

“Screening and Optimization of Agro-industrial wastes for glycoprotein biosurfactant production from *Sphingobacterium thalpophilum* DP9”

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Research

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Abstract

Background

Commercialization of biosurfactant production is a big challenge due to high production cost. Biosurfactant production can be made economic by using low cost agro-industrial wastes or byproducts as media supplement. It also solves the problem of environmental pollution through waste management. In the present study attempt was made to produce biosurfactant from *Sphingobacterium thalpophilum* DP9 using various agro-industrial wastes or byproducts at optimum fermentation conditions evaluated by traditional and statistical methods. Partial characterization of biosurfactant was also carried out through qualitative chromatographic techniques; quantitative spectroscopic method and functional group identification by Fourier transform infrared spectroscopy.

Results

One factor at a time optimization experimentation showed highest emulsification in media supplemented with potato peel powder ($43.12 \pm 3.41\%$), urea ($46.00 \pm 3.09\%$), pH 7 ($41.74 \pm 1.15\%$) at 40% aeration ($47.41 \pm 1.62\%$). 1% Inoculum ($O.D._{600}=1.00$) size favored highest emulsification ($26.39 \pm 1.60\%$). Plackett-Burman design experimentation showed carbon source (potato peel powder), nitrogen source (urea) and temperature significantly affect on biosurfactant production. Response surface experimentation by central composite design showed interaction between nitrogen source and temperature significantly influenced on biosurfactant production. Validation of model was showed increase in emulsification from $43.62 \pm 2.55\%$ to $86.11 \pm 3.47\%$ on fourth day of incubation. Partial characterization showed biosurfactant contains 439.58 ± 0.0129 $\mu\text{g/ml}$ carbohydrate and 507.41 ± 0.0064 $\mu\text{g/ml}$ of protein. Lipid was absent in it. Fourier transform infrared spectroscopy chromatogram showed peaks at 1652.17 cm^{-1} , 1541.01 cm^{-1} , 1463.71 cm^{-1} and 722.32 cm^{-1} which indicated presence of peptide bond, peptide moiety, carbohydrate protons and primary as well as secondary amines.

Conclusion

Potato peel powder, urea and temperature have significant influence on biosurfactant production from *Sphingobacterium thalpophilum* DP9. When potato peel powder and urea supplied at optimum level, biosurfactant production was increased by two fold. Biosurfactant produced by *S. thalpophilum* DP9 was belongs to glycoprotein class and as per our best knowledge, this is the first report on glycoprotein biosurfactant produced from *Sphingobacterium* genus.

Background

Biosurfactants are biologically produced surface active amphiphilic molecules which find their applications in various industries viz. medical (Henkel et al. 2012; Radzuan et al. 2017), pharmaceutical (Bhardwaj et al. 2013; Hu et al. 2015), petroleum (Henkel et al. 2012; Singh et al. 2019), food (Armendáriz et al. 2019; Moshtagh et al. 2019), cosmetic (Vera et al. 2018; Joy et al. 2020) and personal health care industries. They are superior to chemical surfactants because of their biodegradable (Makkar and Cameotra 1997a), biocompatible (Rodrigues and Dourado 2014), non/less toxic (Rodrigues and Dourado 2014), action specific (Rosenberg et al. 1979) and stable nature (Kumaran et al. 2015). Major challenge about the utilization of biosurfactant is the production cost at industrial level and demand of purity in some sectors. For instance, the biosurfactant used for microbial oil enhanced recovery requires large amount of biosurfactant with less purity, however, the biosurfactant employed for cosmetics and personal health care products needs high purity as they might elicits immunological reactions (Mukherjee et al. 2006; Helmy and Kardena 2011; Soares da Silva et al. 2017).

Major challenges for industrial production are high capital cost, less efficient biosurfactant producing strains, limited knowledge of genes and pathways for biosurfactant biosynthesis which may lead to restricted gene manipulation in existing strains, less technological advances and less efficient downstream processing for biosurfactant purification (Makkar and Cameotra 1997b; Thavasi et al. 2008; Helmy and Kardena 2011; Yañez-Ocampo et al. 2017; Radzuan et al. 2018).

One of the possible solution for industrialization of biosurfactant production, is the use of agricultural waste and/or by products as cheaper alternative production media supplements which lower the capital cost and make the process economic (Nitschke et al. 2004; Rivera et al. 2019; Nogueira et al. 2020). Moreover, it also serves the purpose of waste disposal to many agro, brewing and milk or milk product based industries in addition to environmental concern (Mercade and Manresa 1994; Banat et al. 2014). The choice of right agricultural waste and/or byproduct with correct balance of carbohydrate, lipid, protein and other mineral salts is important not only to support organisms growth but also helps in production of secondary metabolites like biosurfactants (Helmy and Kardena 2011). On the basis of such background knowledge the present study was conducted to screen various agricultural wastes or byproducts for the biosurfactant production at optimum fermentation conditions as per the traditional as well as statistical method. These are the conventional but effective strategies for improvement of production (Singh et al. 2019).

Methods

Microorganisms and its maintenance

In the present study, *Sphingobacterium thalpophilum* DP9 – Gram negative bacteria (GenBank accession number: MG–000135) isolated in our laboratory from automobile workshop soil (Anand, Gujarat, India N 22°54' and E 72°95') was used. The bacterial culture was maintain in Luria Bertani (LB) agar slants at 4 °C and was sub-cultured at every three month after confirming purity of culture by Gram's staining.

Inoculum preparation, Medium composition and culture conditions

Inoculum was prepared by adding a well isolated colony from L.B agar plate in to 100 ml of LB medium maintained at 30 °C with 150 rpm shaking condition overnight. Next day, required amount of inoculum (after O.D.₆₀₀ reaches to 1.00) was added to production medium.

Bushnell–Haas medium (BHM) added with carbon and nitrogen source was used as production medium. Media were sterilized by autoclaving 121 °C for 15 min. The screening of factors viz. carbon source, nitrogen source, temperature, pH, inoculum size and aeration were carried out primarily by One–Factor–At–Time (OFAT) method. Afterwards, Plackett–Burman design made by Microsoft Office-2007 with Excel add-on , was employed to screen significant factor.

Carbon source used were rice straw, corn straw, wheat straw, maize bran, wheat bran, potato peel powder, cotton seed cake, sugarcane husk, ground nut husk, coconut oil cake, and sugarcane molasses.

Nitrogen sources used were urea, peptone, ammonium sulfate, ammonium nitrate, ammonium chloride, sodium nitrate, yeast extract and malt extract.

Screening of agricultural wastes or byproducts by One–Factor–at A –Time [OFAT] method

OFAT method was used to screen optimum carbon source (at 1% concentration), nitrogen source (0.1% concentration), inoculum size (0.5 % to 4 % when 1.00 O.D.₆₀₀), pH (5 to 11), temperature (25 °C to 45 °C) and aeration (by varying the headspace volume in 250 ml conical flask by changing the medium volume in the flask by 10 % to 50 % v/v of flask i.e. 250 ml as suggested by Abdel-mawgoud et al. 2008)). Carbon sources and nitrogen sources were screened by separately added in 100 ml of production medium (i.e. BHM). Screening of aeration was carried out by preparing production media with different volume viz. 25 ml, 50 ml, 75 ml, 100 ml, 125 ml and 150 ml in 250 ml Erlenmeyer flask. Later on, pH, inoculum size and temperature were screened sequentially. All media were kept under 150 rpm shaking condition and response was checked for 10 days by emulsification index (E24% test) as suggested by Shahaliyan et al. (2015).

Plackett–Burman Design

Plackett–Burman design was used to find out important medium component (carbon or nitrogen source) and production parameter (temperature and pH). Plackett–Burman design matrix also assumes that there is no interaction between variables considered in present study in the range. It consents to explore up to 'N–1' variables with N experiments without inter-component interactions. In present study, four variables were selected viz. potential carbon source, potential nitrogen source, pH and temperature. Each variable was checked at two level (–1, +1 or low level and high level) as given in Table-1. Plackett–Burman design matrix is a full factorial design and main effects of design is simply calculated as per the difference between average value made at the high level values (+) of the factor and average of measurement at the low level values (–). The critical factors are identified through this experimental matrix and then Central Composite Design (CCD) was used to obtain quadratic model.

Table.1 The various media components including in Plackett-Burman experiments and their corresponding higher, medium and lower concentration

Table.2 Plackett–Burman Design for biosurfactant production from *S. thalpophilum* DP9.

