An updated overview on the regulatory circuits of polyhydroxyalkanoates synthesis

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Summary
Polyhydroxyalkanoates (PHA) are a promising and sustainable alternative to the petroleum-based synthetic plastics. Regulation of PHA synthesis is receiving considerable importance as engineering the regulatory factors might help developing strains with improved PHA-producing abilities. PHA synthesis is dedicatedly regulated by a number of regulatory networks. They tightly control the PHA content, granule size and their distribution in cells. Most PHA-accumulating microorganisms have multiple regulatory networks that impart a combined effect on PHA metabolism. Among them, several factors ranging from global to specific regulators, have been identified and characterized till now. This review is an attempt to categorically summarize the diverse regulatory circuits that operate in some important PHA-producing microorganisms. However, in several organisms, the detailed mechanisms involved in the regulation of PHA synthesis is not well-explored and hence further research is needed. The information presented in this review might help researcher to identify the prevailing research gaps in PHA regulation.

Introduction
Polyhydroxyalkanoates (PHA) are natural polymers of hydroxyalkanoates produced by microorganisms, including bacteria and archaea, usually under conditions of limited nutritional supply with excess carbon source (Reddy et al., 2003; Chen and Wu, 2005). They are accumulated by some microorganism as intracellular carbon and energy reserve to combat adverse environmental conditions (Khanna and Srivastava, 2005). PHA are bioplastics and are gaining considerable prominence in both the environmental and medical fields. Synthesis of PHA is a well-regulated process that involves a number of enzymes and regulatory proteins (Sagong et al., 2018). One of the approaches to maximize PHA production and synthesize novel PHA, is to engineer the biosynthetic pathway (Steinbüchel, 2001). Another approach is to manipulate the regulatory elements controlling PHA synthesis. To realize the latter approach, a better understanding of the regulatory circuits involved in PHA synthesis is necessary. The present review attempts to provide an overview on the regulation of PHA to the readers. It mainly focuses on the current knowledge on PHA regulation in several model species of bacteria and haloarchaea. Understanding the PHA regulation is a progressive topic, and this review might help to identify the prevailing research gaps. Reviews on the regulation of PHA synthesis is limited. The few already published reviews are focussed on the various types of regulations affecting PHA synthesis (Kessler and Witholt, 2001; Velázquez-Sánchez et al., 2020). This review has been presented to the readers from a different point of view. The present review has provided a vivid description of the various regulation systems affecting PHA synthesis mainly with the help of some model organisms.

An overview on PHA biosynthesis pathways
The type of PHA accumulated is closely related to the microbial species and carbon sources. More than 150
monomeric units of hydroxyalkanoates have been identified as PHA monomers (Steinbüchel and Lütke-Eversloh, 2003). Depending upon the (carbon atom) chain length in the monomers, PHA are classified as short-chain length comprising of C3-C5 monomer (SCL-PHA), medium-chain length comprising of C6-C14 monomer (MCL-PHA) and long-chain length consisting of more than 14 carbons in monomer (LCL-PHA) (Sagong et al., 2018). Most researches are focussed on SCL and MCL-PHA. Synthesis of SCL-PHA from natural and engineered strains is receiving great attention from researchers due to their wide distribution and high production level (Wang et al., 2016). Various Gram negative bacteria genera like Cupriavidus, Burkholderia and Azotobacter, and Gram positive bacteria from genera like Bacillus, Nocardia and Rhodococcus, produce SCL-PHA. Additionally, a few haloarchaeal species including Haloferax mediterranei are SCL-PHA producers. Three monomers of SCL-PHA which have received significant consideration are 3-hydroxybutyrate (3HB), 3-hydroxyvalerate (3HV) and 4-hydroxybutyrate (4HB). MCL-PHA have gained considerable attention as they are more flexible in nature. Most fluorescent Pseudomonas species belonging to rRNA homology group I accumulate MCL-PHA by incorporating 3-hydroxyalkanoate monomers including 3-hydroxyhexanoate, 3-hydroxyoctanoate, 3-hydroxydecanoate and 3-hydroxydodecanoate into homo, block- or random copolymers (Fiedler et al., 2000; Chen and Jiang, 2017).

For synthesis of SCL-PHA, several PHA monomer supplying pathways using sugars, alcohols and fatty acids as carbon sources have been identified in bacteria and haloarchaea (Fig. S1) (Han et al., 2013; Jiang et al., 2016; Ye et al., 2018; Mozejko-Ciesielska et al., 2019). Intermediates derived from these substrates are converted to hydroxyacyl-CoA, which are polymerized by PHA synthase to form PHA (Sagong et al., 2018). Most SCL-PHA producers accumulate PHBV, while some species accumulate copolymers such as poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) or poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P3HB4HB) or poly(3-hydroxybutyrate-co-3-hydroxyvalerate-co-4-hydroxybutyrate) (PHBV4HB) (Doi et al., 1990; Koller et al., 2007). The precursor molecules for 3HB, and 4HB are usually acetyl-CoA, and succinyl-CoA, respectively. For 3HV, acetyl-CoA and propionyl-CoA are the common precursors. In some cases, intermediates of the β-oxidation of valerate or other odd-numbered fatty acids serves as the 3HV precursor (Khanna and Srivastava, 2007). Acetyl-CoA is a common metabolic intermediate, obtained from glycolytic pathway. Propionyl-CoA is produced by catabolism of specific amino acids like methionine, threonine by Aspartate/2-oxobutyrate pathway or by oxidation of odd-chain fatty acids (Wongkittichote et al., 2017). In H. mediterranei, four propionyl-CoA supplying pathways are identified which includes, citramalate/2-oxobutyrate pathway, the aspartate/2-oxobutyrate pathway, the methylmalonyl-CoA pathway and 3-hydroxypropionate pathway (Han et al., 2013). Synthesis of 3HB or 4HV monomers proceed under the action of β-ketothiolase enzyme, which condenses acetyl-CoA molecule either with another acetyl-CoA molecule or propionyl-CoA molecule to form acetoacetyl-CoA and/or 3-ketovaleryl-CoA (Slater et al., 1998). 3-ketoacyl-CoA reductase then reduces them to (R)-3-HB-CoA and/or (R)-3-HV-CoA, respectively. Besides, succinyl-CoA is an intermediate of the TCA cycle. Succinate semialdehyde dehydrogenase catalyzes the conversion of succinyl-CoA to succinate semialdehyde, which is further oxidized to 4HB by 4-hydroxybutyrate dehydrogenase (Ye et al., 2018). 4HB is further converted to 4HB-CoA by 4HB-CoA transferase (Chen et al., 2017). Finally, these generated monomers (3HB-CoA, 3HV-CoA and 4HB-CoA) are polymerized to form PHB or its copolymers, PHBV, P3HB4HB and PHBV4HB, catalysed by PHA synthases (Nomura and Taguchi, 2007).

