Apex Patadia and Bharti P Dave. / *International Journal of Biological & Pharmaceutical Research.* 2016; 7(2): 65-73.

e- ISSN 0976 - 3651
Print ISSN 2229 - 7480

International Journal of Biological & Pharmaceutical Research
Journal homepage: www.ijbpr.com

IJBPR

MEDIA OPTIMIZATION FOR Β-KETOTHIOLASE IN PRODUCTION OF POLY Β-HYDROXYBUTYRATE FROM *HALOARCULA* SP. 1, AN EXTREME HALOPHILIC ARCHAEA

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ABSTRACT

One of the eye catching subjects in the scientific community is environmental friendly and economic production of bioplastics [Poly β-hydroxybutyrate (PHB)]. Most of the research on PHB using microbes has been directed towards the processes which are costly. However, PHB productions from Halophilic Archaea which are extremophiles have been completely overlooked. Halophilic archaea offer certain advantages as requiring non sterile conditions for growth which makes the process completely cost effective. The present work aims at PHB production by halophilic archaea with optimization of the enzymes β-ketothiolase as primary precursor of PHB production. Amongst the three key enzymes for PHB biosynthesis, β-ketothiolase is a primary enzyme required for PHB synthesis. The information on β-ketothiolase from the archaeal domain, especially from Haloarchaea, is very sparse. Hence the present study is directed towards detection of β-kethiolase from *Haloarcula* sp. 1., and its optimization. Optimization has been carried out by using fifteen different parameters. As a result of optimization, maximum enzyme activity (0.047EU/ml) was exhibited by *Haloarcula* sp. 1., Which is also the maximum PHB producer.

Key Words: β-ketothiolase, poly β-hydroxybutyrate, Extremely halophilic Archaea.

INTRODUCTION

Among the interesting products reported to be produced by Halophilic archaea is poly- hydroxybutyrate (PHB). PHB is polymer synthesized intracellulary and stored as carbon and energy reservoir by several bacteria, usually when cellular growth is restricted by the lack of nutrients like O, P, N, S and in the presence of excess carbon sources. The interest in PHB has been due to its unique characteristic of being biodegradable thermo polyester that can be produced from renewable resources, and has properties mostly similar to those of petroleum derived plastics (Margesin R and Schinner F, 2001).

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The pathway for production of PHB and its regulation have been studied extensively in *Alcaligenes eutrophus* H16 and *Azotobacter beijerinckii* (Ritchie *et al*., 1971; Senior and Dawes, 1972). The pathway consists of a biosynthetic portion and a degradative portion and is made up of five enzymes. In the biosynthetic part of the pathway, β-ketothiolase catalyzes the reversible condensation of two acetyl coenzyme A (CoA) to acetoacetyl – CoA. Acetoacetyl – CoA is subsequently reduced to D-(-)-3-hydroxybutyryl – CoA and PHB is then produced by the polymerization of β – hydroxybutyryl – CoA via the action of PHB synthetase. β – Ketothiolase is the enzyme participating in the formation of acetoacetyl – Co A, a precursor for (Poly – β – hydroxybutyrate) PHB synthesis.

Compared with what is known about thiolases in *Bacteria* and *Eukarya*, the information on thiolases from the archaeal domain, especially from haloarchaea, is very

sparse. The successful purification of thiolases from haloarchaea has not yet been reported, and its catalytic properties are not known. To extend further the knowledge of thiolases, the present study aims to describe (i) the detection of the enzyme β-ketothiolase from the four potent haloarchaeal PHB producing isolates and (ii) the effect of various cultural conditions on its biosynthesis by the maximum enzyme producing isolate.

MATERIALS AND METHODS

Collection of samples

Samples (Water and sediment) were collected aseptically in sterile glass bottles and plastic bags from salt pans at Newport and Nari, Bhavnagar, Gujarat, India (Latitude 21º45' N and Longitude 72º14' E and 22º10' N and 72º15 E).

Enrichment of Halophilic Archaea

Halophilic Archaea were enriched in Tryptone yeast extract salt (TYES) (Krieg and Holt, 1984), Mullakhan and Larsen, 1975 (M & L) and Larsen (Larsen, 1981) media containing 100µg/ml each of penicillin G, erythromycin and cycloheximide to inhibit growth of bacteria and fungi and incubated at 37 °C for 15 – 20 days (Oren and Litchfield, 1999). From enriched halophilic Archaeal broth organisms were streaked on respective agar plate for the purpose of isolation of pure culture (Upasani et al., 1996). A total of 13 isolates were obtained designated as NPW-1 to NPW -13 and preserved. All the isolates were preliminary screened for PHB production. Potent four PHB producers were used further to study enzyme activity.

Growth and kinetics of β – ketothiolase Producers

The maximum four PHB producing isolates were inoculated in to 100 ml of TYES medium and incubated at 37 °C on shaker at 180 rpm. The cells were harvested by centrifugation at 10,000 rpm for 15 min after 14 days incubation. The cells were lysed in 2% sodium hypochlorite solution and again centrifuged. The supernatant was then assayed for the presence of β – ketothiolase as mentioned below (Nishimura *et al*., 1978; Satoh *et al*., 2002).

β – ketothiolase assay

β – ketothiolase activity was assayed by the thiolysis of acetoacetyl – CoA. The assay mixture (a total volume of 1 ml) contained 100mM Tris – HCl buffer (pH 8.1) (750 µl), 60mM $MgCl_2$ (100 µl), 0.05mM acetoacetyl – CoA (10 µl), 0.05mM CoA (30 µl) and β – ketothiolase (10µl). The culture supernatant containing the enzyme was added after preincubation of the reaction mixture at 25°C for 2 min. The decrease in acetoacetyl CoA was then measured spectrophotometrically at 303nm at 30°C using a millimolar extinction coefficient of 12.9$mM^{-1}cm^{-1}$.One unit of β – ketothiolase was defined as the amount of enzyme that catalyzes the cleavage (conversion) of 1 µmol of acetoacetyl CoA in 1 min. The absorption at 303nm is due to the chelation of magnesium to the enolate form of acetoacetyl – CoA (Stern, 1956).