The linear approach was considered to be sufficient for screening

[Please see the supplementary files section to view the equation.]

Where Y is the response in terms of protein assayed as per the method suggested by Lowery et al. (1951), β_i are regression coefficients, f_i is the level of the independent variable. The experiments were conducted three times and results were noted in mean \pm standard deviation (Mnif et al. 2012; Anvari et al. 2015; Ekpenyong et al. 2017).

Central Composite Design (CCD)

Following the screening of significant media components by Plackett-Burman design, relationship among the quantitative factors and the response (biosurfactant production) was carried out by central composite design (CCD) under response surface methodology (RSM). Best response was evaluated by permutation of the factor levels (El-Gherab et al. 2019). Significant factor obtained after analysis by Plackett-Burman design, was used to create CCD matrix and statistical analysis. Most optimum level, impact and interaction of factors i.e. carbon source (X_1), nitrogen source (X_2) and temperature (X_3) at three coded level (-1, 0 and +1) as shown in the table-3 by full factorial (2^3) CCD using Design s (X_2) and pH (X_3) were optimized by full factorial (2^3) central composite design (CCD) using Design Expert software® (Stat-Ease Inc. Minneapolis, MN, USA, version–6.0.8). 2^3 full factorial design was generated in to 20 experimental runs. The experiments were carried out in 250 ml Erlenmeyer flasks containing 100ml of medium. Each production medium was inoculated with 1% overnight grown bacterial cells (O.D.₆₀₀ = 1.00) and incubated for four days. Interaction experimentations were designed as per CCD model based on three factors. According to CCD design, second order polynomial regression equation was

[Please see the supplementary files section to view the equation.]

Where, Y – Predicted response (Emulsification index; indirectly biosurfactant production); β_0 – Intercept; X_1 – carbon source concentration (g/l); X_2 – Nitrogen source (%w/v); X_3 – Temperature; β_1 , β_2 and β_3 – linear co-efficient; β_{12} , β_{23} and β_{13} – interaction coefficient; X_1^2 , X_2^2 , X_3^2 , X_1X_2 , X_2X_3 and X_1X_3 – interaction between the variables as significant terms.

Experimental Validation of statistical model

From the response surface design the optimum experimental conditions were tested and validated three times. Results were recorded as in terms of mean \pm standard deviation.

Extraction and purification of biosurfactants

Cell free supernatant was collected from each flask prepared as per the optimized and validated process for biosurfactant production by centrifugation (6000 rpm for 20 min) and pH was set 2.00 using 6.0 N HCl. Broth was preserved at 4 °C till visible precipitates observed. Precipitates were collected by centrifugation and kept for air drying. Biosurfactant was collected as dry powder by scraping.

Partial characterization

Qualitative analysis by thin layer chromatography

Biosurfactant produced from optimized medium was qualitatively analyzed through thin layer chromatography for presence of carbohydrate, protein and lipid. Solvent systems used were ethyl acetate: acetic acid: methanol: water (12:3:3:2); n-butanol: acetic acid: water (4:1:1) and chloroform: methanol: water (65: 25: 4) for carbohydrate, protein and lipid, respectively. Carbohydrate was detected by treating the TLC with α -naphthol followed by sulfuric acid. Protein was detected by spraying 0.3% ninhydrine solution and for lipid content iodine vapor was used.

Spectrophotometric qualitative test

Biosurfactant powder was dissolve in distilled water to get 10 mg/ml of concentration. Carbohydrate, protein and lipid contents were estimated by phenol-sulfuric acid test, Folin-Lawry method and GPO/POD method (according to diagnostic triglyceride kit procured from Sigma Diagnostic Pvt. Ltd. Vadodara, India), respectively.

Electrophoretic studies

Biosurfactant was further analyzed through sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by coomassie brilliant blue stain, silver stain. Native-PAGE followed by Periodic acid-Schiff (PAS) stain as per the standard protocol.

FTIR [Fourier- Transform Infrared] spectroscopic study

Presence of chemical groups in biosurfactant was evaluated by Bruker FTIR (Alpha-T) spectrophotometer (Bruker Optics Inc., USA and M/s Lab India Analytical Instruments Pvt. Ltd.). Briefly, the pellet was made with 1 mg dry biosurfactant grinded in 100 mg (final volume) of IR grade KBr under mechanical hydraulic. KBr pellet without biosurfactant serve as control or blank. Both, KBr and biosurfactant were dried in hot air oven at 65 °C for complete removal of moisture content overnight. Chromatogram generated by FTIR spectrophotometer was compared with standard table and presence of possible chemical groups was recorded.

Results And Discussion

The perspective of maximizing biosurfactant yield can be fulfill by utilizing agricultural waste or byproducts which not only help to cut down the capital cost but also helps in waste management. In

present study, we have screen eleven agricultural wastes or byproducts for their efficacy as media supplement. The right choice of agricultural waste is very important because the biosurfactant yield, its type and purity is solely depends on the media constituents (Helmy and Kardena, 2011). Agricultural waste or byproducts provides adequate balance of carbohydrate and lipid to the bacteria along with other nutrients like protein, mineral salts etc. for their growth and also for production of secondary metabolites like biosurfactant (Mercade and Manresa, 1994). Therefore, by using a single agricultural waste or byproduct, the production of biosurfactant can be made economic. In present study, we have screened eight different nitrogen sources for their impact on biosurfactant. They are either organic compound or inorganic salt.

In order to maximize production two methods were adopted. First OFAT to screen effective carbon source, nitrogen source, pH, temperature, % inoculum and aeration, which is followed by statistical approach i.e. Plackett-Burman Design using Microsoft Office-2007 Excel add-on and CCD matrix using Expert Design software for optimum concentration of media constitutes and cultural conditions (Nurfarahin et al. 2019).

OFAT analysis

One factor at a time method for optimization is traditional technique used to screen effective agricultural waste or byproduct. The major advantage of this method is its simplicity (because it does not require any statistical analysis) and results can be narrated by simple graphs (Hema et al. 2019). In present study, among the all agricultural wastes or byproducts listed above highest emulsification index was observed in potato peel powder containing medium ($E_{24\%} = 43.12 \pm 3.41\%$), which was followed by deoiled coconut oil cake ($E_{24\%} = 31.00 \pm 8.27\%$) and wheat bran ($E_{24\%} = 31.47 \pm 3.41\%$) (Fig.1(a)). *Bacillus subtilis* DDU20161 was reported to produce 253.79 gm/L of biosurfactant when cultured in media containing potato peel and pulp during 40 h of incubation time under stirred tank bioreactor (Pande et al. 2020); while potato peel significantly effect on emulsification of biosurfactant from *Bacillus subtilis* SNW3 (Naeem 2018). The emulsification index of biosurfactant produced by *B. subtilis* SNW3 strain was 15. 48% when bacteria were grown in media supplemented with potato peel (Naeem 2018). In case of *B. licheniformis* J1 potato peel was act as biostimulator to increase the petroleum oil degradation potential (Jyoti and Rajesh 2018). According to Javed et al. (2019) potato peel is an economy booster for developing countries because it is inexpensive waste of food processing industry and can support many of the industries viz. backing industry, biogas production industry, lactic acid production industry, enzyme production industry, bio-fertilizer industry, bio-fuels industry, bio-sorbent industry, pharmaceutical industry and biosurfactant production industry.

Similarly, highest emulsification index $46.00 \pm 3.09\%$ was observed in media containing urea, followed by yeast extract ($E_{24\%} = 38.00 \pm 0.10\%$) and ammonium sulfate ($35.33 \pm 0.90\%$) (Fig.1(b)). *Serratia nematodiphila* produced 20% more glycolipid biosurfactant when it grown under media containing 0.1% w/v of urea (Panjjar et al. 2020). *Bacillus cereus* produced biosurfactant effectively reduced surface tension of water when it was cultured in presence of urea (Durval et al. 2018). Whereas, urea was less

preferred by *B. subtilis* RSL2 for the production of biosurfactant (Sharma and Pandey 2020) while, for *Arthrobacter paraffines* ATCC 19558 urea was preferable organic source for biosurfactant production (Ezebuoro et al. 2019).

