



**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

**SYNTHESIS OF BIOPOLYMER POLYHYDROXYALKANOATES (PHA) FROM
HALOFERAX ALEXANDRINUS STRAIN WSP1 ISOLATED FROM SALTERNS
OF MITHAPUR, GUJARAT**

PANDYA B¹ AND PATADIA A^{2*}

- 1:** Ph.D. Research Scholar, Department of Microbiology, Atmiya University Rajkot
2: Former Assistant Professor Atmiya University, Current Assistant Professor Department of
Microbiology, M.B. PATEL science College Anand, Gujrat India

***Corresponding Author: Dr. Apexa Patadia: E Mail: apexasoni6@gmail.com**

Received 15th July 2023; Revised 19th Aug. 2023; Accepted 1st Dec. 2023; Available online 15th Dec. 2023

<https://doi.org/10.31032/IJBPAS/2023/12.12.1081>

ABSTRACT

Polyhydroxyalkanoates (PHAs) may hold the answer to addressing the pollution issues brought on by petrochemical-derived plastics. Archaea represent a vast untapped source of potential for such metabolic products with interesting uses in the environment and industrial importance. Halophilic archaea have drawn a lot of attention among archaea because they are easier to handle and cultivate. Additional benefits of using haloarchaea for PHA generation include the ability to easily harvest intracellularly stored PHA from the cells by suspending them in low osmolarity solutions, such as water. This study's main objective was to check for polyhydroxy butyrate (PHB) accumulation in salt pans in the Mithapur, Surajbari (Kutch), Newport, and Nari (Bhavnagar) regions of Gujarat. 33 isolates, including extremely halophilic archaeon *Haloferax alexandrines strain wsp1* isolated from Mithapur, Gujarat showed a maximum PHA accumulation of 4.37% ±0.17 of cell dry weight (CDW) at optimized conditions. The FT-IR, 1H NMR and 13C NMR analysis revealed that the polymer was a co-polymer of poly(3-hydroxybutyrate-co-3-hydroxy valerate) [P(HB-co-HV)].

Keywords: polyhydroxy alkenoates (PHA), Halophilic archaea, NMR, FTIR

INTRODUCTION

From freshwater ecosystems to hypersaline lakes, life can be found in all salt concentrations seen in natural habitats. In hypersaline conditions, the aerobic halophilic archaea make up the majority of the microbial biomass. These organisms primarily store KCl at a level equal to the external concentration of NaCl in order to withstand the denaturing effects of salt [1]. In India, there are about 10,000 salt producing facilities. Sambhar Salt Lake in Rajasthan, as well as the coastal areas of Gujarat, Tamil Nadu, Maharashtra, Andhra Pradesh, Orissa, and West Bengal, are among the most researched salty habitats in the nation. Gujarat's coastal Kutch and Saurashtra regions, as well as Kandla, Jamnagar, Maliya, Mithapur, Porbandar, and Bhavnagar, are major salt-producing regions. Hypersaline settings are becoming more prevalent as a result of both natural and human-made global changes. Furthermore, it is simple to produce hypersaline settings via the concentration of sea water in arid environments. These facts, together with the occurrence of novel and stable biomolecules in halophilic archaea, suggest that these microorganisms will prove even more valuable in the future. Characterization of such extremely halophilic archaea from newer habitats is, therefore, necessary to explore their biotechnological

potential. Halophilic microorganisms, able to live in saline environments, offer a multitude of potential applications in various fields of biotechnology [2]. Halophiles are currently used in the manufacture of stable hydrolytic enzymes (DNAases, lipases, amylases, gelatinases, and proteases), the fermentation of soy and fish sauce, the production of β -carotene, and the bioremediation of hypersaline brine that has become contaminated. Bacteriorhodopsin for biocomputing, food colouring pigments, and suitable solutes as stress protectants are a few examples of novel halophilic biomolecules employed in specialised applications. Bioplastics, however, is a halophile biotechnology success story. Environmental pollution, which is becoming worse every year and harming the earth severely and permanently, is one of the largest issues the entire world is currently facing. Rapidly developing obsession with plastic among people [2]. Due to their inability to degrade, plastics made from non-renewable petrochemical resources pose a number of environmental issues. Many bacteria and archaea produce a class of biodegradable polymers called polyhydroxyalkanoates (PHAs) that are used as carbon and energy storage materials [3-5]. Poly (3-

