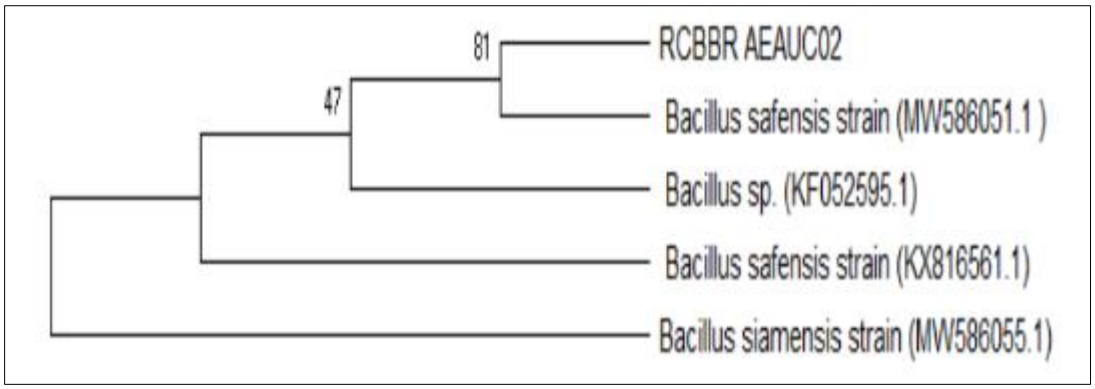

Figure 1 Phylogenetic tree of Bacillus safensis

3.3. Optimization of EPS using Plakett–Burman (PB) design

Ten factors of media components were examined with twelve different trials, and maximum EPS production was obtained for trial number (4), while the lowest production was obtained for trial number (1,3 and 5). The Model F-value of 252.20 implies the model is significant. Investigating each independent term, result indicates factor K_2HPO_4, $MgSO_4H_2O$, $CaSO_4H_2O$, NaCl, Potato Peel, Bean Bran are significant ($p<0.05$) model terms. The ANOVA of Plackett–Burman (PB) design for EPS production is shown in Table 4 where the "Pred R-Squared" of 0.9429 is in reasonable agreement with the "Adj R-Squared" of 0.9956; i.e. the difference is less than 0.2. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Result showing a ratio of 47.639 indicates an adequate signal, thus this model can be used to navigate the design space. The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor.

The model is given as

$Y = -0.0287 + 0.00417(pH) + 0.000555(\text{Incubation time}) - 0.00833(KH_2PO_4) + 0.0144(K_2HPO_4) - 0.2166(MgSO_4H_2O) + 0.2833(CaSO4H2O) - 2.50(FeCl3) + 0.2416(NaCl) + 0.0045(\text{Potato Peel}) - 0.0566(\text{Bean Bran})$

Table 1 Plackett-Burman experimental design for screening of culture conditions affecting EPS production

Std	Run	A	B	C	D	E	F	G	H	I	J	K g/100ml	L g/100ml
7	1	1	-1	-1	-1	1	-1	1	1	-1	1	0.08	0.08
3	2	1	-1	1	1	-1	1	1	1	-1	-1	0.18	0.19
2	3	-1	1	1	-1	1	1	1	-1	-1	-1	0.08	0.08
8	4	1	1	-1	-1	-1	1	-1	1	1	-1	0.21	0.23
12	5	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	0.08	0.08
6	6	-1	-1	-1	1	-1	1	1	-1	1	1	0.14	0.16
11	7	1	-1	1	1	1	-1	-1	-1	1	-1	0.13	0.14
5	8	-1	-1	1	-1	1	1	-1	1	1	1	0.15	0.17
4	9	-1	1	-1	1	1	-1	1	1	1	-1	0.17	0.18
9	10	1	1	1	-1	-1	-1	1	-1	1	1	0.10	0.12
1	11	1	1	-1	1	1	1	-1	-1	-1	1	0.09	0.10
10	12	-1	1	1	1	-1	-1	-1	1	-1	1	0.12	0.13

A (pH), B (Incubation period), C (KH4PO4), D (K_2HPO_4), E ($MgSO_4H_2O$), F ($CaSO_4H_2O$), G ($FeCl_2$), H (NaCl), I (Potato Peel), J (Bean Bran), K (Observed ESP Production), L (Predicted ESP Production).

