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Saccharification of macroalgal polysaccharides through prioritized cellulase producing bacteria

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Abstract

Marine macroalgal cell wall is predominantly comprised of cellulose (polysaccharide) with the complex chain of glycosidic linkages. Bioethanol production from macroalgae entails breaking this complex chain into simple glucose molecule, which has been the major challenge faced by the industries. Cellulases have been preferred for hydrolysis of cellulose due to the absence of inhibitors affecting the subsequent fermentation process. Cellulose degrading bacteria were isolated from wide-ranging sources from marine habitats to herbivore residues and gastrointestinal region. The investigation reveals that *Vibrio parahaemolyticus* bacteria has higher hydrolytic capacity with salt tolerance up to 14% and 3.5% salinity is optimum for growth. Higher hydrolytic activity of 2.45 was recorded on carboxymethyl cellulose medium at 48 h and hydrolytic activity of 2.46 on *Ulva intestinalis* hydrolysate, 3.06 on *Ulva lactuca* hydrolysate at 72 h of incubation. Total activity of enzyme of 2.11 U/ml and specific activity of 6.05 U/mg were recorded at 24 h. Enzyme hydrolysis of macroalgal biomass; *U. intestinalis* and *U. lactuca* produced

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135.9 mg/g and 107.6 mg/g of reducing sugar respectively. The study reveals that the enzyme extracted from salt tolerant *Vibrio parahaemolyticus* bacteria is suitable for optimal saccharification of seaweed polysaccharides towards biofuel production.

Keywords: Biochemistry, Bioengineering, Biotechnology, Ecology, Environmental science, Microbiology

1. Introduction

Biofuels produced through contemporary biological processes, rather than geological processes based fossil fuels (diesel, etc.) are carbon neutral and sustainable, while mitigating greenhouse gas (GHG) footprint in an era of burgeoning oil demand and rapidly dwindling stock of fossil fuels (Ramachandra et al., 2009, 2015; Ramachandra and Shwetmala, 2012; Wang et al., 2017). Three main biofuel variants are ethanol, biodiesel, and bio-jet fuel. Biofuels are classified into first, second and third generation based on the feedstock's carbon source. First generation feedstock was based on the starch based food sources such as wheat, corn, barley etc. The second generation feedstock included biomass rich in lignin and cellulose such as rice straw, wheat straw, sugarcane bagasse etc. Feedstock of the first and second generation received wider resistance due to the conflict of food or land for fuel (Ramachandra et al., 2009). In this context, third generation feedstock involving algae, composed of mostly cellulose and other polysaccharides like starch, laminarin, floridean starch etc. gained significance as feedstock for sustainable production of biofuel with the reduced greenhouse gas (GHG) footprint. The major constituent of the macroalgal biomass is cellulose, which is a complex chain involving β-1, 4-glycosidic linkages (Kumar and Sahoo, 2012) Conversion of cellulose into simple glucose molecule is the major challenge in the ethanol production (Kumar and Sahoo, 2012; Hebbale et al., 2017; Swain et al., 2017). Algal biomass pretreatment for ethanol production involves reduction in size, loosening of complex structures (e.g. lignin or cellulose fiber). Processes such as steam treatment at 200 °C, ammonia fibre explosion (AFEX) at moderate temperatures of 90−100 °C at high pressure (17−20 bar) and cooking with warm dilute acid have been tried for the release of sugars from the biomass. However, dilute acid pretreatment results in inhibitors such as 5-hydroxymethul furfurals and levulinic acid from sugar degradation, whereas in AFEX little degradation of sugars occurs forming low concentrations of inhibitors (Laureano-Perez et al., 2005; Teymouri et al., 2005; Henning et al., 2012). These inhibitors inhibit subsequent fermentation process. Hence, enzymes are being opted to treat the solid portion (cellulose) of the treated biomass. Enzyme treatment is advantageous due to the lower inhibitor production (Goldemberg et al., 2007). Algal cell wall is composed of cellulose

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Iα (triclinic crystalline form) unlike the cellulose Iβ (monoclinic crystalline form) in plant cell wall. Cellulose Iα consists of weaker hydrogen bonds resulting from spatial arrangement of individual cellulose chains, resulting in easy access to endocellulases enzymes during enzyme hydrolysis (Daroch et al., 2013). Conversion of cellulose to simple sugars (glucose) obtained through enzymes is considered as environmentally friendly pretreatment process for biomass conversion. However this research is at nascent stage towards isolating efficient enzyme systems (Swain et al., 2017), and the major challenge is enzyme vary with the feedstock types. Table 1 lists the common enzymes being used in the ethanol production from biomass.

