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An overview on use of recombinant bacteria in polyhydroxyalkanoate production with prominence on the recombinant strains of *Escherichia coli, Bacillus substilis* and *Pseudomonas putida*

Athulya.M.S and Dr. Banhni Das
Department of Biotechnology, Mount Carmel College, Bangalore University, Bangalore, Karnataka

ABSTRACT
The synthetic plastics have wide range of applications in the modern era.The alarming increase in the commercial demands for these non-biodegradable plastics make them a potent agent of environmental pollutions .This paved way for the development of environmental friendly bioplastics like Polyhydroxyalkanoates(PHAs). The PHAs are natural polyesters produced by microorganisms under unbalanced nutritional conditions. Later this capability of microorganisms are used up for the commercial production of bioplastic by several companies like Metabolix, Danimer scientific etc. However the cost of production became a major threat in the industrial production of PHA .The developments in molecular techniques have brought about several approaches for the cost effective production of PHAs and the salient among them is the use of metabolic engineering to generate recombinant bacterial strains. The recombinant bacteria could result in rapid & high yielding production of different PHAs in an economical manner. This review paper emphasis briefly on the features of three common recombinant bacteria used in commercial production of PHA as Escherichia coli, Bacillus subtilis & Pseudomonas putida.

KEYWORDS: *Bioplastics, PHA, metabolic engineering, recombinant bacteria, Escherichia coli, Bacillus subtilis, Pseudomonas putida.*

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I. INTRODUCTION

Plastics are an indispensable part of our daily life. The synthetic plastics are usually produced from fossil feedstock & have a devastating environmental effect due to their non- degradable property [27]. Consequently, the world is in search of alternatives for synthetic plastics that are eco-friendly & have comparable physical and chemical characteristics to synthetic plastics. The biological polyesters like PHAs are major group of bioplastics that have been considered as one of the best substitutes to petroleum derived synthetic plastics[18] .PHAs serve the purpose of bioplastics with their properties of biodegradability , biocompatibility & thermal processability [1,39].These polyesters have wide range of applications especially in packaging products and in the field of medicine to manufacture cardiac stents for treatment of coronary heart disease etc. [19].PHAs are biopolymers produced by diverse microorganisms as reserved energy source under varied sets of stresses in their ecologically niches. These stresses include excess amount of carbon sources along with scarcity of nitrogen, sulphur, phosphorus or oxygen in their growth media [1].Higher PHA accumulation can also be achieved by exposing microorganisms to anoxic conditions [35].The various microorganisms such as bacteria , yeast, some fungi and archaea located in diverse ecological niches like marine regions ,rhizopheric soils, activated sludge of treatment plants , industrial effluents etc.are the important natural producers of PHA [14,10,26,46].The common PHA producing bacteria include *Alcaligenes latus, Ralstonia eutropha, Azotobacter beijerinckii, Bacillus megaterium, Cupriavidus necator ,Pseudomonas oleovorans* etc.(as given in table 1).

The increasing demands of PHAs were met currently by their exclusive production at the industrial level.This is done by using different pure microbial cultures isolated from various ecological niches and different carbon sources as substrate for the fermentative pathway. However the process of isolation & the maintenance of the microbial culture under aseptic conditions for indefinite time, usage of expensive fermentative substrates,poor efficiency of production & purification had led to the high cost of commercial PHA production[15] .Several efforts have been made in recent decades to achieve low cost production of PHA like discovery of microbial strains that could utilize cheap carbon sources(industrial waste water , agro wastes , whey etc.) and efficiently produce intracellular PHAs with minimal degree of purification[20, 48].Once such approach is to use metabolic engineering techniques to create recombinant strains of commonly used and well

studied bacteria. These recombinant bacterial strains have modified PHA synthetic pathways to get high yielding PHAs [9].The recombinant bacterial strains offer several advantages over pure microbial cultures isolated from different sources. This includes their efficiency in using inexpensive & renewable carbon sources with minimal fermentative requirements for the rapid growth and accumulation of PHA. For example, the recombinant *E.coli* produce PHB greater than 50 % of their cell weight [16].In addition to that the recombinant strains could also avoid the wastage of time and resources in the identification , isolation and screening of pure microbial strains from mixed cultures .This review aim to discuss briefly on the three common recombinant bacterial strains used in industrial production of PHAs like *E.coli, Bacillus subtilis & Pseudomonas putida* and how they are metabolically engineered to result in enhanced PHA production[17,46] .
Table 1 depict various natural PHA producing bacteria with their corresponding substrates & PHA yield%

Table 1: Natural PHA producing bacteria, Substrate, PHA yield %.