Dissolve oxygen influences of biosurfactant production (Kronemberger et al. 2007). Effect of aeration on biosurfactant was assayed by filling different volume of media in 250 ml flasks and it was observed that 40 % of aeration (100 ml media in 250 ml flask) was most suitable ($E_{24\%} = 47.41 \pm 1.62\%$), which followed by 30 % ($E_{24\%} = 38.00 \pm 2.30\%$) and 20% ($E_{24\%} = 27.825 \pm 1.73\%$) (Fig.2(a)). *Bacillus mojavensis* A21 produced highest surfactin when cultured in 10% flask (25 ml media in 250 ml of flask) while the production in decreased when culture was kept in 70% aeration (75 ml media in 250 ml flask) (Hmidet et al. 2017). *Bacillus subtilis* BS5 produced maximum surfactin 10% media containing flask where as production is decline sharply when media volume was increased from 10% (Abdel-mawgoud et al. 2008). Optimum pH and temperature for maximum biosurfactant production was 7 ($E_{24\%} = 41.74 \pm 1.15\%$) and 30 °C ($E_{24\%} = 44.00 \pm 2.36\%$) (Fig.2(b) and (c)). Bacterial inoculum ($O.D._{600}=1.00$) size of maximum biosurfactant production observed was 1% ($E_{24\%} = 26.39 \pm 1.60\%$) (Fig.2(d)).

Therefore, potato peel powder and urea were used as carbon and nitrogen sources respectively, under 40% of aeration for further studies. Bacterial inoculum used was 1% with $O.D._{600}=1.00$, pH 7 and incubation temperature was kept 30 °C.

Optimization by Plackett-Burman Design

Once the optimum media components and production parameter was screened by traditional OFAT method, Plackett-Burman design was used for find out most influencing factor on biosurfactant production among the carbon source, nitrogen source, temperature and pH. The design generated by Microsoft Office-2007 with Excel add-on given in Table-2. The Plackett-Burman design for eleven experiments with four variables (i.e. carbon source, nitrogen source, temperature and pH) shown in Table-2. The protein concentration (indirectly biosurfactant production) was considered as response. The results achieved from the experimental run was noted as in Table-2 and it showed variation from 38.4 µg/ml (run no: 12) to 265.9 µg/ml (run no: 5). Data was subjected to statistical analysis using Microsoft Office-7 Excel add-on, to calculate main effect, standard error F-value, *p*-value and 90% clearance. Analysis of the data showed in Table-3. Components showed more than 90% clearance were considered as most influencing factor on biosurfactant production.

From the obtained data it was clear that carbon source, temperature and nitrogen source showed clearance value above 90% than i.e. 97.93%, 92.35% and 92.25%, respectively. Therefore, most influencing factor was carbon source (in present study potato peel powder) followed by temperature and nitrogen source (in present study urea).

After finding the critical factors i.e. potato peel powder, temperature and nitrogen source, response surface methodology was used to study interactions between factors and to find out exact concentration or values of the variables for biosurfactant production through CCD.

Optimization of media component and production parameter by Central composite Design (CCD)

Selected variables which gave significantly influence on biosurfactant production were optimized. Experiment was carried out using CCD which consisted of three levels i.e. low level, high level and central point for each variable. The design and obtained values are described in Table-4. Highest response (E24% = 50%) was obtained in run-4 (5 gm% potato peel powder, urea 1.5 gm% and temperature 45 °C) and lowest response (E24% = 52%). ANOVA analysis was carried out to scrutinize the variability produced by a factor. Regression analysis showed urea (X_2) and temperature (X_4) were significant while potato peel powder (X_1) was insignificant ($p < 0.05$) variables. Linear positive co-efficient values of all three variables were non-significant. The interaction between $X_2 - X_4$ (urea and temperature; $p=0.0248$) was significant while $X_1 - X_2$ (potato peel powder and urea) as well as $X_1 - X_4$ (potato peel powder and temperature) were insignificant. The correlation co-efficient R^2 indicates accuracy of model and it was 76.94% and adjusted R^2 value was 56.19%. Second-order polynomial equation was used to study effect of factors on E24% (indirectly biosurfactant production). The equation for present study was as follow:

[Please see the supplementary files section to view the equation.]

Here, Y is response i.e. E24% (indirectly biosurfactant production); X_1 carbon source (potato peel powder), X_2 nitrogen source (urea) and X_4 temperature.

To evaluate the optimum value of each factor for highest E24% (or biosurfactant production), 3D response surface plots (Figure-3) were created in Design Expert software by plotting the response function of two factors while keeping another at central point. The significant interaction between X_2 and X_4 was obtained from response surface plot.

It was clear from the 3D plots that, interaction between X_1-X_2 and X_1-X_4 the center point of carbon source is 3.2 gm%. Beyond this concentration the predicted value of E24% was increased but, technically, the values of E24% never go beyond 100%. Therefore, values of variables were selected in such a way that response resided below 100%. Similarly, as the values of nitrogen source goes near to 4 mg% and temperature near to 28 °C, the response or value or E24% was predicted approximately to be 100%. Hence, for validation purpose, the E24% (response) was predicted 100% under optimized condition and verified by experimentation carried out in triplicates. Under un-optimized conditions, the E24% was $43.62 \pm 2.55\%$; which was increased after optimization to $86.11 \pm 3.47\%$. The achieved results proposed considerable accuracy in developed model and model validation under the prescribed conditions.

The data are comparable with the results reported in case of *B. aryabhatai* strain ZDY2 by (Yaraguppi et al. 2020). Author documented that, *B. aryabhatai* ZDY2 produced 2.51 fold higher biosurfactant under optimized media when supplied with 4.0% crude oil, 0.7% yeast extract and 3.0 % NaNO_3 . *Bacillus subtilis* SPB1 produced 1.65 fold higher biosurfactant with optimized media supplemented with glucose, urea and K_2HPO_4 at 15 g/L, 6 g/L and 1g/L of concentration respectively (Mnif et al. 2012). Optimum conditions for biosurfactant production by *B. subtilis* N3-1P was 7% v/v

brewery waste, 6.22 mg/L NH_4NO_3 containing media with pH 6.41 which was maintained at 27 °C (Moshtagh et al. 2019).

Partial characterization of biosurfactant

Biosurfactant produced by *S. thalophilum* DP9 from optimized media was extracted simply by acid precipitation. Partial purified biosurfactant was analyzed for presence of carbohydrate, protein and lipid qualitatively by TLC and quantitatively by spectrophotometric methods. Thin layer chromatography results indicated presence of carbohydrate ($R_f = 0.71$) and protein ($R_f = 0.31$). Lipid was absent in the biosurfactant. Spectroscopic qualitative analysis revealed that biosurfactant contain 439.58 ± 0.0129 $\mu\text{g/ml}$ of carbohydrate and 507.4 ± 0.0064 $\mu\text{g/ml}$ of protein, while lipid was absent. On the basis of qualitative and quantitative tests, it was assumed that the biosurfactant was glycoprotein in nature.

Glycoprotein nature of biosurfactant was confirmed by PAS staining Native-PAGE gel. Moreover, the size of the protein part present in biosurfactant was evaluated by SDS-PAGE electrophoresis followed by Coomassie Brilliant Blue (CBB) staining and Silver staining. Approximate size of protein part carried by biosurfactant was found between 42 kDa to 51 kDa. We assumed that it was near to 48 kDa of size. The presence of single band in all three staining i.e. PAS staining, CBB staining and silver staining at almost same place indicated that the biosurfactant obtained was relatively pure. Further purity can be confirmed by High Pressure Liquid Chromatography (HPLC). Results are contradictory to previous reports. Burgos-Díaz et al. (2011) have reported that the *Sphingobacterium* strain 6.2S produces mixture of biosurfactant which belongs to lipopeptide, phospholipid or glycolipid. Similarly, Noparat et al. (2014) have documented that *S. spiritivorum* AS43 produced lipopeptide biosurfactant which having 50.2% lipid and 38.5% or protein in its structure. To our best knowledge, this is the first report of glycoprotein biosurfactant production from *Sphingobacterium* genus.