Biotic components of an ecosystem such as animals, plants, bacteria, fungi, aerobes, anaerobes, mesophiles and thermophiles produce cellulolytic enzymes (Bhat and Bhat, 1997; Niehaus et al., 1999; Zhang and Kim, 2010) in their metabolic processes. Bacteria and fungi produce relatively higher extracellular cellulase, solubilizing crystalline cellulose. Enzymes secreted by these microorganisms are feasible for large scale production due to their broad biochemical diversity, with the possibility of generating mass cultures and also ease of genetic manipulation (Zhang and Kim, 2010). Annexure I lists the different sources used in the earlier studies (Mutalik et al., 2012; Erasmus et al., 1997; Hakamada et al., 1997; Li et al., 2003; Verma et al., 2012; Lo et al., 2009; Hussain et al., 2017; Liang et al., 2009; Gupta et al., 2012; Salah et al., 2007; Gao et al., 2008; Ojumu et al., 2003; Bajaj et al., 2003; Liang et al., 2014; Mata et al., 2010; Ventura and Castañón, 1998; Antonio et al., 2010; Gautam et al., 2011; Bairagi et al., 2002; Dar et al., 2015; Philip et al., 2016; Kamara et al., 2008; Reddy et al., 2003; Krishna, 1999; Dabhi et al., 2014; Maki et al., 2011) about the successful isolation of cellulose degrading bacteria.

Cellulase enzymes are induced by synthetic compounds of disaccharides and thiocellulose such as palmitate, acetate esters, etc. Conventionally, cellulase is induced

Table 1. Common enzymes used for biomass conversion for production of bioethanol.

Feedstock	Enzymes
First generation	
Wheat, Corn, Barley, Rye, Sorghum, Potatoes, Cassava, Sweet potatoes	α- amylase, Pullulanases Glucoamylases, Amyloglucosidase
Second generation	
Rice straw, Wheat straw, Sugarcane bagasse Corn stalk, Saw dust, Cotton stalk, Elephant grass, Napier grass, Switch grass	Endo-β-1,4-glucanase, Cellobiohydrolases, Xylanases β-glucosidases, β-Xylosidases
Third generation	
Macroalgae	Cellulase, Carragenase, Agarase

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by cellulose itself, where low levels of cellulase from the microbes are produced for hydrolyzing the cellulose to soluble sugars, which are transformed into inducers in the cell and influence DNA binding protein for cellulase gene expression. Bacteria produce mostly endoglucanases that cleave cellulose glyosidic chain at random sites. Synthetic cellulose such as carboxymethyl cellulose (CMC) have been utilized for screening cellulose degrading bacteria. Endoglucanase activity (CMCase) are determined by using soluble substrate like CMC (Percival Zhang et al., 2006), endoglucanase enzyme cleaves the intermolecular β-1-4-glycosidic bonds present in cellulose. Cellulase enzyme have wide range of potential applications in food, animal feed, textile, fuel, chemical industries, paper industries, waste management (Bhat and Bhat, 1997), etc.

Several diverse bacteria and fungi have been isolated for cellulase enzymes. However, the studies related to isolation of marine organism for the production of cellulase enzymes is still in the nascent stages. Marine environment is a large reservoir of microbes with well-developed cellular machinery thriving in extreme conditions like salt concentration of 1.7M, temperature stability of 80−108 °C and high pressure of 60MPa (Trivedi et al., 2016). Marine organisms thriving in extreme conditions and tolerance to salts, alkali and other reagents are of particular interest due to the presence of stable enzymes in those microorganisms (Gao et al., 2008). Marine microbial enzymes has potential to offer novel biocatalyst with extraordinary properties (Zhang and Kim, 2010). In this context, the current study focuses on the isolation, identification and characterization of bacteria from different sources for production of cellulase enzyme.