hydroxybutyrate, or PHB), one of the more than 150 PHA monomer subunits that have been discovered so far, is a frequent biopolymer that can be found in nature [5, 6]. PHAs are valuable because they are plastics that are recyclable, biocompatible, and degradable after purification. Due to these characteristics, they are currently quite competitive with some synthetic plastics made from petrochemicals. In the current study, halophilic archaea were isolated from various salt pans, characterised, and screened for the production of intracellular PHB. Additionally, purified PHB from halophilic archaea was chemically characterised by nuclear magnetic resonance (^1H , ^{13}C -NMR) spectroscopy and Fourier transform infrared spectroscopy (FT-IR).

MATERIALS AND METHODS

Geography Brine samples were taken from salt pans situated on the Gujarati coast in Mithapur, Newport, Suraj Bari (Kutch), and Nari (Bhavnagar).

Physicochemical evaluation of brine

Understanding the ecosystem and the species that live there requires research into the physicochemical properties. Additionally, it explains the aquatic habitat and the needs of that habitat for growth under lab settings. In order to determine the pH and the ion concentrations of Na^+ , K^+ , Mg^+ , Ca^{2+} , Cl ,

CaCO_3 , HCO_3 , and SO_4 , the brine samples were examined as described in [7].

Medium for growth and culture conditions

Extremely halophilic archaea from sun salterns were identified and grown on TYES (Tryptone yeast extract salt) media. media's components (gL^{-1}) are NaCl 250; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 20.0; KCl 5.0; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.2; Tryptone 5.0; yeast extract 5.0; [8]. The pH of the medium was adjusted to 7.0–7.2 with 1 N NaOH.

Isolation of halophilic archaea

1 ml of brine and 1 gm of sediment sample were inoculated into 100 ml of TYES media and incubated at 37°C for 7-10 days on a rotary shaker (Orbitek, LEIL) at 150 rpm. 100 μl of enriched samples were used to inoculate solid agar media described as above and incubated at 37°C for 8-10 days. Morphologically different colonies were subcultured for further study.

Screening of halo archaeal strains for PHA accumulation and quantification

A total of 33 dominant archaeal isolates were chosen based on their ability to grow on TYES agar plates, and their colonies were then analysed using Sudan Black B dye in accordance with the instructions in [9] to determine whether they were capable of producing PHB. Briefly, 2 mL of Sudan Black B stain (0.05%) was placed on top of the

colonies that were flourishing on the TYES plate. It was then incubated at room temperature for 30 minutes before being sprayed with 60% ethanol. The stained culture plates were then incubated once more for 30 min, and when the colour changed to a dark greenish-blue, the PHB was deemed positive as part of the initial screening.

The polymer content in the isolates was determined by Law and Slepecky acid hydrolysis method [10]. Briefly, The cultures were centrifuged at 10,000 rpm for 15 min. The pellets were incubated at 37°C for 1 h with 5 ml of 0.2% (v/v) sodium hypochlorite for cell lysis. PHB granules were collected by centrifugation, washed with water, and then with acetone and alcohol for the removal of cell lipids and other molecules. The final pellet was dissolved in 3 small portions of boiling chloroform to dissolve PHB and filtered. The chloroform extract was dried by evaporation and the residue was hydrolyzed in 10 ml of concentrated sulphuric acid by heating at 100°C in a water bath for 20min to convert the polymer to crotonic acid and the absorbance was recorded at 235 nm using a UV-visible spectrophotometer. The amount of crotonic acid produced was extrapolated from the standard curve which was obtained by using PHB (Sigma chemicals).

Quantification of polymers and growth kinetics for the potential haloarchaeal strain wsp1

The following results were found for growth rate and intracellular PHA concentration. As a starter culture, the strain wsp1 was cultivated in TYES medium for 4-5 days (mid-log phase). The production medium [TYES] was inoculated with a 6% inoculum of strain wsp1, which was supplemented with 2% (w/v) Xylose. The flasks were shaken at 110 rpm while being incubated at 37 °C. The following parameters, (i) absorbance at 600 nm, (ii) cell dry weight (CDW), and (iii) PHA concentration in the cells, were examined after regular intervals of 24 h. The pellet from 100 ml of the culture broth was centrifuged at high speed (10,000 rpm for the CDW) and rinsed twice with distilled water.

