Journal of Pharmaceutical Research International

33(60A): 218-225, 2021; Article no.JPRI.80953
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Biosynthesis of Polyhydroxyalkanoate by Recombinant Bacteria: A Review

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i60A34478

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/80953

Review Article

Received 10 October 2021
Accepted 17 December 2021
Published 19 December 2021

ABSTRACT

Polyhydroxyalkanoates (PHAs), a biodegradable polymer, has many industrial and medical applications such as heart valves, scaffold, suture and drug delivery. Because of their structural diversity and close analogy to plastics, PHAs have gained major interest over the world. Natural isolates, recombinant bacteria, and plants have all been found to improve the quality, quantity, and economics of PHA production. Their biodegradability makes them an especially appealing synthetic plastic substitute. PHB biosynthetic genes phbA, phbB, and phbC are grouped and systematised into a single phbCAB operon. In terms of alignment and clustering of the relevant genes, the PHB pathway varies greatly across bacterial genera. In this regard, the enzymes appear to have a high degree of sequence preservation.

The structural studies further improve the mechanism of action of these enzymes and helped in improving and choosing the good candidates for increased production of PHB.

Keywords: Bioplastics; PHA; polyhydroxyalkanoates; recombinant bacteria.

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1. INTRODUCTION

Plastics are utilized in a wide range of industries, from automobiles to medicine. Because of its synthetic polymers, plastics have several advantages, including a wide range of forms and strengths with molecular weights ranging from 50,000 to 1,000,000 Da [1,2]. In the production of plastics, synthetic polymers such as polyethylene, polyvinyl chloride, and polystyrene are widely employed. Plastics can be easily folded into any desired shape as fibres and thin films, and because they are chemically resistant and less elastic, they are widely used in a variety of disposal items, as well as as packaging materials [3]. Recently, the replacement of the non-biodegradable by degradable plastic is more focusing for the decision-makers and for investors in the plastic industries [4]. The manufacturing of eco-friendly bioplastics can help us solve the pollution problems caused by non-degradable plastics. PHA is a bioplastic that have gained recent interests due to its properties which are safety engineering practice, being ethical and environmental-friendly [5, 6]. The previous studies reported that, the discovery of bioplastics as PHAs from microbial sources had important role on decreasing the environmental pollutions [7]. Moreover, PHAs with the same properties of synthetic plastics have also wide range of biomedical field applications such as tissue engineering, drug delivery system, cardiac stents, and medical packaging purposes [8]. The currently research studies are being executed to improve the PHAs productivity, decrees the production costs, and produce PHA with specific functions. The using recombinant *E. coli* strains for the biopolymers production has many of advantages, including growing fast, using wide range of carbon sources, the genetics and metabolic pathways are well understood, and less costly downstream processing lines [9]. The common PHA producing bacteria includes *Ralstonia eutropha, Alcaligenes latus, Azotobacter chroococcum, Azotobacter beijerinckii, Cupriavidus necator, Bacillus megaterium,* and *Pseudomonas oleovorans* (Table 1).

Recently approach is to use metabolic engineering methods to create recombinant strains of commonly used bacteria. These recombinant bacterial strains have modified metabolic pathways to produce high yields of PHA [10, 11]. The resulted recombinant strains will be having many of different advantages than the wild one, includes their high efficiency in utilizing inexpensive and different sources of renewable carbon with minimal fermentative supplies for giving rapid growth and high PHA accumulation.

The previous studies reported that, the recombinant *E. coli* has commonly used as PHA production due to its suitability for genetic handling, high rate of growth, high cell density cultivation and ability to used inexpensive sources of carbon. The *E. coli* strain has been reported to produce different types of short-chain length (scl) polyesters having C4 or C5 monomers, such as poly(4-hydroxybutyrate) P(4HB) homopolymer, P (3HB), poly(3-hydroxyvalerate) P(3HV), P(3HB-co-3HV) copolymer and P(3HB-co- 4HB) [11]. However, the recombinant strain of *E. coli*, having the phaC1 gene from *Pseudomonas aeruginosa*, was capable to produce medium chain- length (mcl) of PHAs, which having C6 to C14 monomers such as homopolymers of 3-hydroxydodecanoate (3HDD), 3-hydroxyhexanoate (3HHx), and terpolymer of P(3HB-co-3HV-co-3HHx), poly (3-hydroxybutyrate-co-3- hydroxyvalerate-co-hydroxyhexanoate) [18]. Furthermore, the hybrid polymers which containing both of the scl- and mcl-monomer units such as P(3HB-co-3HHx), poly(3-hydroxybutyrate-co-hydroxyhexanoate), naturally produced by *Aeromonas caviae*, can be also synthesized in the recombinant *E. coli* by genetic manipulation [19].