In the case of MCL-PHA, most monomeric building blocks are R-configured chiral 3-hydroxalkanoates. The (R)-3-hydroxyacyl precursors for the MCL-PHA monomer in Pseudomonas spp. is supplied by two pathways, β-oxidation pathway and fatty acid de novo biosynthesis pathway (Fig. S2) (Mozejko-Ciesielska et al., 2019). In β-oxidation pathway, acyl-CoA, generated from fatty acid, is oxidized to enoyl-CoA, which is converted into (R)-3-hydroxyacyl-CoA by enoyl-CoA hydratase (PhaJ) for PHA synthesis (Tsuge et al., 2000). In fatty acid de novo biosynthesis pathway, (R)-3-hydroxyacyl-CoA precursors are generated from unrelated carbon sources, such as glucose and gluconate. These substrates, by series of reactions, are converted into acetyl-CoA which is further carboxylated to malonyl-CoA (Rehm et al., 2001). The latter enters the fatty acid de novo biosynthesis pathway where it condenses with acyl-ACP to generate 3-ketoacyl-ACP, which is converted to (R)-3-hydroxyacyl-ACP by β-ketoacyl-ACP reductase (FabG). 3-hydroxyacyl-ACP:CoA transacylase (PhaG), actually functions as a 3-hydroxyacyl-ACP thioesterase and converts (R)-3-hydroxyacyl-ACP to (R)-3-hydroxyalkanoic acid. Finally, the latter is converted to (R)-3-hydroxyacyl-CoA by 3-hydroxyacyl-CoA ligase (Wang et al., 2012; Hokamura et al., 2015). The resulting (R)-3-hydroxyacyl-CoA is then polymerized to form MCL-PHA by PHA synthase.

Regulation of PHA synthesis

PHA have a high demanding global market owing to their several potential benefits like biodegradability,
PHA regulation in Cupriavidus necator

Cupriavidus necator is conveniently handled and cultured on cheap feedstock to produce PHB (Raberg et al., 2018). The designation of C. necator has undergone series of changes. Initially, known as Hydrogenomonas eutrophus, Ralstonia eutrophus, Wautersia eutrophpa and finally as Cupriavidus necator. PHB synthesis in this organism is controlled by at least three regulatory factors, PhaR, PhaM and PTS system (Fig. 1).