2. Materials and methods

2.1. Isolation of bacteria

Bacteria were isolated from natural sources such as marine (M) (water column and mangrove sediment), Gastrointestinal region (GI) (fish gut, sheep and goat rumen), Herbivore (H) animal residue (Nilgai, Bison, Elephant, Spotted deer, Sambhar deer, Cattle). Serial dilution was performed from 10^{-1} to 10^{-7} using 0.89% saline solution. Tryptone Soya agar, TSA (Tryptone 15 g/l, Soya peptone 5 g/l, NaCl 5 g/l and Agar 15 g/l, pH (at 25 °C) 7.3 ± 0.2) and Zobell Marine agar, ZMA (Peptone 5 g/l, Yeast extract 1 g/l, Ferric citrate 0.1 g/l, NaCl 19.45 g/l, $MgCl_2$ 8.8 g/l, Na_2SO_4 3.24 g/l, $CaCl_2$ 1.8 g/l, KCl 0.55 g/l, $NaHCO_3$ 0.16 g/l, KBr 0.08 g/l, Strontium Chloride 0.034 g/l, Boric acid 0.022 g/l, Sodium silicate 0.004 g/l, Sodium fluorate 0.0024 g/l, Ammonium nitrate 0.0016 g/l, Disodium phosphate 0.008 g/l, Agar 15 g/l, pH 7.6 ± 0.02) for isolation and enumeration of heterotrophic marine bacteria and Carboxymethyl Cellulose agar (KH_2PO_4 0.5 g/l, $MgSO_4$ 0.25 g/l, Cellulose 2 g/l, Agar 15 g/l, Gelatin 2 g/l and pH 6.8−7.2) for other samples.

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2.2. Estimation of hydrolytic activity

(i) *CMC medium:* Cellulose degrading ability of bacterial strains were confirmed by streaking on CMC medium with composition KH_2PO4 0.5 g/L, $MgSO_4$ 0.25 g/L, Gelatin 2 g/L, Agar 15 g/L, CMC-Na salt 2 g/L, which were incubated for 24 h, 48 h and 72 h at 35 °C. Endoglucanase activity of the enzyme was ascertained by using CMC (Juturu and Wu, 2014). After incubation, lugol's iodine was added so as to cover the samples in the petriplates, which aids as an indicator for cellulose degradation (in an agar medium) and provides the basis for a rapid and sensitive screening test for cellulolytic bacteria. Cellulose degrading potential of positive isolates were estimated by calculating hydrolysis capacity as given in Eq. (1) (Gupta et al., 2012). The colonies that degraded cellulose forms clear zones, which were considered for further studies. Average values of diameters were considered for the colonies with uneven zones. Cellulolytic activity was identified as low (if the diameter of the zone was between 0.5-1.9), medium (2−3.9) and high (above >4) (Dar et al., 2015)

$$Hydrolysis\ capacity = \frac{diameter\ of\ zone}{diameter\ of\ colony} \quad (1)$$

(ii) *Seaweed hydrolysate: Ulva intestinalis* (UI) (previously known as *Enteromorpha intestinalis*) and *Ulva lactuca* (UL) were treated with 0.7 N and 0.5 N dilute acid and the hydrolysate obtained was supplemented with Agar 20 g/L, which was autoclaved for 121 °C for 15 min. Later the seaweed medium was incubated for 24, 48 and 72 h and hydrolytic capacity of each strain determined.

2.3. Monitoring of bacterial growth

Bacterial strains were prioritized based on the hydrolytic capacity, and were chosen for further study. Bacterial growth was monitored through absorbance of 600 nm at every 24 h interval upto 72 h. Based on this, enzyme activity was calculated with the plot of growth curve considering absorbance vs time. Protein concentration of the crude enzyme was measured by Bradford method and standard plot was prepared taking bovine serum albumin (BSA) as standard (Bradford, 1976). The cellulase activity was quantified by spectrometric determination of reducing sugars by 3, 5-dinitrosalicylic acid (DNS) method (Miller, 1959) at different incubation time of 30 min, 24, 48 and 72 h. The release of reducing sugar was measured through the measurement of absorbance at 546 nm. Enzymatic activity refers to the amount of enzyme that releases 1 μmol of reducing

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sugar per minute. Salt tolerance for the selected bacteria was determined by monitoring the growth (recorded the absorbance at 600 nm) in a broth medium at different NaCl concentrations (of 3.5−14%).

2.4. Crude enzyme production, growth condition and biochemical characterization

The inocula with higher activity of cellulase was transferred to the production medium containing salts (0.5% Yeast extract, 3.5% artificial sea water medium (NaCl 24.6 g/l, KCl 0.67 g/l, $CaCl_2.2H_20$ 1.36 g/l $MgSO_4.7H_2O$ 6.29 g/l $MgCl_2.6H_2O$ 4.66 g/l, $NaHCO_3$ 0.18 g/l Final pH at 25 °C 7.5 ± 0.5) supplemented with 1.5% CMC as a sole source of carbon and pH was adjusted to 7.5−8.0 before sterilization at 121 °C for 15 min. The culture was incubated at 35 ± 2 °C on rotary shaker at 150 rpm. After 24 h of incubation, the production medium was centrifuged at 12,000 rpm for 30 min at 4 °C and supernatant was treated as crude enzyme (Trivedi et al., 2011). Biochemical and morphological analysis were done according to Bergey's Manual of Systematic Bacteriology.

