



iJRASET

International Journal For Research in
Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 9 Issue: X Month of publication: October 2021

DOI: <https://doi.org/10.22214/ijraset.2021.38520>

www.ijraset.com

Call:  08813907089

E-mail ID: ijraset@gmail.com

An *In-Silico* Approach of Polyhydroxybutyrate Synthesis and Phylogeny Study for Degradation of Polyhydroxybutyrate in Organisms from Lower to Higher Organization

Shivangi Shrivastava¹, Dr. Mritunjai Singh², Dr. Archana Tiwari³
^{1, 2, 3}School of Biotechnology, Rajiv Gandhi Prodhogiki Vishwavidhyalaya

Abstract: *The in-silico approach is common in today's world. As it provide a vast knowledge of hypothetical world which have to be proven by undergoing in in-vitro conditions. There are much data is available on databases which helps to complete the future study related to medicine, environment and nano-technology. The study covered the new ideas which can able to change the approach of biosynthesis of PHB in microorganisms and degradation of biopolymer without any harmful effect on environment as well as ecosystem.*

Keywords: *Polyhydroxybutyrate, Phylogeny tree, Polyesters, Biodegradable, Biosynthesis, Rlstonia eutropha*

I. INTRODUCTION

In-silico approach is necessary to start any project in medical as well as environmental field. As it contains data which is required for many studies. This work is done with the help of online tools and databases. PHB biosynthesis is important to understand the basic need and requirement of microorganisms for their survival in non favourable conditions. PHB is storage material which is synthesised by acety co-A moiteies as their raw material (Luengo *et al.*, 2003). Acetyl co-A undergoes in condensation and produces acetoacety co-A with the help of various enzyme activities. The sole purpose of biosynthesis od storage material is, limitation o required macromolecules whih stops the nitrogenous enzyme to synthesis protein and further go for cell division. When microbial enzyme activity stops, microbes start synthesis of polyhydroxyalkanoates in the cell which are polyesters for their survival (Luengo *et al.*, 2003). These polyhydroxyalkanoates are of many types which depends on cell type and their habitate. As polyhydroxyalkanoates contains many type of polyesters but this study was foused on only one type of polyesters which is polyhydroxybutyrate which is highly synthesised bu *Ralstonia eutrophus* which is a gram negative, non-spore forming bacilli. This study's solely focussed on polyhydroxybutyrate because this polyester showing major ressemblence with single use polymer i.e., polyethylene which is synthetically synthesiised and are not able to degrade arter and after several years (Bhat *et al.*, 2020). This single use polymer polluted the area on earth with degrade the quality of environment and ecosystem as many animals and ocean animals are died by eating it (Hayden *et al.*, 2013). These study will hange the future world's appraoh for focussing on degradation as well. These biopolymer are not poisonous for mistakenly eating by animals as well as humans becaue humans contains higher enzymes which are able to degrade these biopolymers in the body and remove out without any gene manipulation.

DATABASES: NCBI, BIOCYC, METACYC, MUSCLE/ CLUSTAL W

TOOLS: Comparative analysis, MEGA X

II. METHODS

A. Selection of Suitable Strain of Microorganism

- 1) Search the site of NCBI (ncbi.nih.nlm.in).
- 2) Open the home page of NCBI.
- 3) Choose the 'all genome' option from left search column.
- 4) Choose the 'bacterial name' in right search option.
- 5) Result shows bacterial FASTA sequence.
- 6) Select the BLAST program.
- 7) Enter a query sequence or upload a file containing sequence.
- 8) Select the database to search.

- 9) Select the algorithm and the parameters of the algorithm for the search.
- 10) Run the BLAST program.
- 11) Optimise the similar of Bacterial genome and select the perfect one.

B. Study of Biosynthesis of Polyhydroxy Butyrate/ butyric acid in Ralstonia eutropha by Biocyc

- 1) Search the online tool: biocyc.org
- 2) Type 'Polyhydroxybutyrate' on search column display on right side (up) on the page.
- 3) Choose the option 'Polyhydroxybutanoate biosynthesis (polyhydroxybutyrate biosynthesis)' out from three results.
- 4) Study the results of reaction with enzymatic pathways.
- 5) Select the option 'Multiple Database' from right side (down) the page.
- 6) Collect the data of same reaction in multiple databases.

C. Use of metacyc tool for study of Polyhydroxybutyrate synthesis in Microorganisms

- 1) Search metacyc.org
- 2) Enter Polyhydroxybutyrate in search column
- 3) Click on pathway of Polyhydroxybutanoate biosynthesis (polyhydroxybutyrate biosynthesis).
- 4) Retrieve the pathway and collect the data
- 5) Search this pathway in Multiple Database

D. Comparative Analysis for Cupriavidus necator H 16

- 1) Search the online tool biocyc.org.
- 2) Enter polyhydroxybutyrate in search column.
- 3) Click on pathway of Polyhydroxybutanoate biosynthesis (polyhydroxybutyrate biosynthesis).
- 4) Run the species comparison
- 5) Go on comparative analysis start page option given on last of the page.
- 6) Select Pathways: breakdown by pathway class, information on pathway holes.
- 7) Select 'choose organism' for comparative analysis
- 8) Add microorganisms according to taxonomy
- 9) Select pathway option and optimize the data

III. PHYLOGENETIC TREE PRODUCTION BY MEGA X SOFTWARE

For alignment

- 1) Go to "Align (dropdown) --> Edit/Build Alignment --> Retrieve sequences from a file --> OK".
- 2) Selected the input file which was in fasta format. A new window was open showing all the sequences.
- 3) Go to "Edit --> Select All" or simply press Ctrl+A.
- 4) Go to "Alignment --> Align by MUSCLE --> Align Protein --> OK". This software can align sequences by ClustalW by selecting "Align by ClustalW" instead of selecting "Align by ClustalW" from the *Alignment* option at the top menu bar.
- 5) After processing, it was showed the aligned sequences in the same window.
- 6) If wanted then saved the session, then go to "Data --> Save Session". Select the appropriate folder and click *Save*.

A. Exporting into the MEGA format

- 1) Go to Data --> Export Alignment --> Mega Format. DATA was also export into other formats such as FASTA, Phylip/Paup at this step.
- 2) Selected the appropriate folder and clicked *Save*.

B. Constructing the Phylogenetic Tree

- 1) Go to the main window of MEGAX. Click Phylogeny --> Construct/Test Maximum Likelihood Tree.
- 2) Select the converted file (.meg) and click *Open*.
- 3) A new window will appear '*Analysis Parameters*'. Here, set the different values such as bootstrapping value, substitution model, etc., It is recommended to test phylogeny by bootstrapping for 500-1000 times. Additionally, selected the substitution model appropriately.
- 4) After setting parameters, click *Compute*. It was time taken which depending upon the number of sequences and bootstrap values.
- 5) Finally, it would showed the constructed tree. Save the tree session and export it into Newick format.