Characterization of the potential haloarchaeal strain wsp1 from a morphological and biochemical perspective

The proposed minimum requirements for the family Halobacteriaceae were followed in the characterization of the haloarchaeal strain wsp1 [11]. Cellular morphology, pigmentation, staining reactions, Various NaCl concentrations (1-4.5M) and Mg²⁺ concentrations (5-85mM) have been taken to observe the physiological nature of isolate. Physiological and biochemical characteristics

were examined by standard methods as described in [12].

Molecular characterization

Using archaeal primers, the 16S rRNA gene of isolates was amplified. The reverse primer, primer, R15215'AGG AGG TGA TCC AGC CGC AG3' (positions 1540-1521), was referred from [13], and the forward primer, F27: 5'ATT CCG GTT GAT CCT GCC GAAG3' (positions 6-27), was constructed in the lab in accordance with [14]. Sequencing was done in SLS Research Pvt Lid, Surat. using DNA sequencing reaction of PCR amplicon was carried out with archaea specific primer ARCHEA_F/ ARCHEA_R using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer an automated DNA sequencer (SLS Surat, Gujrat). The sequences were analyzed by NCBI BLAST search and were submitted to the NCBI Gene Bank database.

Extraction of Polymer

At stationary phase, *Haloferax alexandrinus* strain *wsp1* cells were collected by centrifuging the culture broth at 10,000 rpm for 10 minutes. After quick rinsing with distilled water, acetone, and alcohol, the cell pellet was dried at 70°C. The polymer was recovered by chloroform extraction and storage in a water bath for 12 to 20 hours at 60 to 65 C. The remaining 5% of the chloroform-

containing polymer was poured in a clean glass petri dish and left undisturbed for total evaporation in order to generate a polymer film. Up to 95% of the chloroform was collected by distillation on a rotary evaporator under vacuum at 60°C.

Characterization of polymers

Nuclear magnetic resonance (NMR) and Fourier transform infrared (FT-IR) spectroscopy were each used to analyse the polymer.

Fourier Transform Infrared (FT-IR) Spectroscopy

Cells were separated from 1L of the broth by centrifugation at 4 °C at 10,000 rpm for 10min (Eppendorf – 5804R), suspended in distilled water and lyophilized (OPERON – FDU - 7003) at -80 °C. 2 mg lyophilized cells were removed and thoroughly mixed with 100 mg spectroscopic grade KBr with the help of mortar and pestle. From this mixture, 15mg was used for making KBr pellets. The pellets were kept in an oven at 100°C for 4h to remove atmospheric moisture from the sample. FTIR spectrum was recorded on Thermo Nicolet IR 200 spectrophotometer. The samples were scanned between 400 and 4000 wave numbers (cm⁻¹). PHB obtained from Sigma chemicals was considered as standard.

Spectroscopy using nuclear magnetic resonance

The polymer was dissolved in high quality deuteriochloroform (CDCl₃), and the ¹H NMR spectra of the polymer were acquired using a Bruker Advance II 500 NMR spectrometer at 500 MHz while the instrument was operating at room temperature. At 80 MHz, the ¹³C NMR spectral analysis was carried out. Parts per million (ppm) was the scale used for chemical shifts.

RESULTS AND DISCUSSION

Physical and chemical analyses of brine

The predominant ions in the analysed brine samples were sodium and chloride, which were identical to those documented for thalasso haline settings. For all salterns, the pH ranged from 6 to 7.5 and the concentration of chloride ions was greater than that of other ions (**Table 1**). The samples of brine ranged in colour from pink to colourless.

Isolation of halophilic archaea

All the colonies obtained showed different shades of pink, orange and red colours indicating the presence of halophilic archaea. Total 33 halophilic archaea were isolated from the salt pan of Mithapur Surajbari and Bhavnagar, out of which 16 were from Mithapur salt pans, 9 from Surajbari (kutch) salt pan, 8 from Bhavnagar salt pan 5 and 3

from Nari salt pan and Newport salt pan respectively.