Phasins are low-molecular weight amphiphilic proteins, predominantly present on the surface of PHA granules (Steinbüchel et al., 1995; Maestro and Sanz, 2017). The hydrophobic domain of PhaP phasin effectively binds to the hydrophobic surface of PHA inclusion body, forming a network-like layer on PHA granules (McCool and Cannon, 1999). The hydrophilic domain of PhaP remains exposed to the cytoplasm (Maestro and Sanz, 2017). The PhaP expression is regulated by a DNA-binding protein named PhaR. Co-occurrence of phaR gene and phaP gene has been revealed in many bacteria and halohararcha (Maehara et al., 2001; Pötter et al., 2002; Segura et al., 2003; Chou et al., 2009; Kadowaki et al., 2011; Cai et al., 2012; Nishihata et al., 2018). PhaR homologs are well-distributed among SCL-PHA-producing microorganisms and the phaR gene is located near the other PHA-synthesis genes, including phaC, phaA, phaB and phaZ in the genome (Maehara et al., 2002). The phaR gene in C. necator is located adjacent to its phaCAB gene cluster (Pötter et al., 2002). C. necator has seven phasin proteins (PhaP1-PhaP7), among which PhaP1 is the major phasin affecting PHB synthase and granule size (Pötter et al., 2005; Pfeiffer and Jendrossek, 2012). Deletion of only phaP1 impaired PHB production whereas the other six phaP genes had no significant effect (York et al., 2001; Pötter et al., 2005; Pfeiffer and Jendrossek, 2012). PhaR represses the expression of phaP1 by binding to its promoter, under PHB non-accumulating conditions in this strain (Pötter et al., 2002; York et al., 2002). Additionally, it binds to an intragenic region of phaP3 and represses its transcription (Pötter et al., 2005). Such a regulation of phaP genes by PhaR is necessary at the onset of PHB biosynthesis.
| Organism name | Regulator | Description of regulator | Effect on target genes | Effect on PHA metabolism | Reference |
|---------------|-----------|--------------------------|------------------------|-------------------------|-----------|
| Azotobacter vinelandii | PhbR | AraC family regulatory protein | PhbR activates the transcription of the phbBAC operon. | Inactivation of the phbR gene reduced PHB accumulation by 70%. | Peralta-Gil et al. (2002) |
| | RpoS | Global regulator | RpoS acts as an activator of phbR expression. Inactivation of rpoS reduced phbR and phbB transcript level. | ΔrpoS showed reduced PHB accumulation. | Hernandez-Eligio et al. (2011) |
| | CydR | Oxygen-dependent global regulatory protein | CydR positively impacts the pha gene expression. | PHB accumulation in the cydR mutant occurred during the exponential phase instead of the stationary phase in wild-type strain. | Wu et al. (2001) |
| | PTS system | Multicomponent system responsible for uptake and concomitant phosphorylation of carbohydrates | PTS genes affect transcription levels of the phbR and phbBAC genes possibly by modulating RpoS. | ΔptsP and ΔptsO genes reduced PHB accumulation; ΔptsN synthesized PHB twice. | Segura and Espín (1998); Noguez et al. (2008); Muriel-Millán et al. (2017) |
| | GacS/GacA system | Two-component system | Gac/Rsm system modulates the expression of phbR and post-transcriptionally regulates the expression of pha genes. | Deletion of gacS gene reduced PHB accumulation. | Castañeda et al. (2000); Hernandez-Eligio et al. (2012) |
| | Arf sRNA | Azotobacter regulatory RNA involving Fe; iron responsive sRNA | Positive regulator of phbR expression during iron limitation; arf inactivation reduced phbR and phbB transcripts level. | arf inactivation reduced PHB accumulation. | Muriel-Millán et al. (2014) |
| Azospirillum brasilense | NtrB/ NtrC system | Two-component system | / | ΔntrBC and ΔntrC mutants produced PHB in both exponential phase and stationary phase, irrespective of the nitrogen concentration in the medium. | Sun et al. (2000), Sun et al. (2002) |
| Bacillus megaterium | PhaQ | DNA-binding, PHA-responsive autoregulated repressor | PhaQ negatively regulates phaP expression. | PHB possibly acted as an inducer for the PhaQ-mediated phaP regulation. | Lee et al. (2004) |
| Bradyrhizobium diaeofficiens USDA110 | PhaR | DNA-binding, PHA-responsive repressor but not autoregulated | PhaR represses and activates almost 28 genes and 42 genes including pha genes; PhaR negatively regulated phaP1, phaP4, phaP5, phaA1 and phaA2, phaZ1 and phaZ3, phaC1 and phaC2 and fixK2 expression; PhaR activates the expression of phaB2. Upregulation of fixK2 in ΔphaR mutant further stimulates the transcription of phaC2 which results in formation of inactive PHB synthase. | Deletion of PhaR impaired PHB synthesis and increased EPS production. Contrarily, ΔfixK2 mutant accumulated more PHB compared with wild-type strain. | Queglas et al. (2016); Nishihata et al. (2018) |
| Organism name              | Regulator | Description of regulator               | Effect on target genes                                      | Effect on PHA metabolism                                                                 | Reference                                                                 |
|---------------------------|-----------|---------------------------------------|-------------------------------------------------------------|----------------------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Burkholderia thailandensis | Quorum sensing | Global regulator | Loss of QS system reduced scmR gene expression which reduced the phaC and phaZ expression. | Deletion of QS system or scmR gene reduced PHA synthesis. | Martinez et al. (2020)                                                   |
| Cupriavidus necator       | PhaR      | DNA-binding, PHA-responsive autoregulated repressor | PhaR negatively regulates expression of phaP1 and phaP3. PhaM acts as a physiological activator of PhaC1. | \(\Delta\)phaR mutant led to defective PHA synthesis. Deletion of phaM gene generated fewer large-sized PHB granule; distribution of the granules among daughter cells were hampered in \(\Delta\)phaM mutant. | Potter et al. (2002); Potter et al. (2005); Pfeiffer and Jendrossek (2014); Wahl et al. (2012) |
| PhaM                      |           | Novel DNA-binding protein             |                                                             |                                                                                          |                                                                          |
| PTS system                |           | Multicomponent system responsible for uptake and concomitant phosphorylation of carbohydrates | /                                                           | Inactivation of the ptsN gene increased PHB accumulation; inactivation of ptsI and ptsH led to a reduced PHB accumulation. | Kaddor and Steinbüchel (2011)                                           |
| Halophaga mediterranei    | PhaR      | DNA-binding, PHA-responsive autoregulated repressor | PhaR negatively regulates the phaRP operon. | \(\Delta\)phaRP mutant produced significantly reduced amount of PHBV than the \(\Delta\)phaP mutant. Complementation with phaP in the \(\Delta\)phaRP mutant partially restored PHBV. Complementation with phaR in \(\Delta\)phaRP mutant completely restored PHBV accumulation. However, complementation of both phaP and phaR resulted into formation of regularly shaped PHBV granules. | Cai et al. (2015)                                                   |
| PPS-like protein          |           | PPS-like protein possibly evolved as a regulator protein from the PEP synthetase protein | Deletion of pps-like gene activated the transcription of the three cryptic genes, especially phaC1. | Deletion of the pps-like gene resulted to 70.46% increase in PHBV accumulation. PHB synthesis required expression of only PhaP1. However, PhaP2 acts as a backup phasin and ensured PHB synthesis to some extent in \(\Delta\)phaP1 mutant. | Chen et al. (2019); Chen et al. (2020); Kadowaki et al. (2011); Alves et al. (2016) |
| Herbaspirillum seropedicae SmR1 | PhaR      | DNA-binding, PHA-responsive autoregulated repressor | PhaR represses the expression of phaP1 and phaP2. In the wild-type strain, the two phasins were differentially expressed. The expression level of phaP2 was 8-fold lower than phaP1. However, phaP2 expression increased by 6-fold in the \(\Delta\)phaP1 mutant. | Deletion of the three Fnr encoding genes reduced PHB accumulation. | Batista et al. (2018)                                                   |
| Fnr                       |           | Redox-responsive transcriptional regulator | Deletion of the three Fnr encoding genes reduced the transcription levels of the three phaCs (phaC1, phaC2, and phaC3). | Deletion of the three Fnr encoding genes reduced PHB accumulation. | Batista et al. (2018)                                                   |
| NtrB/ NtrC system         |           | Two-component system | Deletion of ntrC upregulated zwf gene expression. | Deletion of ntrC increased PHB accumulation. | Sacomboio et al. (2017)                                                   |
| Organism name                        | Regulator | Description of regulator                           | Effect on target genes                                                                 | Effect on PHA metabolism                                                                 | Reference                      |
|-------------------------------------|-----------|----------------------------------------------------|----------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|--------------------------------|
| *Pseudomonas aeruginosa*            | RpoN      | Global regulator                                   | Deletion of *rpoN* gene inhibited *phaG* gene expression; negative regulator of *phaF* expression. | Δ*rpoN* mutant was unable to accumulate PHA from unrelated carbon sources like gluconate and octanoate, irrespective of nitrogen supply. | Hoffmann and Rehm (2004, 2005) |