IV. RESULTS AND DISCUSSIONS

Descriptions						Graphic Summary	Alignments	Taxonomy
Sequences producing significant alignments						Download ▼ New Select columns		
<input type="checkbox"/> select all 0 sequences selected								
	Description	Scientific Name	Max Score	Total Score	Query Cover	E value		
<input type="checkbox"/>	acetyl-CoA acetyltransferase, cytosolic [Bactrocera dorsalis]	Bactrocera dors...	347	759	75%	7e-171		
<input type="checkbox"/>	PREDICTED: acetyl-CoA acetyltransferase, cytosolic [Bactrocera latifrons]	Bactrocera latifr...	360	735	75%	1e-169		
<input type="checkbox"/>	acetyl-CoA acetyltransferase, cytosolic [Zeugodacus cucurbitae]	Zeugodacus cuc...	327	686	75%	1e-155		
<input type="checkbox"/>	acetyl-CoA acetyltransferase, cytosolic [Bactrocera oleae]	Bactrocera oleae	320	680	72%	1e-154		
<input type="checkbox"/>	acetyl-CoA acetyltransferase, cytosolic [Rhagoletis pomonella]	Rhagoletis pom...	311	655	72%	3e-147		
<input type="checkbox"/>	PREDICTED: acetyl-CoA acetyltransferase, cytosolic [Rhagoletis zephyria]	Rhagoletis zeph...	310	654	72%	1e-146		
<input type="checkbox"/>	unnamed protein product [Ceratitis capitata]	Ceratitis capitata	307	659	75%	7e-146		
<input type="checkbox"/>	acetyl-CoA acetyltransferase, cytosolic [Ceratitis capitata]	Ceratitis capitata	300	642	75%	8e-144		
<input type="checkbox"/>	acetyl-CoA acetyltransferase, cytosolic [Lucilia cuprina]	Lucilia cuprina	272	618	74%	1e-131		
<input type="checkbox"/>	acetyl-CoA acetyltransferase, cytosolic [Lucilia sericata]	Lucilia sericata	273	617	74%	2e-131		
<input type="checkbox"/>	hypothetical protein DOY81_003424 [Sarcophaga bullata]	Sarcophaga bull...	272	608	73%	7e-129		
<input type="checkbox"/>	PREDICTED: acetyl-CoA acetyltransferase, cytosolic [Musca domestica]	Musca domestica	276	607	75%	2e-128		
<input type="checkbox"/>	LOW QUALITY PROTEIN: uncharacterized protein LOC110186976 [Drosophila serrata]	Drosophila serrata	259	568	74%	1e-124		
<input type="checkbox"/>	acetyl-CoA acetyltransferase, cytosolic [Teleopsis dalmanni]	Teleopsis dalma...	257	589	75%	3e-124		

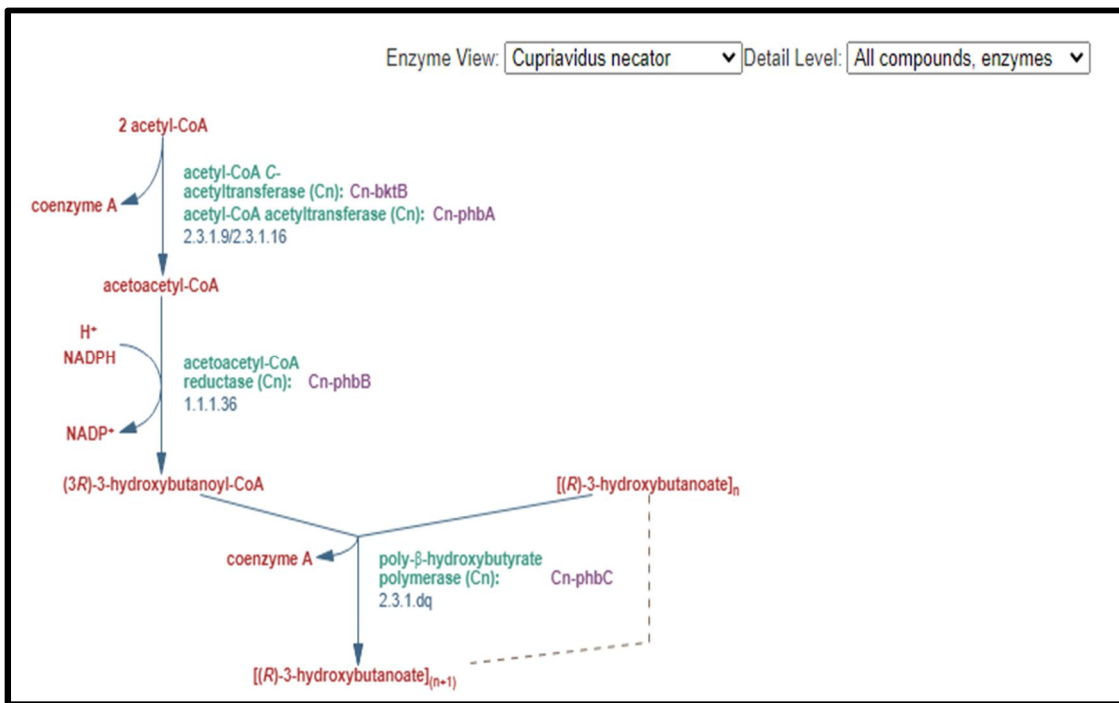
Result 1: BLAST results of strain selection for PHB synthesis

Ralstonia eutropha H16 was taken for this study because this strain of organism produces polyhydroxybutyrate in large amount than other strain. They are facultative aerobes that synthesize Polyhydroxybutyrate keto-acids in the absence of Oxygen and higher Carbon amount. The role of PHB synthesis is, it produces energy for microbial survival in such conditions. Another major advantage of the selected strain was, it is a non-spore-forming, non-pathogenic gram-negative bacteria.

Organism (Database) ▲ ▼	Pathway ▲ ▼
[Pseudomonas] pictorum JCM 9942	polyhydroxybutanoate biosynthesis
Acetobacteraceae bacterium AT-5844	polyhydroxybutanoate biosynthesis
Acetobacteraceae bacterium AT-5844	polyhydroxybutanoate biosynthesis
Achromobacter denitrificans NBRC 15125	polyhydroxybutanoate biosynthesis
Achromobacter denitrificans USDA-ARS-USMARC-56712	polyhydroxybutanoate biosynthesis
Achromobacter insuavis AXX-A	polyhydroxybutanoate biosynthesis
Achromobacter piechaudii ATCC 43553	polyhydroxybutanoate biosynthesis
Achromobacter ruhlandii SCCH3:ACH 33-1365 (GCF_002082135.1)	polyhydroxybutanoate biosynthesis
Achromobacter sp. DMS1	polyhydroxybutanoate biosynthesis
Achromobacter sp. HMSC070F04	polyhydroxybutanoate biosynthesis
Achromobacter spanius DSM 23806 (GCF_002812705.1)	polyhydroxybutanoate biosynthesis
Achromobacter xylosoxidans C54	polyhydroxybutanoate biosynthesis
Achromobacter xylosoxidans HMSC056C09	polyhydroxybutanoate biosynthesis
Achromobacter xylosoxidans HMSC057D05	polyhydroxybutanoate biosynthesis
Achromobacter xylosoxidans HMSC15D03	polyhydroxybutanoate biosynthesis
Achromobacter xylosoxidans HMSC18C08	polyhydroxybutanoate biosynthesis
Achromobacter xylosoxidans NH44784-1996	polyhydroxybutanoate biosynthesis
Achromobacter xylosoxidans serovar "not known" str. NCTC10807 (GCF_001457475.1)	polyhydroxybutanoate biosynthesis
Acidibrevibacterium fodinaquatle G45-3 (GCF_003352165.1)	polyhydroxybutanoate biosynthesis
Acidiphilium angustum ATCC 35903 (GCF_000701585.1)	polyhydroxybutanoate biosynthesis
Acidiphilium cryptum JF-5	polyhydroxybutanoate biosynthesis
Acidiphilium multivorum AIU301	polyhydroxybutanoate biosynthesis
Acidiphilium sp. PM	polyhydroxybutanoate biosynthesis
Acidisphaera rubrifaciens HS-AP3	polyhydroxybutanoate biosynthesis
Acidocella aminolytica 101 = DSM 11237	polyhydroxybutanoate biosynthesis

Result 2: Pathway in multiple database by BioCyc

Metabolic pathway of any organism shows its whole process of synthesizing and degradation as per requirement of survival. Metabolic pathways the utilisation of macromolecules for further reactions. Metabolic pathway for polyhydroxybutyrate synthesis *in-vivo* was observed and studied with BioCyc.