Screening of halo archaeal strains for PHA accumulation and quantification

Polyhydroxyalkanoates (PHAs) are produced and accumulated in prokaryotes as carbon and energy storage materials. Primary Screening was done with Sudan Black B dye method to qualitatively screen the pure bacterial isolates for PHB polymer accumulating abilities. Among the all isolates 24 isolates i.e., WSP1, MIT-5, MIT-6, MIT-8, MIT-9, MIT-11, MIT-12, MIT-13, MIT-14, MIT-15, SUJ-1, SUJ-3, SUJ-4, SUJ-5, SUJ-6, SUJ-8, SUJ-9, SUJ-10, SUJ-11, NAR-1, NAR-3, NEW-1, NEW-2, and NEW-3 showed the presence of brown black granules when stained with Sudan black B. Which indicates as positive for PHB accumulations in test isolates [15]. Thus, these isolates were further selected for quantitative secondary screening by Law and Slaughter method.

The five halophilic archaeal isolates that accumulate PHA are (i.e. SUJ-8, SUJ-9, SUJ-10, SUJ-11 and wsp1) showed significant PHB production in secondary screening. The growth as cell dry weight (CDW) (mg/100ml), PHA content (mg/100ml), and yield (%) for all the haloarchaeal isolates are represented in **Table 2**. The strains with the highest biomass content as CDW (mg/100ml)

was SUJ-8(155±77) > SUJ-11 (116 ± 11) > SUJ-8 (100±0.08) > SUJ-9(25 ± 0.7) > wsp1 (20 ± 0.04) > SUJ-10 (10 ± 0.43). The intracellular accumulation PHA content in terms of Yield (%) was highest in strain wsp1 (4.37±0.17) > SUJ-9(3.2±0.009) > SUJ-8(3.0±0) > SUJ-11 (2.6 ± 0.8).

The growth kinetics and polymer quantification for promising haloarchaeal strain wsp1

Figure 1 displays the time-course of strain wsp1's development in TYES with Xylose. With an initial optical density (OD at 600 nm) of -0.145 ± 0.02 , Haloferax alexandrinus strain wsp1 initially did not develop for 4 to 5 days before increasing to 1.145 ± 0.003 on day 8 and reaching its maximum of 2.106 ± 0.07 on day 16. Similar to this, the CDW of the culture slowly grew throughout time, peaking at $78 \text{ (mg/100ml)} \pm 0.08$ on the 28th day from $13 \text{ (mg/100ml)} \pm 0.005$ on the 4th day to $31 \text{ (mg/100ml)} \pm 0.002$ on the 12th. Growth rate (μ) for the culture was 0.839 ± 0.01 . On the 12th day, active intracellular polymer production of $0.03\% \pm 0.76$ of the CDW was seen; it gradually increased and reached a maximum of $4.37\% \pm 0.17$ of the CDW on the 20th day.

Characterization of the potential haloarchaeal strain wsp1 from a morphological and biochemical perspective

The strain wsp1 produced colonies that were very shiny, viscous, circular with an entire edge, convex/raised elevation, and red in colour when cultivated on TYES medium. The colonies also appeared sticky after a lengthy incubation period of roughly 20 days. Under a 100 phase contrast microscope, the cells of strain wsp1 were Gram-negative and pleomorphic, either alone or in groups. In the presence of different NaCl and Mg²⁺ concentrations, the isolate WSp1 demonstrated growth. 2M is the bare minimum amount of salt needed. The isolate showed substantial growth at 4.5 M NaCl (saturating concentration), with optimal growth occurring at 3 M NaCl. The wsp1 strain was catalase and oxidase positive, and it also shown activity for a number of hydrolytic enzymes such as amylase, protease, lipase, and gelatinase. The strain was able to utilize various carbohydrates such as glucose, sucrose, fructose, maltose, xylose, acetate, and glycerol (**Table 3**).

Molecular characterization

The BLAST analysis of the 16S rRNA gene fragment of the haloarchaeon strain wsp1 showed 99.80% similarity to Haloferax alexandrinus (**Figure 2**). The sequence was deposited in GenBank/DDBJ database with accession number OP458567.

Extraction of Polymer

Using a soxhlet extractor, the concentrated polymer that had been dissolved in chloroform was poured onto a spotlessly clean, dry petri plate to create the polymer film from the *Haloferax alexandrinus* strain wsp1. Initial cultivation of the *Haloferax alexandrinus* strain wsp1 results in the production of a pink to orange carotenoid pigment, a crucial chemotaxonomic marker for the family Halobacteriaceae. After being rinsed with acetone for 10 minutes to remove the pigment, the polymer was produced in a white hue.