2.5. Enzyme saccharification of macroalgal polysaccharide

Dilute acid pretreated macroalgal biomass *U. intestinalis* and *U. lactuca* were subjected to enzyme hydrolysis at 55 °C pH 6.8 for 36 h and. The reducing sugar released was estimated every 6 h using DNS method (Miller, 1959).

2.6. 16S rDNA sequencing for strain identification

Identification of bacterial strain with highest hydrolytic activity was done through using 16S rDNA sequencing. Genomic DNA was isolated and quantity was measured using Nano Drop Spectrophotometer and the quality was determined using 2% agarose gel. A single band of high-molecular weight DNA was observed. 16S rRNA gene was amplified by 16S rRNAF and 16S rRNAR primers. A single discrete PCR amplicon band of 1500 bp was observed when resolved on Agarose gel. The PCR amplicon was purified to remove contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with forward primer and reverse primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of 16S rRNA gene was generated from forward and reverse sequence. 16S rRNA gene sequence was used to carry out BLAST with the database of NCBI genbank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated and the phylogenetic tree was constructed by using MEGA 7 (Tamura et al., 2004; Kumar et al., 2016).

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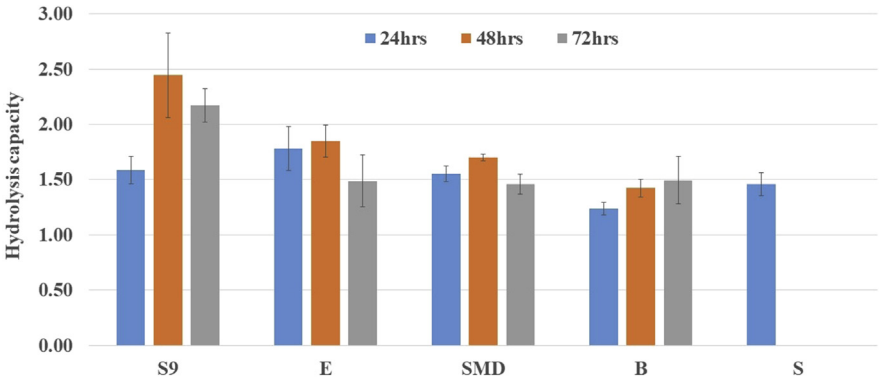

Fig. 1. Hydrolytic activities of all the Cellulose degrading bacteria.

3. Results and discussions

3.1. Isolation of cellulose degrading bacteria

Bacterial samples from marine sources were grown initially on ZMA and TSA and then 9 different colonies (4 isolates from water column and 5 isolates from sediment) were chosen for prioritizing cellulose degrading bacteria. Among these, one strain (S9) exhibited maximum zone of clearance. Identical colonies were observed in gastrointestinal and herbivore residue samples, therefore one isolates each was considered. Herbivore and GI region bacterial samples were directly plated on CMC medium, among herbivore samples maximum zone of clearance was observed from the bacteria isolated from Sambhar deer (SMD), Elephant (E) and Bison (B), whereas in gastrointestinal sample, sheep (S) rumen bacterial strain exhibited maximum zone of clearance. All samples of bacterial colonies positive on CMC and produced clear zone were inoculated on fresh CMC plates to determine hydrolytic activity at different incubation period of 24, 48 and 72 h and flooded with Lugol's iodine. Fig. 1 illustrates the stain wise hydrolytic capacity.

All the bacterial strain exhibited positive zone of clearance at 24 h incubation period with highest hydrolytic capacity recorded for E − 1.78 followed by S9-1.59. While, at 48 h clearance zone increased in all the samples indicating production of extracellular cellulase enzyme, highest hydrolytic capacity was recorded for S9-2.45 (Fig. 2)

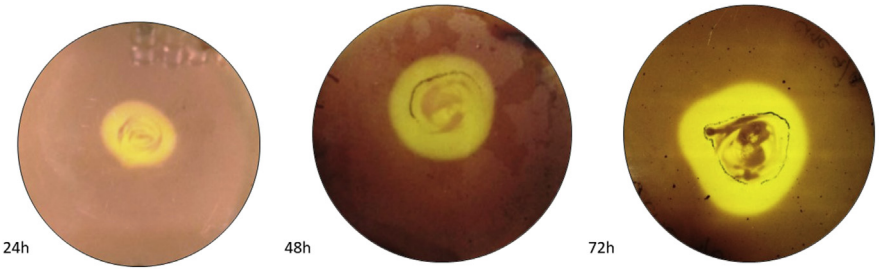

Fig. 2. Hydrolytic activity observed for S9 on CMC medium (24 h, 48 h, and 72 h).