Table 1. Different wild bacterial strains for PHA production

PHA producing bacteria	Carbon Source	PHA Yield (wt %)	References
Bacillus megaterium	Glycerol	73%	[12]
Azotobacter vinelandii	Sucrose	62%	[13]
Azotobacter chroococcum	Molasses	31%	[14]
Haloferax mediterrane	Glucose	61%	[15]
Pseudomonas oleovorans	n-alkanes	60%	[16]
Ralstonia eutropha	Glucose	69%	[17]

2. PHA SYNTHESIS AND PRODUCTION BY RECOMBINANT BACTERIA

The biosynthetic pathway of P(3HB) consists of three enzymatic reactions catalyzed by three different enzymes (Fig. 1). The first reaction consists of the condensation of two acetyl coenzyme A (Acetyl-CoA) molecules into acetoacetyl-CoA by b-ketoacyl CoA thiolase (encoded by *phb*A). The second reaction is the reduction of acetoacetyl-CoA to (R)-3-hydroxybutyryl-CoA by an NADPH-dependent acetoacetyl-CoA dehydrogenase (encoded by *phb*B). Lastly, the (R)-3-hydroxybutyryl- CoA monomers are polymerized into PHB by P(3HB) polymerase, encoded by *phb*C [20].

The comparative analysis between the natural and recombinant strains of PHA production clearly indicated that the wild strains of PHA producers synthesized high amounts of PHA with definite restrictions which includes low rate of growth, cell lysis difficulty for more extraction and purification and low genetic manipulations, while, the recombinant strains of PHA producers solved all these restrictions [22]. The bacterial strain *Cupriavidus necator* showed over expressed *phaCAB* gene from a plasmid showed increase in P3HB production by 40% of cell dry weight and reduction in the time of fermentation by 20% with the decrease in the production cost compared with the wild strain [23]. The interest now is the reducing of the production cost of PHA produced by the recombinant DNA technology. To achieve this, the PHA synthesizing gene isolated from a natural PHA producing bacteria as *Ralstonia eutropha* is inserted into a host bacterium of PHA producing as *Pseudomonas* to enhance the PHA biosynthesis or insert into a non-PHA producing bacterial strains as *E. coli* to get the capability biosynthesis of the PHA.

The metabolically engineered of *Ralstonia eutropha* strain gain the capability to expression of the L-arabinose hydrolyzing enzyme which let the recombinant strain to metabolize L-arabinose as carbon source to give high amounts of intracellular PHB accumulation [24]. Earlier studies reported that, different natural PHA producing bacteria such as *Pseudomonas olevorans, Methylobacterium, Bacillus megaterium,* and *Rastonia eutropha* were used mainly for production of PHA at commercial scales. Nowadays, many of recombinant bacterial strains are replaced to produce of PHAs at the industrial scales because the recombinant strains are found with many advantageous than the wild one due to its high yield of production, fast production, easy manipulation, ability to use broad range of inexpensive carbon source and the low production costs [12]. The bacterial strains as *E. coli, Bacillus subtilis* and *Pseudomonas putida* are the common used as recombinant bacterial strains for PHA production at the industrial scales [22].