Result 2.1: Biosynthesis of Polyhydroxybutyrate in *Cupriavidus necator* H16 by MetaCyc

Metacyc online tool provided data of different pathways for multiple reactions at a time which a microbial cell facilitates. Acquired vast knowledge from initial to the final stage. As it cleared all the queries related to Polyhydroxybutyrate synthesis *in-vivo*. For example, acetyl co-enzyme plays the role of substrate for Biosynthesis of PHB with multiple enzyme activities in multiple stages but when and how acetyl co-enzyme undergo for the further reaction of producing PHB *in-vivo*. Synthesis of PHB in microbes complete in 3 steps which occurs in hypoxia condition or facultative microbes undergo fermentation during starvation. These steps are: **Step 1:** Acetyl Co-A synthesized from a different metabolic reaction, undergo the condensation process in which two moieties of acetyl Co-A condense with the utility of 3- Ketothiolase to produce a molecule Acetoacetyl Co-A. **Step 2:** In the second step, Acetoacetyl Co-A reduces by the process of NADPH- dependent Acetoacetyl Co-A reductase to produce (R)- 3- hydroxybutyrate Co-A. **Step 3:** In the last step, PHB synthase synthesis and merge 3 hydroxybutyrate moieties to produce the Poly 3- hydroxybutyrate backbone.

Comparative Analysis Summary Results

Note: In addition to reflecting differences in biology of different organisms, these statistics will reflect differences in the levels of curation, data availability, and completeness of the PGDBs for these organisms.

Comparative analysis and statistics were computed for the following organism databases:

- *Aquabacterium olei* NBRC 110486
- *Aquabacterium parvum* B6
- *Aquabacterium* sp. NJ1
- *Aquincola tertiarycarbonis* MIMtkpLc11
- *Cupriavidus necator* H16
- *Cupriavidus necator* H16
- *Cupriavidus necator* N-1
- *Ideonella sakaiensis* 201-F6
- *Ideonella* sp. B508-1
- *Inhella inkyongensis* IMCC1713
- *Leptothrix cholodnii* SP-6
- *Methylibium petroleiphilum* PM1
- *Methylibium* sp. CF059
- *Methylibium* sp. T29-B
- *Mitsuaria chitosanitabida* NBRC 102408
- *Mitsuaria* sp. 7
- *Paucibacter* sp. KCTC 42545 KCTC 42545
- *Ralstonia insidiosa* ATCC 49129
- *Ralstonia insidiosa* FC1138
- *Ralstonia mannitolilytica* MRY14-0246
- *Ralstonia mannitolilytica* SN82F48
- *Ralstonia pickettii* 12D
- *Ralstonia pickettii* 12J
- *Ralstonia pickettii* 5_7_47FAA
- *Ralstonia solanacearum* CFBP2957
- *Ralstonia solanacearum* CMR15
- *Ralstonia solanacearum* EP1
- *Ralstonia solanacearum* FJAT-1458

Result 3.1: Comparative Analysis Summary Results

Pathway Class	<i>A. olei</i> NBRC 110486	<i>A. parvum</i> B6	<i>Aquabacterium</i> sp. NJ1	<i>A. tertiarycarbonis</i> MIMtkpLc11	<i>C. necator</i> H16	<i>C. necator</i> H16	<i>C. necator</i> N-1	<i>I. sakaiensis</i> 201-F6	<i>Ideonella</i> sp. B508-1	<i>I. inkyongensis</i> IMCC1713	<i>L. cholodnii</i> SP-6	<i>M. petroleiphilum</i> PM1	<i>Methylibium</i> sp. CF059	<i>M. sp. T29-B</i>	<i>M. chitosanitabida</i> NBRC 102408	<i>M. sp. 7</i>	<i>Paucibacter</i> sp. KCTC 42545 KCTC 42545
Biosynthesis	167	160	163	175	194	192	223	164	160	167	173	173	175	118	128	128	163
Amine and Polyamine Biosynthesis	3	4	5	5	5	5	4	6	3	2	2	6	5	2	1	1	5
Amino Acid Biosynthesis	26	25	22	25	30	28	36	24	27	29	27	23	26	19	23	23	27
Aminoacyl-tRNA Charging	2	2	2	2	3	1	3	2	2	3	2	2	2	2	3	2	2
Aromatic Compound Biosynthesis	4	4	3	3	3	4	5	3	3	3	4	4	3	3	3	3	3
Carbohydrate Biosynthesis	14	10	11	16	14	15	16	15	9	8	14	11	17	10	6	7	11
Cell Structure Biosynthesis	6	5	5	5	4	5	6	4	5	5	6	5	5	2	5	3	5
Cofactor, Carrier, and Vitamin Biosynthesis	51	52	55	55	59	56	65	49	53	52	56	61	58	32	36	33	52
Fatty Acid and Lipid Biosynthesis	16	15	16	17	16	17	19	19	18	16	17	17	18	14	12	14	16
Metabolic Regulator Biosynthesis	4	1	3	4	5	5	5	4	1	1	1	3	4	2	2	2	1
Nucleoside and Nucleotide Biosynthesis	15	15	15	14	21	18	20	15	13	16	14	14	16	12	15	16	16
Other Biosynthesis	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Polyphenyl Biosynthesis	3	2	4	2	3	3	5	3	2	2	4	2	2	2	2	1	2
Secondary Metabolite Biosynthesis	4	4	4	7	7	6	7	4	5	6	5	5	5	1	2	5	5
Storage Compound Biosynthesis	2	2	2	2	0	1	1	2	2	1	2	2	1	1	0	0	2
Tetrapyrrole Biosynthesis	3	4	4	6	4	4	4	6	4	4	4	5	4	3	3	2	4

Pathway Class: Biosynthesis - Storage Compound Biosynthesis	<i>A. olei</i> NBRC 110486	<i>A. parvum</i> B6	<i>Aquabacterium</i> sp. NJ1	<i>A. tertiarycarbonis</i> MIMtkpLc11	<i>C. necator</i> H16	<i>C. necator</i> H16	<i>C. necator</i> N-1	<i>I. sakaiensis</i> 201-F6	<i>Ideonella</i> sp. B508-1	<i>I. inkyongensis</i> IMCC1713	<i>L. cholodnii</i> SP-6	<i>M. petroleiphilum</i> PM1	<i>Methylibium</i> sp. CF059	<i>M. sp. T29-B</i>	<i>M. chitosanitabida</i> NBRC 102408	<i>M. sp. 7</i>	<i>Paucibacter</i> sp. KCTC 42545 KCTC 42545	<i>R. insidiosa</i> ATCC 49129
Cyanophycin metabolism	X	X	X	X			X	X	X	X	X	X					X	
polyhydroxybutanoate biosynthesis	X	X	X	X		X		X	X		X	X	X	X			X	X

