Karuppiah *et al. Bioresour. Bioprocess.* (2015) 2:48
DOI 10.1186/s40643-015-0077-1

Bioresources and Bioprocessing
a SpringerOpen Journal

RESEARCH Open Access

A statistical approach on optimization of exopolymeric substance production by *Halomonas* sp. S19 and its emulsification activity

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Abstract

An exopolymer producing bacterial strain was identified as *Halomonas* sp. S19 by 16S rRNA gene sequencing isolated from Mandapam, Southeast coast of India. Strain S19 produces a significant amount of exopolymer (320 mg L^{-1}) in a medium optimized with 2.5 % glucose, 0.6 % peptone, 7.5 % salt and pH 7.5 at 35 °C. The exopolymer consists of total sugars (65 %), proteins (4.07 %), uronic acids (8.08 %) and sulphur contents (6.39 %). FT-IR and ^{1}H NMR analysis revealed the presence of functional groups corresponding to carbohydrates, proteins and sulphates. The exopolymer of *Halomonas* sp. S19 emulsifies different oils. However, 10 % exopolymer shows 55.18, 55.18, 49.81 and 24.62 % of emulsifying activity for sesame oil, coconut oil, paraffin and kerosene. The present study was focused on optimisation of exopolymer production using Box–Behnken experimental design and its possibility for potential emulsification index.

Keywords: *Halomonas* sp., Exopolymer, Optimisation, NMR, DSC, Emulsification

Background

The genus *Halomonas* consists of a broad range of taxonomically and physiologically differed organisms growing at a diverse range of salinities (Kushner and Kamekura 1988). In marine environment, most of the bacteria exclusively secrete exopolymeric substances outside the cell, which may be tightly or loosely bounded on the cell surface and assist the cell to survive (Sutherland 2001). The exopolymeric substances are composed of sugars and non-sugar components (proteins, uronic acids, sulphates and acetyl group) (Llamas et al. 2012).

The presence of proteins, sulphates and uronic acids in bacterial exopolymeric substance confers anticancer, immune modulatory and emulsification activity (Ruiz Ruiz et al. 2011; Perez Fernandez et al. 2000; Bouchotroch et al. 2000). Bio-emulsifiers are higher molecular weight compounds consisting complex mixtures of heteropolysaccharides, lipopolysaccharides, lipoproteins and proteins (Perfumo et al. 2009). Bio-emulsifiers efficiently emulsify two immiscible liquids even at low concentrations, but in contrast is less effective at reduced surface tension. In an oil-polluted environment, the emulsifier plays a significant role in dispersing the hydrocarbons by binding and preventing from merging. It has been attributed to the presence of high number of reactive groups exposed in their structures.

Many microbial polysaccharides serve as emulsifiers due to their ability to stabilise emulsions between water and hydrophobic compounds. When compared with the chemically derived compounds, the bacterial exopolymeric substances were found to be stable in extreme conditions like temperature, pH and salinity (Banat et al. 2000). Hence, an interest has been focused towards the production of biologically derived compounds. Bio-emulsifiers are essential in the formation and stabilisation of emulsion. It reduces the surface tension of oil

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and water interface by forming a protective layer around emulsion droplets and blocks coalescence by adsorbing with the oil/water interface.

Most of the research fields are bound with factorial assessments. Box–Behnken experimental design is used to assess the optimal level of variables. Response surface methodology (RSM) is a vital one in research field and a combination of mathematical and statistical approaches are gainful for analysing and modelling experiments. This technique is a resourceful one to optimise various parameters resulting in response surface (Kadirgama et al. 2007; Abou-El-Hossein et al. 2007; Nguyena and Borkowskib 2008). The present study was focused to optimise the exopolymer production by *Halomonas* sp. S19 using Box–Behnken model and its significant feature of emulsification activity on various oils.

Methods

Isolation and characterisation of the strain

The exopolymer producing bacteria was isolated from soil sample collected from Southeast Coastal area of Mandapam, India. The bacteria were screened based on the mucoidal appearance on zobell marine agar plates and by staining with Sudan Black B. The DNA samples were collected and amplified in PCR. The 16S rRNA sequencing was performed using 20 µL of purified PCR products (50 ng/µL), the primers 518F (CCAGCAGCCGCGGTAATACG) and 800R (TACCAGGGTATCTAATCC) and Big Dye terminator cycle sequencing kit (Applied BioSystems, USA) (Neefs et al. 1990). The most similar sequences to the sequence of the isolated strain were obtained using BLAST similarity search tool, and the multiple sequence alignment was performed using the program MUSCLE by progressive alignment method. A computer-based program, Gblocks, was used for alignment refinement. The phylogenetic tree was constructed using the programme PhyML 3.0 approximate likelihood-ratio test (aLRT) with HKY85 substitution model for the neighbour-joining method.

Optimization of exopolymer production

The carbon (glucose, sucrose, lactose and galactose) and nitrogen sources (peptone) were separately provided in the basal salt medium at different concentrations and the best source was selected for further studies. The optimum carbon-to-nitrogen ratio was determined by providing the nitrogen at different concentrations (0.5–1 %) and with constant concentration of carbon source (2.5 %) in 250-mL Erlenmeyer flasks containing 100 mL of basal salt medium (BSM). After determining the optimum carbon-to-nitrogen ratio, the medium was statistically optimised by Box–Behnken model with salt concentration, pH and growth temperature as variables; totally, seventeen runs were studied at low and high levels of the variables. The low and high levels of sea salt were 2.5 and 7.5 %. In the case of pH, 6.5 and 7.5 were observed as low and high levels. In the case of temperature, the low and high values were 25 and 45 °C, respectively. 2 ml aliquots of 24 h isolated strain was used as inoculum and allowed to grow for 72 h on a rotary shaker at 110 rpm. The experimental design, statistical and graphical analysis of the data were performed using 'Design Expert' software (version 9, Stat-Ease, Inc., Minneapolis, MN, USA).