PHA producing bacteria	Type of PHA	Substrate	PHA Yield (wt%)	References
Bacillus megaterium	PHB	Glycerol or glucose	70%	[14]
Azotobacter vinelandii	PHB	Sucrose	66%	[45]
Cupriavidus necator	PHB	Jatropha oil	20.2%	[41]
Haloferax mediterrane	PHB	Glucose	60-65%	[42]
Pseudomonas oleovorans	PHB	n-alkanes	63%	[44]
Ralstonia eutropha	PHBV	Glucose	68.8%	[40]
Alcaligenes latus	P3HB	Sucrose	50-60%	[43]

II. AN OUTLINE ON PHA SYNTHESIS IN BACTERIA

The bioplastics synthesized by the bacteria, archaea and fungi belongs to a group of polyester known as PHAs .Bacteria are one of the main candidates for industrial production of PHAs .Most of the bacteria could intracellularly accumulate PHA upto 90% (w/w) of their dry cell mass [57].Bacteria naturally produce PHAs as a group of insoluble hydroxy fatty acids & store them within their lipid inclusions as endocellular carbon reserve .Most of the times unbalanced nutritional conditions and environmental stresses promotes bacterial PHA synthesis .The PHA accumulation in bacteria can occur in two growth phases. The stationary phase of bacteria mostly exhibit non growth associated PHA accumulation with excess of carbon sources, limited nitrogen, phosphorus, magnesium & oxygen. Bacteria also exhibit growth associated PHA accumulation during exponential growth phase under balanced conditions [25]. In PHA production bacterial cells can be considered as a biochemical catalyst with high substrate specificity even in terms of the substrate‘s optical isomers. Depending on the bacterial strain, the genes and enzymes regulating the biogenesis of PHA have different characteristics. The ability of a bacterial isolate to synthesize a particular PHA is due to substrate specificity of the key enzyme PHA synthase [4].Also the biodegradable property of PHA are attributed by the bacterial enzymes like PHA hydrolases or PHA depolymerases [4,9].

Bacterial PHAs can be classified into different groups based on their size (short, medium & long chain polymers), arrangement and number of carbon atoms in the polymeric forms(homo& heteropolymer).Different kinds of bacterial PHAs include P3HHx [poly(3-hydroxylhexanoate)], P3HO[poly(3-hydroxyoctoate)] P3HD [poly(3-hydroxydodecanoate)], P3HB [poly(3-hydroxybutyrate)] & P3HV [poly(3-hydroxyvalerate)].The diversification in bacterial PHA products arise mainly due to the different type of bacterial strains , metabolic pathways, fermentative substrates & upstream and downstream methods employed in the PHA production process[2,21,23,24]. The biosynthetic pathway for PHA production in bacteria varies as bacteria could metabolize carbon sources differentially [4]. Therefore, it is very important to understand the common PHA synthetic pathways present in bacteria as it could assist in the process of bacterial strain improvement for enhanced bioplastic production[9].The following are some of the common & well studied metabolic pathways followed by the bacteria for the synthesis of PHA monomers(as given in figure 1).