Identification of isolate producing maximum β – ketothiolase enzyme activity

One of the isolate which showed maximum β – ketothiolase enzyme activity had been identified by its molecular characterization i.e., 16S rDNA partial sequencing (Xcelris Labs., Ahmedabad, Gujarat, India).

Optimization of Media for β – ketothiolase production

Optimization of pH

Optimization of pH i.e., 5, 6, 7, 8, 9 was performed in liquid media (adjusted by adding 1N HCl or 20% Na_2CO_3) (Oeding and Schlegal, 1973).

Optimization of temperature

Temperature was optimized by incubating inoculated media at different temperature i.e., 25 ºC, 30 ºC, 37 ºC, 45 ºC, 55ºC (Berndt H and Schlegal HG, 1975).

Optimization of salt

Optimization of salt for maximum enzyme production was carried out by varying concentration of NaCl and KCl i.e., 5%, 10%,15%,20%, 25%, 30% and 35% in liquid media (Kyriakidis *et al.,* 2005).

Optimization with Inoculums sizes

For optimization organisms were grown in liquid media with different inoculums sizes i.e., 10^4, 10^5, 10^6, 10^7, 10^8 and 10^9 cells/ml (Mahishi LH, 2001).

Optimization with Carbon source as Substrate

Optimization with different carbon sources i.e., glucose (0 – 6 g/l), sucrose (0 – 6 g/l), arabinose (0 – 6 g/l), starch (0 – 6 g/l), glycerol (0 – 6 g/l), acetate (0 – 6 g/l), cane molasses (0 – 6 g/l) and bagasse (0 – 6 g/l) was carried out for maximum enzyme production Chen et al., 2004.

Optimization with Nitrogen Sources as Substrate

Optimization with different nitrogen source i.e., ammonium sulphate (0-0.2g/l) , ammonium nitrate (0-0.2g/l), ammonium chloride (0-0.2g/l), ammonium phosphate (0-0.2g/l), corn steep liquor (0-0.2g/l), tryptophan (0-0.2g/l), protease peptone (0-0.2g/l) and potassium nitrate (0 – 0.2g/l) was carried out for maximum enzyme production (Celik et al., 2005).

After optimization with individual carbon and nitrogen sources different Carbon:Nitrogen ratios as substrate were added as follows 12: 1, 6: 1, 3: 1, 0.3: 1 and 0.15: 1 .

Optimization with phosphate as Substrate

Optimization with phosphate as substrate for

maximum enzyme production was carried out were following substrate at different concentration were added i.e., KH_2PO_4 (0.000937 – 1 g/l) and K_2HPO_4 (0.000937 – 1 g/l) (Liu et al., 2002).

Statistical analyses

Data were analyzed by one sample T – test for the effect of various pH, temperature, NaCl and KCl concentrations and inoculum size. Paired T – test was performed for the effect of starch, glucose, acetate, sucrose, cane molasses, bagasse, ammonium nitrate, ammonium sulphate, ammonium chloride, ammonium phosphate, corn steep liquor, tryptone, protease peptone, KH_2PO_4, K_2HPO_4, carbon: nitrogen ratio and the β – ketothiolase activity after optimization of the cultural conditions using SPSS. 17. Standard deviation was calculated by Microsoft Excel 2007.

RESULTS AND DISCUSSION

Among the various media (TYES, M & L and Larsen) used for the isolation of extremely halophilic Archaea, TYES supported maximum growth of isolates. After enrichment, thirteen haloarchaeal strains were obtained from respective agar medium by streaking method. Organisms were designated as NPW-1 to NPW-13. All the isolates were screened for maximum PHB production by Law and Seplecky spectrophotometric assay method. On the basis of assay four strains viz., *Haloarcula sp. 1, Halorubrum sp. 2, Halobaculum sp.* and *Halobacterium salinarium* (One identified by 16S rRNA sequencing and other three by biochemical test, and lipid content in their cell wall (data not shown))showing maximum PHB production were further selected for enzyme assay.

Detection of β – ketothiolase enzyme

Maximum β – ketothiolase activity was exhibited by *Haloarcula* sp. 1(0.047 EU/ml) which was also the maximum PHB producer, followed by *Halorubrum* sp. 2 (0.021 EU/ml) and *Halobacterium salinarum* (0.011 EU/ml) while, least was from *Halobaculum* sp (0.007 EU/ml) (Table1). Hence *Haloarcula* sp. 1 was selected for examining the effect of various cultural conditions on β – ketothiolase activity.

Identification of isolate producing maximum β – ketothiolase enzyme activity

Results of 16S rDNA partial sequencing (642 bp) of the strain NPW-9 showed maximum sequence identity (100%) with the complete sequence of *Haloarcula* sp. AB19 (GenBank Accession No. DQ471854.1) (Fig. 1). Thus, the isolate NPW-9 is affiliated to *Haloarcula* species and hence in the present study it is referred to as *Haloarcula* sp.1 as referred to previously.

Effect of optimization on β – ketothiolase enzyme activity

Effect of pH

Fig. 2 shows the time course activity of β – ketothiolase at pH (5 – 9) by *Haloarcula* sp. 1. The enzyme activity increased with increase in pH reaching maxima at pH 7 (0.440 EU/ml) on 20th day with a steep decline at pH – 8 (0.041 EU/ml). However, at alkaline pH i.e. 9, the enzyme activity was completely suppressed. Thus pH might be an important factor in regulating enzyme activity, since thiolase activity falls with increasing pH from an optimum at pH – 7.