Extraction and characterization of exopolymer

The exopolymeric substance was extracted (Parthiban et al. 2014), and the total sugar (Dubois et al. 1956), proteins (Lowry et al. 1951), uronic acids (Filisetti-Cozzzi and Carpaita 1991) and sulphates (Dodgson 1961) were determined spectrophotometrically. The monosaccharide constituent was determined by hydrolysing the exopolymer in 4 mol·L^{-1} Trifluoroacetic acid (TFA) and heated at 100 °C for 10 min and filtered through 0.45-µm syringe filter. Distillation using methanol removed residual acid from the filtrate. Finally, the sugars were analysed by HPLC (Thermo Scientific, Model Accela) and compared with the standard (Freitas et al. 2009). The dried sample was mixed with potassium bromide and analysed at spectral range of 4000–400 cm^{-1} using Fourier transform infrared spectrophotometer (FT-IR Bruker IFS 85). ^{1}H NMR spectrum (Bruker AVANCE III 500 MHz AV 500) was recorded by dissolving 20 mg of pure exopolymer in 1 mL of D_2O at room temperature. The thermal properties of bacterial exopolymer were studied by differential scanning colorimetric (DSC) (Mettler Toledo DSC 822e) analysis at 40–450 °C (10 °C/min) under nitrogen atmosphere.

Emulsification index (EI_{24})

Emulsification index was determined briefly by adding 1 mL of exopolymer solution (5 and 10 %) to 0.5 mL of various hydrocarbons (vegetable oils, paraffin and kerosene) in test tubes and mixed vigorously, and allowed to stand for 24 h. Emulsifying activity was expressed as percentage (%) by measuring the total height occupied by emulsion. Tween 20 was used as a control in this study (Cooper and Goldenberg 1987). The emulsification index was calculated by the formula mentioned below:

$$\text{Emulsification index } [EI_{24}] = \text{Height of emulsifying layer/Total height of layer} \times 100.$$

Results and discussion

Identification of exopolymer producing strain

In the marine environment, most of the bacteria produce exopolymeric substances probably associated with the sticky texture. Previously, the exopolymer production by

sticky colonies of *Pseudomonas aeruginosa* (Uhlinger and White 1983; Roberson and Firestone 1992) and *E. coli* (Junkins and Doyle 1992) has been reported. The blast similarity search and phylogenetic analysis of 16S rRNA sequence revealed that the isolated bacterial strain had close resemblance with *Halomonas* sp. (Accession no JX569798.1) (Fig. 1).

Optimization of exopolymer production by Box–Behnken model

The production of exopolymer was found to increase with increasing concentration of sugars from 29.6 mg L^{-1} (0.5 %) to 51.5 mg L^{-1} (2.5 %). The maximum exopolymer production (51.5 mg L^{-1}) was observed with 2.5 % glucose provided in basal salt medium (Table 1) and the peptone (1 %) with a yield of 39 ± 0.15 mg L^{-1}. The C:N was optimised using 2.5 % of glucose and the peptone at different concentrations from 0.5 to 1 %. The optimum C:N ratio was found to be 11.90:1.0 with an yield of 218.66 ± 0.57 mg L^{-1} of exopolymer (Table 2).

The interaction between carbon and nitrogen sources (peptone) plays a significant role in exopolymer production. The higher concentration of peptone was found to play an inhibitory role on exopolymer production. However, the carbon:nitrogen ratio has a vital role in exopolymer production. The high amount of nutrients might somehow reduce the exopolymer production. Apart from nutritional conditions, exopolysaccharide synthesis and yields largely depend on the environmental condition like pH, temperature, aeration, etc. However, higher or lower the optimal range resulted in decreasing of exopolymeric substances (EPS) yield (Kumar et al. 2007; Gandhi et al. 1997).

Despite carbon and nitrogen sources, the cultural condition has a significant role in the exopolymer production; the deviation in cultural condition might affect the

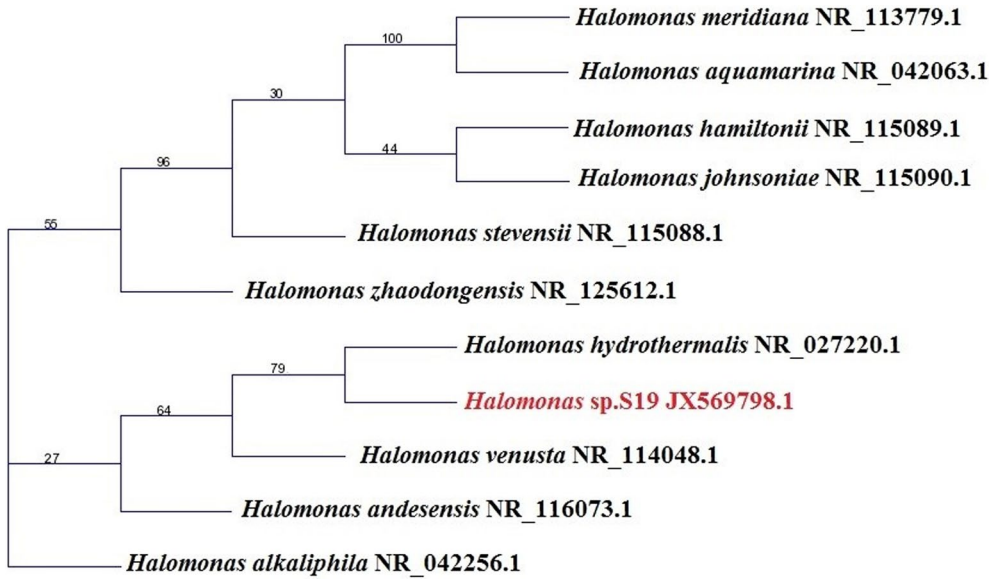

Fig. 1 Phylogenetic tree of *Halomonas* sp. S19 and their closest strains of blastn result based on 16S rRNA sequences. It was constructed using the programme PhyML 3.0 approximate likelihood-ratio test (aLRT) with HKY85 substitution model for the neighbour-joining method