Table 2 Identification of significant factors for EPS production using Placket-Burman Design

	Coefficient		**Standard**	**95% CI**	**95% CI**	
Factor	Estimate	df	Error	Low	High	VIF
Intercept	0.13	1	8.333E-004	0.12	0.14	
A-pH	4.167E-003	1	8.333E-004	-6.422E-003	0.015	1.00
B-Incubation Time	8.333E-004	1	8.333E-004	-9.755E-003	0.011	1.00
C-KH2PO4	-8.333E-004	1	8.333E-004	-0.011	9.755E-003	1.00
D-K2HPO4	0.011	1	8.333E-004	2.448E-004	0.021	1.00
E-MgSO4H2O	-0.011	1	8.333E-004	-0.021	-2.448E-004	1.00
F-CaSO4H2O	0.014	1	8.333E-004	3.578E-003	0.025	1.00
G-FeCl3	-2.500E-003	1	8.333E-004	-0.013	8.089E-003	1.00
H-NaCl	0.024	1	8.333E-004	0.014	0.035	1.00
J-Potato Peel	0.022	1	8.333E-004	0.012	0.033	1.00
K-Bean Bran	-0.014	1	8.333E-004	-0.025	-3.578E-003	1.00

Table 3 Identification of significant factors for EPS production using Placket-Burman Design

ANOVA for selected factorial model						
Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		**Mean**	**F**	**p-value**	
Source	**Squares**	**df**	**Square**	**Value**	**Prob > F**	
Model	0.021	10	2.102E-003	252.20	0.0490	significant
A-pH	*2.083E-004*	*1*	*2.083E-004*	*25.00*	*0.1257*	
B-Incubation Time	*8.333E-006*	*1*	*8.333E-006*	*1.00*	*0.5000*	
C-KH2PO4	*8.333E-006*	*1*	*8.333E-006*	*1.00*	*0.5000*	
D-K2HPO4	*1.408E-003*	*1*	*1.408E-003*	*169.00*	*0.0489*	
E-MgSO4H2O	*1.408E-003*	*1*	*1.408E-003*	*169.00*	*0.0489*	
F-CaSO4H2O	*2.408E-003*	*1*	*2.408E-003*	*289.00*	*0.0374*	
G-FeCl3	*7.500E-005*	*1*	*7.500E-005*	*9.00*	*0.2048*	
H-NaCl	*7.008E-003*	*1*	*7.008E-003*	*841.00*	*0.0219*	
J-Potato Peel	*6.075E-003*	*1*	*6.075E-003*	*729.00*	*0.0236*	
K-Bean Bran	*2.408E-003*	*1*	*2.408E-003*	*289.00*	*0.0374*	
Residual	8.333E-006	1	8.333E-006			
Cor Total	0.021	11				

Table 4 Correlation

Std. Dev.	**2.887E-003**	**R-Squared**	**0.9996**
Mean	0.13	Adj R-Squared	0.9956
C.V. %	2.26	Pred R-Squared	0.9429
PRESS	1.200E-003	Adeq Precision	47.639
-2 Log Likelihood	-136.11	BIC	-108.77

3.4. Response surface methodology

3.4.1. Four factors pH (A), Incubation Time

(B), Potato Peel (C) and Bean Bran (D) were selected for further analysis using the Box-Behnken Design (Table 2). The Model F-value of 6.72 implies the model is significant ($p<0.05$). Investigating each independent term, result indicates factor A, D, AC, AD, A^2 are significant ($p<0.05$) model terms. The Adeq Precision revealed a ratio of 9.24 indicating an adequate signal, thus this model can be used to navigate the design space. More so, R-squared and Adj R-square of 0.90 and 0.77 was obtained respectively. The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. The normality plot showed the normality of residuals, while the model contour and surface plot revealed the spread of the predicted values in the design space.