Effect of temperature

Fig. 3 shows the time course activity of β – ketothiolase at temperatures (25 – 55°C). The enzyme activity increased with increase in temperature from 25°C reaching maxima at 37°C (0.469 EU/ml) with a decline at 45°C. However at 55°C, the enzyme activity was completely suppressed. The isolate also exhibited a delay in the biosynthesis of the enzyme at 25°C and 30°C.

Since the thiolytic enzyme activity usually is measured by UV registration of the disappearance of the commonly used Mg^{2+}–enolate complex at 303nm at 25°C, the present study examines the effect of temperature on the thiolytic activity of β – ketothiolase enzyme. It also has been demonstrated that incubation temperature influences the activity pattern of β – ketothiolase and that individual enzyme of a strain may be affected differently by temperatures

Effect of NaCl and KCl concentration

Figs. 4 and 5 show the time course activity of β – ketothiolase at 5 – 35% NaCl and KCl concentrations. Enzyme activity increased with increase in NaCl and KCl concentrations from 10% reaching maxima at 25% NaCl concentration (0.388 EU/ml) and 25% KCl concentration (0.488 EU/ml) on 20th day and decreasing at 30 and 35% NaCl concentration. Least activity was at 35% NaCl and KCl concentrations. But both NaCl and KCl at 5% completely suppressed enzyme activity.

Thiolase activity in halophilic Archaea is highly dependent on the concentration of NaCl and KCl in the buffer, being optimal at saturated NaCl and KCl concentrations (4.5M). At various concentrations of salts it was observed that effect of KCl was higher than the effect of NaCl. Higher NaCl and KCl concentrations i.e., 30 and 35% decreased the activity of halophilic thiolases.

Effect of Inoculums size

Fig. 6 shows the time course activity of β – ketothiolase at inoculum sizes of 10^4 – 10^9 cells/ml. The isolate showed maximum activity at 10^7 cells/ml (0.528 EU/ml) on 20th day with a decline at 10^8 and 10^9 cells/ml, while least activity was observed at lowest inoculum sizes 10^4 and 10^5 cells/ml. Inoculum concentrations are important for growth and productivity. Too high or too low inoculum concentrations cause low growth and

productivity. A large inoculum size in culture will rapidly lead to crowded and nutritional deficiency, poor aeration and substrate availability.

Effect of Starch

Fig. 7 shows the effect of starch (0 – 6 g/l) on enzyme activity. Enzyme activity was maximum at 1 g/l of starch (0.678 EU/ml) on 20th day. Higher concentrations of starch i.e. 2 – 6 g/l and the absence of starch in the medium (0 g/l) suppressed enzyme activity. Thus, higher concentration of starch although were not inhibitory, did not prove to favor enzyme activity.

Effect of Glycerol

Fig. 8 shows the effect of glycerol (0 – 6 g/l) on β – ketothiolase activity. Activity increased from 1 g/l reaching maxima at 5 g/l (0.845 EU/ml) on 20th day. However highest concentration i.e., 6 g/l suppressed enzyme activity. Thus, glycerol proved to induce enzyme activity.

Effect of Acetate

Fig. 9 shows the effect of acetate (0 – 6 g/l) on enzyme activity. The maximum activity was at 1 g/l (0.971 EU/ml) on 20th day. However higher concentration of acetate i.e., from 2 – 6 g/l proved to be inhibitory to enzyme activity as evidenced by a steep decline in the figure.

Effect of Glucose

Fig. 10 shows the effect of glucose (0 – 6 g/l) on enzyme activity. Addition of glucose increased enzyme activity from 1 g/l and reaching maximum at 5 g/l (2.388 EU/ml) of glucose on 20th day with a decrease at 6 g/l.

Effect of Sucrose

Fig. 11 shows the effect of sucrose (0 – 6 g/l) on enzyme activity. There is a progressive increase in enzyme activity on addition of 1 g/l of sucrose, reaching maxima at 3 g/l (1.1844 EU/ml) on 20th day. A higher increase in concentration of sucrose decreased enzyme activity.

Effect of natural substrates

Figs. 12 and 13 show the effect of natural substrates as cane molasses and Bagasse respectively on enzyme activity. The addition of cane molasses and bagasse increased enzyme activity from 1 g/l reaching maxima at 4 g/l i.e., 0.612 EU/ml and 0.769 EU/ml respectively with a decline at 5 and 6 g/l respectively. However, higher enzyme activity (0.769 EU/ml) was exhibited by addition of bagasse as compared to cane molasses.

Effect of Nitrogen sources

Figs. 14 – 17 show the effect of various concentrations (0 – 0.2 g/l) of synthetic N sources as ammonium nitrate, ammonium sulphate, ammonium chloride and ammonium phosphate on enzyme activity. Enzyme activity increased on addition of synthetic N sources reaching maxima at 0.04 g/l (0.093 EU/ml) with NH_4NO_3, 0.08 g/l (0.08 EU/ml) with $(NH_4)_2SO_4$, 0.02 g/l (0.02 g/l EU/ml) with NH_4Cl and 0.1 g/l (0.657 EU/ml) with $(NH_4)_2PO_4$ on 20th day. Thus, maximum activity was exhibited with addition of NH_4Cl as N source.

Effect of Natural Nitrogen sources

Fig. 18 show the effect of natural N source i.e., corn steep liquor (0 – 0.2 g/l) on enzyme activity. However, as compared to synthetic N sources, corn step liquor exhibited maximum enzyme activity at highest concentration i.e., 0.2 g/l (1.214 EU/ml).

Figs. 19 and 20 show the effect of two nitrogen sources tryptone and protease peptone in concentrations (0 – 0.2 g/l) on β – ketothiolase enzyme activity by *Haloarcula* sp. 1. The enzyme activity was maximum at 0.04 g/l (0.741 EU/ml) on 20th day with major decline at rest of the concentrations whereas, with protease peptone the enzyme activity was maximum at 0.08 g/l (0.257 EU/ml).