Table 1 Effect of various carbon sources on exopolymer production

Carbon source	Exopolymer production for different concentrations of sugars (mg L^{-1})				
	0.50 %	1.00 %	1.50 %	2.00 %	2.50 %
Glucose	29.63 ± 0.11	35.43 ± 0.11	41.40 ± 0.17	43.43 ± 0.11	51.53 ± 0.05
Sucrose	28.40 ± 0.10	34.10 ± 0.10	40.16 ± 0.15	42.16 ± 0.15	47.50 ± 0.10
Lactose	28.13 ± 0.15	31.33 ± 0.15	35.43 ± 0.11	39.13 ± 0.15	42.43 ± 0.15
Galactose	27.60 ± 0.17	30.10 ± 0.10	33.46 ± 0.05	35.36 ± 0.11	37.15 ± 0.15

Results represent the means of three experiments ± SD

Table 2 Effect of carbon:nitrogen ratio on exopolymer production

Sucrose (carbon source) g L^{-1}	Peptone (nitrogen source) g L^{-1}	C:N ratio	Exopolymer (mg L^{-1})
25	5	11.90:1.00	218.66 ± 0.57
	6	14.28:1.00	204.86 ± 0.23
	7	10.20:1.00	185.10 ± 0.17
	8	8.92:1.00	114.13 ± 0.15
	9	7.93:1.00	72.10 ± 0.10
	10	7.14:1.00	65.36 ± 0.11

Results represent the means of three experiments ± SD

exopolymer production and growth (results not shown). The Box–Behnken model of RSM was validated in shake flask level by the conditions predicted by the software. The experimental results showed the values which were closer to the predicted values supporting the data and model as valid (Table 3). The polynomial equation is shown below, which was derived based on the experimental factors, where Y is the response (exopolymer):

$$[Y] = 284.443 + 29.5735A + 8.06373B + 4.75C + 8.75AB + 3.01471AC + 5.61275BC + -9.125A^2 + -7.125B^2 + -9.81771C^2.$$

Table 3 Experimental design generated with Design Expert 9.0: the predicted and actual values of production of exopolymer by *Halomonas* sp. S19

Run	A: sea salt (%)	B: pH	C: Temp °C	Predicted value exopolymer (mg)	Actual value exopolymer (mg)
1	5.0	7.0	35	285.00	285
2	5.0	7.5	45	285.93	280
3	2.5	7.0	45	237.66	240
4	5.0	7.0	35	285.00	285
5	5.0	7.0	35	285.00	285
6	7.5	6.5	35	280.99	278
7	7.5	7.0	25	287.31	285
8	5.0	7.0	35	285.00	285
9	5.0	6.5	25	260.30	265
10	2.5	7.0	25	234.19	232
11	7.5	7.0	45	302.84	305
12	5.0	6.5	45	258.57	260
13	5.0	7.0	35	285.00	285
14	2.5	7.5	35	239.01	242
15	5.0	7.5	25	265.20	265
16	7.5	7.5	35	316.86	320
17	2.5	6.5	35	238.14	235

A high value of CV indicates lower reliability of the experiment. In the present analysis, lower value of CV 1.50 indicated a greater reliability of the experiments performed. The probability values were found to be 0.0001, 0.0009 and 0.0134 for sea salt (*A*), pH (*B*) and temperature (*C*), respectively, ensuring the factors to be significant in exopolymer production (Table 4). The Pred R-Squared and Adj R-Squared were 0.8002 and 0.9722, respectively; the Pred R-Squared was found to be in a reasonable agreement with the Adj R-Squared. The difference is less than 0.2. Adeq precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. A ratio of 26.350 indicates an adequate signal. In this case, *A*, *B*, *C*, *AB*, *BC*, A^2, B^2, C^2 are significant model terms. The response of the RSM was shown as 3D response surface graphs (Fig. 2), and contour plots resulted in an infinite number of combinations of the two factors, upon keeping the other constant. *Halomonas* sp. S19 produces 320 mg L^{-1} in a medium containing 7.5 % sea salt, pH 7.5 incubated at 35 °C. The ANOVA showed the interaction effect of variables on exopolymer production. There is only 0.01 % chance that an *F* value of this large could occur due to noise. Values of "Prob > *F*" less than 0.0500 indicate model terms are significant. The goodness of fit of the model was checked by the determination coefficient (R^2). In this case, the value of the determination coefficient ($R^2 = 0.99$) indicated the significance of the model.

In the present study, *Halomonas* sp. S19 produced 110 mg L^{-1} exopolymer in basal salt medium containing 2.5 % glucose, and 320 mg L^{-1} exopolymer in a medium optimised with glucose: peptone (14.28:1.00), 7.5 % salt and pH 7.5 at 35 °C. These results corroborated

Table 4 ANOVA for response surface quadratic model

Source	Sum of squares	*Df*	Mean square	F value	*p* value Prob > *F*
Model	9521.10	9	1057.90	63.22	<0.0001
A-sea salt	6862.20	1	6862.20	410.08	<0.0001
B-pH	510.19	1	510.19	30.49	0.0009
C-temp	180.50	1	180.50	10.79	0.0134
AB	306.25	1	306.25	18.30	0.0037
AC	37.08	1	37.08	2.22	0.1802
BC	128.53	1	128.53	7.68	0.0276
A^2	350.59	1	350.59	20.95	0.0026
B^2	213.75	1	213.75	12.77	0.0090
C^2	366.31	1	366.31	21.89	0.0023
Lack of fit	117.14	3	39.05		
Std. dev.	4.09		R-squared		0.9878
Mean	272.47		Adj R-squared		0.9722
C.V. %	1.50		Pred R-squared		0.8002
PRESS	1925.58		Adeq precision		26.350