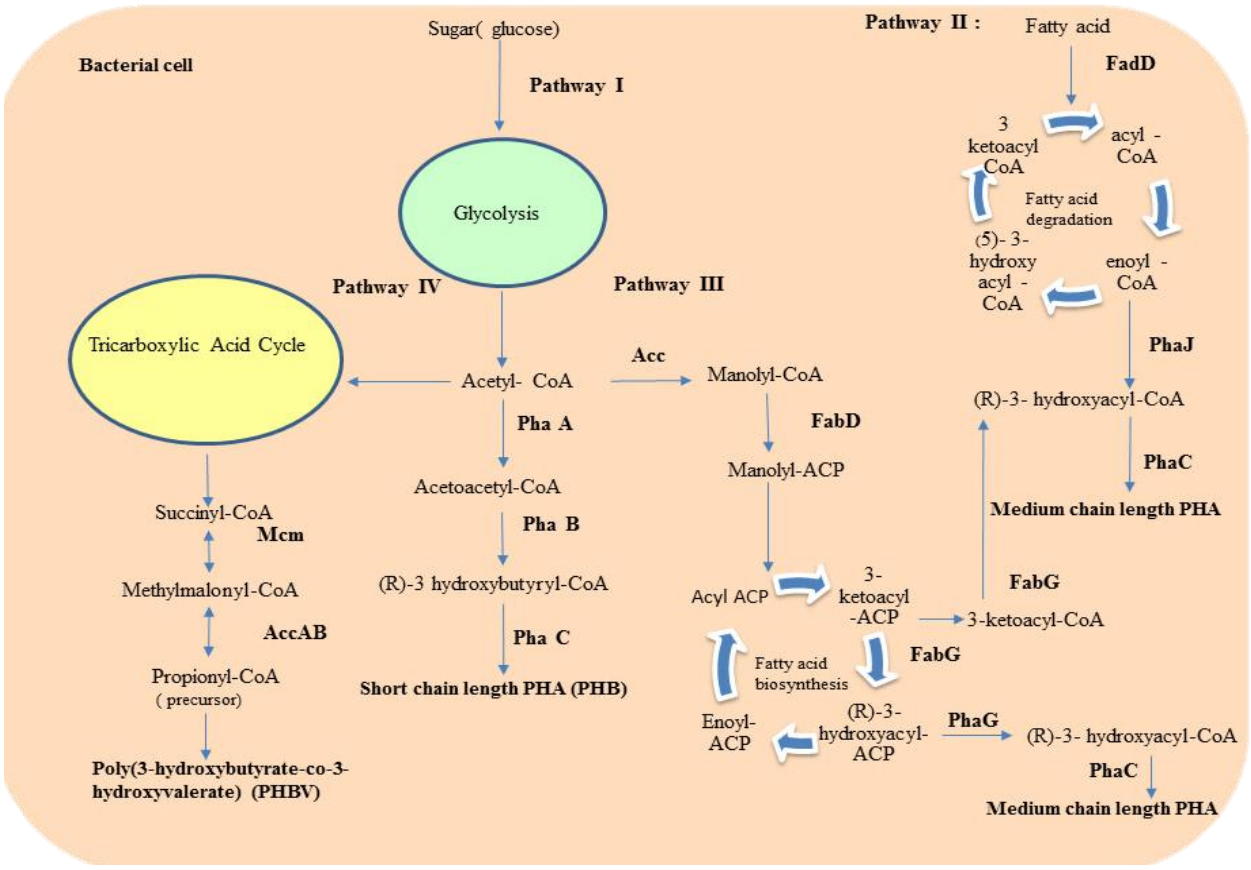
Pathway I : The first & most widely studied PHA is the homopolymer polyhydroxybutyrate (PHB).In this pathway the bacteria initially metabolize sugars like glucose & fructose to produce acetyl coenzyme A and NADPH.This is achieved by following the glycolytic and pentose phosphate pathways .Later the resulting two molecules of acetyl -CoA s are converted to PHB via three metabolic steps each catalysed by a specific enzyme .In the first step, 3- ketothiolase (PhaA) combines two molecules of acetyl-CoA to form acetoacetyl- CoA .Subsequently this acetoacetyl- CoA is reduced in the 2nd step to 3- hydroxybutyryl- CoA using NADH as a cofactor with the enzyme acetoacetyl- CoA reductase (PhaB) . Finally, PHB synthase (PhaC) polymerizes 3- hydroxybutyryl- CoA to short chain length PHA as PHB with liberation of coenzyme A. In this pathway the enzyme PHA synthase (PhaC) act as the key enzyme in allowing the bacterial isolate to produce a specific kind of PHA.This enzyme is highly substrate specific as it can only accept (R)- isomers as substrate [2,3,4].

Pathway II: In this pathway the bacteria produce medium chain length PHAs by metabolizing fatty acids (palmitic acid, oleic acid etc.) via fatty acid β-oxidation pathway. Here the PHA is derived from the enoyl-

CoA derivatives of the fatty acid β-oxidation pathway by the overexpression of enoyl-CoA hydratase (PhaJ) or 3-ketoacyl-ACP reductase (FabG) enzymes [2,3,4].