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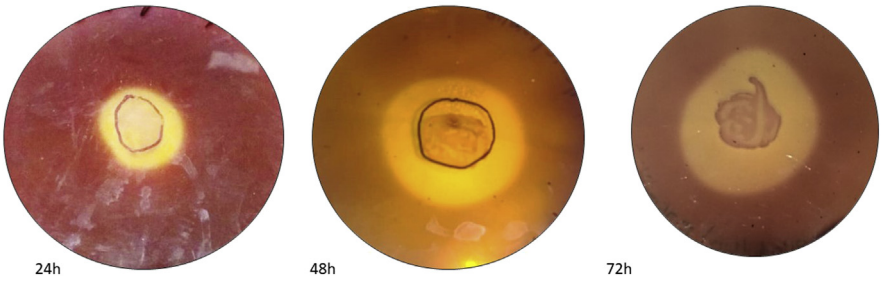

Fig. 3. Hydrolytic activity observed for S9 on Seaweed (*U. intestinalis,* UI) hydrolysate (24 h, 48 h, and 72 h).

followed by E-1.85. However, no zone was observed for sample S at 48 h and 72 h. Earlier study (Gupta et al., 2012) recorded hydrolytic activity of 9.8 for bacterial strain isolated from snail gut, which thrives on cellulosic biomass as their major feed.

As part of bioethanol production process from macroalgae, algal biomass is treated with dilute acid to release its constituents consisting of oligosaccharides and monosaccharides. Macroalgal sample *U. intestinalis* and *U. lactuca* were hydrolysed using dilute acid, and the acid hydrolysate was used for determining the hydrolytic activity. Bacteria isolates were inoculated onto seaweed acid hydrolysate sample supplemented with agar. Sugar present in the feedstock (seaweed) served as the source of carbon for the bacteria, which were incubated for 24, 48 and 72 h. Maximum hydrolytic activity for *U. intestinalis* was 2.45 at 72 h (Fig. 3) and *Ulva lactuca* was 2.39 at 48 h (Fig. 4).

Strain S9 from marine sources exhibited largest clearance zone with the highest hydrolytic activity of 2.45 at 72 h on *U. intestinalis* agar medium when compared to herbivore residue and gastrointestinal region bacterial strains (Fig. 2). Hence, marine strain (S9) was chosen for further enzyme production (scale up).

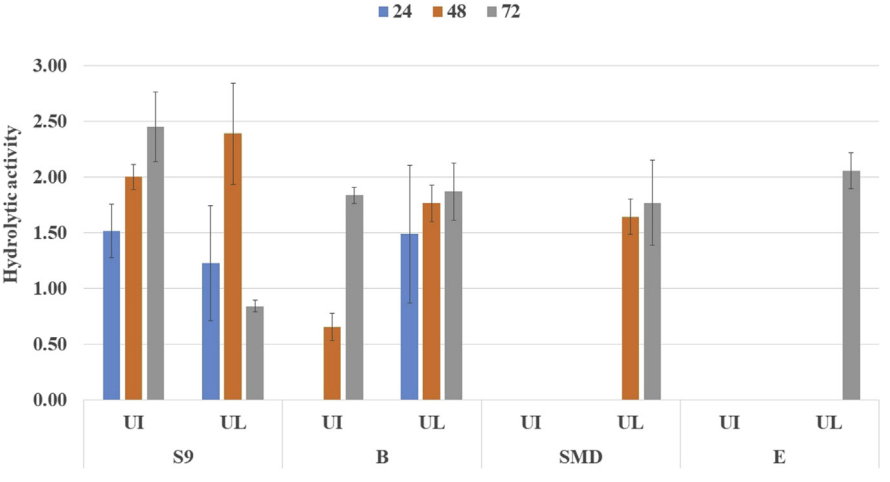

Fig. 4. Hydrolytic activity recorded on *U. intestinalis* (UI) and *U. lactuca* (UL) hydrolysate.