<i>R. insidiosa</i> FC1138	<i>R. mannitolilytica</i> MRY14-0246	<i>R. mannitolilytica</i> SN82F48	<i>R. pickettii</i> 12D	<i>R. pickettii</i> 12J	<i>R. pickettii</i> 5_7_47FAA	<i>R. solanacearum</i> CFBP2957	<i>R. solanacearum</i> CMR15	<i>R. solanacearum</i> EP1	<i>R. solanacearum</i> FJAT-1458	<i>R. solanacearum</i> FJAT-91	<i>R. solanacearum</i> FQY_4	<i>R. solanacearum</i> GM1000	<i>R. solanacearum</i> HA4-1	<i>R. solanacearum</i> KACC 10722	<i>R. solanacearum</i> KACC10709
X															
X	X	X	X	X	X	X			X			X			X

R	R	R	R	R	R	R	R	R	R	R	R sp	R	Rhizobacter	Rhizobacter	Rhizobacter
<i>solanacearum</i>	<i>solanacearum</i>	<i>solanacearum</i>	<i>solanacearum</i>	<i>solanacearum</i>	<i>solanacearum</i>	<i>solanacearum</i>	<i>solanacearum</i>	<i>solanacearum</i>	<i>solanacearum</i>	<i>solanacearum</i>	5_2_56FAA	<i>gummiphilus</i>	sp	sp	sp
MolK2	OE1-1	PSI07	Po82	SL3022	T12	T25	UW386	UW551	YC40-M			NS21	Root1221	Root29	Root404
			X						X	X		X	X	X	X

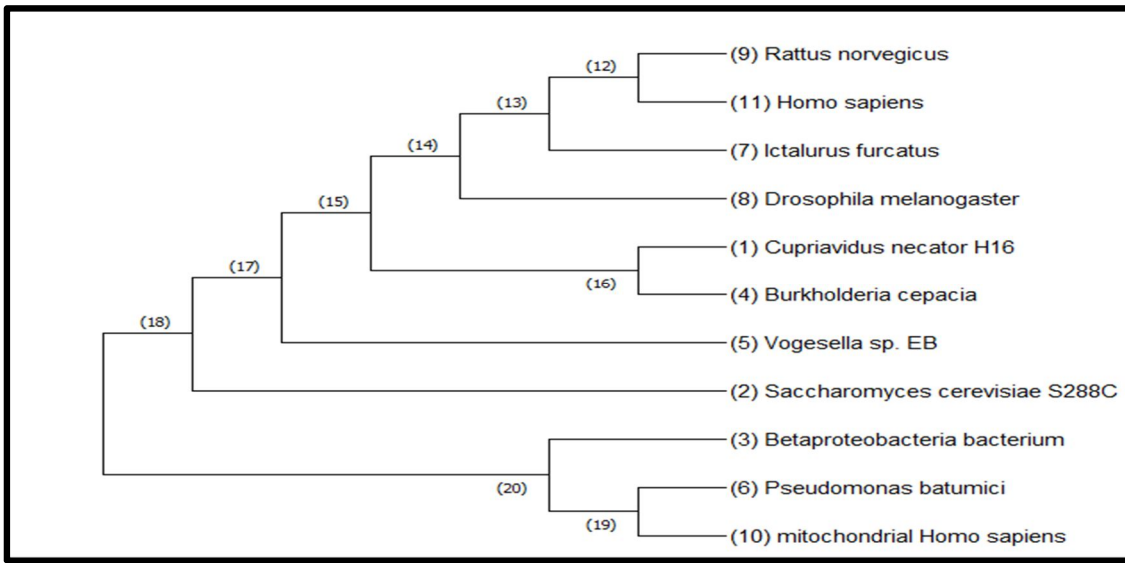
Rhizobacter	Rhizobacter	R	R	R	S	S. natans	T	T	T	T	T	T	T	T	X	Xylophilus	P
sp. Root29	sp. Root404	<i>depolymerans</i>	<i>benzoatilyticus</i>	<i>gelatinosus</i>	<i>natans</i>	<i>sulfidivorans</i>	<i>fonticaldi</i>	<i>taiwanensis</i>	<i>arsenitoxydans</i>	<i>bhubaneswarensis</i>	<i>intermedia</i>	<i>intermedia</i>	sp	<i>ampelinus</i>	<i>Lea#220</i>	<i>brachysporium</i>	
		KCTC 42856	JA2	IL144	DSM 6575	subsp. sulfidivorans	PL17	VT154-175	3As	DSM 18181	ATCC 15466	K12	FB-6	Cd	CCH5-B3	DSM 7029	
		X		X	X	X		X							X	X	
X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	

Result 3.2: Outcomes of Storage Compound Biosynthesis

The major aim of comparative analysis is to identify similarities and differences between different species/taxonomy. Investigation of bacterial communities and diversity is very important as these microbes exert direct beneficial or pathogenic effects on other species. Comparison of the culturable and non-culturable community will help to determine the structurally abundant, functionally viable, and potentially valuable bacteria that can ultimately be used as inoculum for the desired product. This studied was required to check whether the strain selected for study is suitable or not. This study concluded that there are many taxonomy and species which are available for higher productivity nonetheless productive more than *Ralstonia eutropha*.



Result 4.1: Aligned Hydroxybutyrate dehydrogenase Enzyme of different species



Result 4.2: Evolutionary Analysis by Maximum Likelihood Method

The roots of a phylogenetic tree represent the common ancestor of the sequences. Some trees are unrooted, and thus do not specify the common ancestor. A tree can be rooted using an outgroup (that is, a taxon known to be distantly related from all other Operational taxonomic units). Bootstrapping is a statistical technique that tests the sampling errors of a phylogenetic tree. It does so by repeatedly sampling trees through slightly perturbed datasets.

V. CONCLUSION

Ralstonia eutropha H16 was taken for this study because this strain of organism produces polyhydroxybutyrate in large amount than other strain. They are facultative aerobes that synthesize Polyhydroxybutyrate keto-acids in the absence of Oxygen and higher Carbon amount. The role of PHB synthesis is, it produces energy for microbial survival in such conditions. Another major advantage of the selected strain was, it is a non-spore-forming, non-pathogenic gram-negative bacteria. Metabolic pathway of any organism shows its whole process of synthesizing and degradation as per requirement of survival. Metabolic pathways the utilisation of macromolecules for further reactions. Metabolic pathway for polyhydroxybutyrate synthesis *in-vivo* was observed and studied with BioCyc. Metacyc online tool provided data of different pathways for multiple reactions at a time which a microbial cell facilitates. Acquired vast knowledge from initial to the final stage. As it cleared all the queries related to Polyhydroxybutyrate synthesis *in-vivo*. For example, acetyl co-enzyme plays the role of substrate for Biosynthesis of PHB with multiple enzyme activities in multiple stages but when and how acetyl co-enzyme undergo for the further reaction of producing PHB *in-vivo*. The major aim of comparative analysis is to identify similarities and differences between different species/taxonomy. Investigation of bacterial communities and diversity is very important as these microbes exert direct beneficial or pathogenic effects on other species. Comparison of the culturable and non-culturable community will help to determine the structurally abundant, functionally viable, and potentially valuable bacteria that can ultimately be used as inoculum for the desired product. This studied was required to check whether the strain selected for study is suitable or not. This study concluded that there are many taxonomy and species which are available for higher productivity nonetheless productive more than *Ralstonia eutropha*. The roots of a phylogenetic tree represent the common ancestor of the sequences. Some trees are unrooted, and thus do not specify the common ancestor. A tree can be rooted using an outgroup (that is, a taxon known to be distantly related from all other Operational taxonomic units). Bootstrapping is a statistical technique that tests the sampling errors of a phylogenetic tree. It does so by repeatedly sampling trees through slightly perturbed datasets. Data were collected from NCBI for producing a phylogeny tree. Each enzyme (Protein) was selected from different species. Collected data is in FASTA sequence form. For MegaX, a sheet was generated and uploaded according to the MegaX sheet format. Sequence after upload was sequence aligned with the help of Muscle/ ClustalW. After all these steps data sheet was prepared for phylogeny tree analysis for evolutionary.