Pathway III : The acetyl CoA produced as the result of glycolytic pathway can enter into a direct branch of de novo fatty acid synthesis pathway to produce medium chain length PHA . In fatty acid biosynthesis pathway (R)-3-hydroxyacyl-ACP(Acyl Carrier Protein) intermediates are converted to (R)-3-hydroxyacyl-CoA by the enzyme acyl-ACP :CoA transacylase (PhaG) . Later this (R)-3-hydroxyacyl-CoA could serve as the monomer for PHA biogenesis in bacteria[3].

Pathway IV: In bacteria the methylmalonylCoA pathway also results in PHA biosynthesis. This pathway links PHA production to TCA cycle in the presence of glucose. In glycolytic pathway glucose get converted to acetyl-coA which then enters the TCA cycle. The succinyl CoA as one of the intermediates of TCA cycle can then follow an alternative pathway called methylmalonyl pathway for biosynthesis of PHA like PHBV. This pathway cause succinyl CoA to get converted to methylmalonylCoA by the action of the enzyme methylmalonylCoA mutase (Mcm) .The methylmalonylCoA is then converted to propionyl CoA by the enzyme (AccAB) methylmalonyl-CoA carboxytransferase. Later this propionyl CoA can serve as the precursor for PHBV synthesis [4].



- PhaA: β-ketothiolase, PhaB: PHA-specific acetoacetyl-CoA reductase ,PhaC: PHA synthase
- FadD: fatty acyl-CoA synthetase , PhaJ : (R)-specific enoyl-CoA hydratase
- Acc: acetyl-CoA carboxylase , FabD: malonyl CoA:ACP transacylase ,FabG: 3-ketoacyl-ACP reductase , PhaG: acyl-ACP:CoA transacylase
- Mcm: methylmalonyl-CoA mutase ,AccAB: methylmalonyl-CoA carboxytransferase

Figure 1: Common metabolic pathways for PHA biogenesis in bacteria [2,9,10,31,32,44].

III. RECOMBINANT BACTERIA IN PHA PRODUCTION

Comparative analysis between natural and recombinant PHA producers showed that the natural PHA producers synthesised high level of PHA with certain limitations. These limitations include slow growth rate , difficulty in cell lysis for further extraction and purification process and poor genetic characterization of natural strain affecting their genetic manipulations .On the other hand the recombinant strains of the natural PHA producers overcome all these limitations[17]. The natural PHA producer *Cupriavidus necator* showed all the above mentioned limitations while producing PHA like P3HB. However the recombinant strain

with over expressed *phaCAB* gene from a plasmid showed increased P3HB production by 33-40% of cell dry weight .In addition to that the recombinant strain of *Cupriavidus necator* also showed a reduction in the fermentation time by 20 % for the same level of productivity .Altogether the recombinant strain resulted a decrease in the overall production cost compared to wild type strain of *Cupriavidus necator*[50,51].One of the strategies for the enhanced and cost effective production of PHA is the development of better bacterial strains by recombinant DNA technology. In this method the high efficacy of foreign gene from a natural PHA producing bacteria like *Ralstonia eutropha* is introduced either into a PHA producing host bacteria (*Pseudomonas*)for enhanced PHA biosynthesis or into a non - PHA producing host bacteria(*E.coli*) to gain the capability of PHA biosynthesis. Recombinant bacterial strains for PHA biosynthesis can also be made to optimize the activity of the key enzyme like PHA synthase or to optimize the upstream processes of PHA production [17].The recombinant strain of *Halomonas* showed increased production of PHB with simplified downstream processing. This recombinant strain accumulated the PHB within their inclusion bodies in such a way that it could be easily purified. Thereby decreasing the overall cost of production. This is accomplished by the overexpression of a cell division inhibitor protein MinCD within the bacteria[46].The bacteria can also be metabolically engineered to create improved strains that favour the utilisation of cheap & broad spectrum of carbon sources for enhanced PHA production .The common pathways in bacteria which are mostly modified for the above purpose are the ED pathway(Enter Doudoroff) & SD pathway(Serine Deaminase) .The metabolically engineered strain of *Ralstonia eutropha* acquire the ability to express the L-arabinose hydrolyzing enzyme.The L-arabinose hydrolyzing enzyme allow the recombinant strain to metabolize L-arabinose as carbon source for high level of intracellular PHB accumulation[10,14]. Earlier many natural PHA producing bacteria including *Pseudomonas olevorans, Bacillus megaterium ,Methylobacterium, Rastonia eutropha* etc. were used mostly for the commercial production of PHA .Nowadays several recombinant bacterial strains replaced many of these wild type bacterial strains in industries for the production of PHA. The recombinant strains are found to be more advantageous than the nature strains of PHA producers due to their following features like increased yield , the rapid production , simplified downstream processing, ability to utilize broad spectrum of inexpensive substrates & decreased overall production costs [14,17]. *E.coli* , *Bacillus subtilis & Pseudomonas putida* are some of the commonly used & industrially important recombinant bacterial strains for PHA production [17].