Polymer characterization

The ester carbonyl group (C=O) stretching is seen in the polymer's FT-IR spectra (**Figure 3**) as a strong absorption band at 1774 cm⁻¹ [12, 16, 17]. The band at 1504.53 cm⁻¹ is caused by the asymmetric bending of -CH₃, whereas the band at 1234.48 cm⁻¹ represents the stretching of C-O-C and peaks at 2900 cm⁻¹, or 2972 cm⁻¹ and 3055.35 cm⁻¹, respectively [18]. The carboxyl group's internal OH vibration is what causes the absorptions at 3410.26 cm⁻¹ [18]. The polymers' FT-IR spectra compared favourably to the reference PHB (Sigma). The polymer's

monomeric composition was identified using the ¹H NMR spectra (**Figure 4**). The spectra revealed a signal for the methyl groups (-CH₃) of hydroxy valerate (HV) and hydroxybutyrate (HB), respectively, at 0.87–0.91 ppm and 1.29–1.32 ppm. The methylene CH₂ group of HV was represented by a different signal at 1.7 ppm. The signal at 7.26 ppm matches the CDCl₃ signal [19]. In addition, there was no HV peak and only one conspicuous peak of methyl (CH₃) from the HB unit at 1.25 ppm in the ¹H NMR spectra of a homopolymer of HB (PHB) [20].

The methine group (-CH-) group of HV and HB were identified in the E3 polymer's ¹³C NMR scans as signals at 76.2 ppm and 76.84 ppm, respectively (Fig. 6). The methylene (-CH₂) group of HV and HB was represented by the signals at 30.44 ppm and 42.08 ppm. Another signal at 29.20–29.54 ppm represents the -CH₂ group and -CH₃ group of HV [21]. The homopolymer of HB exhibits prominent peaks at 20 ppm of the (-CH₃) group, 40 ppm of methylene (-CH₂-) group, 67–68 ester (-O-CH-) group, and 170 ppm of carbonyl carbon (-C-) group [22].

Table 1: Physical and chemical analysis of brine

Parameters	Mithapur	New Port	Nari	Suraj Bari	Unit
Temperature	30.33±0.57	35.66±0.57	34±0.57	35±0.81	°C
pH	6.16±0.28	7.5±0.43	7.2±0.52	7.12±0.25	
Chloride	2011±73.90	1934.66±115	2016±39.8	1877±122	mg/l
Total Hardness	1839±116.0	1830.66±56	904±38.1	663±53	mg/l
Alkalinity (as CaCo3)	73.33±10.50	34.33±11.5	30±6.35	95±4.92	mg/l
Calcium (as Ca)	320±17	296.33±49.4	163±7.50	231±69.7	mg/l
Magnesium (as Mg)	8.7±2.94	5.6±2.51	4±0.9	3±2.66	mg/l
Sodium (as Na)	255±48.2	33.33±2.64	26±1.73	205±19.7	mg/l
Potassium (as K)	91.66±10.40	206.66±9.45	216±6.24	44±26.2	mg/l
Phosphate	10.7±1.21	53.53±7.81	23.66±3.05	0±0.05	mg/l
Sulphate (as SO4)	239.5±17.38	0.023±0.023	0.01±0.01	35±2.87	mg/l
Salinity	3.63±0.13	3.494±0.20	3.642±0.07	3.39±0.2	ppt

Table 2: DCW, PHB Production and Yield of isolates

Isolates	DCW (mg/100ml)	PHB (mg/ml)	Yield(%)
SUJ-8	155±77	1.09±0.3	3.0±0
SUJ-9	25±07	0.8±02	3.2±0.009
SUJ-10	10±0.43	0.02±0	0.2±0
SUJ-11	116.66±11	2.7±0.87	2.6±0.8
WSp1	20±0.04	0.88±0.042	4.37±0.17