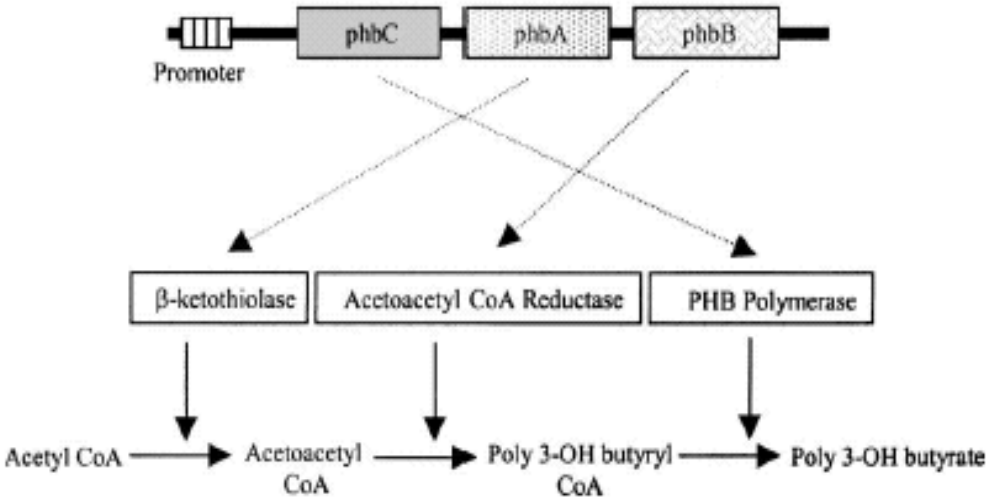

Fig. 1. Biosynthetic pathway of poly(3-hydroxybutyrate). P(3HB) is synthesized by the successive action of ß-ketoacyl-CoA thiolase (phbA), acetoacetyl-CoA reductase (phbB) and PHB polymerase (phbC) in a three-step pathway. The genes of the phbCAB operon encode the three enzymes. The promoter (P) upstream of phbC transcribes the complete operon (phbCAB) [21]

The mechanisms of biosynthesis of PHA inside the bacterial strains is the conversion of different carbon sources into PHAs. The use of recombinant strain, can be activated to produce PHAs without convincing stressed conditions, such as phosphorus or nitrogen starvation, which lead to decrease the production cost. Furthermore, the recombinant strains are well-defined, they could be further modified for PHA production optimization. In addition, *E. coli* is the common organism used as recombinant strain for producing PHAs. It utilizes high lactose containing dairy whey as a carbon source with the PHA-producing genes (the *pha* operon) from *C. necator*. The traditional strains of *E. coli* available in molecular biology labs include DH5α, JM, or XL1-Blue often lack the capability to use lactose as a nutrient carbon source. When nine different wild-type cell strains were tested for their ability to produce PHA using lactose as the sole carbon source, it was discovered that the strains GCSC6576 and GCSC4401, which are transformed with a high-copy-number plasmid, and pSYL107, which contains the A. eutrophus PHA biosynthesis operon, are the most capable of producing PHAs [25].The *E. coli* strains GCSC6576 (pSYL107) was grown on a high concentration of whey as a carbon substrate, yields 87 g/L of dry cell weight with 79% content of PHB [26]. As the same, recombinant *E. coli* is the excellent PHAs producer when using the *pha* operon from *C. necator* bacterial strain [27]. Several of *E. coli* strains were known to be able to apply lactose when transformed with the plasmid pJC4 and *A. latus pha* genes. Also, the strain CGSC 4401 was the best strain for PHB production with 119.5 g/L dry cell weight and 80.5% PHB content [28].

In two prior investigations, the recombinant E. coli was employed to produce PHAs from whey. Pais et al. [29] employed proton suicide to select a recombinant E. coli strain that generated a minimal amount of organic acids after transformation with operon of the *C. necator pha*. The study results obtained the low production of organic acid resulted in slow growth of the bacterial strain with high production of PHB accounted as 18.88 g PHB/L for the recombinant strain vs 7.8 g PHB/L for the wild strain [29]. The maximal cell density and PHB concentrations were found to be 70.1 g/L and 73 percent, respectively [30].