The phylogeny tree was constructed in between enzymes that present in multiple organisms from microbial species to higher eukaryotes. That enzyme was responsible for the synthesis of keto-acids (hydroxybutyrate). Results were showed that positively define the evolution of genes responsible for an enzyme present in almost all organisms. For example, homo sapiens' liver cells also produce hydroxybutyrate in starvation conditions.

REFERENCES

- [1] Ahn WS, Park SJ, Lee SY. Production of poly(3-Hydroxybutyrate) by fed-batch culture of recombinant Escherichia coli with a highly concentrated whey solution. *Applied Environmental Microbiology*, **2000**; 66: 3624- 7.
- [2] Akiyama M, Taima Y, Doi Y. Production of poly(3- hydroxyalkanoates) by a bacterium of the genus Alcaligenes utilizing long-chain fatty acids. *Applied Microbiology and Biotechnology*, **1992**; 37:698–701.
- [3] Albuquerque M G E, Eiroa M, Torres C, Nunes B R, Reis M A M. Strategies for the development of a side stream process for polyhydroxyalkanoate (PHA) production from sugar cane molasses. *Journal of Biotechnology*, **2007**; 130: 411-21.
- [4] Albuquerque M, Torres C, Reis M. Polyhydroxyalkanoate (PHA) production by a mixed microbial culture using sugar molasses: effect of the influent substrate concentration on culture selection. *Water Resource*, **2010**; 44:3419–3433.
- [5] Allen A, Anderson W, Ayorinde F, Eribo B. Biosynthesis and characterization of copolymer poly(3HB-3HV) from saponified Jatropha curcas oil by Pseudomonas oleovorans. *Journal of Industrial Microbiology and Biotechnology* **2010**; 37: 849-56.
- [6] Arcos-Hernandez M.V., Laycock B., Pratt S., Donose B.C., Nikolie M.A.L., Luckman P., Werker A., and Lant P.A., Biodegradation in a soil environment of activated sludge derived polyhydroxyalkanoate (PHBV). *Polymer Degradation and Stability*, **2012**.
- [7] Ashby RD, Foglia TA. Poly(hydroxyalkanoate) biosynthesis from triglyceride substrates. *Applied Microbiology Biotechnology*, **1998**; 49: 431-7.
- [8] Bassas M, Marqués AM, Manresa A. Study of the crosslinking reaction (natural and UV induced) in polyunsaturated PHA from linseed oil. *Biochemical Engineering Journal* **2008**; 40: 275-83.
- [9] Begun G, Palko A, Brown L. The ammonia-ammonium carbonate system for the concentration of nitrogen-15. *Journal of Physical Chemistry*, **1956**; 60:48–51.
- [10] Bengtsson S, Pisco AR, Reis MAM, Lemos PC. Production of polyhydroxyalkanoates from fermented sugar cane molasses by a mixed culture enriched in glycogen accumulating organisms. *Journal of Biotechnology*, **2010**; 145: 253-63.
- [11] Bertrand J-L, Ramsay BA, Ramsay JA, Chavarie C. Biosynthesis of Poly-Hydroxyalkanoates from Pentoses by Pseudomonas pseudoflava. *Applied Environmental Microbiology*, **1990**; 56: 3133-8.
- [12] Bhat R. A., Qadri H., Wani K. A., Dar G.H., Mehmood M. A., *Innovative Waste Management Technologies for Sustainable Development*. IGI Global: International Publisher of Information Science and Technology Research, **2020**; 4: 52-81.
- [13] Bhatt R., Patel K., Trivedi U., *A Handbook of Applied Biopolymer Technology: Synthesis, Degradation and Applications*. **2013**; 10: 51: 35: 311- 331.
- [14] Bhubalan K, Lee W-H, Loo C-Y, et al. Controlled biosynthesis and characterization of poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyhexanoate) from mixtures of palm kernel oil and 3HV-precursors. *Polymer Degradation Stability* **2008**; 93: 17-23.