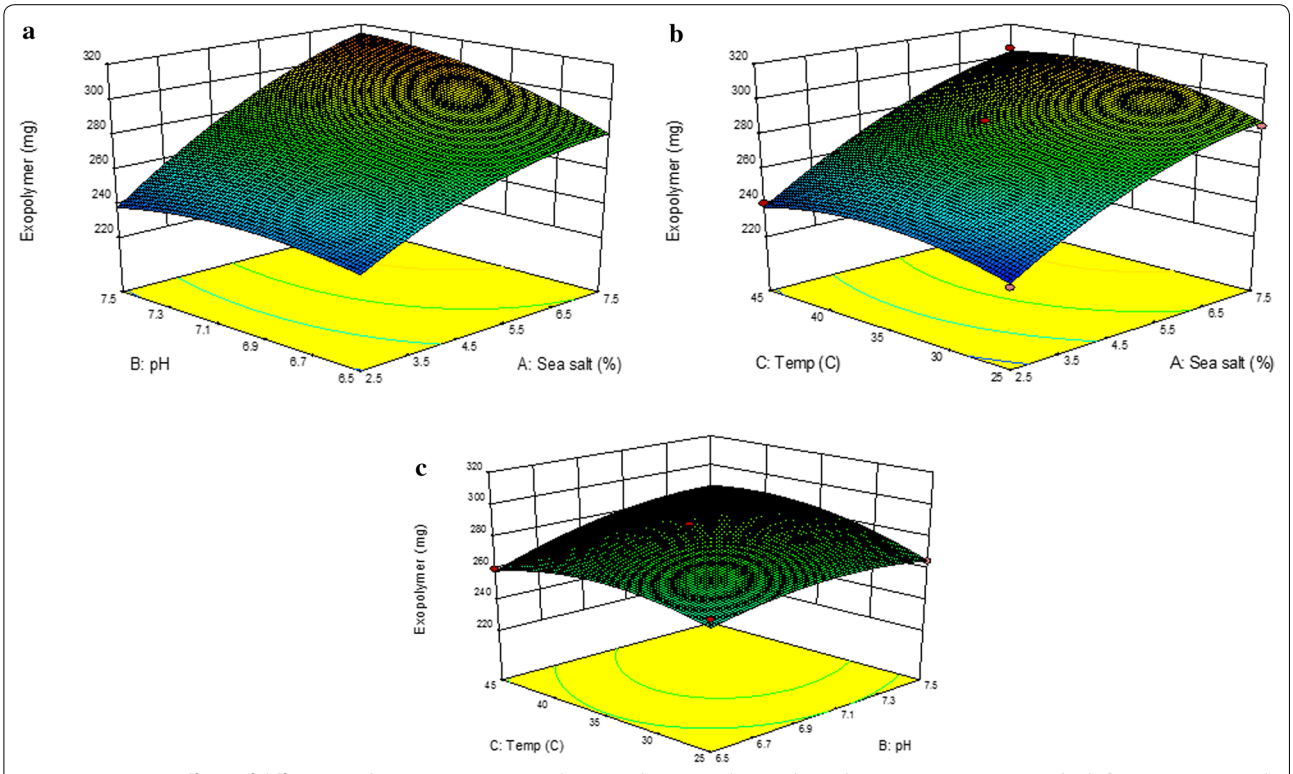

Fig. 2 Interactive effects of different medium components on the exopolymer production by *Halomonas* sp. S19. **a** pH and salt; **b** temperature and salt; **c** pH and temperature. The maximum production of exopolymer (218.66 mg L^{-1}) for *Halomonas* sp. S19 was observed in a medium supplied with glucose (2.5 %) and peptone (0.5 %), sea salt (7.5 %), pH 7.5 and incubation temperature 35 °C

with the production by *H. ventosae* and *H. anticariensis*, which produced 283.5 and 289.5 mg L^{-1} exopolymer in a medium containing 7.5 % sea salt, 1 % glucose (Mata et al. 2006). However, *H. almeriensis* produces 1.7 g L^{-1} exopolymer in MY complex medium containing 1 % glucose and 7.5 % total salts at 32 °C (Llamas et al. 2012). As far as production is concerned, *Halomonas maura* gives the highest yield (4.28 g L^{-1}) at a salt concentration of 5 % in MY medium containing 1 % glucose and 0.3 % yeast extract at pH 7 (Arias et al. 2003).

The salt concentration and temperature have a significant role in exopolymer production by halophilic bacteria. *I. fontislapidosi* F23Tl showed the best growth and high yield of exopolymer (1.50 g L^{-1}) at sea salt concentrations of 7.5 % and 32 °C, whereas the exopolymer production decreased (1.25 g L^{-1}) at lower salt concentration (2.5 %) (Mata et al. 2008).

Characterization of exopolymeric substance

The characterisation studies showed that the exopolymer contains total sugars (65 %), proteins (4.07 %), uronic acids (8.08 %) and sulphur contents (6.39 %). HPLC analysis revealed that exopolymer consists of glucose, mannose and galactose (Fig. 3). The exopolymer of *Halomonas* sp. S19 comprised 61.4 % of sugars; this result was in agreement with *Halomonas maura* (65.34 %) and *Halomonas* sp. S31 (60.2 %) (Bouchotroch et al. 2000; Mata et al. 2008). However, it contradicted with *H. almeriensis* (30.5 %), *H. eurihalina* (37.5 %), *H. anticarinesis* (33.7 %), *H. ventosae* Al 16 (30.8 %) and *Volcaniella eurihalina* (37 %) consisting of low sugar (Llamas et al. 2012; Mata et al. 2006; Quesada et al. 1993). The presence of proteins, uronic acids and sulphates contributed polyanionic nature to exopolymer (Decho 1990).