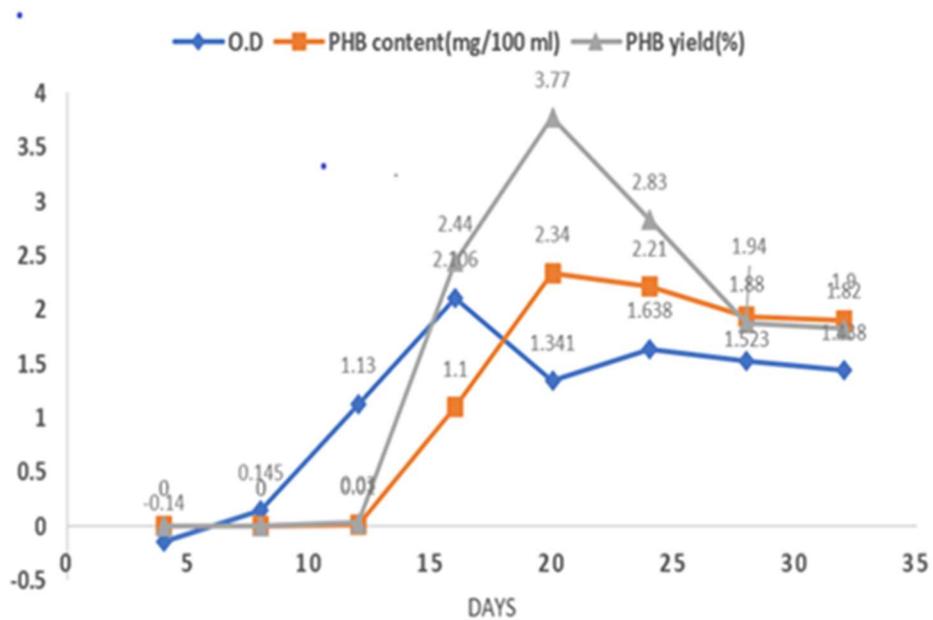


Figure 1: The growth kinetics and polymer quantification

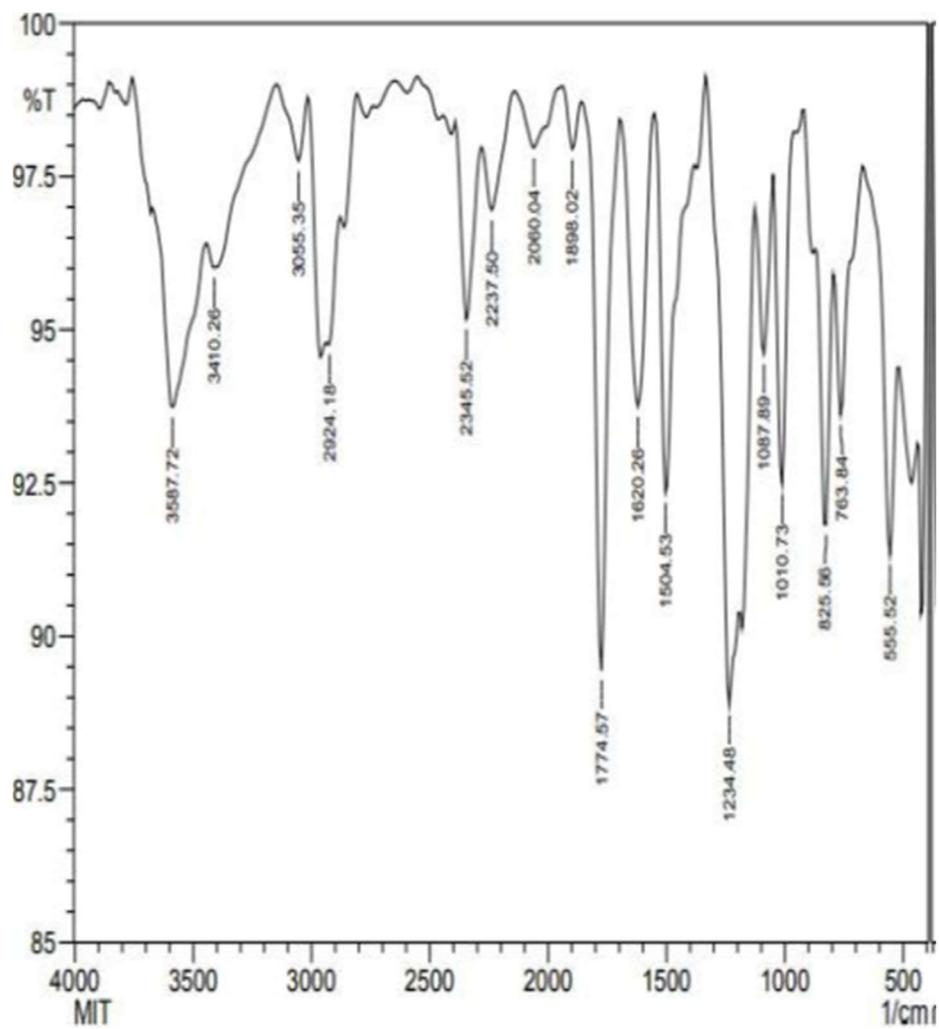


Figure 3: FTIR spectrum of polymer

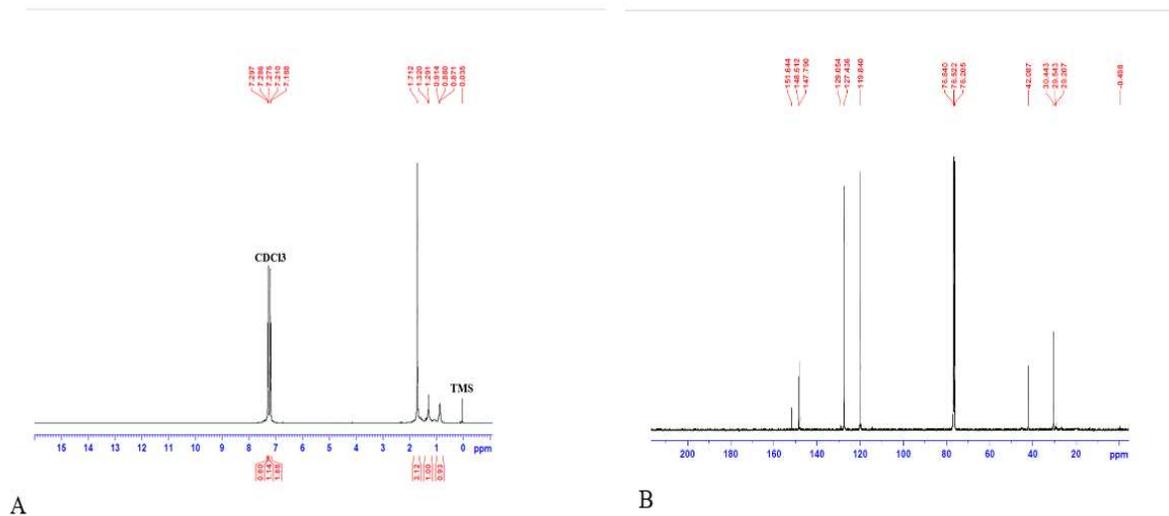


Figure 4: A ¹H NMR spectra of wsp1 polymer.

B ¹³C NMR spectra of wsp1 polymer

CONCLUSION

The haloarchaeon *Haloferax alexandrinus* strain wsp1 was identified for the first time as the creator of the copolymer P(HB-co-HV), which has potential applications in pharmaceuticals for targeted medication delivery and other medical fields. The production of nanocapsules using this polymer and another copolymer as a blend is one of the many medical applications that can be further researched. Additionally, this archaeon's capacity for flock formation for prawn farming is being investigated.

ACKNOWLEDGEMENT

BSP is Thankful to SHODH scheme India for funding to support the research work. The authors are grateful to DPC members Dr. Bharti P. Dave and Dr. Devyani Tipre to review the research progress. My Heartiest thanks to Dr. Apexa Patadia for continuous guidance and supervision of research work. Also thankful to Atmiya University Rajkot, India, for providing lab facilities and guidance.