|                                     |           | Quorum sensing                                     | Deletion of *lasI* or *lasR* or both significantly reduced expression of *phaC*.           | Mutants exhibited reduced PHA content.                                                   | Xu et al. (2011)               |
| *Pseudomonas chlororaphis PA23*     |           | QS and Anr cross-regulation                         | *PhzR* mediates anr gene regulation that modulates *pha* gene expression.                 | QS-deficient strain and Δ*anr* mutant accumulated less PHA.                               | Mohanan et al. (2019)          |
| *Pseudomonas extremaustralis*       | Anr       | Oxygen-sensitive global regulator                   | Anr inactivation decreased *phaR* and *phaC* expression by 6,000- and 1,380-fold, respectively. | PHB accumulation under microaerobic and anaerobic conditions reduced significantly.       | Tribelli et al. (2010)         |
| *Pseudomonas oleovorans*            | PhaD      | TetR-like transcriptional regulator                 | PhaD acts as the transcriptional activator of *phaF*; facilitates *pha* transcription by binding to its promoter. | Deletion of *phaD* decreased PHA accumulation, reduced granule size and increased granule number. | Klinke et al. (2000); Sandoval et al. (2007) |
| *Pseudomonas putida CA-3*           | GacS/GacA system | Two-component system           | Disruption of gacS inhibited PhaC1 protein expression.                                      | Δ*gacS* was incapable of PHA accumulation.                                               | Ryan et al. (2013)             |
| *Pseudomonas putida GPo1*           | PhaF      | Intrinsically disordered protein, nucleoid binding ability, PHA granule binding ability, involved in PHA granule segregation | PhaF acts as a negative regulator of *phaC*1 gene and *phaF* operon in PHA non-accumulating conditions. | Disruption of *phaF* did not affect PHA accumulation under favourable condition.         | Prieto et al. (1999)          |
| *Pseudomonas putida KT2440*         | RpoN      | Global regulator                                   | RpoN acts as a negative regulator of *phaF* expression under nitrogen excess conditions. | Δ*rpoN* mutant accumulated more PHA during nitrogen limitation compared with nitrogen excess. | Hoffmann and Rehm (2004, 2005) |
|                                     | RpoS      | Global regulator                                   | RpoS might serve as a negative regulator of *phaC1* promoter.                            | Deletion of *rpoS* gene increased PHA degradation.                                       | Raiger-Lustman and Ruiz (2008) |
|                                     | PsrA      | Transcriptional regulator                           | PsrA is involved in the fatty acid – PHA metabolic network.                                | Deletion of *psrA* gene reduced the PHA production and also changed the monomer composition. | Fonseca et al. (2014)          |
|                                     |           | Stringent response                                 | Deletion of the *relA* and *spoT* genes significantly increased expression of the *phaF* genes. | PHA production in the Δ*relA*Δ*spoT* mutant was similar under both nitrogen limiting and non-limiting conditions. | Mozekjo-Ciesielska et al. (2017) |
|                                     |           | PTS system                                         | Multicomponent system responsible for uptake and concomitant phosphorylation of carbohydrates | *psbP* or *psbO* deletion impaired PHB accumulation; *ptaW* mutation increased PHB synthesis. | Velázquez et al. (2007)        |
| Organism name          | Regulator | Description of regulator                                                                 | Effect on target genes                                                                                      | Effect on PHA metabolism                                                                 | Reference                     |
|-----------------------|-----------|------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|--------------------------------|
| *Pseudomonas putida*  | PhaF      | Intrinsically disordered protein, nucleoid binding ability, PHA granule binding ability, involved in PHA granule segregation | Deletion of *phaF* reduced transcription of *phaC1* gene by 3.5-fold; highest transcriptional level of *phaI* differed from mid-exponential phase in wild type to stationary phase in the Δ*phaF* mutant. | PhaF acted as an activator of PHA synthesis; PHA content was reduced in the Δ*phaF* mutant during continuous fermentation. | Galán et al. (2011)            |
|                       | PhaD      | TetR-like transcriptional regulator                                                      | PhaD acts as a carbon-dependent activator of the *pha* gene cluster.                                       | Disruption of *phaD* significantly affected PHA accumulation in the presence of octanoate. | De Eugenio et al. (2010)        |
|                       | Crc       | Global regulator                                                                        | Crc represses *phaC1* expression post-transcriptionally by inhibiting its translation in nutritionally balanced medium. | Inactivation of crc gene increased PHA accumulation in nutritionally balanced medium.       | La Rosa et al. (2014)           |
| *Pseudomonas putida*  | GacS/GacA system | Two-component system                                                                     | Disruption of gacS reduced transcription rate of the entire *pha* cluster.                                  | Disruption of the GacS sensor kinase was linked to reduced PHA production.                  | Prieto et al. (2016)            |
| KT2442/KT2440         | PhaF      | Intrinsically disordered protein, nucleoid binding ability, PHA granule binding ability, involved in PHA granule segregation | PhaF might act as a transcriptional activator of *phaC1* gene or enhancer of polymerase activity.          | Deletion of *phaF* reduced aliphatic PHA synthesis and completely abolished aromatic PHA synthesis. | Sandoval et al. (2007)          |
|                       |           |                                                                                         |                                                                                                            |                                                                                             |                                |
| *Rhizobium etli*      | Ani       | Shows 31% and 29% sequence identity with PhaR from *P. denitrificans* and *C. necator*, respectively; possesses putative DNA-binding domain | Deletion of aniA led to disappearance of 795 proteins, including PhaB, resulting into altered global protein expression. | ΔaniA showed significant decrease in PHB content and increase in EPS production.        | Encarnación et al. (2002)       |
|                       |           |                                                                                         |                                                                                                            |                                                                                             |                                |
| *Sinorhizobium*       | MmgR sRNA | Makes more granules Regulator; noncoding 77-nucleotide transcript; Belongs to the orthologous sRNAs rbs1 subfamily | MmgR negatively controls phasin gene expression at post transcriptional level.                              | MmgR finely controlled PHB accumulation. ΔmmgR mutant produced abnormally large amounts of PHB and irregularly shaped granules. | Lagares et al. (2017)           |
| *melloti*             |           |                                                                                         |                                                                                                            |                                                                                             |                                |
| Organism name | Regulator | Description of regulator | Effect on target genes | Effect on PHA metabolism | Reference |
|---------------|-----------|--------------------------|------------------------|--------------------------|-----------|
| Synechocystis sp. PCC 6803 | Slr0058 | Novel regulatory protein, structurally similar to PhaF but lacked the DNA binding domain | Slr0058 regulates PHB synthesis initiation and granule formation. | During vegetative growth, no visible PHB granules were detected in Δslr0058 mutant; during chlorosis, the mutant had abnormally increased number of PHB granules. | Koch et al. (2020b) |
| | PirC | Novel PII interactor of carbon metabolism | PirC negatively regulates PGAM, encoded by slr1945 gene | Deletion of pirC increased PGAM activity, resulting into increased acetyl-CoA production and thus over-accumulation of PHB. | Orthwein et al. (2021); Koch et al. (2020a) |
| | SigE | Group 2 RNA polymerase sigma factor | SigE engineering modified the metabolic pathway from glycogen to PHB biosynthesis during nitrogen depletion, overexpression of sigE increased the levels of enzymes involved in glycogen catabolism and oxidative pentose phosphate pathway. | Overexpression of sigE increased the PHB production levels under nitrogen depletion. | Osanai et al. (2013) |
| | Rre37 | OmpR-type response regulator | Rre37 regulates carbon storage distribution. Overexpression of rre37 accelerated glycogen catabolism at pathway-level. | Overexpression of rre37 enhanced PHB accumulation. | Osanai et al. (2014) |