3.1 Recombinant *Escherichia coli*

E.coli are gram negative rods that belong to large and diverse group of bacteria . *E .coli* are one of the extensively used model organisms for genetic studies as they are more susceptible for genetic manipulations. As a well-studied bacteria their entire genome is sequenced and molecular tools are available for their genetic manipulation .Therefore *E.coli* can be genetically modified either to overexpress a desired foreign gene or to induce a specific metabolic pathways that enable them to utilize cheap carbon sources like waste water, molasses, whey etc. as starting material to produce desirable chemical end products [30]. *E.coli* are non- natural PHA producer which also lack the depolymerase activity to degrade intracellularly accumulated PHA.The metabolically engineered recombinant *E.coli* harbouring the PHA biogenesis pathway are good candidate for the industrial production of PHAs. Recombinant *E.coli* could provide several advantages over wild type PHA producers. These include:

- Faster growth rate
- high cell density fermentation
- high level of productivity
- ability to metabolize inexpensive, renewable & sustainable fermentative media for cost effective production
- reduce downstream processing costs by developing a secretion method that eliminates the processes of separation and purification steps
- easy polymer recovery
- Lack of depolymerase activity which facilitate the storage of PHA within inclusion bodies without being degraded [5, 21].

For instance, the recombinant *E. coli* with deleted genes such as *mtgA* and *mreB* showed enhanced PHAs production by increasing the volumetric capacity of intracellular PHA [10].In a study *E. coli* was engineered with genes cluster for biosynthesis of the common PHA that is PHB *from Bacillus aryabhattai* PHB10. This recombinant *E.coli* strain do not require any external induction for production of good quality PHB with similar properties to commercial graded PHB. The polymer accumulation level of this recombinant *E.coli* was estimated to be as 6.22 ± 0.08 g/L which corresponds to 83.18% of cell dry mass (w/w)[36] . In another study recombinant *E. coli* engineered with PHA biosynthetic genes from the bacteria *Cupriavidus necator* produced P(3HB) with a yield of 80–90% dry cell weight in fed-batch cultivation and with a yield of 76% dry cell weight in a pH-stat fed-batch culture[47,54]. It is also noticed that the same recombinant strain of *E.coli* produced a yield of 80% dry cell weight of P (3HB) when grown on molasses [47, 55]. The fadB mutant strain of *E.coli*(LS1298) was genetically engineered to express PHA synthases like phaC1 and phaC2 from

Pseudomonas aeruginosa. When grown in media containing C8-C14 fatty acids(LB medium + decanoate) this recombinant *E.coli* strain resulted in the accumulation of medium side chain PHA .On the other hand acrylic acid can be used to channel the intermediates of fatty acid synthesis to PHA synthesis in recombinant *E. coli* harbouring the *phaC1Pa* gene[56].