FT-IR spectroscopy of exopolymer

The stretching at 705.62, 745.02 and 865.35 cm^{-1} corresponds to the presence of sulphates, and an intense peak between 1100 and 1200 cm^{-1} (1040.68, 1073.35, 1123.70 cm^{-1}) attributes to the characteristic sugar (carbohydrate) derivative named fingerprint region of sugars. The CH_3CH_2 stretching of the characteristic sugar was observed at 1286.96 and 1398.46 cm^{-1}. The peaks at 1727.72 and 1641.34 cm^{-1} indicated the presence of COOH groups. The NH_2 and the OH stretchings were observed at broad and narrow stretchings on 3152.15 and 3774.03 cm^{-1}, respectively (Fig. 4).

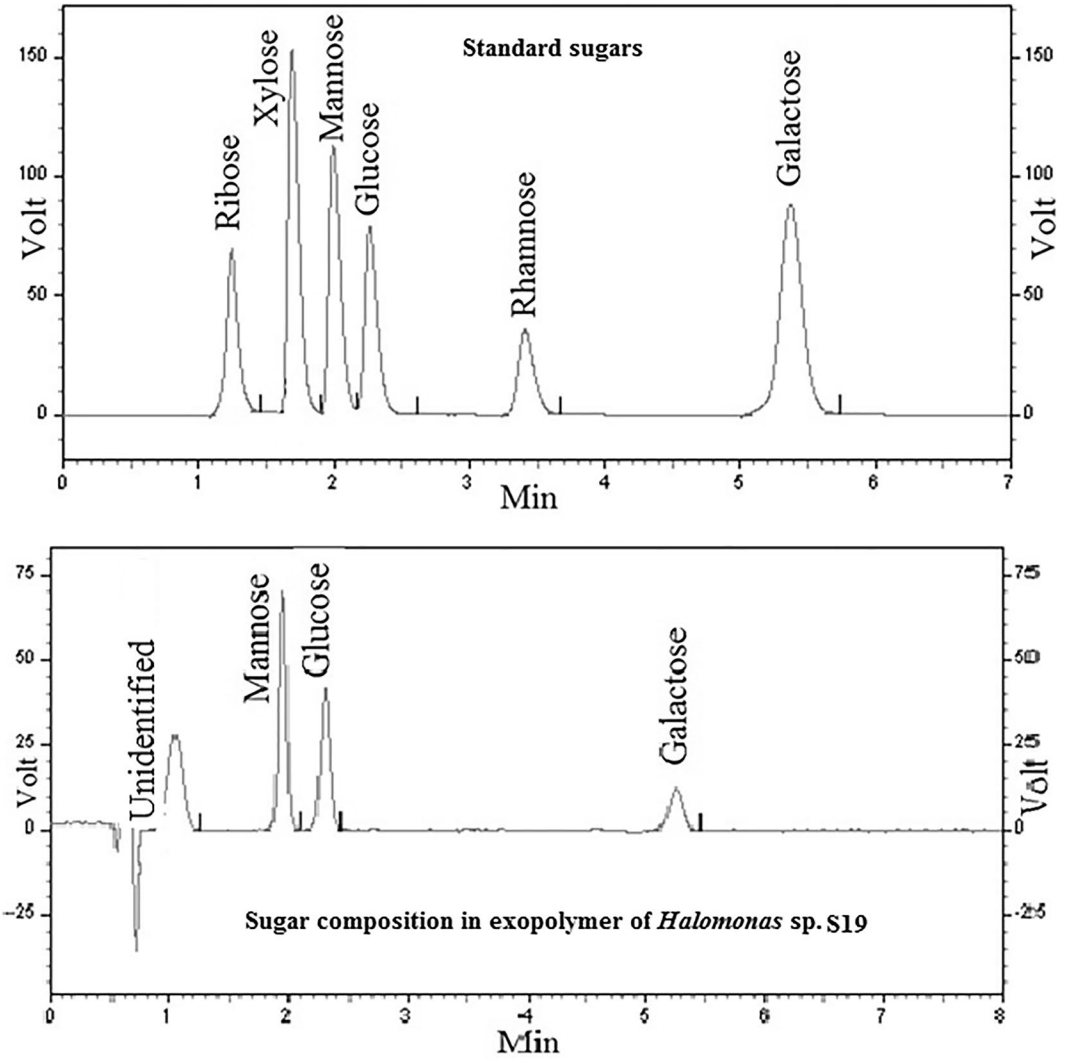

Fig. 3 HPLC analysis of exopolymer produced by *Halomonas* sp. S19. The exopolymer contains glucose, galactose and mannose as major sugars

^{1}H NMR study of exopolymer

The ^{1}H NMR (Fig. 5) study was complex due to the convergence of most sets of the signals in the spectrum. In comparison with the NMR data published in carbohydrate research database (http://www.glyco.ac.ru), the signals often served as signatures for differentiating complex carbohydrate structures. The signals between 0.798 and 0.857 ppm in ^{1}H NMR corresponded to alkane (Singh et al. 2011). The proton signals arising from the methyl protons of the 6-deoxy sugars were observed at 1.279–2.0 ppm. A peak at 2.022 ppm indicated the presence of sulphates. The presence of N–H group of proteins was observed at 1.307 ppm (Jain et al. 2012; Mishra and Jha 2009). The signals at 0.8–1.2 and 1.1–1.5 ppm represented alkanes and alkenes, respectively. The signals observed in 3.3–4.2 ppm correspond to the fingerprint region of sugar moieties due to the protons attached to C_2–C_6 and poorly resolved because of the overlapping chemical shifts. Similar peaks were observed in the exopolymer produced by *B. flexus* (Singh et al. 2013), *Bacillus licheniformis* (Spano et al. 2013) and *Amphidinium carterae Hulburt* 1957 (Mandal et al. 2011).