The following second-order polynomial equation was;

$$Y= +111.4 - 33.3(A) + 0.46(B) - 4.98(C) - 1.99(D) - 0.04(AB) + 1.59(AC) + 0.46(AD) - 0.04(BC) -0.0003(BD) + 0.0006(CD) + 2.22(A^2) - 0.004(B^2) - 0.49(C^2) - 0.05(D^2) \quad (3)$$

where *Y* represents EPS production (g/L); 111.4 is the intercept; -33.3, 0.46, -4.98 and -1.99 are the linear coefficients; -0.04, 1.59, 0.46,-0.0003 and 0.0006 are the interactive coefficients, 2.22, -0.004, -0.49 and -0.05 are the quadratic coefficients; and A, B, C and D are the concentrations pH, Incubation Time, Potato Peel and Bean Bran respectively. The statistical significance of Eq. (3) was evaluated by *F*-test and ANOVA analysis (Table 5).

Table 5 Experimental design and results of Box-Behnken optimization experiment

	Run	**F1A: pH**	**F 2 B: Potato Peel (g)**	**F 3 C: Bean Bran (g)**	**F 4 D: Incubation (Hours)**	***B. safensis* EPS (g/l)**	***B. safensis* Predicted EPS (g/l)**
22	1	0	1	0	-1	4.75	4.43
12	2	1	0	0	1	4.82	5.75
10	3	1	0	0	-1	5.05	4.57
3	4	-1	1	0	0	7.05	7.70
2	5	1	-1	0	0	5.86	5.48
23	6	0	-1	0	1	2.72	2.68
25	7	0	0	0	0	4.44	4.44
9	8	-1	0	0	-1	10.2	9.36
4	9	1	1	0	1	5.28	4.95
14	10	0	1	-1	1	4.2	3.76
1	11	-1	-1	0	1	6.22	6.82
5	12	0	0	-1	-1	2.42	3.89
17	13	-1	0	-1	0	9.32	8.79
11	14	-1	0	0	1	4.47	5.04

7	15	0	0	-1	1	2.91	2.28
21	16	0	-1	0	-1	4.97	4.23
19	17	-1	0	1	0	5.49	5.03
8	18	0	0	1	1	3.73	2.53
20	19	1	0	1	0	6.79	6.96
24	20	0	1	0	1	2.46	2.84
13	21	0	-1	-1	0	2.5	2.53
18	22	1	0	-1	0	2.67	2.77
6	23	0	0	1	-1	3.15	4.06
15	24	0	-1	1	0	3.27	3.80
16	25	0	1	1	0	2.85	2.91

Table 6 ANOVA for Response Surface Quadratic model

Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		**Mean**	**F**	**p-value**	
Source	**Squares**	**df**	**Square**	**Value**	**Prob > F**	
Model	92.01	14	6.57	6.72	0.0023	significant
A-pH	*12.57*	*1*	*12.57*	*12.84*	*0.0050*	
B-PP	*0.092*	*1*	*0.092*	*0.094*	*0.7656*	
C-BB	*0.13*	*1*	*0.13*	*0.14*	*0.7208*	
D-IT	*7.41*	*1*	*7.41*	*7.57*	*0.0204*	
AB	*0.50*	*1*	*0.50*	*0.51*	*0.4924*	
AC	*15.80*	*1*	*15.80*	*16.14*	*0.0024*	
AD	*7.56*	*1*	*7.56*	*7.73*	*0.0195*	
BC	*1.12*	*1*	*1.12*	*1.15*	*0.3091*	
BD	*4.000E-004*	*1*	*4.000E-004*	*4.087E-004*	*0.9843*	
CD	*2.025E-003*	*1*	*2.025E-003*	*2.069E-003*	*0.9646*	
A^2	*13.89*	*1*	*13.89*	*14.19*	*0.0037*	
B^2	*0.49*	*1*	*0.49*	*0.50*	*0.4936*	
C^2	*1.68*	*1*	*1.68*	*1.72*	*0.2190*	
D^2	*0.65*	*1*	*0.65*	*0.66*	*0.4354*	
Residual	9.79	10	0.98			
Cor Total	101.80	24				