Another study successfully cloned the *pha* operon from *C. necator* into *E. coli* XL1-Blue with utilizing soy waste for PHB production, the soy waste was hydrolyzed by NaOH for 8h and 27.83% of the accumulated PHB was observed [31]. Furthermore, when this recombinant strain grown in glucose, about 43% dry cell weight of PHAs are produced [32]. This confirm that, the waste of soy and malt are good sources of carbon for PHA production.

Another study used restaurant waste that use the anaerobically digestion to produce lactic as a carbon source for the recombinant *E. coli* pnDTM2, the PHB accumulation content was 44% [33]. In addition to *E. coli*, another *Aeromonas* sp. (strain KC007-1) has also been used and accumulate 32.7% PHA [34]. With increasing of the genetic tools and techniques, it helps in optimizing of the synthesis pathways for non-native bacterial hosts to utilize and consume the substrates for PHAs production [35].

Table 2. Different recombinant bacterial strains for PHA production

Strain	Plasmid	PHA operon origin	Dry cell weight (g/L)	PHA production (g PHA/g dcw)	Reference
E. coli GCSC 657	pSYL107	*C. necator*	6.6	82%	[25]
E. coli GCSC 657	pSYL107	*C. necator*	88	81%	[26]
E. coli	pSYL107	*C. necator*	34	81%	[27]
E. coli CML3-1	pMAB26	*C. necator*	33	28%	[29]
E. coli CGSC 4401	pJC4	*Alcaligenes latus*	119	80%	[28]
E. coli CGSC 4401	pJC4	*Alcaligenes latus*	192	86%	[36]
E. coli K24K	pJP24K	*Azotobacter* sp. FA8	NA	72%	[30]
E. coli	pUC19/PHA	*C. necator*	71	24%	[32]
E. coli XL1-Blue	pKS	*C.necator*	3	27%	[31]
Aeromonas KC007-R1	pRK415H16	*C.necator*	1.86	32%	[34]

3. GENETIC BASIS OF PHA SYNTHESIS

In the synthetic pathway of the PHB formation in the recombinant strains, the biosynthetic genes including, *phbA* (for 3-ketothiolase), *phbB* (NADPH-dependent acetoacetyl-CoA reductase), and *phbC* (PHB synthase) from Acetyl-CoA are clustered and apparently systematized in only one operon named *phbCAB* [21]. The multiplicity of the P(3HB) biosynthetic pathways indicates how far the *pha* loci have differed. Meanwhile, the genes encoding enzymes of ssc-PHA (*phb*) production and the genes encoding enzymes for msc-PHA (pha) production are not necessarily to clustered and the gene organization differs from one species to another. In Acinetobacter sp.: *Pseudomonas acidophila, Alcaligenes latus*, and *Ralstonia eutropha*, the phbCAB genes are in tandem on the chromosome and also not necessarily to clustered. While, *Rhizobium meliloti, Paracoccus denitrificans*, and *Zoogloea ramigera*, the phbAB and phbC loci are not linked [37]. In addition to, the *Chromatium vinosum*, *Synechocystis*, and *Thiocystis violacea*, the PHA polymerase has two sub-units encoded by phbE and phbC genes, while, the phbAB and phbEC genes are located in one locus but divergently oriented [38]. The phb loci in *P. acidophila, C. vinosum*, *R. eutropha*, *T. violacea* and *R. meliloti* have an additional gene named phbF, its function not defined in the PHA metabolism [39], while part of the gene encoding a protein homologous to the hypothetical *E. coli* protein yfiH is located upstream of the *P. acidophila*, *Z. ramigera*, and *R. eutropha* P(3HB) polymerase genes. On the other hands, the *P. oleovorans* and *Pseudomonas aeruginosa* bacterial strains producing msc-PHA, the pha loci contain two phaC genes separated by phaZ, which encodes an intracellular PHA depolymerase [38].