"/" indicates no target genes reported.
synthesis to avoid the interference by PhaP in the initiation process of PHB synthesis (York et al., 2002). Deletion of phaR in C. necator reduced PHB yield. Intriguingly, the ΔphaRΔphaP1 double mutant produced further reduced PHB yield compared with ΔphaP1 mutant, suggesting PhaR also contributes in promoting PHB synthesis in a PhaP-independent manner in C. necator (York et al., 2002). Possibly, PhaR represses additional PHA-synthesis proteins such as PHA synthase whose inaccurate expression might have hindered PHB production or led to premature PHB utilization (York et al., 2002; Quelas et al., 2016). Once the cells start PHB synthesis, PhaR has the ability to sense the presence of PHB granules and binds to the native PhaP-free PHB granules, thus releasing the inhibitory effect on phaP1 and phaP3 transcription. It was observed that ΔphaP1 mutant synthesized naked PHB granules with their exposed hydrophobic surfaces coalescing to form only one large PHB granule. On the other hand, ΔphaR mutant synthesized large amounts of PhaPs that covered the PHB granule and stabilized them, forming smaller sized PHB granules than the wild-type strain. Thus, it suggests that PhaR-mediated controlled expression of phaP proteins is critical for PHB granule morphogenesis (Pöpper et al., 2002). Finally, at the end of PHB synthesis process, the PHB granules attain a critical volume and the surface area becomes limited (Quelas et al., 2016). Thus, the continuously expressed PhaP1 and PhaP3 proteins displace PhaR, thereby, increasing the cytosolic concentration of free PhaR. These unbound PhaR binds to the phaP1 and phaP3 promoter, inhibiting their transcription. As PhaP increases, it displaces PhaR from PHB granules. As free PhaR increases, it again binds to the phaR and phaP promoter, inhibiting their transcription.