FTIR analysis

Molecular composition of partially purified biosurfactant was carried out by Fourier transform infrared spectroscopy. Weak stretching peak at 3292.61 cm^{-1} indicated presence of stretching vibrations from –NH of peptide (Noparat et al. 2014). Strong peaks at 3009.51 cm^{-1} and 2927.05 cm^{-1} indicated –CH stretching vibrations from – CH_2 and – CH_3 group of aliphatic chain (Burgos-Díaz et al. 2011; Noparat et al. 2014; Yaraguppi et al. 2020). Stretching at 1746.68 cm^{-1} indicated presence of ester or carbonyl –C=O of –COOH (Yaraguppi et al. 2020) and 1463.71 cm^{-1} described –C–O–H planar bending of carboxylic acid or carbohydrate protons (Panjiar et al. 2020) or –C–O stretch of ester (Burgos-Díaz et al. 2011). 1652.17 cm^{-1} peak indicated presence of –CO–N bond stretching (Noparat et al. 2014) or –N–H stretching of – NH_3^+ from peptide group (Burgos-Díaz et al. 2011) which gave confirmation of presence of peptide bond (Hema et al. 2019). Peak at 1541.01 cm^{-1} designated to –NH bond at deformation mode combined with –CN stretching or aliphatic and peptide like moiety (Noparat et al. 2014). Peaks at 1378.85 cm^{-1} , 1239.47 cm^{-1} and 1164.67 cm^{-1} were observed due to ester –CO bond stretching, –CH stretching and lactones –C–O bond stretching or pyranose –C–O–C– symmetric stretching, respectively

(Hema et al. 2019; Yaraguppi et al. 2020; Panjiar et al. 2020). Peak at 1099.02 cm^{-1} indicated the stretch of $-\text{C}-\text{N}$ group of aliphatic amines or $-\text{C}-\text{O}-\text{H}$ stretch of alcohol or phenol or $-\text{C}-\text{O}$ of ester, ether, alcohol or carboxylic acids. $77=22.32\text{ cm}^{-1}$ peak occurred due to vibrations of primary or secondary amines or due to methylene scissoring vibrations of protein moiety (Yaraguppi et al. 2020). Hence, from the FTIR chromatogram (peaks at 1652.17 cm^{-1} , 1541.01 cm^{-1} , 1463.71 cm^{-1} and 722.32 cm^{-1}) the presence of peptide bond, peptide moiety, carbohydrate protons and primary as well as secondary amines were confirmed which again indicated that the biosurfactant is belongs to glycoprotein class.

Conclusions

To make the biosurfactant production economic, agro-industrial waste or byproducts can be use as media supplement. In present study, eleven carbon agro-industrial waste or byproducts used as carbon sources and eight (organic as well as inorganic) nitrogen sources were screen for better biosurfactant production by conventional one factor at a time method. Other parameters screened by OFAT were pH, temperature, inoculum size and aeration. OFAT experimentations showed potato peel powder and urea were good carbon and nitrogen sources for biosurfactant production when it supplied in media with 40% of aeration and pH 7. Inoculum size for optimum biosurfactant production was 1% (v/v $\text{OD}_{600} = 1.00$) and temperature was $30\text{ }^{\circ}\text{C}$. To find out most significant factor influencing on biosurfactant production Plackett-Burman matrix design was used. Results showed, out of four variables (potato peel powder, urea, temperature and pH) considered carbon source, nitrogen source and temperature were most influencing factors on biosurfactant production. Later on interactions studies was carried out to find optimal level of variables for maximum biosurfactant production. Study showed nitrogen and temperature were significantly effect on biosurfactant production. Experimental validation based on second order polynomial equation for quadratic model showed, biosurfactant production increased to $86.11 \pm 3.47\%$ (emulsification) from $43.62 \pm 2.55\%$ (emulsification) when supplied with 3.2 gm% potato peel powder as carbon source and 4.0 gm% urea as nitrogen source and incubated at $28\text{ }^{\circ}\text{C}$ for four days. Qualitative analysis by thin layer chromatography showed presence of carbohydrate and protein moiety in biosurfactant, which was confirmed by quantitative analysis which showed presence of $439.58 \pm 0.0129\text{ }\mu\text{g/ml}$ carbohydrate and $507.41 \pm 0.0064\text{ }\mu\text{g/ml}$ protein part. Lipid was absence in it. Native-PAGE staining followed by PAS staining confirms that the biosurfactant was glycoprotein in nature. SDS-PAGE study reveals that protein moiety have approximately 48 kDa of size. FTIR chromatogram picks occurred at 1652.17 cm^{-1} , 1541.01 cm^{-1} , 1463.71 cm^{-1} and 722.32 cm^{-1} which indicated presence of peptide bond, peptide moiety, carbohydrate protons and primary as well as secondary amines which also confirms glycoprotein class of biosurfactant from *S. thalpophilum* DP9. As far as our best knowledge this is the first report for production of glycoprotein biosurfactant from *Sphingobacterium* genus.

Abbreviations

ANOVA: analysis of variance; BHM: Bushnell-Haas Medium; CCD: central composite design; cm: centimeter; $^{\circ}\text{C}$: degree centigrade; E24%: emulsification at 24hin percentage; FTIR: Fourier Transform

Infra-Red; gm: gram; DPO/POD: glycerol phosphate oxidase-peroxidase; KBr: Potassium bromide; kD: kilo Dalton; LB: Luria Bertani; ml: milliliter; OFAT: one factor at a time; O.D.: optical density; PAGE: poly acrylamide gel electrophoresis; pH: potential of hydrogen; R_f: retention factor; SDS: sodium dodecyl sulfate; TLC: thin layer chromatography

Declarations

Authors' contributions

JS designs and carry out all the experimentations mentioned in the manuscript. DP contributed in manuscript design, formatting and data analysis. SI helps in statistical optimization and finalizes the data. MN helps in final output of manuscript by correcting in scientific manner. All authors directly participated in the planning and execution of this study. All authors read and approved the final manuscript.

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Competing interest

The authors declare that they have no competing interests.

Availability of data and materials

The data sets supporting the conclusions of this article are included in the article.

Consent for publication

All authors read the final manuscript and approved its submission to *Bioresources and Bioprocessing*.

Ethical approval and consent to participate

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References

- Abdel-mawgoud AM, Aboulwafa MM, Hassouna NA (2008) Optimization of Surfactin Production by *Bacillus subtilis* Isolate BS5. *Applied Biochemistry and Biotechnology* 150:305–325 . doi: 10.1007/s12010-008-8155-x
- Anvari S, Hajdarajollah H, Mokhtarani B, Noghabi KA (2015) Physicochemical and thermodynamic characterization of lipopeptide biosurfactant secreted by *Bacillus tequilensis* HK01. *RSC Advances* 5:91836–91845 . doi: 10.1039/C5RA17275F
- Armendáriz BP, Cal-y-Mayor-Luna C, Girgis El-Kassis E, Ortega-Martínez LD (2019) Use of waste canola oil as a low - cost substrate for rhamnolipid production using *Pseudomonas aeruginosa*. *AMB Express* 9:1–9 . doi: 10.1186/s13568-019-0784-7
- Banat IM, Satpute SK, Cameotra SS, et al (2014) Cost effective technologies and renewable substrates for biosurfactants' production. *Frontiers in Microbiology* 5:1–18 . doi: 10.3389/fmicb.2014.00697
- Bhardwaj G, Cameotra SS, Chopra HK (2013) Utilization of oleo-chemical industry by-products for biosurfactant production. *AMB Express* 3:68 . doi: <http://www.amb-express.com/content/3/1/68> MINI
- Burgos-Díaz C, Pons R, Espuny MJ, et al (2011) Isolation and partial characterization of a biosurfactant mixture produced by *Sphingobacterium* sp. isolated from soil. *Journal of Colloid and Interface Science* 361:195–204 . doi: 10.1016/j.jcis.2011.05.054
- Durval IJB, Resende AHM, Figueiredo MA, et al (2018) Studies on biosurfactants produced using *Bacillus cereus* isolated from seawater with biotechnological potential for marine oil-spill bioremediation. *Journal of Surfactants and Detergents* 18:101071 . doi: 10.1002/jsde.12218
- Ekpenyong MG, Antai SP, Asitok D, Ekpo BO (2017) Plackett-Burman design and response surface optimization of medium trace nutrients for glycolipopeptide biosurfactant production. *Iranian Biomedical Journal* 21:249–260 . doi: 10.18869/acadpub.ibj.21.4.249
- El-Gherab FZ, Hassaine O, Zadi-Karam H, Karam N-E (2019) Statistical optimization for the development of a culture medium based on the juice of waste-dates for growth of *Lactococcus lactis* LCL strain by using the Plackett – Burman and Response Surface Methodology. *Waste and Biomass Valorization* 10:2943–2957 . doi: 10.1007/s12649-018-0283-0
- Ezebuio V, Otaraku IJ, Oruwari B, Okpokwasili GC (2019) Effects of nitrogen and carbon sources on biosurfactant production by hydrocarbon-utilizing *Stenotrophomonas* sp. *Microbiology Research Journal International* 29:1–10 . doi: 10.9734/MRJI/2019/v29i530177