Differential scanning calorimetric analysis

In DSC analysis, 22.46 % weight loss was observed during phase I of degradation at 60–120 °C and 67.23 % at ≥287 °C due to evaporation of water during the heating process, while the phase II stage of degradation was attributable to thermal decomposition as another study (Parikh and Madamwar 2006). The exothermic curve profiles

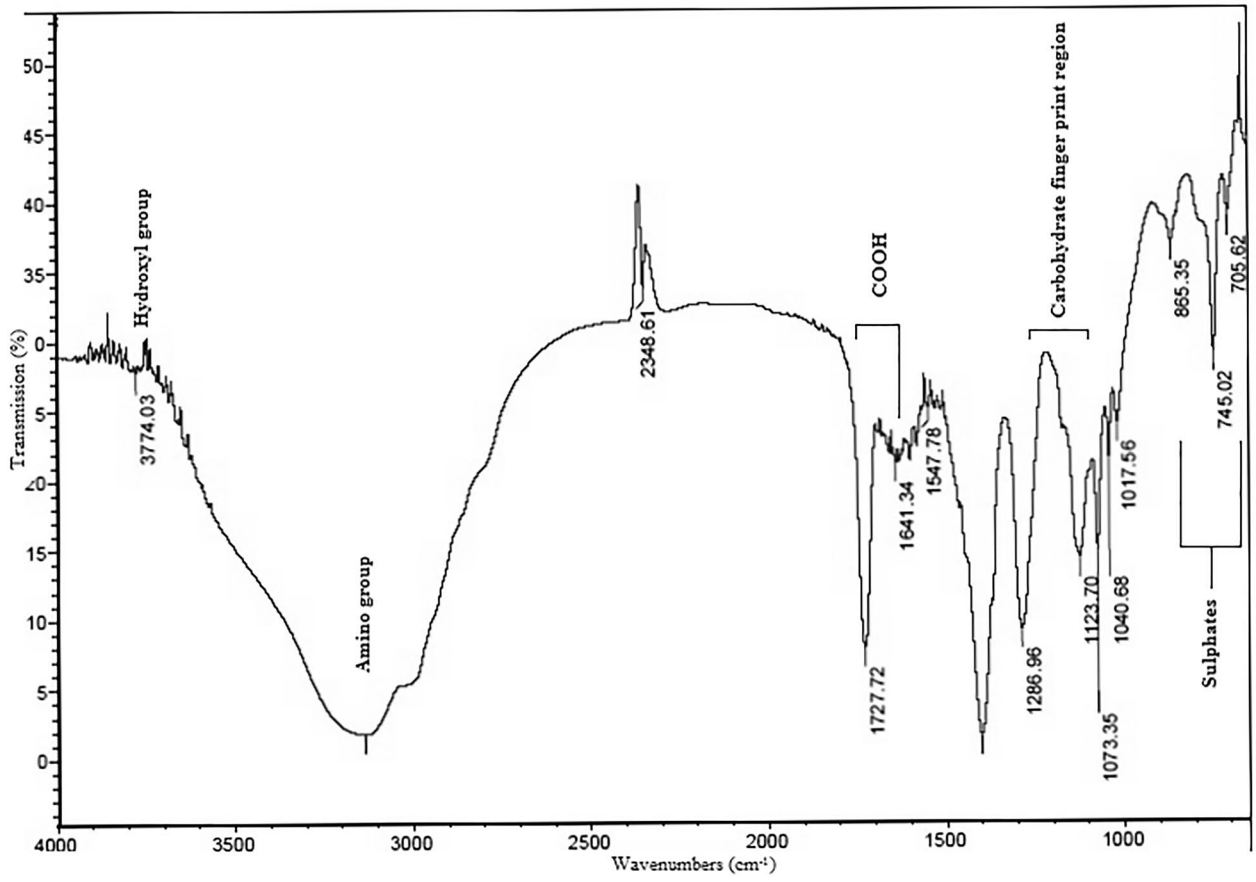

Fig. 4 FT-IR analysis of exopolymer produced by *Halomonas* sp. S19. FT-IR spectrum shows the characteristic stretching for carbohydrate fingerprint region at 1100–1200 cm^{-1}, sulphates at 705.62–865.35 cm^{-1} and amino group of proteins at 3152.15 cm^{-1}

exhibited two peaks at 268.93 and 340.78 °C (Fig. 6). The onset transition temperatures for the exopolymer of *Halomonas* sp. S19 were observed at 240.58 and 316.91 °C. This transition of crystalline solid to amorphous solid was an exothermic process, and differential scanning calorimetric analysis showed a significant thermal transition of low and high.

Emulsification index (EI_{24})

The bacterial exopolymer (5 %) showed 49.44 % (coconut oil), 49.81 % (sesame oil), 38.51 % (paraffin) and 16.48 % (kerosene) emulsification index. However, Tween 20 excellently emulsifies (>65 %) at 5 %. In order to check the emulsion to be an emulsifier having the stabilizing ability, it must retains at least 50 % of the original emulsion volume after 24 h of its preparation (Willumsen and Karlson 1997). Considering this criterion, the bacterial exopolymer at 10 % has proven to possess emulsion forming and stabilising capacity for the oils tested. The exopolymer stabilises different oils and water in which the hydrophobic phase was a hydrocarbon or a vegetable or mineral oil. The exopolymer of *Halomonas* sp. S19 exhibited (55.18, 55.18, 49.81 and 24.62 %) for sesame oil, coconut oil, paraffin and kerosene, respectively (Table 5).