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Table 2. Morphological characteristic and biochemical tests.

Morphological and biochemical tests		
Colony morphology		**Strain 9**
Gram reaction		Gram negative rods
Shape		Irregular
Margin		Curled
Elevation		Raised
Size		moderate (1 cm)
Texture		Smooth
Appearance		Shiny
Pigmentation		non-pigmented
Colour		cream
Optical property		translucent
Motility test		
Biochemical test		
Indole test		-
Methyl red test		-
Voges-Proskauer test		-
Citrate utilization test		-
Catalase		+
Oxidase		+
Carbohydrate fermentation test		
Glucose	Acid	+
	Gas	-
Galactose	Acid	+
	Gas	-
Xylose	Acid	*
	Gas	-
Lactose	Acid	*
	Gas	-
Sucrose	Acid	*
	Gas	-
Starch utilization		+
*partial consumption		

3.2. Bacterial identification

The biochemical characterization showed the strain 9 to be gram negative, motile rods, and aerobic in nature (Table 2). The phylogenetic analysis of S9 with 16S rDNA sequence exhibited 99% homology with MF278586.1 (*Vibrio parahaemolyticus* strain ukmVp1) (Fig. 5). Vibrio belong to *Vibrionaceae* family, commonly inhabiting halocline aquatic environments especially brackish waters and oceans. These are associated with aquatic animals such as parasites of fish, crustaceans

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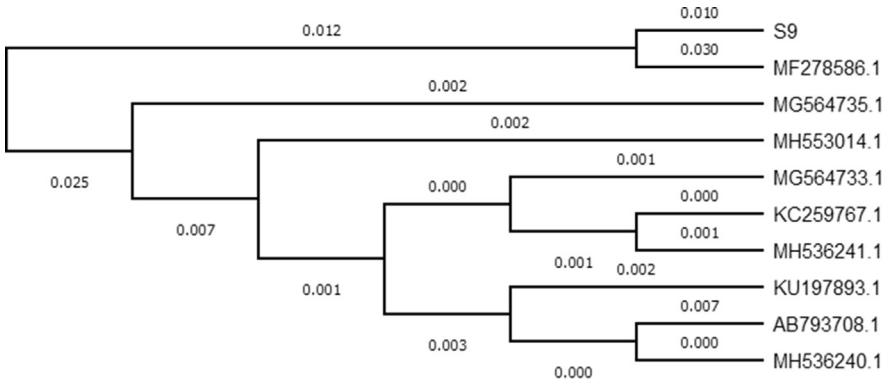

Fig. 5. Phylogenetic tree of S9 (*Vibrio parahaemolyticus*) associated with other members of genus *Vibrio* using 16S r DNA sequence.

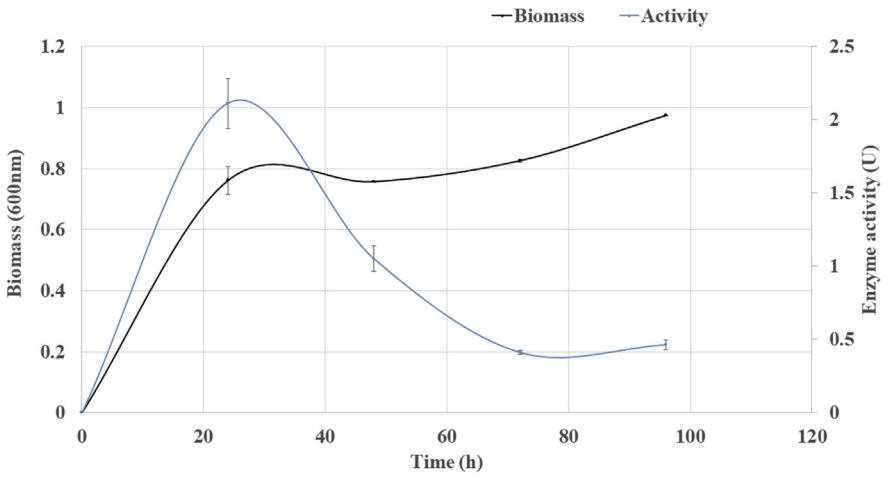

Fig. 6. Growth curve and enzyme activity of Strain 9.

and molluscs. *Vibrio* like bacterium strain G21 was isolated from mangrove soil that produced cellulase enzyme (endo-β-1, 4-glucanase, Cel5A) (Gao et al., 2010), similarly *Vibrio* sp. LX-3 bacteria was isolated from soil for cellulase enzyme production (Li et al., 2003).