- Helmy Q, Kardena E (2011) Strategies toward commercial scale of biosurfactant production as potential substitute for its chemically counterparts. *International Journal of Biotechnology* 12:66–86
- Hema T, Kiran GS, Sajayyan A, et al (2019) Response surface optimization of a glycolipid biosurfactant produced by a sponge associated marine bacterium *Planococcus* sp. MMD26. *Biocatalysis and Agricultural Biotechnology* 18:101071 . doi: 10.1016/j.bcab.2019.101071
- Henkel M, Müller MM, Kügler JH, et al (2012) Rhamnolipids as biosurfactants from renewable resources: Concepts for next-generation rhamnolipid production. *Process Biochemistry* 47:1207–1219 . doi: 10.1016/j.procbio.2012.04.018
- Hmidet N, Ayed H Ben, Jacques P, Nasri M (2017) Enhancement of surfactin and fengycin production by *Bacillus mojavensis* A21 : Application for diesel biodegradation. *BioMed research international* 2017:5893123 . doi: 10.1155/2017/5893123
- Hu X, Wang C, Wang P (2015) Optimization and characterization of biosurfactant production from marine *Vibrio* sp. strain 3B-2. *Frontiers in Microbiology* 6:976 . doi: 10.3389/fmicb.2015.00976
- Javed A, Ahmad A, Tahir A, et al (2019) Potato peel waste – its nutraceutical , industrial and biotechnological applications. *AIMS Agriculture and Foods* 4:807–823 . doi: 10.3934/agrfood.2019.3.807
- Joy S, Khare SK, Sharma S (2020) Synergistic extraction using sweep-floc coagulation and acidification of rhamnolipid produced from industrial lignocellulosic hydrolysate in a bioreactor using sequential (fill-and-draw) approach. *Process Biochemistry* 90:233–240 . doi: 10.1016/j.procbio.2019.11.014
- Jyoti A, Rajesh D (2018) Bioslurry phase remediation of petroleum-contaminated soil using potato peels powder through biosurfactant producing *Bacillus licheniformis* J1. *International Journal of Environmental Science and Technology* 15:525–532 . doi: 10.1007/s13762-017-1410-3
- Kronemberger FA, Santa Anna LM, Fernandes ACLB, Ramos deMenezes R, Borges CP, Freire DMG (2007) Oxygen-controlled biosurfactant production in a bench scale bioreactor. *Biotechnology for Fuels and Chemicals* 401:413. https://doi.org/10.1007/978-1-60327-526-2_39
- Kumaran MDB, Kalaichelvan PT, Santhi R (2015) Exploitation of agro-industrial wastes as substrates for cellulase production by *Bacillus licheniformis* MTCC 429. *Microbiology Journal* 5:36–42 . doi: 10.3923/mj.2015.36.42
- Lowery OH, Rosebrough NJ, LewisFarr A, Randall RJ (1951) Protein measurement with Folin phenol reagent. *Journal of Biological Chemistry* 265–275
- Makkar RS, Cameotra SS (1997a) Utilization of molasses for biosurfactant production by two *Bacillus* strains at thermophilic conditions. *Journal of the American Oil Chemists' Society* 74:887–889 . doi: 10.1007/s11746-997-0233-7

- Makkar RS, Cameotra SS (1997b) Utilization of molasses for biosurfactant production by two *Bacillus* strains at thermophilic conditions. *Journal of the American Oil Chemists' Society* 74:887–889 . doi: 10.1007/s11746-997-0233-7
- Mercade ME, Manresa MA (1994) The use of agroindustrial by-products for biosurfactant production. *Journal of the American Oil Chemists' Society* 71:61–64 . doi: 10.1007/BF02541473
- Mnif I, Chaabouni-ellouze S, Ghribi D (2012) Optimization of the nutritional parameters for enhanced production of *B. subtilis* SPB1 biosurfactant in submerged culture using response surface methodology. *Biotechnology Research International* 2012:795430 . doi: 10.1155/2012/795430
- Moshtagh B, Hawboldt K, Zhang B (2019) Optimization of biosurfactant production by *Bacillus Subtilis* N3-1P using the brewery waste as the carbon source. *Environmental Technology* 40:3371–3380 . doi: 10.1080/09593330.2018.1473502
- Mukherjee S, Das P, Sen R (2006) Towards commercial production of microbial surfactants. *Trends in Biotechnology* 24:509–515 . doi: 10.1016/j.tibtech.2006.09.005
- Naeem AH (2018) Biotechnological approaches for enhanced production of biosurfactants by *Bacillus subtilis* SNW3 Department of Microbiology Faculty of Biological Sciences. Quaid-i-Azam University, Islamabad
- Nitschke M, Ferraz C, Pastore GM (2004) Selection of microorganisms for biosurfactant production using agroindustrial wastes. *Brazilian Journal of Microbiology* 35:81–85 . doi: 10.1590/S1517-83822004000100013
- Nogueira IB, Rodríguez DM, Da Silva Andradade RF, et al (2020) Bioconversion of agroindustrial waste in the production of bioemulsifier by *Stenotrophomonas maltophilia* UCP 1601 and application in bioremediation process. *International Journal of Chemical Engineering* 9434059:1–9 . doi: 10.1155/2020/9434059
- Noparat P, Maneerat S, Saimmai A (2014) Application of biosurfactant from *Sphingobacterium spiritivorum* AS43 in the biodegradation of used lubricating oil. *Applied biochemistry and biotechnology* 172:Noparat, P., Maneerat, S., Saimmai, A. (2014). A . doi: 10.1007/s12010-014-0829-y
- Nurfarahin AH, Mohamed MS, Phang LY (2019) Development of palm fatty acid distillate-containing medium for biosurfactant production by *Pseudomonas* sp. LM19. *Molecules* 24:2613 . doi: 10.3390/molecules24142613
- Pande V, Patel V, Salunke P, Patel U (2020) Biosynthesis and development of novel method for commercial production of biosurfactant utilizing waste potato peels. *Indain Drugs* 57:59–65
- Panjiar N, Mattam AJ, Jose S, et al (2020) Valorization of xylose-rich hydrolysate from rice straw, an agroresidue, through biosurfactant production by the soil bacterium *Serratia nematodiphila*. *Science of*

the Total Environment 729:138933 . doi: 10.1016/j.scitotenv.2020.138933

Radzuan MN, Banat IM, Winterburn J (2017) Production and characterization of rhamnolipid using palm oil agricultural refinery waste. *Bioresource Technology* 225:99–105 . doi: 10.1016/j.biortech.2016.11.052

Radzuan MN, Banat IM, Winterburn J (2018) Biorefining palm oil agricultural refinery waste for added value rhamnolipid production via fermentation. *Industrial Crops and Products* 116:64–72 . doi: 10.1016/j.indcrop.2018.02.045

Rivera AD, Urbina MAM, Lopez-y-Lopez VE (2019) Advances on research in the use of agro - industrial waste in biosurfactant production. *World Journal of Microbiology and Biotechnology* 35:1–18 . doi: 10.1007/s11274-019-2729-3

Rodrigues LR, Dourado F (2014) Functionalization of silicone rubber surfaces towards biomedical applications. *Concise Encyclopedia of High Performance Silicones* 111–122 . doi: 10.1002/9781118938478.ch8

Rosenberg E, Perry A, Gibson DT, Gutnick DL (1979) Emulsifier of *Arthrobacter* RAG-1 : Specificity of Hydrocarbon Substrate. *Applied and Environmental Microbiology* 37:409–413

Shahaliyan F, Safahieh A, Abyar H (2015) Evaluation of emulsification index in marine bacteria *Pseudomonas* sp . and *Bacillus* sp . *Arabian Journal of Science and Engineering* 40:1849–1854 . doi: 10.1007/s13369-015-1663-4

Sharma S, Pandey LM (2020) Production of biosurfactant by *Bacillus subtilis* RSL-2 isolated from sludge and biosurfactant mediated degradation of oil. *Bioresource Technology* 307:123261 . doi: 10.1016/j.biortech.2020.123261

Singh P, Patil Y, Rale V (2019) Biosurfactant production: emerging trends and promising strategies. *Journal of Applied Microbiology* 126:2–13 . doi: 10.1111/jam.14057

Soares da Silva R de CF, Almeida DG, Meira HM, et al (2017) Production and characterization of a new biosurfactant from *Pseudomonas cepacia* grown in low-cost fermentative medium and its application in the oil industry. *Biocatalysis and Agricultural Biotechnology* 12:206–215 . doi: 10.1016/j.bcab.2017.09.004