The results of monomeric sugar composition of *Halomonas* sp. S19 are similar to exopolymer of *H. almeriensis* that contains glucose and mannose, and small quantities of rhamnose (Llamas et al. 2012). However, in *Halomonas* sp HE67, the detected monosaccharides were glucuronic acid, glucosamine, mannose, rhamnose, galactose, galactosamine and glucose (Gutierrez et al. 2007). The exopolymer produced by *H. anticarinesis* consists of glucose (17 %), mannose (43 %), rhamnose (1.5 %), xylose (1.5 %) and galacturonic acid (37.5 %) (Mata et al. 2006).

A considerable emulsification index observed for the exopolymeric substance produced by *Halomonas* sp. S19 comprised protein (4.1 %), sulphate (6.39 %) and uronic acid (8.08 %). The presence of various groups in the exopolymer allows good adhesion to oil droplets during emulsification and providing steric stability to the emulsion. Such interactions play a significant role in the

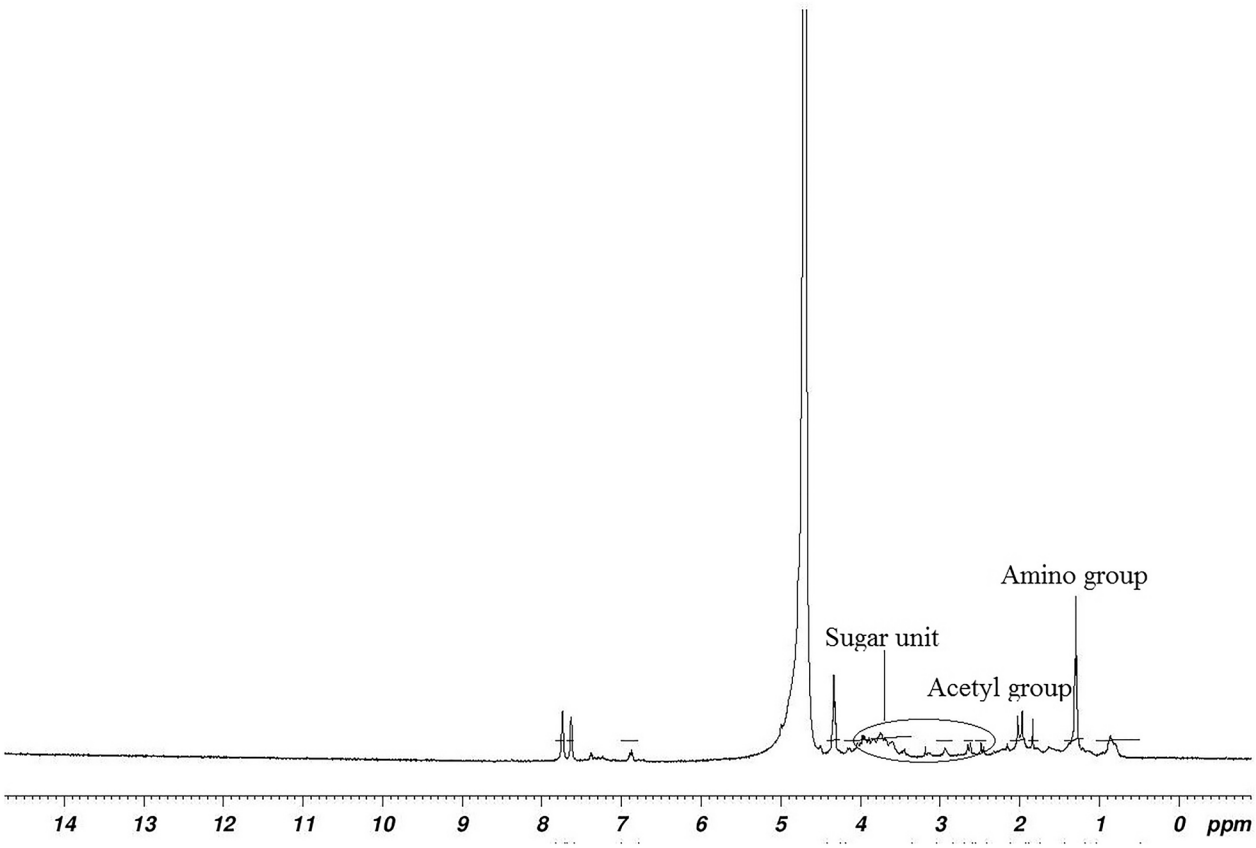

Fig. 5 Proton NMR analysis of exopolymer. The fingerprint region of sugar moieties observed in 3.3–4.2 ppm, sulphates at 2.022 ppm and the NH group of proteins was observed at 1.307 ppm

formation, structure and stabilisation of emulsions (Bach and Gutnick 2005).

The presence of considerable amount of proteins and uronic acids in the exopolymer makes it to play an eminent role in the emulsification of various oils (Mata et al. 2006; Bramhachari et al. 2007). The emulsification index of exopolymer produced by *Halomonas* sp. TG39 (Gutierrez et al. 2008), and *H. almeriensis* M8T (Llamas et al. 2012) was mainly influenced by its proteins, sulphates and uronic acids. The exopolymer produced by *H. almeriensis* effectively emulsifies sunflower (65 %) and mineral oil (67.5 %) (Llamas et al. 2012). The exopolymer producing *H. eurihalina* showed 57.59 % emulsification activity on mineral oil at 0.5 % concentration, which contains proteins (7.27 %) and sulphates (7.15 %) in the exopolymer (Martinez Checa et al. 2007). Mauran, an exopolysaccharide produced by *H. maura*, consists of 2.57 % proteins and 8.14 % uronic acids, reported to produce up to 78 % activity against hexadecane (Bouchotroch et al. 2000). The exopolymeric substance produced by *H. eurihalina* H96 consists of proteins (7 %), uronic acids (7 %) and sulphates (17.6 %); the emulsification index was observed as 73 % in crude oil and 19 % in light oil at 0.5 % concentration (Perfumo et al. 2009).