REFERENCES

- [1] Salgaonkar, Bhakti B., and Judith M. Bragança. "Biosynthesis of poly (3-hydroxybutyrate-co-3-hydroxyvalerate) by *Haloferax alexandrinus* strain E3." *International journal of*

biological macromolecules 78 (2015): 339-346.

- [2] Quillaguaman J, Hashim S, Bento F, Mattiasson B, Hatti-Kaul R, Poly(β hydroxybutyrate) production by a moderate halophile, *Halomonas boliviensis* LC1 using starch hydrolysate as substrate, *J Appl Microbiol* 99, (2005), 151-157.
- [3] Krauss H, Koller M, Muhr A, Fasl H, Stelzer F, Braunegg G, Archaeal production of polyhydroxyalkanoate (PHA) co- and polyesters from biodiesel industry-derived byproducts, *Archaea*, 129268, (2013).
<https://doi.org/10.1155/2013/129268>
- [4] Kalia V.C., Patel S.K.S, Shanmugam R., Lee J.-L, Polyhydroxyalkanoates: trends and advances toward biotechnological applications, *Bioresour. Technol.* 326 (2021), 124737,
<https://doi.org/10.1016/j.biortech.2021.124737>.
- [5] Philip S., Keshavarz T., Roy I., Polyhydroxyalkanoates: biodegradable polymers with a range of applications, *J. Chem. Technol. Biotechnol.* 82 (2007) 233–247,
<https://doi.org/10.1002/jctb.1667>