Another PHA granule-associated protein detected in C. necator is PhaM (Fig. 1) (Pfeiffer et al., 2011). It is a 32 kDa DNA-binding protein possessing phasin-like properties and shows non-significant similarity (15%) to phaP1 and phaP3 transcription. It was observed that PhaM binds to the promoter of phaR and phaP and inhibits their transcription. In the presence of nascent PHB chain, PhaR binds to PHB granules and the negative effect of PhaR on phaR and phaP transcription is released and their transcription continues. As PhaP increases, it displaces PhaR from PHB granules. As free PhaR increases, it again binds to the phaR and phaP promoter, inhibiting their transcription.

Fig. 1. Schematic diagram representing regulation of PHA synthesis in C. necator.
A. Regulation of PHA synthesis by PhaR. In the absence of PHB chain, free PhaR binds to the promoter of phaR and phaP and inhibits their transcription. In the presence of nascent PHB chain, PhaR binds to PHB granules and the negative effect of PhaR on phaR and phaP transcription is released and their transcription continues. As PhaP increases, it displaces PhaR from PHB granules. As free PhaR increases, it again binds to the phaR and phaP promoter, inhibiting their transcription.
B. Association of PHB granule with nucleoid via PhaM in C. necator.
C. Regulation of PHB synthesis by PTS system. PTS system regulates PHB synthesis by modulating PHA mobilization system, possibly by transferring the phosphoryl group or influencing ppGpp level via SpoT1. Inactivated PHB degradation may improve PHA accumulation. The red and dashed red arrows indicate repression and proposed repression effects, respectively.
with PhaR protein (Wahl et al., 2012). The predicted structure of PhaM reveals a carboxy terminal similar to histone proteins, which explains the DNA binding ability of PhaM (Wahl et al., 2012). Its amino terminal contains two potential transmembrane domains which might be involved in the binding with PHB granules. The four lysine residues present in the carboxy terminus PAKKA motifs in PhaM is responsible for attaching PHB granules to the nucleoid region (Bresan and Jendrossek, 2017). Under PHA-accumulating conditions, PHB granules synthesized by wild type are attached to the nucleoid region (Wahl et al., 2012). The association of PHB granules with the nucleoid region is probably related to PhaM. PhaM and PhaC1 are constitutively expressed and PhaM directly interacts with PhaC1 and forms the complex of PhaM-PhaC1 even in the absence of PHB. The interaction between PhaM and PhaC1 is specific as PhaM is unable to interact with PhaC of Aeromonas caviae in vitro although both belong to class I synthases (Ushimaru and Tsuge, 2016). The PhaM-PhaC1 initiation complex bound to nucleoid serve as a scaffold for PHB granules (Pfeiffer and Jendrossek, 2013, 2014). During cell division and prior to PHB formation, this initiation complex is colocalized at least two discrete nucleoid-associated foci. As PHB granules formation begin, they attach to the complex following which they distribute among two daughter cells. Thus, each daughter cell receives at least one formed PHB granule. At the later stages of growth, the PhaM and PhaC1 proteins detach from the granules (Bresan and Jendrossek, 2017). The constitutive overexpression of PhaM increased the number of small PHB granules which completely covered the nucleoid surface (Wahl et al., 2012). Deletion of phaM generated fewer PHB granules with a larger diameter compared with the wild-type strain. This is probably because PhaM acts as a physiological activator of PhaC1 as one molecule of PhaM activated 10–11 molecules of PhaC1 (Pfeiffer and Jendrossek, 2014). Moreover, the distribution of PHB granules among the daughter cells in the ΔphaM mutant was hampered as only one of the daughter cells contained PHB granules (Wahl et al., 2012). Thus, PhaM ensures proper segregation of PHB granules into daughter cells during cell division by facilitating the interaction of PHB granules with the nucleoid region. In addition, PhaM also interacts with PhaP5 and forms a PhaM-PhaP5 complex which are attached to the nucleoid region (Pfeiffer and Jendrossek, 2013). In the wild-type strain, phaP5 expression is very low and its deletion did not affect the granule number and localization (Wahl et al., 2012). Notably, when phaP5 was overexpressed, the number of PHB granules increased but their diameter decreased (Wahl et al., 2012). Moreover, the PHB granules were detached from the nucleoid region and were localized near both the cell poles in the form of clusters. It is possible that binding of the overexpressed PhaP5 to PhaM, restricts the binding of PhaM to DNA and/or to PhaC1, leading to detachment of PHB granules from nucleoid region. Alternatively, it is also possible that, the overexpressed PhaP5 displaces the PhaM molecules from the PHB granule surface leading to altered subcellular localization of the granules. Therefore, subcellular localization of PHB granules is determined by integrated expressions of PhaM, PhaP5 and PhaC1 in C. necator.

Another point of PHA regulation in C. necator is exerted by the PTS system (Fig. 1). The sugar PTS system (sugar phosphotransferase system) is a multicomponent system that participates in uptake and concomitant phosphorylation of carbohydrates (Enri, 2013). Its paralog system is PTS Ntr (nitrogen-related phosphotransferase system). Phosphoenolpyruvate (PEP) serves as a common phosphoryl donor in both the systems. Sugar PTS system is carbohydrate-specific where phosphoryl group from PEP is relayed to enzyme I and then to a histidine protein (HPr). Enzyme I and HPr are carbohydrate non-specific. The phosphoryl group is transferred from HPr to the carbohydrate-specific enzyme IIA (Krauš et al., 2009). Enzyme IIB accepts the phosphoryl group from IIA and transfers to the sugar attached to another enzyme IIc. PTS Ntr system is nitrogen-related where the phosphoryl group from PEP is sequentially transferred to nitrogen-related enzyme I (EINtr), nitrogen-related protein (NPr), and then to nitrogen regulatory enzyme II (EIIANtr) (Wang et al., 2005). C. necator possesses an incomplete PEP-PTS system that is composed of enzyme I, HPr and EIIANtr, EIIAMan encoded by ptsI, ptsH, ptsN and ptsM, respectively (Kaddor and Steinbächel, 2011) (Fig. 1). It lacks EIIb and EIIc components and thus cannot phosphorylate sugars. Intriguingly, the C. necator HPr shows 33% amino acid similarity with the NPr of E. coli and it strongly phosphorylates enzyme IIA Nr rather than EIIANtr (Rabus et al., 1999; Krauš et al., 2009). Interestingly, IIA Nr plays a regulatory role in PHB metabolism (Krauš et al., 2009). Inactivation of the ptsN gene resulted in a higher PHB accumulation in the mutant strain (Kaddor and Steinbächel, 2011). Contrarily, deletion of ptsI or ptsH leads to a reduced PHB accumulation when excess carbon source was present. After carbon source was exhausted, the PHB content rapidly reduced in the ptsI or ptsH mutants. Possibly, the PtsI and PtsH proteins regulate the PHB-mobilizing system in this strain (Pries et al., 1991). In the presence of carbon source, these proteins transfer the phosphoryl group to the PHB mobilizing enzyme system and inactivates the latter. Perhaps, in their absence, the PHB-mobilizing system cannot be inactivated, leading to a faster degradation of the accumulated PHB.
Alternatively, it is also possible that these proteins regulate PHB mobilization at the transcription level. In 2014, it has been further suggested that the PTS system affects the stringent response in C. necator which influences the PHB accumulation (Karstens et al., 2014). The stress response during nitrogen limitation is achieved through synthesis of ppGpp nucleotide. SpoT1 and SpoT2 proteins determine the level of ppGpp in cells in response to nitrogen availability. SpoT1 is a bifunctional (p)ppGpp synthase/hydrolase enzyme. However, SpoT2 possesses only ppGpp synthase activity. A spoT1 ΔspoT2 double mutant showed strongly impaired PHB accumulation and a more active PHB mobilization in a connection between stress response and PHB accumulation in C. necator which influences the PHB accumulation (Karstens et al., 2014). Notably, the non-phosphorylated EIIA^Ntr resulted from ptsI or ptsH deletion interacts with SpoT1 (Karstens et al., 2014). Thus, it is possible that this interaction changes the ppGpp level which affects the PHB content of the mutants.