Recombinant strains of *E*.coli utilizes glucose with a yield of 61.7 % PHA [49]. The genetically engineered strain of *E.coli* could produce PHA by metabolizing inexpensive carbon sources like acetate instead of glucose. Acetate can be obtained as a by-product from the hydrolysis of lignocellulosic biomass present in agro-waste .The overexpression of phosphotransacetylase or acetate kinase pathway within the recombinant *E.coli* resulted in enhanced acetate assimilation & synthesis of biopolymer such as PHBV,P3HB and P3HB4HB [poly(3-hydroxybutyrate-co-4-hydroxybutyrate)]. PHBV is synthesized by the overexpression of propionyl-CoA transferase & propionate permease enzymes with PHBV titre of 0.33 g/L & 1.09 g/L . This also resulted in 6.58 mol% & 10.37 mol% content of 3-hydroxyvalerate monomer . P3HB is produced by the overexpression of phosphotransacetylase or acetate kinase with PHBV titre of 1.27 g/L. P3HB4HB is synthesized by the overexpression of semi aldehyde dehydrogenase, 4-hydroxybutyrate dehydrogenase and CoA transferase enzymes.As a result the PHBV titre of 1 .71 g/L along with a 4-hydroxybutyrate monomer content of 5.79 mol% were obtained [36]. Recombinant *E. coli* engineered with the PHA biosynthetic gene *orf1* from bacteria *Aeromonas hydrophila* were able to utilize inexpensive decanoate and odd-chain fatty acids as fermentative substrates for enhanced production of terpolymers of P(3-hydroxybutyrate-co-3-hydroxyvalerate-co-3- hydroxyhexanoate) [37].The only limitations of using recombinant *E.coli* for PHA production is that *E.coli* contain pyrogenic LPS endotoxins that could copurify with PHA and is therefore unfavourable for biomedical applications. This limitation need to be taken care of while using the recombinant *E.coli* for commercial PHA production[9].Altogether the recombinant strains of *E .coli* have proven to be a promising source for commercial production of PHA & a powerful tool for the molecular analysis of PHA biosynthesis. As the microbial clones of the recombinant *E.coli* can direct the synthesis of PHA like poly-β-hydroxybutyrate (PHB) to levels as high as 95% of the cell dry weight[6].

3.2 Recombinant *Bacillus subtilis*

Bacillus subtilis are the most studied gram positive rod shaped soil bacteria . They are considered as an important next generation super secreting cell factories for the biogenesis of several prominent recombinant products. This can be achieved by the methods of recombinant DNA technologies & metabolic engineering[12]. *Bacillus substilis* are therefore known as industrial work horses . They were the first soil microorganism to be completely sequenced and are also employed for the functional analysis of other gram positive bacteria. The evidences from genomic (NCBI) & metabolic databases(KEGG) show that certain strains of *B. subtilis* don't possess genes related to PHA biosynthesis like *PhaA* & *PhaB.* This in turn reduces the background effect of homologous genes of the host during heterologous gene expression. However *Bacillus substilis* possess genes(*Phaz* genes) that code for the depolymerase enzymes that could result in hydrolysis of PHA[8] .The heterogeneous bacterial group *Bacillus substilis* consist of subspecies like *B. subtilis subsp subtilis* and *B. subtilis subsp.spizizenii* . Among them only the subspecies *spizizenii* are PHA producers & the subspecies *substilis* are non- PHA producers like *E.coli* .Therefore such subspecies can be genetically modified to express PHA biosynthetic genes from other desired bacteria along with the suppression of the depolymerase *Phaz* gene. Recombinant *Bacillus subtilis* owe certain unique features that make them ideal candidate for commercial production of PHA and they are:

- the lack of the toxic lipo-polysaccharides making them suitable for biomedical applications.
- easy and timely recovery of PHA by expression of self-lysing genes on completion of PHA biosynthetic process.
- usage of bio wastes (industrial wastewater, molasses, agro biomass) as substrate for cost effective PHA production [9].

Few drawbacks of recombinant *Bacillus substilis* strain include the presence of misfolded proteins,the presence of proteases, plasmid instability and lack of suitable expression vectors [8].The initial researches on bioplastics such as PHAs were specifically done in the genus *Bacillus* especially in *Bacillus megaterium* from which the best quality PHA (P3HB)was first isolated. *Bacillus megaterium* is known to naturally harbour class IV PHA synthase complex encoded by the genes *phaC* & *pha R.* This in turn is a key enzyme for the PHA biogenesis by utilizing substrates like acetate and 3 hydroxybutyrate via biosynthetic pathway 1 as mentioned earlier[9]. The commercially important strains of recombinant *Bacillus substilis*(1A304(Φ105MU331) engineered with *phaPQRBC* genes from *Bacillus megaterium* are utilized for the cost effective & large scale production of different PHAs like P3HB & P3HV etc. These recombinant strains could grow easily to very high cell density using inexpensive carbon & nitrogen source like molasses without usage of antibiotics [38] .In a study the recombinant strain of *Bacillus substilis* were constructed by inserting a plasmid named pBE2Cl to the *Bacillus substilis* strain DB104 .The resultant recombinant strain as *Bacillus*