4. APPLICATIONS

Different applications of the PHAs, because of its chemical properties such as they are inert, non-toxic and has antimicrobial activities, therefore, they are used for manufacturing of pharmaceutical items such as the adhesive films and hygiene-related baby items, including nappies [21]. As well as, they can be used in manufacture of electronic products such as mobile phones and computer tablets and due to their amphiphilic properties makes them appropriate for encapsulating seeds and fertilizers, biodegradable films and storage containers of crops. Also, PHAs have many medical applications including orthopaedic straps and pins, adhesive bandages, surgical sutures, repair stents, drug delivery systems, patches and matrix for bone marrow. Previous study reported that, when PHA combined with hydroxyapatite, it becomes a bioactive composite proper for regeneration and replacement of bone tissue [40]. There are other commercial uses of PHAs indicated in Fig. 2.

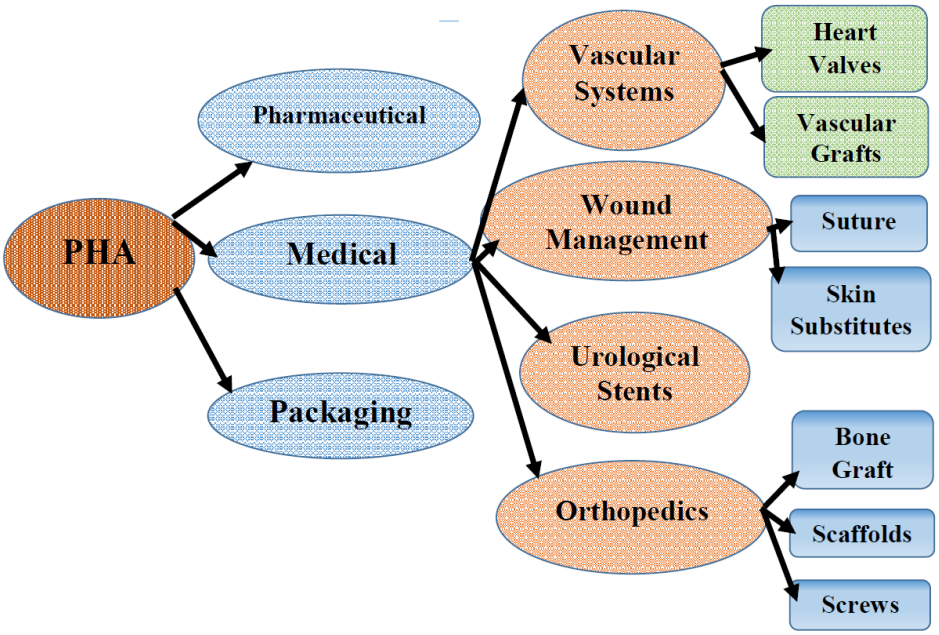

Fig. 2. A flow chart indicating the various uses of PHAs

The review concluded that, the recombinant bacterial strains have numerous of advantages than the wild type of bacterial PHA producers. In addition to, the recombinant bacterial strains have the capability to give large numbers and low production cost of different types of PHAs by using different sources of cheap and renewable carbon such as the industrial waste water, agricultural waste, dairy waste products. The other advantages include high rates of growth to realize high cell density, easy intracellular recovery of PHA, simplified manufacturing methods, effective for genetic and metabolic manipulation.

5. CONCLUSIONS

PHAs are extremely important because they allow society to separate the economic growth from the resource tiredness and the environmental destruction. With increase in the PHAs diversity there will be a significant increase in their application. However, the cost of production of PHAs forces their large-scale commercialization and industrialization. A lower-cost can be attained by participating different scientific fields such as synthetic biology, systematic biology, biotechnology and cultivation of high-cell-density, which will simplify the synthesis of all PHA kinds.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