Thus, amongst all the nitrogen sources, NH_4Cl led to maximum induction of enzyme activity.

Effect of phosphates

Figs. 21 and 22 indicate the effect of addition of phosphates as KH_2PO_4 and K_2HPO_4 at varying concentrations (0.000937 – 1 g/l) on β – ketothiolase activity by *Haloarcula* sp. 1. The addition of both KH_2PO_4 and K_2HPO_4 induced enzyme activity but the induction was higher with KH_2PO_4 (0.810 EU/ml) than K_2HPO_4 (0.588 EU/ml) at 0.00375 g/l and 0.015 g/l respectively. The data clearly indicates that enzyme activity is induced maximally with limiting concentration of PO_4 through the limiting concentration varied with both KH_2PO_4 and K_2HPO_4.

Effect of Carbon and Nitrogen

Fig. 23 show the effect of various carbon: nitrogen ratios from 12:1 to 15:1 on β – ketothiolase activity by *Haloarcula* sp. 1 where glucose was taken as a C source and NH_4Cl as ammonium source. Maximum enzyme activity was exhibited with C: N ratio of ratio 3:1 (0.488 EU/ml). The ratio of 3:1 showed a 2 fold increase enzyme activity over control.

Table 2 shows the summarized effect of all the optimized conditions that have been obtained by *Haloarcula* sp. 1 for maximum β – ketothiolase activity. Table 3 indicates increase in enzyme activity with optimized conditions as compared to unoptimized conditions. The enzyme activity showed 99.27% increase on 8th day as compared to unoptimized conditions where the enzyme activity was 0.025 EU/ml on 8th day. Thus optimization of cultural conditions increased β – ketothiolase activity, the first enzyme involved in biosynthesis of PHB.

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Table 1. β – ketothiolase activity by the isolates

Isolates	β – Ketothiolase activity (EU/ml)
Haloarcula sp. 1	**0.047**
Halorubrum sp. 2	0.021
Halobaculum sp.	0.007
Halobacterium salinarum	0.011

Table 2. Optimized conditions obtained from *Haloarcula* sp. 1 for β-ketothiolase activity

Sr. No.	Cultural conditions	Optimized Value
1	pH	7
2	Temperature	37 °C
3	NaCl%	25%
4	Inoculum Size	10^7 cells/ml
5	Starch g/l	1 g/l
6	Glycerol g/l	5 g/l
7	Acetate g/l	1 g/l
8	Glucose g/l	5 g/l
9	Sucrose g/l	3 g/l
10	Cane molasses g/l	4 g/l
11	Bagasse g/l	4 g/l
12	NH_4NO_3 g/l	0.04 g/l
13	(NH_2) SO_4 g/l	0.08 g/l
14	NH_4Cl	0.02 g/l
15	$(NH_4)_2PO_4$ g/l	0.1 g/l
16	Corn steep liquor g/l	0.2 g/l
17	Tryptone g/l	0.04 g/l
18	Protease peptone g/l	0.08 g/l
19	KH_2PO_4 g/l	0.00375 g/l
20	K_2HPO_4 g/l	0.015 g/l
21	Carbon : Nitrogen ratio	3:1

Table 3. Effect of optimized conditions on β – ketothiolase activity from *Haloarcula* sp.

Isolates		β – ketothiolase activity (EU/ml)								
	Days →	0	2	4	6	8	10	12	14	16
Haloarcula sp. 1	C	0.00 ±0.00	0.004 ±0.003	0.010 ±0.002	0.021 ±0.001	**0.025 ±0.002**	0.020 ±0.005	0.017 ±0.006	0.004 ±0.004	0.003 ±0.004
	T	0.00 ±0.00	0.418 ±0.002	1.674 ±0.002	2.789 ±0.001	**3.453 ±0.002**	2.223 ±0.007	1.234 ±0.005	0.223 ±0.003	0.023 ±0.003
C – Control, T- Test										

Fig 1.Phylogenetic tree of isolate NPW-9 (using neighbor-joining method)

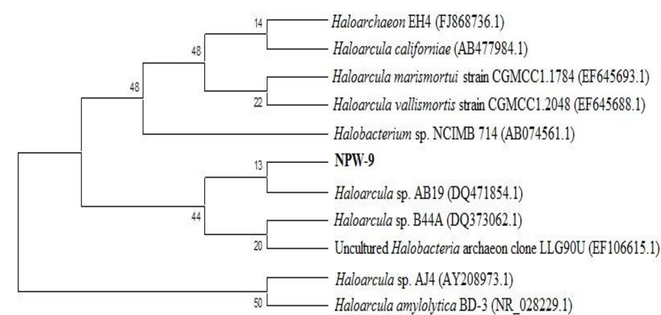

Fig 2. Effect of pH (5 – 9) on β – ketothiolase activity (EU/ml)

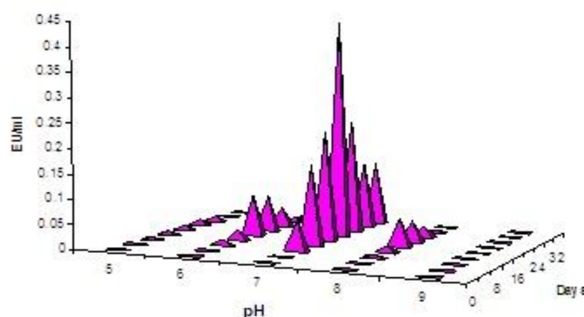

Fig 3. Effect of temperature (25 – 55°C) on β – ketothiolase activity (EU/ml)