- [15] Byrom D. Production of poly- β -hydroxybutyrate: poly- β -hydroxyvalerate copolymers. *FEMS Microbiology Letter*, **1992**; 103:247–250.
- [16] Cai Z., Hou C., and Yang G., Characteristics and blending performance of electroactive polymer blend made with cellulose and poly (3-hydroxybutyrate). *Carbohydrate Polymers*, **2012**; 87: 650-657.
- [17] Ceyhan N, Ozdemir G. Poly-hydroxybutyrate (PHB) production from domestic wastewater using *Enterobacter aerogenes* 12Bi strain. *African Journal of Microbiology Research* **2011**; 5: 690-702.
- [18] Chaijamrus S, Udpuay N. Production and characterization of polyhydroxybutyrate from molasses and corn steep liquor produced by *Bacillus megaterium* ATCC 6748. *Agricultural Engineering International: The CIGR E Journal*, **2008**;X.
- [19] Chan R.T., Garvey C.J., Marcal H., Russell R.A., Holden P.J., and Foster L.J.R., Manipulation of Polyhydroxybutyrate Properties through Blending with Ethyl-cellulose for a Composite Biomaterial. *International Journal of Polymer Science*, **2011**.
- [20] Chander M., Microbial Production of Biodegradable Plastics from Agricultural Waste. *International Journal of Research and Analytical Reviews*, **2019**; 6(2): 2349-5138.
- [21] Chaudhry W, Jamil N, Ali I, Ayaz M, Hasnain S. Screening for polyhydroxyalkanoate (PHA)-producing bacterial strains and comparison of PHA production from various inexpensive carbon sources. *Annals of Microbiology*, **2011**; 61: 623-9.
- [22] Chen G-Q, Page WJ. Production of poly-b-hydroxybutyrate by *Azotobacter vinelandii* in a two-stage fermentation process. *Biotechnology Techniques*, **1997**; 11: 347-50.
- [23] Costa S, Lépine F, Milot S, Déziel E, Nitschke M, Contiero J. Cassava wastewater as a substrate for the simultaneous production of rhamnolipids and polyhydroxyalkanoates by *Pseudomonas aeruginosa*. *Journal of Industrial Microbiology and Biotechnology* **2009**; 36: 1063-72.
- [24] Cromwick AM, Foglia T, Lenz RW. The microbial production of poly(hydroxyalkanoates) from tallow. *Applied Microbiology and Biotechnology*, **1996**; 46: 464-9.
- [25] Cui Y W, Shi Y P, Gong X Y. Effects of C/N in the substrate on the simultaneous production of polyhydroxyalkanoates and extracellular polymeric substances by *Haloferax mediterranei* via kinetic model analysis. *Journal of Chemical Society*, **2017**; 7:18953–18961.
- [26] De Almeida A, Giordano AM, Nickel PI, Pettinari MJ. Effects of aeration on the synthesis of poly(3-hydroxybutyrate) from glycerol and glucose in recombinant *Escherichia coli*. *Applied Environmental Microbiology*, **2010**; 76:2036–2040.
- [27] Du C., Sabirova J., Soetaert W., Lin S.K.C., Polyhydroxyalkanoates Production from Low-cost Sustainable Raw materials. *Current Chemical Biology*, **2012**; 6:1.
- [28] Eggink G, Steinbuchel A, Poirier A, Witholt B. International symposium on bacterial polyhydroxyalkanoates. NRC Research Press, Toulouse, **1997**.
- [29] Fernández D, Rodríguez E, Bassas M, et al. Agro-industrial oily wastes as substrates for PHA production by the new strain *Pseudomonas aeruginosa* NCIB 40045: Effect of culture conditions. *Biochemical Engineering Journal* **2005**; 26: 159-67.
- [30] Fukui T, Doi Y. Efficient production of polyhydroxyalkanoates from plant oils by *Alcaligenes eutrophus* and its recombinant strain. *Applied Microbiology and Biotechnology* **1998**; 49: 333-6.
- [31] Full TD, Jung DO, Madigan MT. Production of polyhydroxyalkanoates from soy molasses oligosaccharides by new, rapidly growing *Bacillus* species. *Letter of Applied Microbiology*, **2006**; 43: 377-84.
- [32] Fuller RC. Microbial inclusions with special reference to PHA inclusions and intracellular boundary envelopes. *International Journal of Biological Macromolecules* **1999**; 25: 21-9.
- [33] Gumel A, Annuar M, Heidelberg T. Growth kinetics, effect of carbon substrate in biosynthesis of mcl-PHA by *Pseudomonas putida* Bet001. *Brazilian Journal of Microbiology*, **2014**; 45:427–438.
- [34] Gustafsson J., Landberg M., Batori V., Akesson D., Taherzadeh M.J., and Zamani A., Development of Bio-Based Films and 3D Objects from Apple Pomace. *Polymers* **2019**; 11: 289.
- [35] Haba E, Vidal-Mas J, Bassas M, Espuny MJ, Llorens J, Manresa A. Poly 3-(hydroxyalkanoates) produced from oily substrates by *Pseudomonas aeruginosa* 47T2 (NCBIM 40044): Effect of nutrients and incubation temperature on polymer composition. *Biochemical Engineering Journal* **2007**; 35: 99-106.
- [36] Hao J, Wang X, Wang H. Overall process of using a valerate-dominant sludge hydrolysate to produce high-quality polyhydroxyalkanoates (PHA) in a mixed culture. *Journal of Natural Products*, **2017**; 7:6939–6943.
- [37] He W, Tian W, Zhang G, Chen G-Q, Zhang Z. Production of novel polyhydroxyalkanoates by *Pseudomonas stutzeri* 1317 from glucose and soybean oil. *FEMS Microbiology Letter*, **1998**; 169: 45-9.
- [38] Hohne G., Hemminger W.F., and Flammersheim H.J., *Differential Scanning Calorimetry*. Springer, **2003**.
- [39] <http://www.ncbi.nlm.nih.gov>
- [40] https://www.researchgate.net/figure/fig-II-3-Pathway-of-PHB-synthesis_fig2_312934859
- [41] https://www.researchgate.net/figure/Updated-prices-of-bioplastics_tbl5_266850155
- [42] Hu D. Biosynthesis and characterization of polyhydroxyalkanoate block copolymer P-3-HB-b-P-4-HB. *Biomacromolecule*, **2011**; 12:3166–3173.
- [43] Iriani E.S., Permana A.W., Yuliani S., Kailaku S.I., and Sulaiman A.A., The effect of Agricultural waste Nanocellulose on The Properties of Bioplastic for Fresh Fruit Packaging. *Earth and Environmental Science*, **2019**; 309: 012035.
- [44] Israni N, Shivakumar S. Evaluation of upstream process parameters influencing the growth associated PHA accumulation in *Bacillus* sp. *Journal of Scientific and Industrial Research*, **2015**;74:290–295.
- [45] Jiang G. Carbon sources for polyhydroxyalkanoates and an integrated biorefinery. *International Journal of Molecular Science*, **2016**; 17:1157.
- [46] kahar P, Tsuge T, Taguchi K, Doi Y. High yield production of polyhydroxyalkanoates from soybean oil by *Ralstonia eutropha* and its recombinant strain. *Polymer Degradation Stability*, **2004**; 83: 79-86.
- [47] Kang M, Peng S, Tian Y, Zhang H. Effects of dissolved oxygen and nutrient loading on phosphorus fluxes at the sediment–water interface in the hai river estuary, China. *Marine Pollution Bulletin*, **2018**; 130:132–139.
- [48] Kaur M., Aggrawal N.K., Kumar V., and Dhiman R., Effects and Management of *Partheniumhysterophorus* : A Weed of Global Significance. *International Scholarly Research Notices*, **2014**.
- [49] Keenan TM, Nakas JP, Tanenbaum SW. Polyhydroxyalkanoate copolymers from forest biomass. *Journal of Industrial Microbiology*, **2006**; 33: 616-26.