ACKNOWLEDGEMENT

This Publication was supported by the Deanship of Scientific Research at Prince Sattam bin Abdulaziz University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Andrady A, Neal MA. Applications and societal benefits of plastics. Phil. Trans. R. Soc. B. 2009;364:1977–1984.
2. Di Bartolo A, Infurna G, Dintcheva NT. A review of bioplastics and their adoption in the circular economy. HWWI Res. 2021; 13:1229.
3. Plastics Europe. Plastics—The Facts 2019. An Analysis of European Plastics Production, Demand and Waste Data. 2019. Available:https://www.plasticseurope.org/en/resources/market-data.
4. Blaithín Mc, Margaret BF, McDonald P, Marija M. Production of Polyhydroxy-butyrate (PHB) and Factors Impacting Its Chemical and Mechanical Characteristics. Polymers. 2020;12:2908.
5. Raza ZA, Abid S, Banat IM. Polyhydroxyalkanoates: Characteristics, production, recent developments and applications. International Biodeterioration & Biodegradation. 2018;126:45-56.
6. Shrivastav A, Mishra SK, Shethia B, Pancha I, Jain D, Mishra S. Isolation of promising bacterial strains from soil and marine environment for polyhydroxyal-kanoates (PHAs) production utilizing Jatropha biodiesel byproduct. International Journal of Biological Macromolecules. 2010;47(2):283-287.
7. Gabr GA. Isolation and identification of bacterial strains able to biopolymer polyhydroxybutyrate (PHB) production from Soil of Al-Kharj probes, Saudi Arabia. Journal of Pharmaceutical Research International. 2018;21(6):1-11.
8. Gomez C, Julia M. Making green polymers even greener: Towards sustainable production of polyhydro-xyalkanoates from agroindustrial by-products. Advances in Applied Biotechnology. 2012;2012.
9. Ren Q. Biosynthesis of medium chain length poly-3-hydroxyalkanoates: from *Pseudomonas* to *Escherichia coli*. Ph.D. thesis. ETH, Zu¨rich, Switzerland; 1997.
10. Björn G, Martina C, Bock E, Stefan J, Peter N, Christopher JB, Sebastian LR. Untargeted metabolomics analysis of *Ralstonia eutropha* during plant oil cultivations reveals the presence of a fucose salvage pathway. Scientific Reports. 2021;11:14267.
11. Dewedar S, Abdel Hamid MS, EL-Ghareeb D, Haroun AA, ElBaz AF, El-Mekawy A. Statistical optimization and chemical characterization of newly extracted siderophore from *Azotobacter chroococcum*. Bioscience Res. 2018; 15(2):1163-1177.