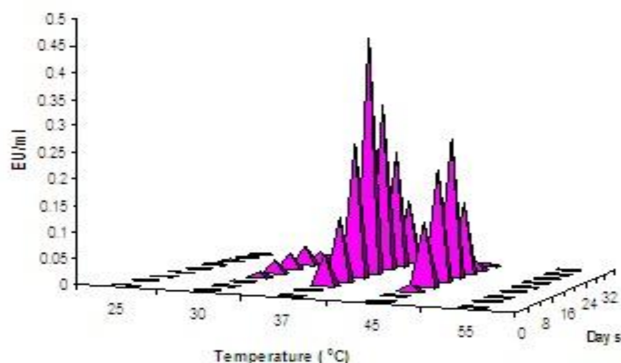

Fig 4. Effect of NaCl (5 – 35%) on β – ketothiolase activity (EU/ml)

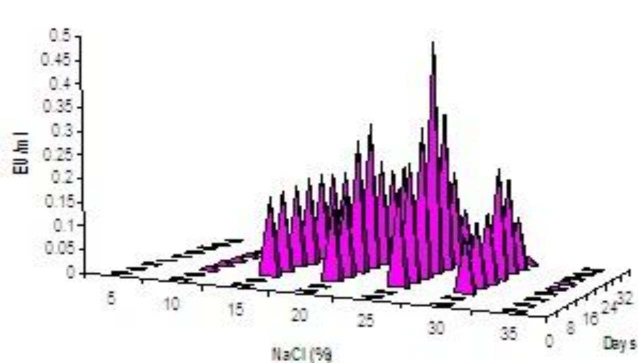

Fig 5. Effect of KCl (5 – 35%) on β – ketothiolase activity (EU/ml)

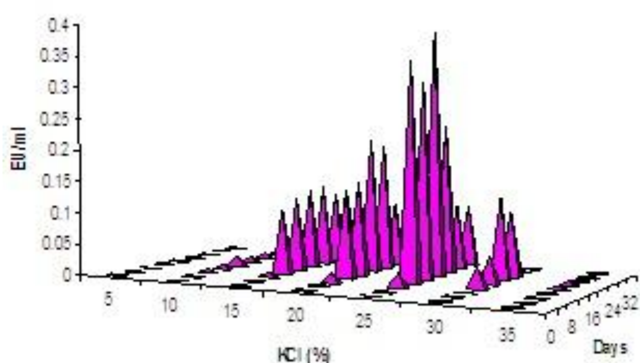

Fig 6. Effect of Inoculum sizes (10^4 – 10^9 cells/ml) on β – ketothiolase activity (EU/ml)

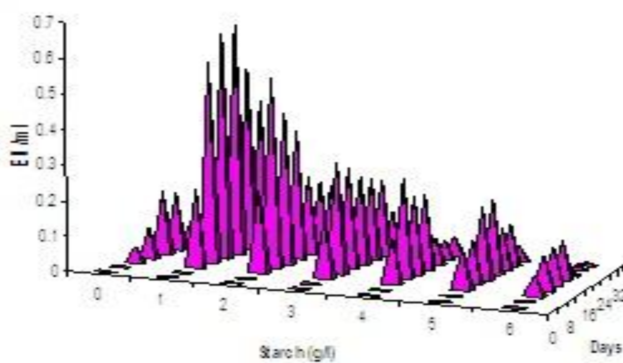

Fig 7. Effect of starch (0 – 6 g/l) on β – ketothiolase activity (EU/ml)

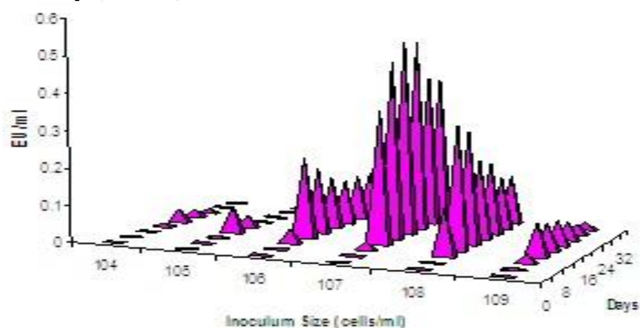

Fig 8. Effect of glycerol (0 – 6 g/l) on β – ketothiolase activity (EU/ml)

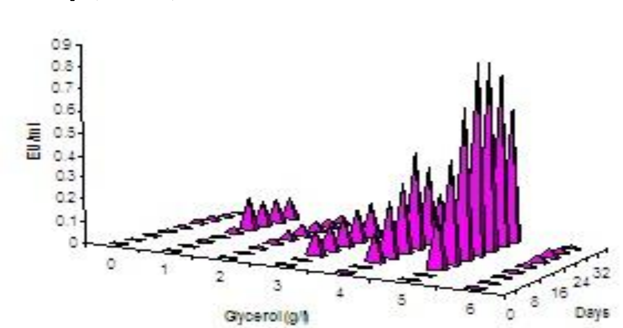

Fig 9. Effect of acetate (0 – 6 g/l) on β – ketothiolase activity (EU/ml)

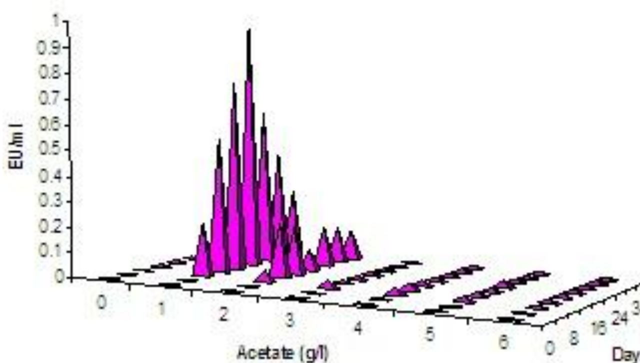

Fig 10. Effect of glucose (0 – 6 g/l) on β – ketothiolase activity (EU/ml)