3.3. Growth estimation

Simultaneous growth estimation with the enzyme activity revealed maximum enzyme activity at 24 h (1 day) (Fig. 6). In a similar study, Trivedi et al. (2011) has reported the optimum time of 72 h for enzyme production through the isolation of marine bacteria *Bacillus flexus* from degrading *Ulva lactuca* for cellulase production. On CMC medium larger zone of clearance was recorded at 48 h of incubation, whereas in liquid production medium higher enzyme activity was recorded at 24 h, this is due to the presence of agar in CMC medium which provide fairly rigid matrix that slows the diffusion of enzyme through the

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Table 3. Enzyme activity of S9 at different incubation period.

Time (h)	Protein (mg)	Total activity (U/ml)	Specific activity (U/mg)
24	348.66	2.11	6.05
48	299.45	1.05	3.51
72	368.95	0.41	1.12

matrix (Hankin and Anagnostakis, 1977). Liquid production medium contained CMC as sole source of carbon with the higher CMCase activity of 2.11 IU/ml was recorded at 24 h. Trivedi et al. (2011) estimated Enzyme activity of 1.35 U/ml and specific activity of 4.14 U/mg from *Bacillus flexus* isolated from degrading *U. lactuca.* Liang et al. (2014) isolated 245 bacterial strains from sugarcane bagasse pulp supplemented with agar and 22 strains out of 245 showed hydrolyzing zone and one strain *Paenibacillus terrae* exhibited highest CMCase activity of 2.08 U/ml in liquid culture. Gupta et al. (2012) isolated cellulose degrading bacteria from invertebrates such as; termites, snail, caterpillar and bookworm, endoglucanase assay ranged between 0.162 to 0.400 IU/ml for 30 mins. *Vibrio harveyi* and *Vibrio fischeri* isolated from gut of *Mugil cephalus* fish, exhibited higher CMCase activity of 0.3 U/ml and 0.35 U/ml at 30 min respectively (Ramesh and Venugopalan, 1988). CMCase activity of *Vibrio LX*-3 strain which was isolated from soil samples were carried out and 15.29 U/ml activity was recorded for an incubation period of 7−8 days (Li et al., 2003). Enzyme was produced from *Vibrio agar-liquefaciens isolated* from deteriorating wooden pilings exposed to seawater on a muddy coast, CMCase or endoglucanase activity was determined at various incubation period and highest activity of 0.09 U/ml was recorded between 8-10 days incubation (Abhaykumar and Dube, 1992). In our study, however enzyme activity of 0.13 U/ml was estimated at 30 min and

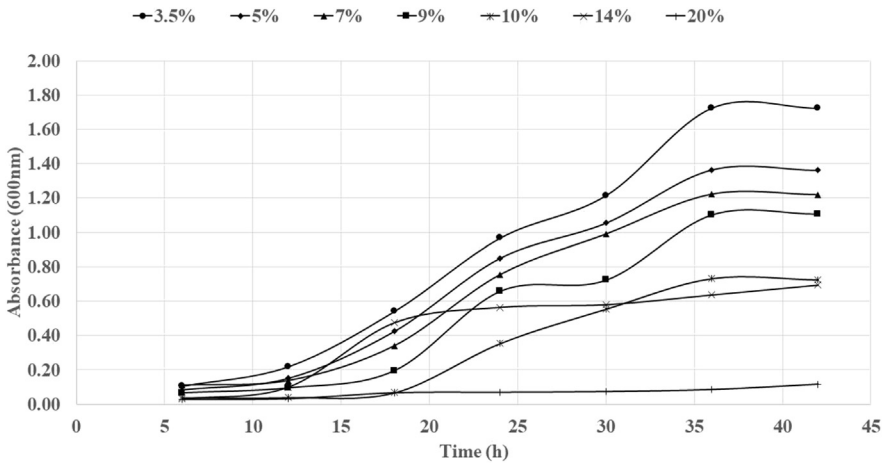

Fig. 7. Bacterial growth curve with different concentration of salt.