- [50] Keenan TM, Tanenbaum SW, Nakas JP. Microbial formation of polyhydroxyalkanoates from forestry-based substrates. ACS Symposium Series, **2006**; 921: 193-209.
- [51] Kellerhals M B, Kessler B, Witholt B, Tchouboukov A, Brandl H. Renewable long-chain fatty acids for production of biodegradable medium-chain-length polyhydroxyalkanoates (mcl-PHAs) at laboratory and pilot plant scales. *Macromolecules*, **2000**; 33:4690–4698.
- [52] Khosravi-Darani K, Mokhtari Z B, Amari T, Tanaka K. Microbial production of poly(hydroxybutyrate) from C1 carbon sources. *Applied Microbiology and Biotechnology*, **2013**; 97:1407–1424.
- [53] Kim J.S., Lee Y.Y., Kim T.H., A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. *Bioresource Technology*, **2015**; 08:084.
- [54] Kim S W, Kim P, Lee H S, Kim J H. High production of poly- β -hydroxybutyrate (PHB) from *Methylobacterium organophilum* under potassium limitation. *Biotechnology Letter*, **1996**; 18:25–30.
- [55] Koller M, Bona R, Chiellini E et al. Polyhydroxyalkanoate production from whey by *Pseudomonas hydrogenovora*. *Bioresource Technology*, **2008**; 99: 4854–63.
- [56] Koller M, Hesse P, Salerno A, Reiterer A, Braunegg G. A viable antibiotic strategy against microbial contamination in biotechnological production of polyhydroxyalkanoates from surplus whey. *Biomass Bioenergy*, **2011**; 35: 748-53.
- [57] Korkakaki E, Van Loosdrecht MC, Kleerebezem R (Impact of phosphate limitation on PHA production in a feastfamine process. *Water Resources* 126:472–480.
- [58] Kulprecha S, Boonruangthavorn A, Meksiriporn B, Thongchul N. Inexpensive fed-batch cultivation for high poly(3-hydroxybutyrate) production by a new isolate of *Bacillus megaterium*. *Journal of Bioscience and Bioengineering*, **2009**; 107: 240-5.
- [59] Kumar B.S., Prabakaran G., Production of PHB (bioplastics) using bio-effluent as substrate by *Alcaligenes eutrophus*. *Indian Journal of Biotechnology*. **2006**; 5: 76-79.
- [60] Kumar M, Singhal A, Verma PK, Thakur I S. Production and characterization of polyhydroxyalkanoate from lignin derivatives by *Pandoraea* sp. *ISTKB. Journal of the American Chemical Society*, **2017**; 2:9156–9163.
- [61] Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular biology and evolution*, 33(7), 1870-1874.
- [62] Lavanya D., Kulkarni P.K., Dixit M., Raavi P.K., Krishna L.N.V., Sources of Cellulose and their Applications- A Review. *International Journal of Drug formulation and Research*, **2011**; 2(6): 2229-5054.
- [63] Law K-H, Leung Y-C, Lawford H, Chua H, Lo W-H, Yu P. Production of polyhydroxybutyrate by *Bacillus* species isolated from municipal activated sludge. *Applied Biochemistry and Biotechnology*, **2001**; 91-93: 515-24.
- [64] Lee SY, Middelberg APJ, Lee YK. Poly(3-hydroxybutyrate) production from whey using recombinant *Escherichia coli*. *Biotechnology Letter*, **1997**; 19: 1033-5.
- [65] Lee W-H, Loo C-Y, Nomura CT, Sudesh K. Biosynthesis of polyhydroxyalkanoate copolymers from mixtures of plant oils and 3- hydroxyvalerate precursors. *Bioresource Technology* **2008**; 99: 6844- 51.
- [66] Li R, Chen Q, Wang PG, Qi Q. A novel-designed *Escherichia coli* for the production of various polyhydroxyalkanoates from inexpensive substrate mixture. *Applied Microbiology and Biotechnology*, **2007**; 75: 1103-9.
- [67] Lin CSK, Luque R, Clark JH, Webb C, Du C. Wheat-based biorefining strategy for fermentative production and chemical transformations of succinic acid. *Biofuels, Bioproducts, Biorefining*, **2012**; 6: 88-104.
- [68] Liu C, Luo G, Wang W, He Y, Zhang R, Liu G. The effects of pH and temperature on the acetate production and microbial community compositions by syngas fermentation. *Fuel*, **2018**; 224:537–544.
- [69] Loo C-Y, Lee W-H, Tsuge T, Doi Y, Sudesh K. Biosynthesis and Characterization of Poly(3-hydroxybutyrate-3-hydroxyhexanoate) from Palm Oil Products in a *Wautersia eutropha* Mutant. *Biotechnology Letter* **2005**; 27: 1405-10.
- [70] Luengo J.M., Garcia B., Sandoval A., Naharro G., and Olivera E.R., Bioplastics from microorganisms. *Current Opinion in Microbiology*, **2003**; 6: 251-260.
- [71] Marangoni C, Furigo Jr A, de Aragão GMF. Production of poly(3- hydroxybutyrate-co-3-hydroxyvalerate) by *Ralstonia eutropha* in whey and inverted sugar with propionic acid feeding. *Process Biochemistry*, **2002**; 38: 137-41.
- [72] Martinez G A, Rebecchi S, Decorti D, Domingos J M B, Rio D D, Bertin L, Porto C D, Fava F. Towards multi-purpose biorefinery platforms for the valorisation of red grape pomace: production of polyphenols, volatile fatty acids, polyhydroxyalkanoates and biogas. *Green Chemistry*, **2016**; 18:261–270.
- [73] Martinez-Toledo MV, Gonzalez-Lopez J, Rodelas B, Pozo C, Salmeron V. Production of poly--hydroxybutyrate by *Azotobacter chroococcum* H23 in chemically defined medium and alpechin medium. *Journal of Applied Microbiology* **1995**; 78: 413-8.
- [74] Mary Siji.K., Pillai P.K.S., Amma D.B., Pothen L.A., Thomas S., *Handbook of Biopolymer-Based Materials: From Blends and Composites to Gels and Complex Network*, **2013**; 26: 777-799.
- [75] Masood F, Abdul-Salam M, Yasin T, Hameed A. Effect of glucose and olive oil as potential carbon sources on production of PHAs copolymer and tercopolymer by *Bacillus cereus* FA11. *Biotechnology*, **2017**; 7:87–101.
- [76] megaX
- [77] Mohanty A.K., Wibowo A., Misra M., and Drzal L.T., Development of Renewable Resource- Based Cellulose Acetate Bioplastic: Effect of Process Engineering on the Performance of Cellulosic Plastics. *Polymer Engineering and Science*, **2003**; 43: 5.
- [78] Mousavioun P., George G.A., and Doherty W.O., Environmental degradation of lignin/poly (hydroxybutyrate) blends. *Polymer Degradation and Stability*, **2012**; 97: 1114-1122.
- [79] Mudenu C., Mondal K., Singh U., Katiyar V., Production of Polyhydroxyalkanoates and its Potential Applications. *Advances in Sustainable Polymer*, **2019**.
- [80] Muhr A. Biodegradable latexes from animal-derived waste: biosynthesis and characterization of mcl-PHA accumulated by *Ps. citronellolis*. *Reactive and Functional Polymers*, **2013**; 73:1391–1398.
- [81] Munoz LEA, Riley MR. Utilization of cellulosic waste from tequila bagasse and production of polyhydroxyalkanoate (pha) bioplastics by *Saccharophagus degradans*. *Biotechnology and Bioengineering*, **2008**; 100: 882- 8.