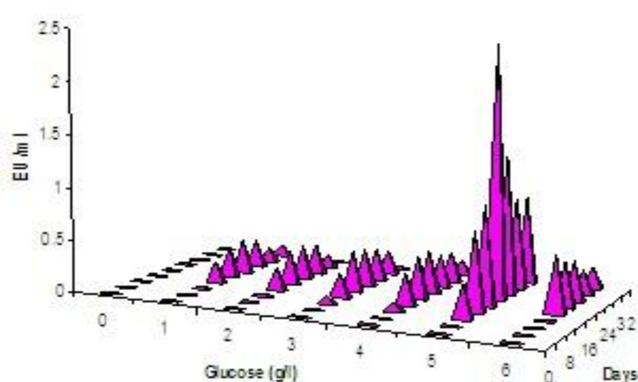

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Fig 11. Effect of sucrose (0 – 6 g/l) on β – ketothiolase activity (EU/ml)

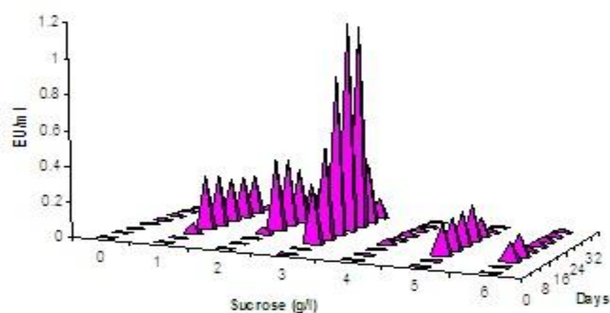

Fig 12. Effect of cane molasses (0 – 6 g/l) on β – ketothiolase activity (EU/ml

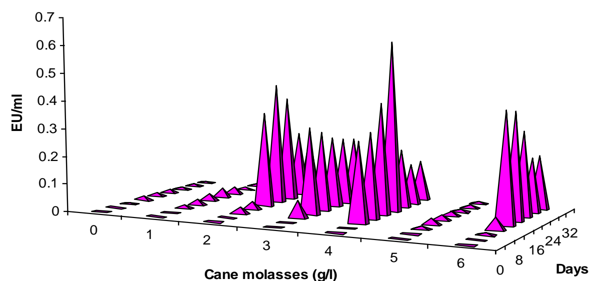

Fig 13. Effect of bagasse (0 – 6 g/l) on β – ketothiolase activity (EU/ml)

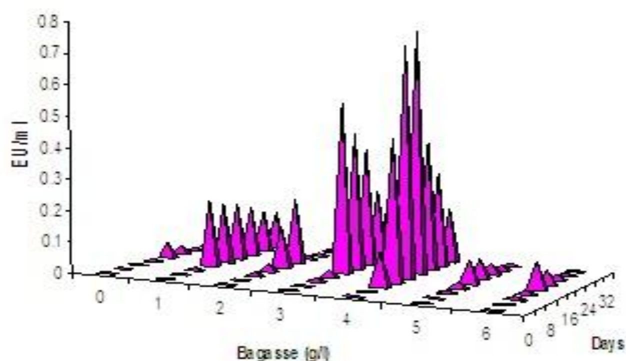

Fig 14. Effect of ammonium nitrate (0 – 0.2 g/l) on β – ketothiolase activity (EU/ml)

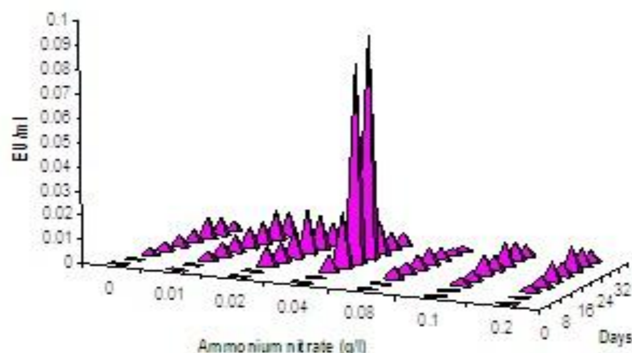

Fig 15. Effect of ammonium sulphate (0 – 0.2 g/l) on β – ketothiolase activity (EU/ml)

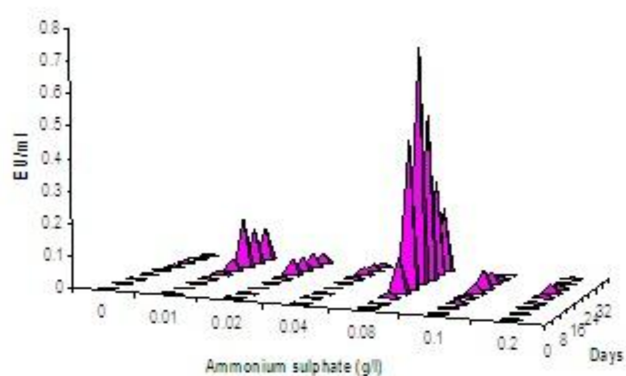

Fig 16. Effect of ammonium chloride (0 – 0.2 g/l) on β – ketothiolase activity (EU/ml)

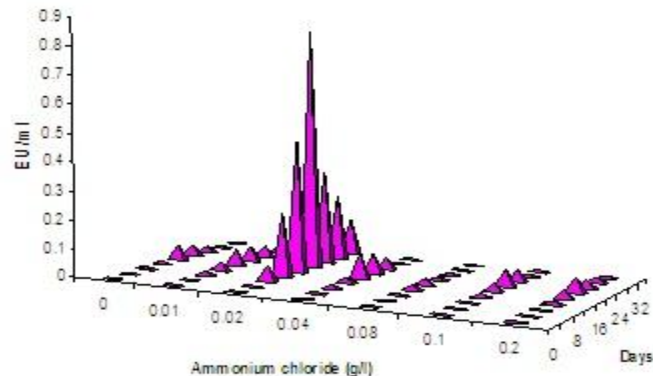

Fig 17. Effect of ammonium phosphate (0 – 0.2 g/l) on β – ketothiolase activity (EU/ml)