subtilis/ pBE2C1 were then employed for the over expression of *phaCAB* genes from *Pseudomonas aeruginosa*.As a result *Bacillus subtilis*/ pBE2C1 produced the biopolymer hydroxydecanoate -co-hydroxydodecanoate [P(HD-co-HDD)]by utilizing malt waste as fermentative substrate. In the same study another recombinant strain of *Bacillus substilis* were constructed by inserting a plasmid named pBE2C1AB to the *Bacillus substilis* strain DB104 .Then the recombinant strain as *Bacillus subtilis*/ pBE2C1AB were used for the over expression of *phaAB* genes from *R. eutropha.* This in turn resulted in the production of the biopolymer hydroxybutyrate-co-hydroxydecanoate-co-hydroxydodecanoate[P(HB-co-HD-co-HDD)]by utilizing malt waste as fermentative substrate. The results of the study also showed that the recombinant *Bacillus substilis* could efficiently metabolize renewable carbon sources like the malt waste in the medium better than that of glucose & thus could substantially lower the cost of PHA production [11].

3.3 Recombinant *Pseudomonas putida*

Pseudomonas putida is a gram negative rod shaped saprotrophic soil bacterium .They harbour the capability of degrading hydrocarbon like toluene .*Pseudomonas putida* were the first microorganisms to be patented in the world and are extensively used in bioremediation. This soil microbe naturally harbour aromatic catabolic pathways that serves to degrade oil present in contaminated sites .This in turn could provide the suitable conditions for PHA biogenesis with excess of carbon & limited nitrogen sources .*Pseudomonas putida* follow the pathway II (beta oxidation pathway)or pathway III(denovo fatty acid synthesis pathway)as mentioned earlier for PHA biosynthesis [14].The key steps within these pathways of *Pseudomonas putida* could be engineered in such a way to produce precise substrate based design of PHA homopolymers, copolymers, and block polymers.This in turn allow the study of structure–property relationship in a clear way [17]. A mutant strain of *P.putida* KT2442 as *Pseudomonas putida* KTOY06DC was cloned with PHA synthesis genes (*phaPCJAc*) from *Aeromonas caviae* to create a recombinant strain . The resultant recombinant strain of *Pseudomonas putida* KTOY06DC could synthesis short chain length and medium-chain-length PHA block copolymer[52].The PHA accumulation was also observed with partial or complete deletion of gene encoding for the enzyme 3-hydroxyacyl-CoA dehydrogenase (which catalyzes the conversion of long-chain-3-hydroxyacyl-CoA to 3-ketoacyl-CoA) from the strain *P. putida* KTOY08 .The PHA thus accumulated contained only two different monomeric structures .Among the two monomeric structures most of them showed the same chain length as that of the substrate fatty acids and the remaining monomers showed the chain length which is shorter by the former by two carbon atoms [17,29].The *Pseudomonas putida* can use multiple carbon sources for the production of medium chain length PHAs . This include fatty acids (directly undergo β- oxidation),sugars and aromatic compounds(subjected to fatty acid de novo biosynthesis) [22].The wildtype *Pseudomonas putida* can be genetically modified to possess altered metabolic pathways that could favour the utilization of broad range of renewable waste products as substrates .Therefore such recombinant strains of *Pseudomonas putida* can be used for the improved production of high quality & low cost mcl-PHAs .This could be achieved by the overexpression & deletion of certain genes of the PHA biogenesis pathway [29]. *Pseudomonas putida* can be engineered in different ways to enhance mcl- PHA production and this consists of :

- knocking down competing β- oxidation pathways
- overexpressing genes within PHA biosynthesis operon via plasmid or chromosomal integration
- inserting different ribosome binding sites (RBS) or promoters
- elevating NADH or NADPH supply for PHA synthesis
- engineering cell morphology to increase cell size
- eliminating the ability to consume PHAs by knocking out depolymerase gene[7 ,13 ,17].

The *Pseudomonas putida* strain KT2440 was genetically engineered to utilize the renewable carbon source like lignocellulosic biomass by overexpression of the genes like *phaG, alkK, phaC1 and phaC2* . This in turn increased the carbon flux into mcl- PHA biosynthesis .In addition to that the deletion of certain genes like the *Phaz* depolymerase genes & those genes in beta oxidation pathway as *fadBA1* and *fadBA2* can result in the prevention of degradation of stored PHA within inclusion bodies[13].As compared to wild type strain recombinant *Pseudomonas putida* showed 53% increase in mcl- PHA titre (g l−1)& 20% increase in PHA yield from p- coumaric acid .Similarly the recombinant *Pseudomonas putida* also showed 20% increase in mcl- PHA titre (g l−1) and 100% increase in PHA yield from lignin. Therefore this strain can be employed for enhancing mcl- PHA production from aromatic compounds and lignocellulose wastes[13].Likewise the recombinant *Pseudomonas putida* showed 2.5- fold increase in mcl- PHA production when grown on nitrogen- rich medium supplemented with heptanoate and octanoate. In a study,the recombinant strain of *Pseudomonas putida* KT2440 produced PHHx with 60% yield by utilizing glucose whereas on utilizing gluconate and glucose it resulted in PHBHHx with 19% yield .However the wild type strain of *Pseudomonas putida* KT2440 could utilize only glycerol to produce PHB with 25.74% yield [32,33,34]. In another study two