Thavasi R, Jayalakshmi S, Balasubramanian T, Banat IM (2008) Production and characterization of a glycolipid biosurfactant from *Bacillus megaterium* using economically cheaper sources. *World Journal of Microbiology and Biotechnology* 24:917–925 . doi: 10.1007/s11274-007-9609-y

Vera ECS, de Azevedo PO de S, Domínguez JM, Oliveira RP de S (2018) Optimization of biosurfactant and bacteriocin-like inhibitory substance (BLIS) production by *Lactococcus lactis* CECT-4434 from agroindustrial waste. *Biochemical Engineering Journal* 133:168–178 . doi: 10.1016/j.bej.2018.02.011

Yañez-Ocampo G, Somoza-Coutiño G, Blanco-González C, Wong-Villarreal A (2017) Utilization of agroindustrial waste for biosurfactant production by native bacteria from chiapas. *Open Agriculture* 2:341–349 . doi: 10.1515/opag-2017-0038

Yaraguppi DA, Bagewadi ZK, Muddapur UM, Mulla SI (2020) Response surface methodology - based optimization of biosurfactant production from isolated *Bacillus aryabhatai* strain ZDY2. *Journal of Petroleum Exploration and Production Technology* 0123456789 . doi: 10.1007/s13202-020-00866-9

Tables

Table: 1 The various media components including in Plackett-Burman experiments and their corresponding higher, medium and lower concentration levels.

Variable codes	Media Constituents	Units	High (+1)	Low (-1)
X_1	Carbon Source (potato peel powder)	gm%	5	1
X_2	Nitrogen Source (urea)	mg%	1	0.1
X_3	pH	pH	9	5
X_4	Temperature	°C	45	25

Table: 2 Plackett-Burman experimental designs for 11 variable and the corresponding responses in µg/ml.

Run	Independent Variables											Response (Protein µg/ml)
	X_1	X_2	X_3	X_4	D_1^*	D_2^*	D_3^*	D_4^*	D_5^*	D_6^*	D_7^*	
1	1	-1	1	-1	-1	-1	1	1	1	-1	1	184.65
2	1	1	-1	1	-1	-1	-1	1	1	1	-1	284.4
3	-1	1	1	-1	1	-1	-1	-1	1	1	1	74.65
4	1	-1	1	1	-1	1	-1	-1	-1	1	1	186.9
5	1	1	-1	1	1	-1	1	-1	-1	-1	1	265.9
6	1	1	1	-1	1	1	-1	1	-1	-1	-1	185.9
7	-1	1	1	1	-1	1	1	-1	1	-1	-1	115.15
8	-1	-1	1	1	1	-1	1	1	-1	1	-1	84.9
9	-1	-1	-1	1	1	1	-1	1	1	-1	1	52.9
10	1	-1	-1	-1	1	1	1	-1	1	1	-1	59.9
11	-1	1	-1	-1	-1	1	1	1	-1	1	1	62.9
12	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	38.4

* Dummy Variable

Table-3 Analysis of Plackett- Burman Design for Biosurfactant Production

Variables	Code	Effect	Mean Square	Mean Square for error	F values	P values	90% clearance
Carbon source	X_1	123.125	15159.766	493.76	30.70	0.02	97.93
Nitrogen source	X_2	63.542	4037.543		8.18	0.08	92.25
pH	X_3	11.292	127.502		0.26	0.84	16.09
Temperature	X_4	63.958	4090.668		8.28	0.43	92.35

* Statistically significant at 90% of probability level. (Note: Only mean values of response were considered for calculation)

Table-4 Central composite design of three variables and predicted as well as obtained values for biosurfactant production in terms of E24%

Run	Potato peel powder (gm %)	Urea (gm %)	Temperature (°C)	Response (E24%)	
				Predicted Values (Y')	Actual Values (Y)
1	3.5	1	35	30.40	30
2	3.5	0.16	35	24.37	25
3	2	0.5	25	25.86	30
4	5	1.5	45	24.34	50
5	2	1.5	25	27.37	22
6	3.5	1	35	24.84	42
7	5	0.5	25	46.33	25
8	0.98	1	35	48.30	25
9	3.5	1	35	22.62	35
10	3.5	1	35	19.22	31
11	6.02	1	35	27.96	15
12	3.5	1	35	43.88	20
13	5	0.5	45	28.61	30
14	5	1.5	25	46.22	25
15	2	0.5	45	30.45	28
16	3.5	1	18.18	30.45	31
17	3.5	1	35	30.45	25
18	2	1.5	45	30.45	47
19	3.5	1	51.82	30.45	42
20	3.5	1.84	35	30.45	45

Table-5 ANOVA analysis for Response Surface Quadratic model for biosurfactant production in terms of E24%

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	1323.842	9	147.0936	3.707858	0.0266	significant
X_1	13.9809	1	13.9809	0.352423	0.5659	
X_2	305.9119	1	305.9119	7.711266	0.0196	
X_4	374.3329	1	374.3329	9.435987	0.0118	
X_1^2	163.609	1	163.609	4.124169	0.0697	
X_2^2	53.89803	1	53.89803	1.358633	0.2708	
X_4^2	87.51186	1	87.51186	2.205953	0.1683	
X_1X_2	10.125	1	10.125	0.255226	0.6244	
X_1X_4	6.125	1	6.125	0.154396	0.7026	
X_2X_4	276.125	1	276.125	6.960414	0.0248	
Residual	396.7077	10	39.67077			
Lack of Fit	103.2077	5	20.64155	0.351645	0.8620	not significant
Pure Error	293.5	5	58.7			
Cor Total	1720.55	19				
	Std. Dev.	6.298474			R-Squared	0.76943
	Mean	31.15			Adj R-Squared	0.561916
	C.V.	20.21982			Pred R-Squared	0.293496
	PRESS	1215.576			Adeq Precision	6.532098

Figures

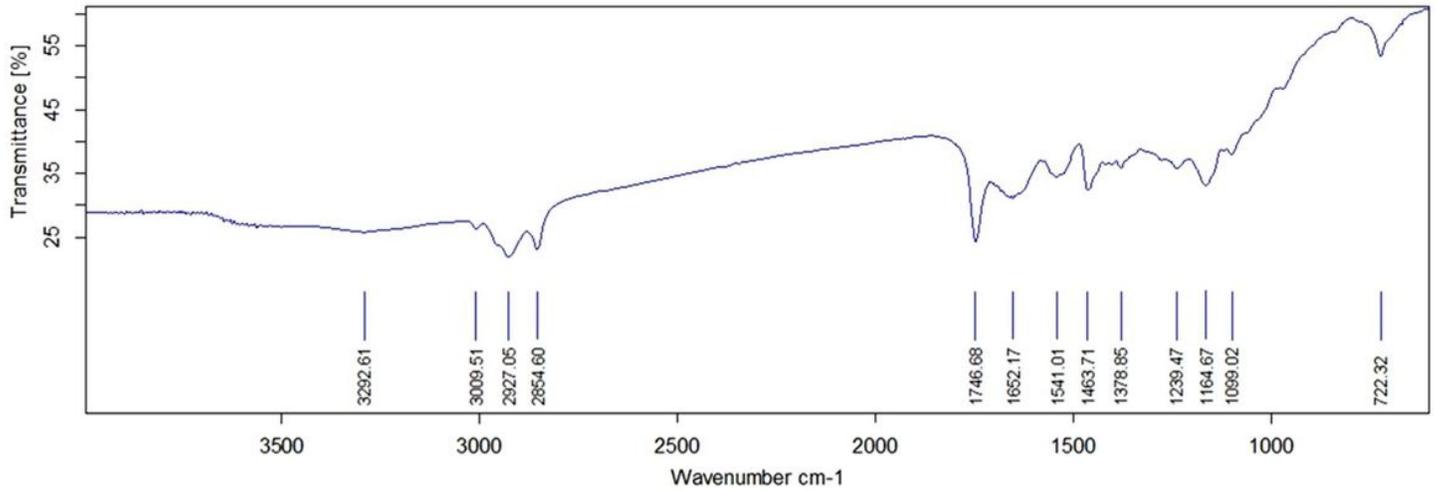


Fig.6 FTIR chromatogram of biosurfactant produced by *Sphingobacterium thalophilum* DP9