**PHA regulation in Pseudomonas putida KT2440 (or KT2442)**

*P. putida* KT2440 and its spontaneous rifampicin resistant mutant *P. putida* KT2442, are natural MCL-PHA producers and possesses almost identical expression profile (Follonier et al., 2011). In *P. putida* KT2442/KT2440, PhaF, PhaD, Crc, PsrA, RpoN, RpoS, stringent response, PTS system and GacS/GacA system plays regulatory role in PHA synthesis (Fig. 2). The particular effect of the different regulatory factors on PHA synthesis is various other strains of *P. putida* such as *P. putida* GPO1, *P. putida* U and *P. putida* CA-3 have been summarized in Table 1.

The PHA gene cluster of *P. putida* KT2440 comprises of two operons, phaC1ZC2D and phaF (Prieto et al., 2016). The latter operon is located downstream of the former one. The transcription of phaC1ZC2D and phaF are driven by the promoters of phaC1 and phaL, respectively (De Eugenio et al., 2010). The phaC1 and phaC2 genes encode two type II PHA synthases with the preference for 3-hydroxyacyl-CoA with length of C6-C14, phaZ encodes an intracellular PHA depolymerase, phaD encodes a TetR-like transcriptional regulator and phaL and phaF encode PHA granule-associated phasin proteins (De Eugenio et al., 2010). PhaF is involved in maintaining the molecular architecture of PHA granules (Galán et al., 2011; Obeso et al., 2015). According to the structural characterization study from Maestro et al., (2013), PhaF belongs to the intrinsically disordered protein family. PhaF has an interesting structural property as it possesses three separate motifs (Galán et al., 2011; Maestro et al., 2013; Tarazona et al., 2019). The amino terminal region containing the long and uninterrupted amphipathic alpha-helical structure is stabilized by the interaction with PHA granules. The carboxy terminus containing histone-like DNA-binding domain is natively unfolded in the absence of DNA but acquires a super helical structure upon its non-specific binding to DNA. It has been demonstrated that PhaF segregates the PHA granules among the two daughter cells during cell division process by binding to both PHA granules and nucleoid region (Galán et al., 2011; Tarazona et al., 2020). The central core of PhaF contains a leucine zipper motif, which is involved in the oligomerization of PhaF protein. Such kind of coiled-coil sequence is also predicted in the primary structure of Phal, which indicated that PhaF and Phal are likely to form a heterodimer or heterotetramers to stabilize the PHA granules (Maestro et al., 2013). *P. putida* KT2442 uses octanoic acid as the preferred carbon source for PHA synthesis since the expression of phaC1 and phal was higher when octanoic acid was fed than glucose, citric acid or gluconate (Galán et al., 2011). PhaF plays a role in regulating the expression of phaC1 and phal. In the ΔphaF mutant, the transcription level of phaC1 was reduced by 3.5-fold and the highest transcriptional level of phal was differed from the mid-exponential phase to the stationary phase compared with the wild type. Moreover, the PHA granules in ΔphaF mutant failed to distribute among the daughter cells, which reduced the PHA content during continuous fermentation where PHA formation and cell division take place simultaneously (Galán et al., 2011). Hence, PhaF maintains the heterogeneity of the cell population with respect to PHA synthesis and granule distribution. Recently, it has been revealed that PhaF stabilizes PHA granules and functions in properly segregating them during cell division by interacting with the Phal located on the granule surface (Tarazona et al., 2020). Additionally, it is also capable of interacting with PhaD and modulates the regulatory activity of PhaD on transcription of pha operon in *P. putida* KT2442. PhaD is not associated with PHA granules rather randomly localized throughout the cytoplasm. PhaD interacts with the phal and phaC1 promoter regions to activate the transcription of the phalF and phaC1ZC2D operon, respectively. (Hoffmann and Rehm, 2004; De Eugenio et al., 2010).
The upregulation in the transcription levels of the PhaD-regulated PHA genes is more pronounced when octanoate is used compared with glucose. Thus, PhaD serves as a carbon-dependent activator of the \textit{pha} gene cluster in \textit{P. putida} KT2442 (De Eugenio et al., 2010). It is likely that PhaF may act as a coactivator of PhaD as it has been found that presence of PhaF affected the unilateral binding of PhaD to \textit{phaI} promoter (Fig. 2) (Tarazona et al., 2020). However, exact interactive role of PhaF with PhaD and \textit{phaI} is yet to be determined.

There are evidences that \textit{phaIF} and \textit{phaF} transcription in \textit{P. putida} KT2440 are differentially regulated. The \textit{phaF} expression is induced by nitrogen limitation in this strain (Hoffmann and Rehm, 2005). RpoN, RNA polymerase sigma factor 54 (\textit{rpoN}), is a global regulator that affects the transcription of nitrogen-regulated genes. Interestingly in the \textit{ΔrpoN} mutant, \textit{phaF} was expressed irrespective of nitrogen status in the culture. Thus, RpoN acts as a negative regulator of \textit{phaF} expression in \textit{P. putida} KT2440, under nitrogen excess conditions.