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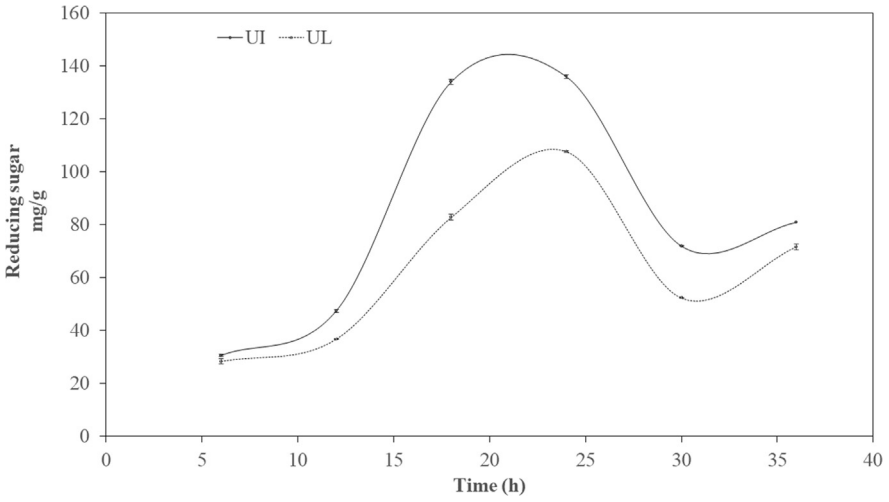

Fig. 8. Enzyme hydrolysis of pretreated *U. intestinalis* (UI) and *U. lactuca* (UL) biomass.

highest enzyme activity of 2.11 U/ml specific activity of 6.05 U/mg were recorded at 24 h incubation (Table 3). In comparison to the other studies, *Vibrio parahaemolyticus* strain showcased higher enzyme activity at lower incubation period indicating its characteristic feature to metabolize complex carbon compounds.

Growth monitoring was carried out at different salt tolerance (Fig. 7) and it was seen that bacterial strain S9 exhibited optimum growth at 3.5% NaCl concentration, with extended exponential phase upto 24 h, where at different salt concentration there was decline of exponential phase. The strain 9 had tolerance level upto 10% revealing its origin from estuarine source indicating halophilic nature. Li et al. (2003) isolated *Vibrio* sp. LX-3 from soil, which was gram negative rod and facultatively anaerobic. Growth of the bacteria was supported by NaCl, bacteria produce enzymes that digested both crystalline cellulose and agar. In *Vibrio sp.* G21, endo β-1, 4-glucanase Cel5A and Egl-AG from *Bacillus agaradhaerens* activity is induced in the presence of NaCl (Hirasawa et al., 2006; Gao et al., 2010; Trivedi et al., 2011).

3.4. Enzyme saccharification of macroalgal polysaccharide

Enzyme hydrolysis was performed for acid pretreated *U. intestinalis* and *U. lactuca* biomass (Fig. 8). Reducing sugar was seen to increase linearly with incubation period from 12 to 24 h ranging from 47.4 mg/g to 135.9 mg/g, and decreased beyond 24 h to 71.9 mg/g in the case of *U. intestinalis.* Enzyme hydrolysis of *U. lactuca* recorded similar linear trend with reducing sugar release of 28.3 mg/g to 107.6 mg/g from 6 to 24 h. Trivedi et al (2015) isolated cellulase enzyme from *Cladosporium sphaerospermum* and subjected *Ulva lactuca,* green seaweed to enzyme hydrolysis and obtained 112 mg/g of reducing sugar. However, in this study highest

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reducing sugar of 107.6 mg/g and was obtained from *U. lactuca* whereas 135.9 mg/g reducing sugar from *U. intestinalis* indicating enzyme ability to hydrolyze the macroalgal polysaccharide.

4. Conclusion

Cellulose degrading bacteria were isolated from wide-ranging sources from marine habitats to herbivore residues and gastrointestinal region. *Vibrio parahaemolyticus* isolated from marine environment is capable of hydrolyzing CMC as well as *U. intestinalis* and *U. lactuca* hydrolysate medium indicating presence of cellulase enzyme capable of hydrolyzing seaweed polysaccharides. Highest enzyme activity was recorded at 24 h and bacteria had salt tolerance up to 14% indicating its marine nature with total activity of 2.11 U/ml and specific activity of 6.05 U/mg. Enzyme hydrolysis for acid pretreated macroalgal biomass *U. intestinalis* and *U. lactuca* was carried out and 135.9 mg/g and 107.6 mg/g of reducing sugar was obtained respectively. The highlight of the current study is the enzyme extracted from salt tolerant *Vibrio parahaemolyticus* bacteria is suitable for optimal saccharification of seaweed polysaccharides towards biofuel production.

Declarations

Author contribution statement

Deepthi Hebbale: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

R Bhargavi: Performed the experiments.

T.V. Ramachandra: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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