12. Singh SB, Grewal A, Kumar P. Biotechnological production of Polyhydroxyalkanoates: A review on trends and latest developments. Chinese Journal of Biology. 2014;2014:1–18.
13. Jiang G, Hill DJ, Kowalczuk M, Johnston B, Adamus G, Irorere V, Radecka I. Carbon sources for polyhydroxyalkanoates and an integrated biorefinery. International Journal of Molecular Sciences. 2016; 17(7).
14. Haroun AA, Abdel-Hamid MS. Evaluation and characterization of polyhydroxybutrate produced by *azotobacter chroococcum*. Biotechnol: An Indian J. 2015;11(9):347-354.
15. Zhang YZ, Liu GM, Weng WQ, Ding JY, Liu SJ. Engineering of *Ralstonia eutropha* for the production of poly(3-hydroxy-butyrate-co-3-hydroxyvalerate) from glucose. Journal of Biotechnology. 2015; 195:82–88.
16. Chen GQ, Wu Q, Jung YK, Lee SY. Pha/Phb. Comprehensive Biotechnology, Second Edition. 2011;3:217–227.
17. Yu J. Microbial production of bioplastics from renewable resources. Bioprocessing for Value-Added Products from Renewable Resources. 2007;585–610.
18. Park SJ, Ahn WS, Green PR, Lee SY. Biosynthesis of poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3-hydroxyhexanoate) by metabolically engineered *Escherichia coli* strains. Biotechnol Bioeng. 2001;74: 81-86.
19. Meereboer KW, Misra M, Mohanty AK. Review of recent advances in the biodegradability of polyhydroxyalkanoate (PHA) bioplastics and their composites. Green Chem. 2020;22:5519–5558.
20. Giubilini A, Bondioli F, Messori M, Nyström G, Siqueira G. Advantages of additive manufacturing for biomedical applications of polyhydroxyalkanoates. Bioengineering. 2021;8:29.
21. Madison LL, Huisman GW. Metabolic-engineering of poly(3-hydroxyalkanoates): From DNA to plastic. Microbiol. Mol. Biol. Rev. 1999;63(1):21–53.
22. Thirumala M, Reddy SV. Production of PHA by recombinant organisms. International Journal of Life Sciences Biotechnology and Pharma Research. 2012;1(2):40–62.
23. Park JS, Park HC, Huh TL, Lee YH. Production of poly-hydroxybutyrate by *Alcaligenes eutrophus* transformants harbouring cloned phaCAB Genes. Biotechnol. Lett. 1995;17:735-740.
24. Liu CC, Zhang LL, An J, Chen B, Yang H. Recent strategies for efficient production of polyhydroxyalkanoates by micro-organisms. Letters in Applied Microbiology. 2016;62(1):9–15.
25. Lee SY, Middelberg APJ, Lee YK. Poly (3-hydroxybutyrate) production from whey using recombinant Escherichia coli. Biotechnol. Lett. 1997;19:1033–5.
26. Wong L. Poly-(3-hydroxybutyrate) production from whey by high-density cultivation of recombinant Escherichia coli. Appl. Microbiol. Biotechnol. 1998;50:30–3.
27. Kim BS. Production of poly(3-hydroxybutyrate) from inexpensive substrates. Enzyme Microb. Technol. 2000;27:774–7.
28. 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. Appl. Environ. Microbiol. 2000;66:3624–7.
29. Pais J, Farinha I, Freitas F, Serafim LS, Martínez V, Martínez JC, et al. Improvement on the yield of polyhydroxyalkanotes production from cheese whey by a recombinant Escherichia coli strain using the proton suicide methodology. Enzyme Microb. Technol. 2014;55:151–8.
30. 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 agro industrial by-products. Appl. Environ. Microbiol. 2006; 72:3949–54.
31. Hong K, Leung YC, Kwok SY, Law KH, Lo WH, Chua H, et al. Construction of recombinant *Escherichia coli* strains for polyhydroxybutyrate production using soy waste as nutrient. Appl. Biochem. Biotechnol. 2000;84–86:381–90.
32. Law KH, Chan PL, Lau WS, Cheng YC, Leung YC, Lo WH, et al. Construction of recombinant *Escherichia coli* strains for production of poly-(3-hydroxybutyrate-co-3-hydroxyvalerate). Appl. Biochem. Biotechnol. 2004;113–116:361–72.
33. Eshtaya MK, Rahman NA, Hassan MA. Bioconversion of restaurant waste into Polyhydroxybutyrate (PHB) by recombinant E. coli through anaerobic digestion. Int. J. Environ. Waste Manag. 2013;11:27–37.

34. Chien CC, Ho LY. Polyhydroxyalkanoates production from carbohydrates by a genetic recombinant *Aeromonas* sp. Lett. Appl. Microbiol. 2008;47:587–93.
35. Gustavsson M, Lee SY. Prospects of microbial cell factories developed through systems metabolic engineering. Microb. Biotechnol. 2016;9:610–7.
36. Ahn WS, Park SJ, Lee SY. Production of poly (3-hydroxybutyrate) from whey by cell recycle fed-batch culture of recombinant *Escherichia coli*. Biotechnol. Lett. 2001; 23:235–40.
37. Umeda F, Kitano Y, Murakami K, Yagi K, Miura Y, Mizoguchi T. Cloning and sequence analysis of the poly(3-hydroxyalkanoicac id)-synthesis genes of *Pseudomonas acidophila*. Appl. Biochem. Biotechnol. 1998;70–72:341–352.
38. Hein S, Iran H, Steinb€uchel A. Synechocystis sp. PCC6803 possesses a two-component polyhydroxyalkanoic acid synthase similar to that of anoxygenic purple sulfur bacteria. Arch. Microbiol. 1998;170:162–170.
39. Povolo S, Casella S, Nuti MP. Involvement of an orf in the synthesis and/or degradation of polyhydroxyalkanoates (PHA) in *Rhizobium meliloti*. In: International Symposium on Bacterial Polyhydroxyalkanoates. Davos, Switzerland. 1996;4/01.
40. Chen GQ, Wu Q. Microbial production and applications of chiral hydroxyalkanoates. Appl Microbiol Biot. 2005;67:592–599.

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