Figure 1

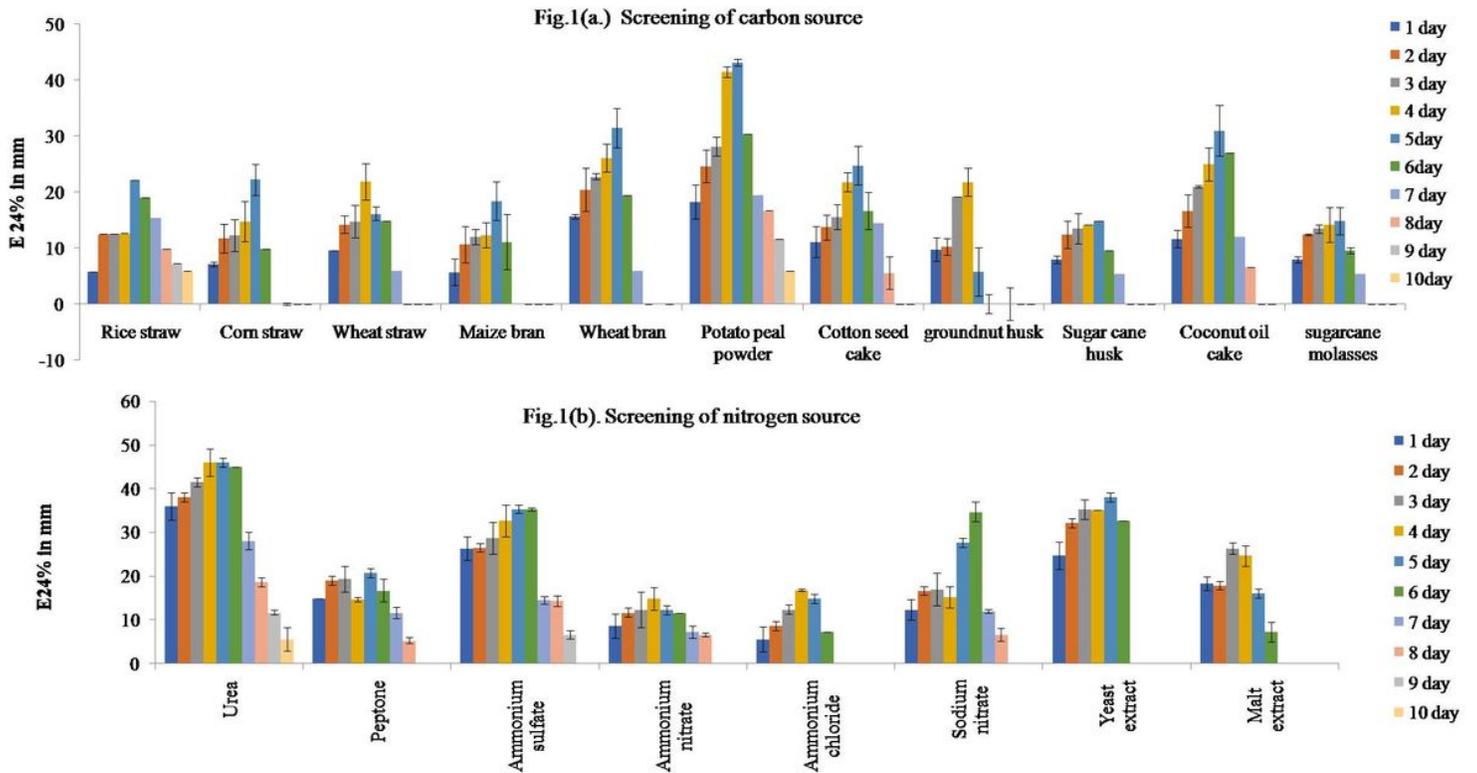


Fig.1 Screening of (a) carbon source and (b) nitrogen source for biosurfactant production

Figure 2

Screening of (a) carbon source and (b) nitrogen source for biosurfactant production

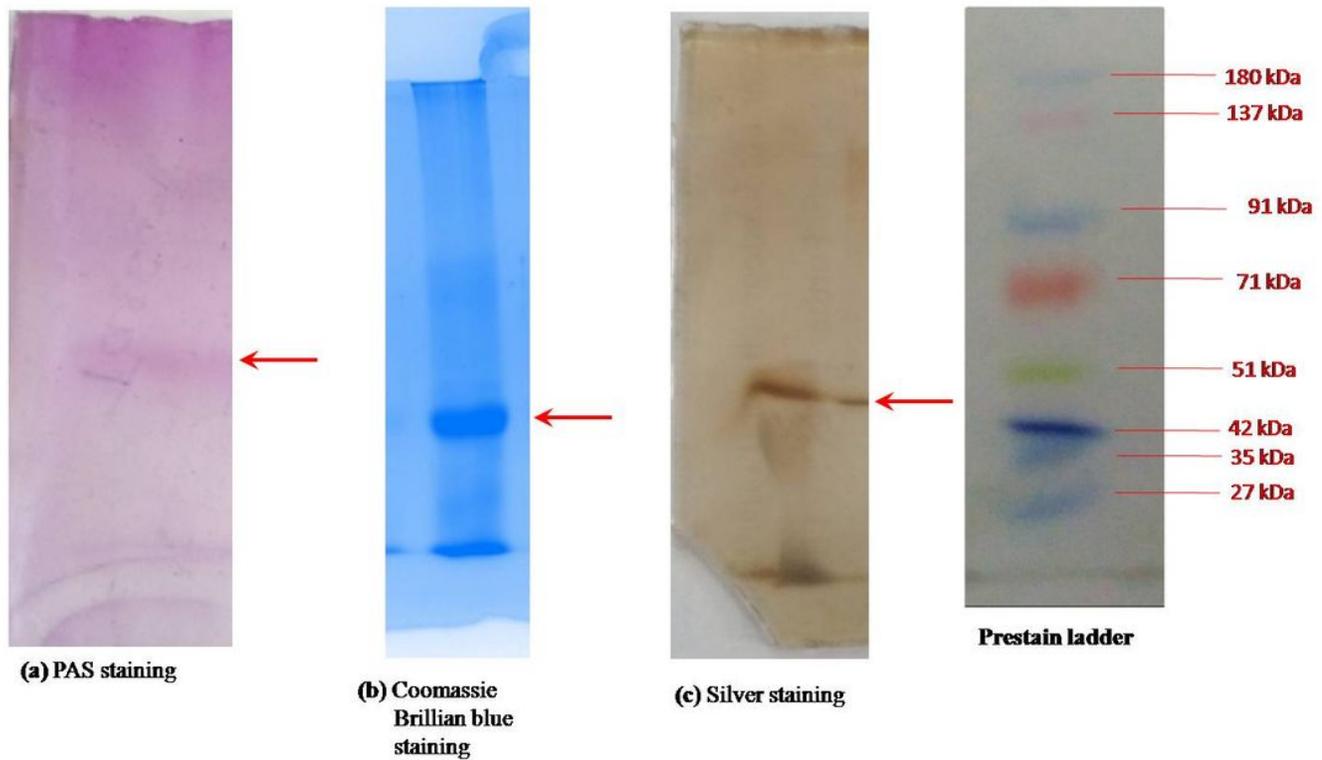


Fig. 5 (a) PAS staining of Native gel electrophoresis ; (b) Coomassie Brilliant Blue and (c) Silver staining of SDS-PAGE gel with prestain ladder on right