A large number of bacteria produce polymers that are emulsifying efficiently at low concentrations and exhibiting considerable substrate specificity. They are composed of polysaccharides, proteins, lipopolysaccharides, lipoproteins, etc., (Banat et al. 2000). The exopolymer reported for the emulsification in the present study can be classified under polysaccharide–protein complex (polymeric microbial surfactant). The presence of carboxyl group and sulphates provides overall negative charge to the polymer, thereby imparting binding and adsorptive properties for divalent cation by electrostatic interactions.

Conclusion

Biopolymers are promising invariably and exploring in the space of synthetic emulsifiers. Chemical analysis revealed that the exopolymeric substance of *Halomonas* sp. S19 in the present study consists of different sugars and non-sugar components that can be used as a safe alternative to chemical emulsifiers. Property of

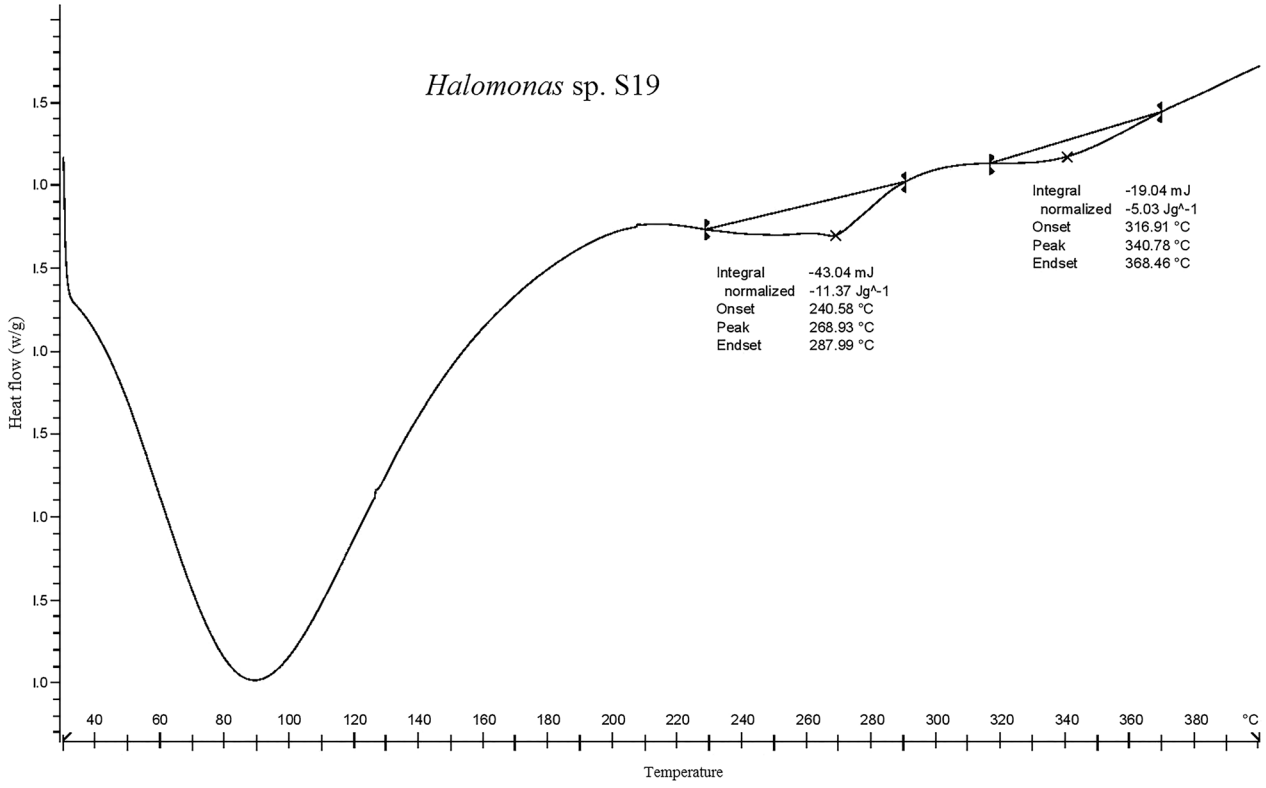

Fig. 6 DSC analysis of exopolymer. The exopolymer loses 22.46 % weight during phase I of degradation (60–120 °C) and followed by 67.23 % weight reduction at second stage of degradation (≥287 °C)

Table 5 Emulsification index of exopolymer and Tween 20 upon different oils

Emulsification index %				
Oils	**Exopolymer**		**Tween 20**	
	5 %	**10 %**	**5 %**	**10 %**
Coconut oil	49.44 ± 0.5	55.18 ± 0.6	67.03 ± 0.6	95.37 ± 1.6
Sesame oil	49.81 ± 0.3	55.18 ± 0.3	72.03 ± 0.3	88.51 ± 0.6
Paraffin	38.51 ± 0.6	49.81 ± 0.3	72.22 ± 0.5	92.22 ± 0.9
Kerosene	16.48 ± 0.3	24.62 ± 0.3	27.40 ± 0.6	78.70 ± 1.6

Results represent the means of three experiments ± SD

the exopolymer emulsifying edible oils makes as lucrative emulsifier and exploited owing to their advantages against synthetic products.

Authors' contributions

RT and KP carried out the synthesis and characterisation of the exopolymer. VV and NV carried out the computational experiments and drafted the manuscript. All authors read and approved the final manuscript.

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Acknowledgements

The authors are grateful to the management of H.K.R.H College, Uthamapalayam, Theni District, DST-FIST and UGC-SAP DRS-II for instrumentation facilities in the Department of Animal Science, Centre of Excellence in Life Sciences, Bharathidasan University, Tiruchirappalli, SAIF-IITM, Chennai for NMR analysis and STIC cochin for DSC analysis.

Competing interests

The authors declare that they have no competing interests.

Received: 15 July 2015 Accepted: 27 November 2015
Published online: 21 December 2015

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