- [6] Kumar M., Rathour R., Singh R., Sun Y., Pandey A., Gnansounou E., Lin Y., Tsang D., Thakur I., Bacterial polyhydroxyalkanoates: opportunities, challenges, and prospects, *J. Clean. Prod.* 263 (2020), 121500, <https://doi.org/10.1016/j.jclepro.2020.121500>
- [7] APHA, Standard Methods for the Examination of Water and Wastewater. 21st Edition, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC (2005)
- [8] Law J.H, Slepecky RA Assay of poly- β -hydroxybutyric acid, *J Bacteriol* **82** (1961), 33-36.
- [9] Zuriani R., Vigneswari S., Azizan M.N.M., Majid M.I.A., Amirul A.A., *Biotechnol. Bioprocess Eng.* 18 (2013) 472–478
- [10] Kansiz M, Billman-Jabobe H, McNaughton D, Quantitative determination of the biodegradable polymer Poly(beta-hydroxybutyrate) in a recombinant *Escherichia coli* strain by use of mid-infrared spectroscopy and multivariate statistics, *Applied and Environmental Microbiology* (2000) Aug;66(8):3415-20, doi: [10.1128/AEM.66.8.3415-3420.2000](https://doi.org/10.1128/AEM.66.8.3415-3420.2000)
- [11] Oren, A. Industrial and environmental applications of halophilic microorganisms. *Environ. Technol.* 31, (2010) 825–834. [CrossRef] [PubMed]
- [12] Krieg RN, Holt GJ (eds) *Bergey's Manual of Systematic Bacteriology Vol III* Williams and Wilkins, Baltimore, London (1984)
- [13] Xu X, Min W, Huang W Isolation and characterization of a novel strain of *Natrinema* containing a bop gene. *J Zhejiang Univ Sci* 6B (2005) :142–146
- [14] Gupta R, Lanter JM, Woese CR Sequence of the 16S ribosomal RNA from *Halobacterium volcanii*, an archaeobacterium. *Science* 221(1983):656–659
- [15] Park Y.L., Song H.S., Choi T.R., Lee S.M., Park S.L., Lee H.S., Kim H.J, Bhatia S. K., Gurav R., Park K., Yang Y.H., Revealing of sugar utilization systems in *Halomonas sp.* YLGW01 and application for poly(3-hydroxybutyrate) production with low-cost medium and easy recovery, *Int. J. Biol. Macromol.* 167 (2021)

- 151–159,
<https://doi.org/10.1016/j.ijbiomac.2020.11.163>
- [16] Han J., Lu Q., Zhou L., Zhou J., Xiang H., Appl. Environ. Microbiol. 73 Intech Open (2007)6058–6065
- [17] Taran M., Amirkhani H., International Journal of Biological Macromolecules,(2010) 632–634
- [18] Hong J.-W., Song H.-S., Moon Y.-M., Hong Y.-G., Bhatia S.K., Jung H.-R., Choi T.-R., Yang S.-Y., Park H.-Y., Choi Y.-K., Yang Y.-H., Polyhydroxybutyrate production in halophilic marine bacteria *Vibrio proteolyticus* isolated from the Korean peninsula, Bioprocess Biosyst.Eng. 42 (2019) 603–610,
<https://doi.org/10.1007/s00449-018-02066-6>
- [19] Van-Thuoc D., Huu-Phong T., Thi-Binh N., Thi-Tho N., Minh-Lam D., Quil-laguamán J., Microbiol. Open 1 (2012) 395–406
- [20] Liu H., Pancholi M., Stubbs III J., Raghavan D., Journal of Applied Polymer Science 116 (2010)3225–3231
- [21] Bluhm T.L., Hamer G.K., Marchessault R.H., Fyfe C.A., Veregin R.P., Macro-molecules 19 (1986) 2871–2876
- [22] Bonthron K.M., Clauss J., Horowitz D.M., Hunter B.K., Sanders J.K.M., FEMSMicrobiol. Rev. 103 (1992) 269–278