Figure 3

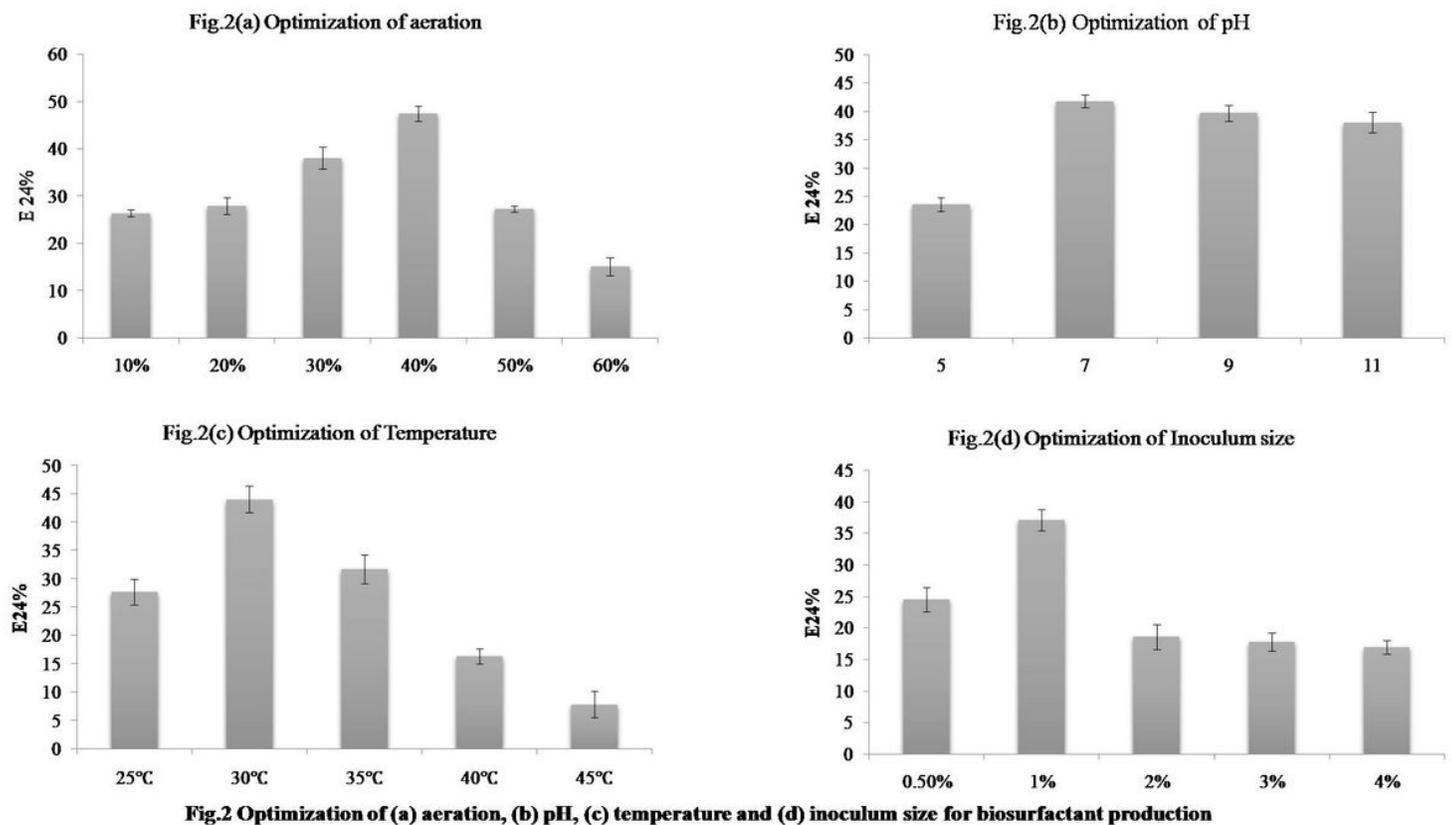
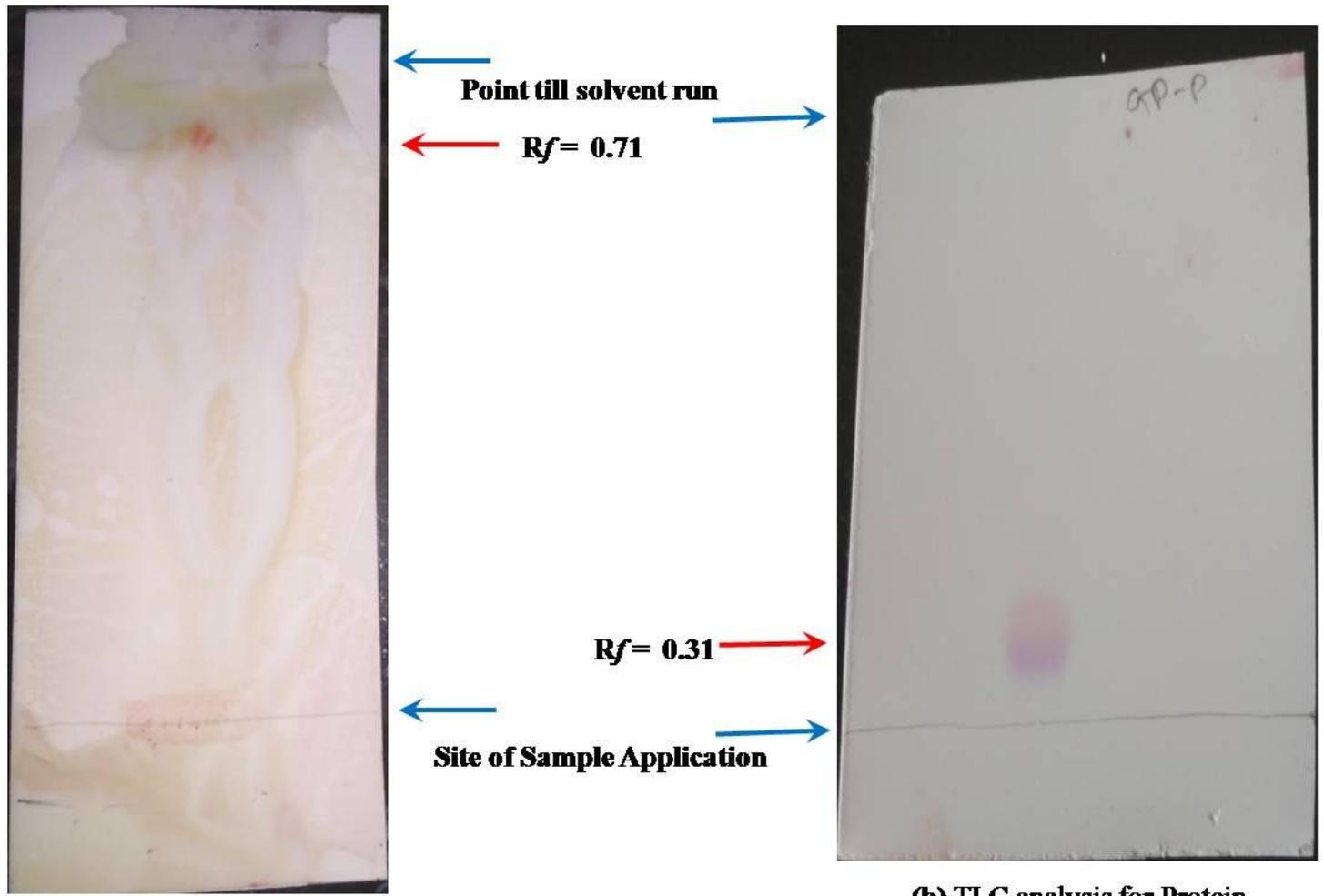


Figure 4

Optimization of (a) aeration, (b) pH, (c) temperature and inoculum size for biosurfactant production



(a) TLC analysis for carbohydrate

(b) TLC analysis for Protein

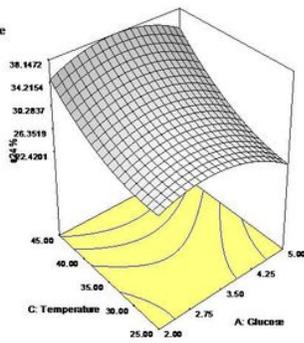
Fig.4 TLC analysis of (a) carbohydrate and (b) protein

Figure 5

DESIGN-EXPERT Plot

e24%
X = A: Glucose
Y = C: Temperature

Actual Factor
B: Urea = 1.00

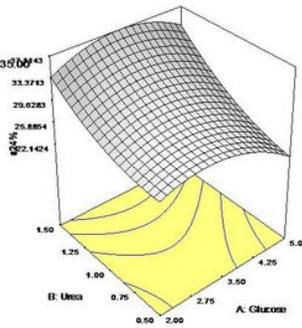


(a) Interaction between X_1 and X_4

DESIGN-EXPERT Plot

e24%
X = A: Glucose
Y = B: Urea

Actual Factor
C: Temperature = 35.00

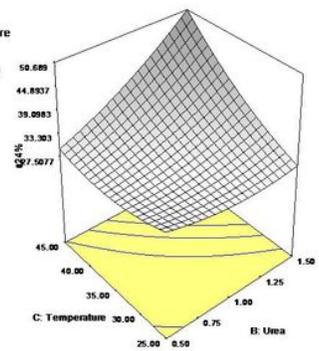


(b) Interaction between X_1 and X_2

DESIGN-EXPERT Plot

e24%
X = B: Urea
Y = C: Temperature

Actual Factor
A: Glucose = 3.50



(c) Interaction between X_2 and X_4

Fig.3 3D response surface plots of interaction between (a) carbon source and temperature; (b) carbon source and nitrogen source; (c) nitrogen source and temperature

Figure 6

3D response surface plots of interaction between (a) carbon source and temperature, (b) carbon source and nitrogen source, (c) nitrogen source and temperature

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