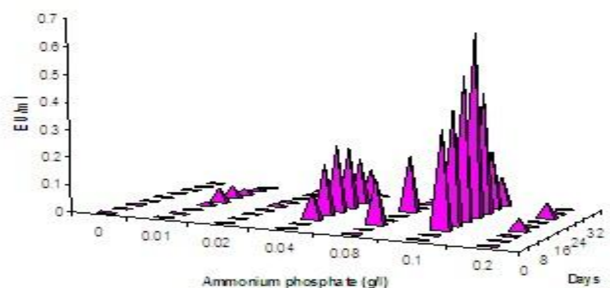

Fig 18. Effect of corn steep liquor (0 – 0.2 g/l) on β – ketothiolase activity (EU/ml)

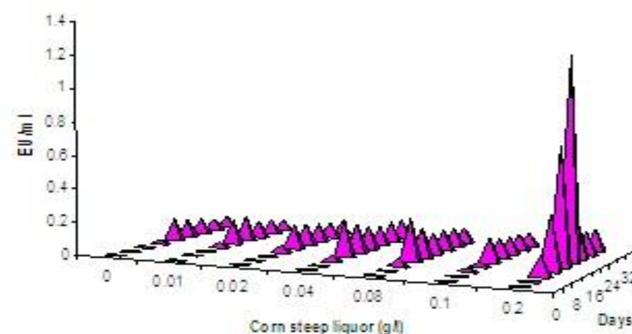

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Fig 19. Effect of tryptone (0 – 0.2 g/l) on β – ketothiolase activity (EU/ml)

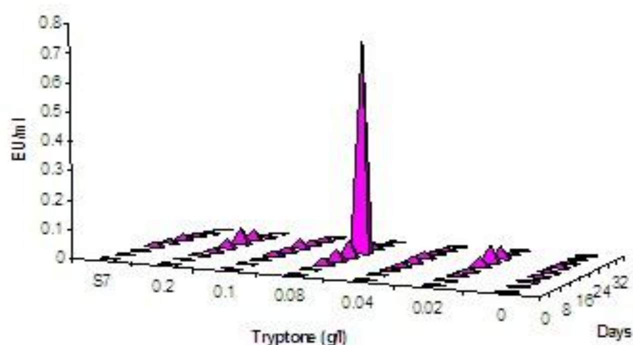


Fig 20. Effect of protease peptone (0 – 0.2 g/l) on β – ketothiolase activity (EU/ml)

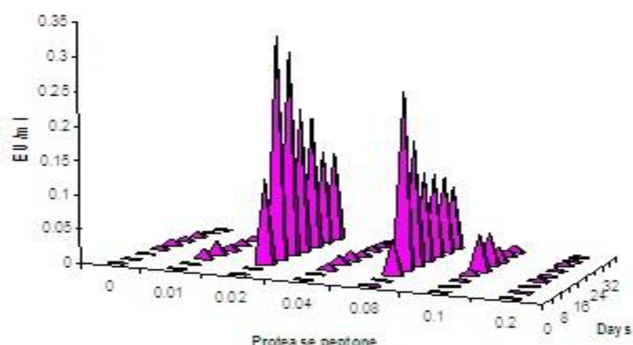


Fig 21. Effect of potassium di-hydrogen phosphate (0 – 1 g/l) β – ketothiolase activity (EU/ml)

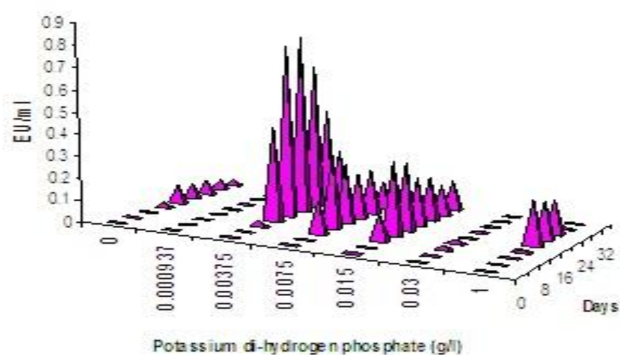


Fig 22. Effect of di-potassium hydrogen phosphate (0 – 1 g/l) on β – ketothiolase activity (EU/ml)

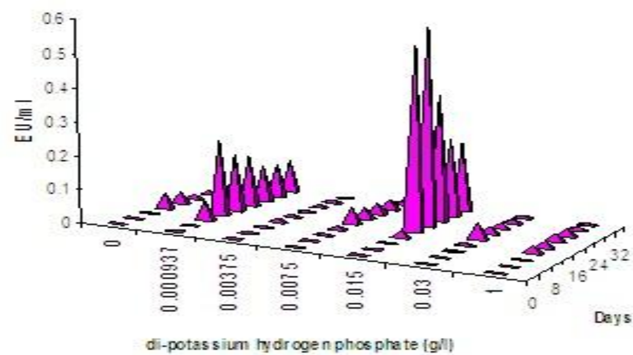
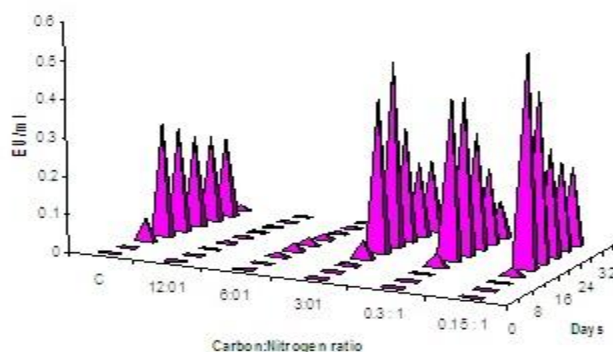