- [82] Nath A, Dixit M, Bandiya A, Chavda S, Desai AJ. Enhanced PHB production and scale up studies using cheese whey in fed batch culture of *Methylobacterium* sp. ZP24. *Bioresource Technology*, **2008**; 99: 5749-55.
- [83] Nikel PI, de Almeida A, Melillo EC, Galvagno MA, Pettinari MJ. New Recombinant *Escherichia coli* Strain Tailored for the Production of Poly(3-Hydroxybutyrate) from Agroindustrial By-Products. *Applied Environmental Microbiology* **2006**; 72: 3949-54.
- [84] Ntaikou I, Kourmentza C, Koutrouli EC et al. Exploitation of olive oil mill wastewater for combined biohydrogen and biopolymers production. *Bioresource Technology*, **2009**; 100: 3724-30.
- [85] Page WJ. Production of polyhydroxyalkanoates by *Azotobacter vinelandii* UWD in beet molasses culture. *FEMS Microbiology Reviews*, **1992**; 103: 149-57.
- [86] Pandian S R, Deepak V, Kalishwaralal K, Rameshkumar N, Jeyaraj M, Gurunathan S. Optimization and fed-batch production of PHB utilizing dairy waste and sea water as nutrient sources by *Bacillus megaterium* SRKP-3. *Bioresource Technology*, **2010**; 101:705-711.
- [87] Park S J, Jang Y A, Noh W, Oh Y H, Lee H, David Y, Baylon M G, Shin J, Yang J E, Choi S Y, Lee S H, Lee S Y. Metabolic engineering of *Ralstonia eutropha* for the production of polyhydroxyalkanoates from sucrose. *Biotechnology and Bioengineering*, **2015**; 112:638-643.
- [88] Pisco AR, Bengtsson S, Werker A, Reis MAM, Lemos PC. Community Structure Evolution and Enrichment of GlycogenAccumulating Organisms Producing Polyhydroxyalkanoates from Fermented Molasses. *Applied Environmental Microbiology*, **2009**; 75: 4676-86.
- [89] Poblete-Castr I, Escapa I E, Jager C, Puchalka J, Lam J M C, Schomburg D, Prieto M P, Dos Santos V A P. The metabolic response of *P. putida* KT2442 producing high levels of polyhydroxyalkanoate under single-and multiple-nutrientlimited growth: highlights from a multi-level omics approach. *Microbial Cell Factories*, **2012**; 11:1-21.
- [90] Ramsay JA, Hassan M-CA, Ramsay BA. Hemicellulose as a potential substrate for production of poly(-hydroxyalkanoates). *Canadian Journal of Microbiology*, **1995**; 41: 262-6.
- [91] Rao U, Sridhar R, Sehgal PK. Biosynthesis and biocompatibility of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) produced by *Cupriavidus necator* from spent palm oil. *Biochemical Engineering Journal* **2010**; 49: 13-20.
- [92] Raza Z A, Abid S, Banat I M. Polyhydroxyalkanoates: Charaterizations, production, recent developments and applications. *Internatinal Biodeterior Biodegradation*, **2018**; 126: 45-56.
- [93] Raza Z.A., Tariq M.R., Majeed M.I., Banat I.M., Recent developments in bioreactor scale production of bacterial polyhydroxyalkanoates. *Bioprocess and Biosystems Engineering*, **2019**; 42: 901-919.
- [94] Rehm B H. Bacterial polymers: biosynthesis, modifications and applications. *Nature Reviews Microbiology*, **2010**; 8:578.
- [95] Rincon J, Camarillo R, Rodriguez L, Ancillo V. Fractionation of used frying oil by supercritical CO₂ and cosolvents. *Industrial and Engineering Chemistry Research*, **2010**; 49:2410-2418.
- [96] Ruiz C., Kenny S.T., Narancic T., Babu R., and Connor K.O., Conversion of waste cooking oil into medium chain polyhydroxyalkanoates in a high cell density fermentation. *Journal of Biotechnology*, **2019**; 8:20.
- [97] Samer M., Khalefa Z., Abdelall T., Moawya W., Farouk A., Abdelaziz S., Soliman N., Solah A., Goma M., Mohamed M., Bioplastics production from agricultural crop residues. *Agricultural Engineering International: CIGR Journal*, **2019**; 21(3): 190-194.
- [98] Saratale G.D., Oh M.K., Characterization of poly-3-hydroxybutyrate (PHB) produced from *Ralstonia eutropha* using an alkali-pretreated biomass feedstock. *International Journal of Biological Macromolecules*, **2015**; 80: 627-635.
- [99] Sartori T., Tibolla H., Prigol E., Colla L.M., Cost J.A.V.C., and Bertolin T.E., Enzymatic Saccharification of Lignocellulosic Residues by Cellulases obtained from Solid State Fermentation Using *Trichoderma viride*. *BioMedical Research International*, **2015**; 342716:9.
- [100]Shahid S, Mosrati R, Ledauphin J, Amiel C, Fontaine P, Gaillard J L, Corroler D. Impact of carbon source and variable nitrogen conditions on bacterial biosynthesis of polyhydroxyalkanoates: evidence of an atypical metabolism in *Bacillus megaterium* DSM 509. *Journal of Bioscience and Bioengineering*, **2013**; 116:302-308.
- [101] Shay E G. Diesel fuel from vegetable oils: status and opportunities. *Biomass Bioenergy*, **1993**; 4:227-242.
- [102]Silva LF, Taciro MK, Michelin Ramos ME, Carter JM, Pradella JGC, Gomez JGC. Poly-3-hydroxybutyrate (P3HB) production by bacteria from xylose, glucose and sugarcane bagasse hydrolysate. *Journal of Industrial Microbiology and Biotechnology*, **2004**; 31: 245-54.
- [103]Singh O.V., Chandel A. K., Sustainable Biotechnology- Enzymatic Resources Renewable Energy , Springer International Publishing AG, **2018**; 15: 399-421.
- [104]Solaiman DKY, Ashby RD, Foglia TA. Medium-Chain-Length Poly(-Hydroxyalkanoate) Synthesis from Triacylglycerols by *Pseudomonas saccharophila*. *Current Microbiology* **1999**; 38: 151-4.
- [105]Solaiman DKY, Ashby RD, Foglia TA. Production of polyhydroxyalkanoates from intact triacylglycerols by genetically engineered *Pseudomonas*. *Applied Microbiology and Biotechnology* **2001**; 56: 664-9.
- [106]Solaiman DKY, Ashby RD, Hotchkiss Jr AT, Foglia TA. Biosynthesis of medium-chain-length Poly(hydroxyalkanoates) from soy molasses. *Biotechnology Letter*, **2006a**; 28: 157-62.
- [107]Song JH, Jeon CO, Choi MH, Yoon SC, Park W: Polyhydroxyalkanoate (PHA) production using waste vegetable oil by *Pseudomonas* sp. strain DR2. *Journal of Microbiology and Biotechnology* **2008**; 18: 1408- 1415.
- [108]Sreekanth M, Vijayendra S, Joshi G, Shamala T. Effect of carbon and nitrogen sources on simultaneous production of α -amylase and green food packaging polymer by *Bacillus* sp. CFR 67. *Journal of Food Science and Technology*, **2013**; 50:404-408.
- [109]Thakor N, Trivedi U, Patel KC. Biosynthesis of medium chain length poly(3-hydroxyalkanoates) (mcl-PHAs) by *Comamonas testosteroni* during cultivation on vegetable oils. *Bioresource Technology*, **2005**; 96: 1843-50.
- [110]Third KA, Newland M, Cord-Ruwisch R. The effect of dissolved oxygen on PHB accumulation in activated sludge cultures. *Biotechnology and Bioengineering*, **2003**; 82:238-250.
- [111]Tsuge T, Yamamoto T, Yano K, Abe H, Doi Y, Taguchi S. Evaluating the ability of Polyhydroxyalkanoate synthase mutants to produce P(3HB-co-3HA) from soybean oil. *Macromolecular Bioscience* **2009**; 9: 71-8.
- [112]Van-Thuoc D, Quillaguam J, Mamo G, Mattiasson B. Utilization of agricultural residues for poly(3-hydroxybutyrate) production by *Halomonas boliviensis* LC1. *Journal of Applied Microbiology*, **2008**; 104: 420-8.



- [113]Volova T.G., Boyandin A.N., Vasiliev A.D., Karpov U.A., Prudnikova S.V., Mishukova O.V., Boyarskikh U.A., Filipenko M.L., Rudnev V.P., Xuan B.B., Dung V.V., Gitelson I.I., Biodegradation of polyhydroxyalkanoates (PHAs) in tropical coastal waters and identification of PHA- degrading bacteria. *Polymer Degradation and Stability*, **2010**; 95: 2350-2359.
- [114]Wu Q, Huang H, Hu G, Chen J, Ho K P, Chen G Q. Production of poly-3-hydroxybutrate by *Bacillus* sp. JMa5 cultivated in molasses media. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, **2001**; 80: 111-8.
- [115]www.biocyc.com
- [116]www.metacyc.com
- [117]Xu Z, Dai X, Chai X. Effect of influent pH on biological denitrification using biodegradable PHBV/PLA blends as electron donor. *Biochem Eng J*, **2018**; 131:24–30.
- [118]Yellore, Desai. Production of poly-3-hydroxybutyrate from lactose and whey by *Methylobacterium* sp. ZP24. *Letter Applied Microbiology*, **1998**; 26: 391-4.
- [119]Young FK, Kastner JR, May SW. Microbial Production of poly-- hydroxybutyric acid from d-xylose and lactose by *Pseudomonas cepacia*. *Applied Environmental Microbiology* **1994**; 60: 4195-8.
- [120]Yu J, Si Y. A dynamic study and modeling of the formation of polyhydroxyalkanoates combined with treatment of high strength wastewater. *Environmental Science and Technology*, **2001**; 35:3584–3588.
- [121]Yu J, Stahl H. Microbial utilization and biopolyester synthesis of bagasse hydrolysates. *Bioresource Technology*, **2008**; 99: 8042-8.
- [122]Zhao D. Improving polyhydroxyalkanoate production by knocking out the genes involved in exopolysaccharide biosynthesis in *Haloferax mediterranei*. *Appl Microbiol Biotechnol*, **2013**; 197:3027–3036.
- [123]Sepe P., and Limited R.T. *Thermal Analysis of Polymers*, Rapra Technology Limited.



10.22214/IJRASET



45.98



IMPACT FACTOR:
7.129



IMPACT FACTOR:
7.429



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Call : 08813907089  (24*7 Support on Whatsapp)