mutant strains of *Pseudomonas putida* KT2442 were constructed with defect in PHA polymerase (*phaC1*)and PHA depolymerase (*phaZ*)encoding genes .The construct was then used for the study of the fundamental role of PHAs in balancing the stored carbon or biomass or number of cells as function of carbon availability .This also demonstrated that PHA metabolism allows *P. putida* to adapt the carbon flux of hydroxyacyl-CoAs to cellular demand . Therefore this could establish a functional PHA turnover cycle in *P. putida* KT2442 through the coordination of PHA synthesis and mobilization pathways [17].

IV. FUTURE PROSPECTS

Recombinant strains of bacteria like *E.coli*, *Bacillus subtilis* and *Pseudomonas putida* became an industrial landmark in the economical production of PHA, as it holds several advantages over natural PHA producing bacteria. However they exhibit several limitations in terms of stability of gene expression, purity of PHA product, storage of PHA etc. This paved way for the future research in developing superior recombinant strains of the above bacteria that could overcome these drawbacks. This could be accomplished mainly by discovering novel PHA biogenesis pathway, modifying the existing pathway, identifying new PHA synthesizing genes and advanced methods of extraction and purifications that could result in optimized production of PHA. Additionally, new PHA producing bacterial strains can be discovered from different ecological niches and their recombinant strains can be developed for the commercial production of best quality PHA[49].

V. CONCLUSION

The progress in science and technology have led to more and more discoveries and inventions within the few decades .However the devastating effect of these innovations mainly affected the nature by resulting in several environmental pollutions and depletion of the natural resources.In order to cope up with current scenario people are mainly focusing on researches that could create eco-friendly products and services. One such approach was the discovery of biodegradable plastics like PHAs from microbial sources. This had a major impact on reducing environmental pollutions. In addition to that PHAs also have comparable properties to synthetic plastics with wide range of applications in biomedical field like tissue engineering , cardiac stents, drug delivery system , packaging purposes etc.[28]. However, the commercial PHA production was not very successful because of the efficiency of the production, purification methods and cost effectiveness. On the other hand these demerits worked as a challenge for the scientists in the future. Some of the biggest challenges included, the process of isolation and maintenance of microbial cultures producing biopolymers under aseptic conditions for indefinite time. Even the requirement of large amount solvents for polymer extraction made the process highly complicated with compromised production of the polymer. These issues make the question of biopolymer production for commercial use more challenging[17, 42] .Since then, there has been a continuous search for organisms that are highly efficient in the production, strains capable of utilizing cheap carbon sources to reduce the production cost and modified purification techniques to make the isolation of PHA produced inside the cells more efficient and cost effective. These avenues have drawn interest widely in the scientific community from the last decade. This then resulted in the new strategy of using of metabolic and genetic engineering methods for the development of recombinant bacteria for enhanced commercial production of PHA. From the review it is concluded that the recombinant strains of industrially important bacteria like *E.coli*, *Bacillus subtilis* & *Pseudomonas putida* provide several advantages over natural wild type PHA producers. The recombinant bacteria were able to provide large scale & low cost production of different PHAs by utilizing broad range of cheap & renewable carbon sources like industrial waste water, agro waste , dairy waste like whey , molasses etc. Other advantages include faster growth rate to achieve high cell density , easy intracellular PHA recovery , simplified downstream processing, effective for genetic & metabolic manipulation etc.[1]. The advancement in metabolic & evolutionary engineering, the demand for bioplastics & the extensive research conducted on the PHAs enabled the discovery of new organisms, genes & metabolic pathways for the efficient production of natural and non-natural PHAs products which are more economically viable with better properties to meet a wide range of applicability [10].

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