Table 7 Correlation

Std. Dev.	**0.99**	**R-Squared**	**0.9039**
Mean	4.70	Adj R-Squared	0.7693
C.V. %	21.03	Pred R-Squared	N/A
PRESS	N/A	Adeq Precision	9.249
-2 Log Likelihood	47.50	BIC	95.78
		AICc	30.83

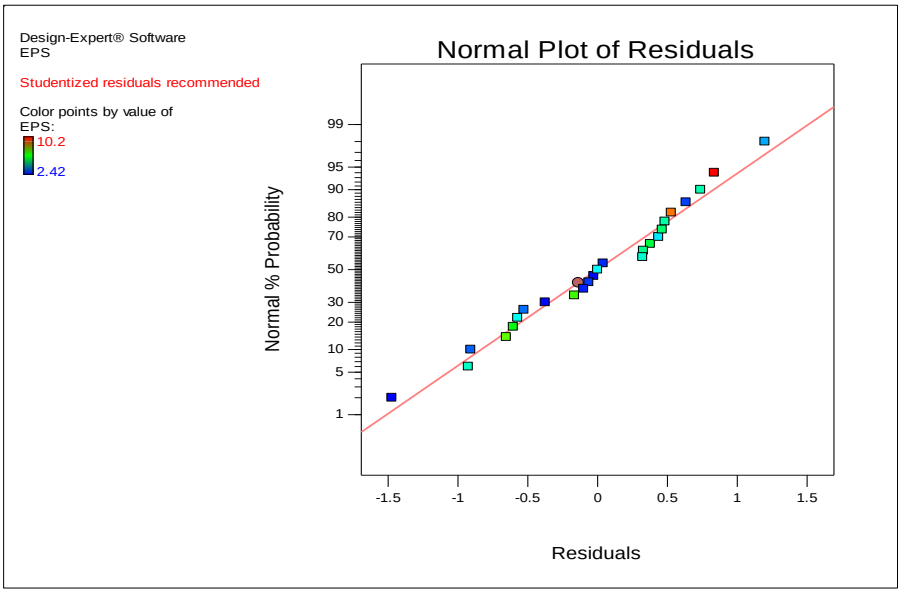

Figure 2 Probability plots of Response Surface Methodology

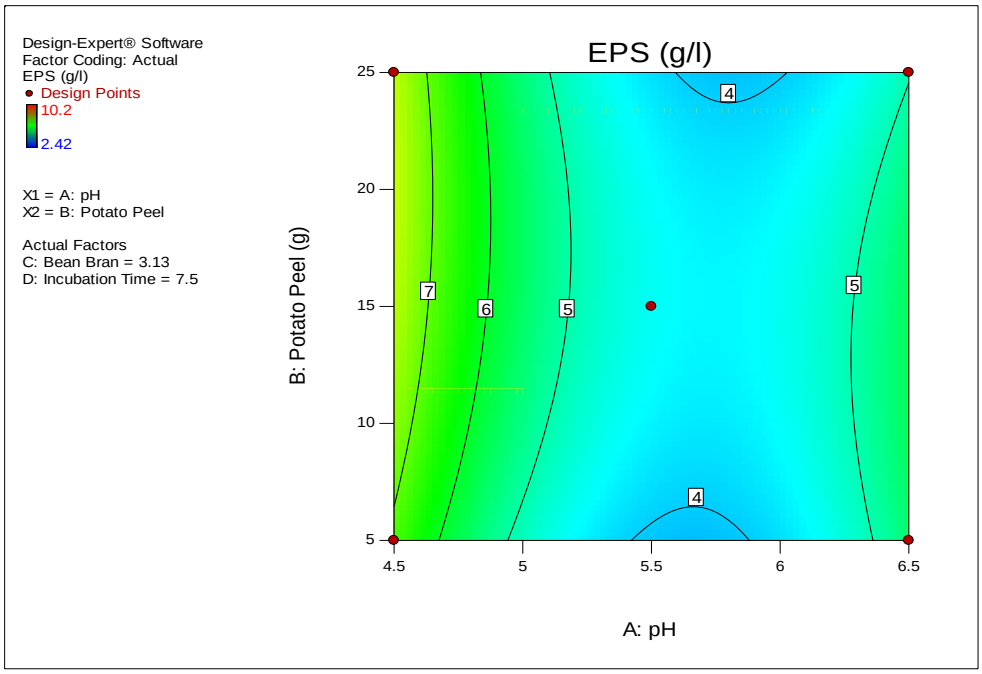

Figure 3 Contour plots of Response Surface Methodology

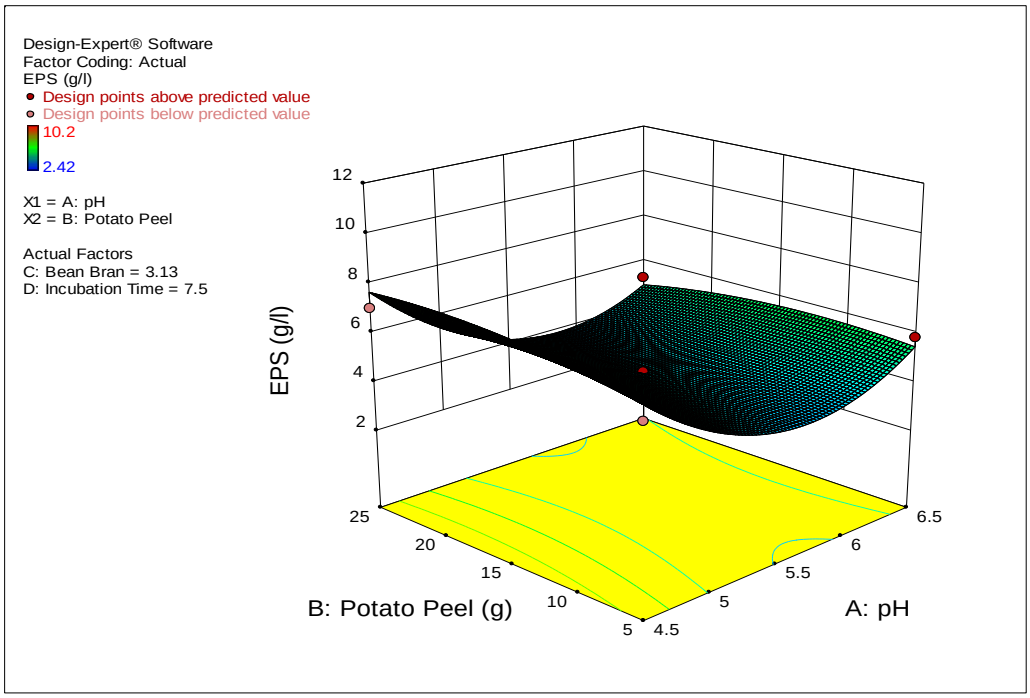

Figure 4 Surface plot of Response Surface Methodology

3.5. Characterization of the exopolysaccharide

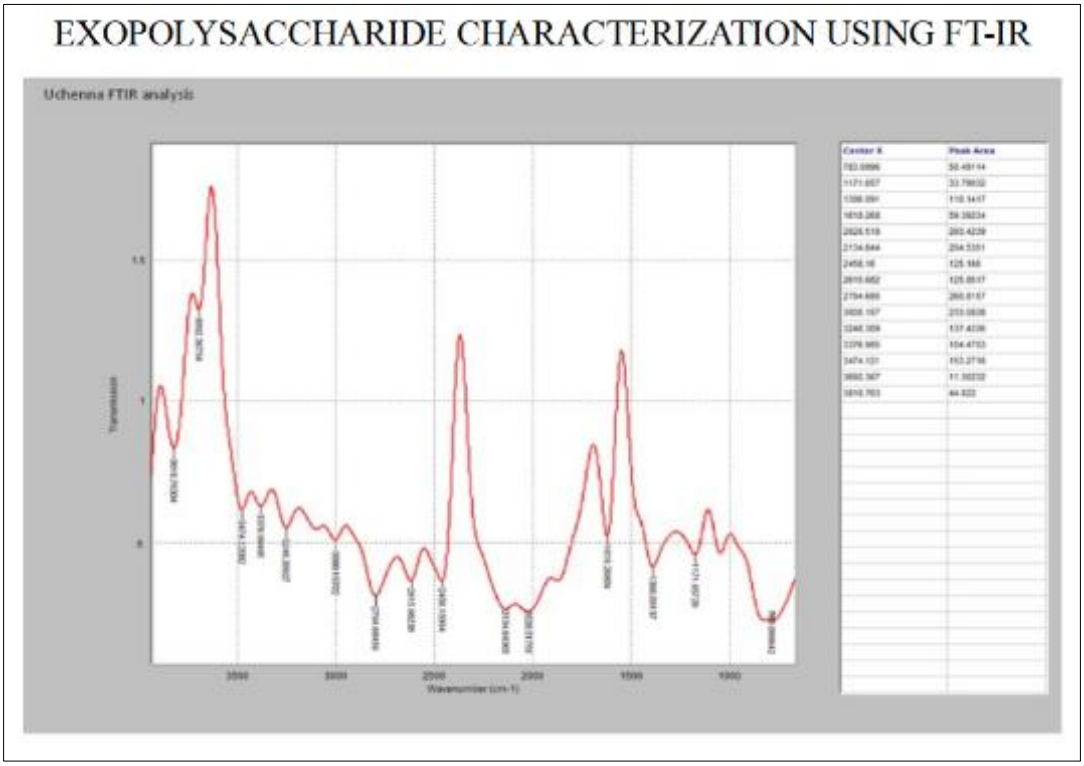

Figure 5 FT-IR characterization of the EPS

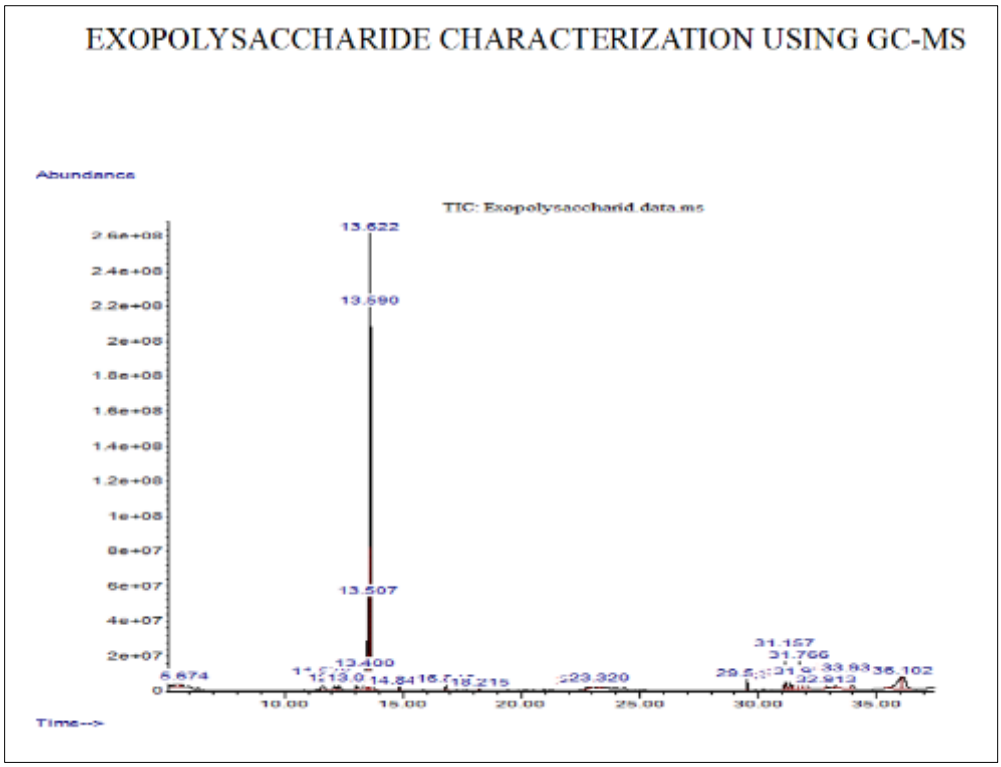

Figure 6 GC-MS Characterization of the Exopolysaccharide

4. Discussion

Exopolysaccharides (EPS) was extracted from *B. safensis* broth media by using ethanol to bacterial culture in ratio 2:1 respectively. The first screening of different bacterial isolates for selection of most potent EPS producing strain, the maximum productivity obtained was 1.02g/l from isolate WZ1. This result agreed with (18) who reported maximum productivity of 3g/l in *Bacillus velensis*.