Fig 23. Effect of carbon:nitrogen ratio on β – ketothiolase activity (EU/ml)



CONCLUSION

The most common PHB-biosynthetic pathway consists of three enzymes; the first is β-ketothiolase, acetoacetyl-Co A reductase and PHB synthase which are encoded in genes designated as *phaA*, *phaB* and *phaC*, respectively (Encarnacion *et al*., 2002). As thiolases are ubiquitous enzymes that play an important role in many biochemical pathways such as poly (hydroxybutyrate) (PHB) synthesis hence, the present work deals with detection of β-ketothiolase from the isolates and its optimization for maximum production. Thus the work aims at optimization of a β – ketothiolase, enzyme of the biosynthetic pathway of PHB from a halophilic archaeon *Haloarcula* sp. 1. This might be the first report on optimization of halophilic β – ketothiolase that acts at high salt concentrations. These thiolases exhibit not only an extreme salt requirement but also has unique kinetic properties that differ significantly from *Bacteria* and *eukarya*. It is possible that there is a special thiolytic mechanism in haloarchaea. As alteration in nutrient conditions like carbon sources, limiting nitrogen and phosphate sources and different ratios of carbon: nitrogen often causes dramatic shifts in intermediary metabolism. Many of these shifts are controlled by global regulatory networks capable of coordinated induction or repression of many enzymes.

ACKNOWLEDGEMENT
None

AUTHOR'S STATEMENT
The authors declare no conflict of interest.

REFERENCES

Berndt H, Schlegal HG. Kinetics and properties of ß-ketothiolase from *Clostridium pasteuranium. Arch Microbial*. 1975; 103: 21-30.

CelikGy, Beyatli Y, Aslin B. Determination of poly-ß-hydroxybutyrate (PHB) production in different media by *Pseudomonas cepacia* G13 strains. *Fresen Environ Bull*. 2005; 14: 954-956.

Chen GQ, Chen JY, Liu T, Zheng Z, Chen JC. Polyhydroxyalkanoate synthases phaC1 and phaC2 from *Pseudomonas stutzeri* 1317 had different substrate specificities. *FEMS MicrobiolLett*. 2004; 234: 231-237.

Halophilic Archaeobacteria from marine Salterns, Mithapur, India. *Biores J.* 1996; 23-32.

Krieg RN, Holt GJ (eds). Bergey's Manual of Systematic Bacteriology Vol III Williams and Wilkins, Baltimore, London. 1984.

Kyriakidis DA, Pantazaki AA, Ioannou AK. Athermostable ß-ketothiolase of Polyhydroxyalkanoates (PHAs) in *Thermusthermophillus*: Purification and biochemical properties. *Mol Cell Biochem*. 2005; 269: 27-36.

Larsen H. The family *Halobacteriacerae* In: The Prokaryotes - A Handbook on Habitats, Isolation and Identification of Bacteria, Vol II, Starr MP, Stolp H, Truper HG, Balows A, Schlegel HG (eds), Springer, Berlin. 1981; 985-994.

Liu T, Liu S-J, Xue Y, Ma Y. Purification and characterization of an extremely Halophilic acetoacetyl-CoA thiolase from newly isolated *Halobacterium* strain ZP-6. *Extremophiles*. 2002; 6: 97-102.

Mahishi LH. Molecular Characterization of Polyhydroxyalkanoates (PHA) Synthesizing Gene(s) from *Streptomyces aureofaciens* NRRL 2209. Ph.D. Thesis, University of Pune, Pune, India. 2001.

Margesin R, Schinner F. Potential of halotolerant and Halophilic microorganisms for biotechnology. *Extremophiles*. 2001; 5:73-83.

Mullakhanbhai MF, Larsen G. *Halobacterium volcanii* sp. nov., Dead sea *Halobacterium* with moderate salt requirement. *Arch Microbiol*. 1975; 104: 207-214.

Nishimura T, SaitoT, Tomita K. Purification and properties of ß-ketothiolase from *Zoogloearomigera. Arch Microbiol*. 1978; 116: 21-27.

Oeding V, Schlegal HG. ß-ketothiolase from *Hydrogenomonaseutropha* H16 and its significance in the regulation of Polyhydroxybutyrate metabolism. *Biochem J.* 1973; 134: 12176-12181.

Oren A, Litchfield CD. A procedure for the enrichment and isolation of *Halobacterium*. *FEMS Microbiol Lett*. 1999; 173: 353-358.

Ritchie GAF, Senior PJ, Dawes EAI. The purification and characterization of acetoacetyl-coenzyme A Reductase from *Azotobacterbeijerinckii*. *J Biochem*. 1971; 121: 309-316.

Satoh Y, Tajima K, Tannai H, Munekata M. Enzyme-catalysed Poly(3- hydroxybutyrate) synthesis from acetate with CoA recycling and NADPH regeneration *invitro*. *J Bioscience Bioengg*. 2002; 95(4): 335-341.

Senior PJ, Dawes EA. The regulation of Polyhydroxybutyrate metabolism in *Azotobacterbeijerinchii. Biochem J*. 1972; 134: 225-238.

Senior PJ, Dawes EA. The role and regulation of energy reserve polymer in microorganism. *Adv Microbial Physiol*. 1973; 10: 135-266.

Stern JR. Optical properties of acetoacetyl-S-coenzyme A and its metal chelates. *J Biol Chem.* 1956; 221: 33-44.

Upasani VN, Kates M, Mullakhanbhai FM, Desai SG. Isolation and characterization of Halophilic Archaeobacteria from marine Salterns, Mithapur, India. *Biores J.* 1996; 23-32.