This was followed by Plackett-Burman design for screening of significant variables. Data analysis obtained from this model indicted the use of Bean Bran as nitrogen source and Potato Peel during production of polysaccharides by isolate code (WZI) in place of Yeast Extract and Sucrose respectively.

With the same consequence; RSM depend on data obtained from Plackett–Burman and the data analysis indicated that the maximum yield was estimated to be 9.36g/L, and the actual yield obtained with the optimal medium was 10.20 g/L (the average value of triple experiments), which was in close accordance with the model prediction. This is obviously higher than what was reported by (18). It has been reported that the production of EPS is a response to nutrient composition of the medium. In this study, high level of EPS was achieved because the medium was rich in organic carbon and nitrogen source. This is in support of study done earlier by (13) which showed that Optimization studies on EPS production using cost effective medium Sweet Potato and Bean Bran produced more EPS than Sucrose and Yeast Extract respectively though the yield of EPS production has been strongly associated to yeast extract as reported by (19) and corroborated by (20) and (21). In general, EPS production is influenced by pH, Temperature and Carbon source (22).

FT-IR for purified EPS showed a characteristic absorption band appeared at 1171.66 cm^{-1} (Fig.4) was assigned to carbohydrate C-O stretching vibrations and dominated by glycosidic linkage (C-O-C) stretching vibration agreed with the findings of (18) and (23). Exopolysaccharides, being complex carbohydrate polymers, often show strong peaks in this region due to the glycosidic linkages between monosaccharide units. The peak above could be specifically associated with glycosidic bond in the exopolysaccharide structure which links the sugar units together. This bond is a defining feature of the polysaccharide's defining molecular structure.

Another absorption band at 1618.27 cm^{-1} is often associated with the stretching vibrations of the carbonyl (C=O) group. It indicates the presence of uronic acid which are common components in the polysaccharide backbone. This agrees with the findings of (18) and (24).

Absorption peak of 3000.16 cm^{-1} is consistent with the (C-H) stretching vibrations found in polysaccharides. This suggest that the EPS has a significant aliphatic or aromatic C-H groups which is typical for polysaccharide structures. This also agrees with the works of (25) and (26).

Absorption peaks at 3248.31 cm^{-1} was attributed to the hydroxyl (O-H) stretching vibration of the polysaccharide which are a notable feature of the EPS. This is consistent with the findings of (25) and (27).

Based on the result of GC-mass analysis, it was concluded that the major monosaccharide for purified EPS obtained from *Bacillus safensis* OPO49917 revealed that the EPS contained Glucose, Mannose and Galactose. (18) and (25) reported that *Bacillus velensis* KY498625 and *Bacillus amyloliquefaciens* RT7 produce EPS that contains glucose, mannose which agreed with our EPS.

5. Conclusion

Industrial production of EPS requires cost effective and optimal culture medium. Two statistical experimental design Plackett–Burman and Box–Behnken were used to improve Exopolysaccharide production from *Bacillus safensis.* The production of EPS under optimal conditions obtained from data analysis revealed significant increase in productivity from 1.20 to 10.20g/L. This has laid credence to importance of utilizing statistical designs to cut cost and ensure improved EPS production.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no conflict of interest exist in the work.

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