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Fig. 2. Schematic diagram representing different types of regulation in \textit{P. putida} KT2442/KT2440.
A. Positive regulation of \textit{phaC1} and \textit{phaI} expression by PhaF.
B. Activation of \textit{phaC1ZC2D} and \textit{phaIF} transcription by PhaD.
C. Negative regulation of \textit{phaF} by RpoN under nitrogen excess condition.
D. Regulation of PHA synthesis by PTS system. Non-phosphorylated EIIANtr in \textit{ΔptsP} or \textit{ΔptsO} inhibits the activity of pyruvate dehydrogenase (PDH) and lowers acetyl-CoA flux for PHA synthesis. \textit{ΔptsN} and the \textit{ΔptsOΔptsN} mutant enhances PDH activity and increases PHA synthesis by channelling more acetyl-CoA.
E. Negative regulation of \textit{phaIF} operon by stringent response.
F. Proposed negative regulation of \textit{phaC1} promoter by RpoS.
G. Repression of \textit{phaIF} operon by Crc protein under conditions of nutrient balance.
H. Proposed involvement of GacS/GacA system in PHA synthesis. Disruption of the \textit{gacS} sensor kinase reduces transcription rate of the entire \textit{pha} cluster.
I. Negative regulation of fatty acid metabolism pathway by PsrA. Deletion of \textit{psrA} genes activates fatty acid metabolism leading to reduced PHA production level. The red and dashed red arrows indicate repression and proposed repression effects, respectively. The green and dashed green arrows indicate activation and proposed activation effects, respectively. The black dashed double arrow represents the existence of interaction.
(Fig. 2). In contrast, the co-transcription of phal and phaF was only induced during nitrogen limitation in both the wild-type and ΔrpoN mutant. Thus, the phaF expression is RpoN-independent. *P. putida* KT2440 possesses a typical PTS\(^{\text{P}}\) system composed of enzyme I\(^{\text{P}}\), histidine protein (NhP) and enzyme II\(^{\text{n}}\), encoded by ptsP, ptsO and ptsN genes, respectively (Fig. 2). PHA accumulation was significantly impaired in the case of ptsP or ptsO deletion (Velázquez et al., 2007). However, PHA synthesis increased for ptsN deletion. Probably, deletion of ptsN gene creates a factual situation of excess carbon source with respect to other limiting nutrients. This channelizes the available carbon source to PHA synthesis. On the contrary, the PHA synthesis machinery of ΔptsP or ΔptsO might sense a shortage of carbon source which directs the carbon source to other metabolic pathways. Intriguingly, the non-phosphorylated EIIA\(^{\text{n}}\) inhibits which directs the carbon source to other metabolic pathways. Interestingly, the *P. putida* as the carbon source, the wild-type showed a significant reduction in PHA degradation rate due to higher expression of *phaZ* gene during the stationary phase (Raiger-Iustman and Ruiz, 2008). Moreover, deletion of the carbon source in this phase lowered the rate of PHA synthesis, resulting in a lower PHA content in the ΔrpoS. Hence, it has been suggested that RpoS negatively regulated *phaC1* promoter, possibly in an indirect manner. *P. putida* KT2442/KT2440 also possesses the GacS/GacA system that is involved in the regulation of its PHA synthesis (Prieto et al., 2016). Disruption of the GacS sensor kinase led to a reduction in PHA synthesis. The transcription rate of the entire *pha* cluster was reduced. However, the underlying mechanism involved in regulating PHA synthesis by GacS/GacA system is not yet determined. In addition, a transcriptional repressor PsrA negatively regulated the genes involved in fatty acid metabolism pathway in this strain (Fonseca et al., 2014). Deletion of *psrA* genes upregulated the *fabB* gene expression and suggested that fatty acid metabolism was more active in ΔpsrA. Thus, less number of intermediates were channelled towards PHA biosynthesis leading to a reduced production level.

Complex regulatory circuit in *Azotobacter vinelandii*

*Azo*to*bat*er *v*inelandii is a free living, nitrogen fixing bacteria capable of accumulating PHB. This species has a complex regulatory circuit which involves PhbR, PEP-PTS system and Gac/Rsm system (Fig. 3). The genome of *A. vinelandii* possesses a biosynthetic phbBAC operon, the regulatory gene phbR encoding the transcriptional regulator of phbBAC (PhbR), the phbP gene encoding the phasin regulator PhbF (known in other bacteria as PhaR) (Segura et al., 2000, 2003; Peralta-Gil et al., 2002). Inactivation of the *phbR* gene reduced PHB accumulation by 70%, and the activity of the acetoacetyl-CoA reductase, β-ketothiolase and PHB synthase enzymes were reduced by 30%, compared with the wild-type strain (Peralta-Gil et al., 2002). Thus, PhbR activates the transcription of the phbBAC operon. Notably, the transcription of phbB gene is driven by two overlapping promoters, pB1 and pB2. In the ΔphbR mutant, the level of transcript corresponding to the pB1 promoter was
significantly reduced and that corresponding to pB2 promoter increased. This indicates that PhbR activates the transcription of \textit{phbB} driven by pB1 promoter. Possibly, in the absence of PhbR, RNA polymerase more efficiently initiates transcription from pB2 promoter.

Similar to \textit{P. putida} KT2440, \textit{A. vinelandii} possesses PTS\textsuperscript{Ntr} system (Enzyme I\textsuperscript{Ntr}, histidine protein NPr and enzyme II\textsuperscript{Ntr}) which is encoded by \textit{ptsP}, \textit{ptsO} and \textit{ptsN} genes. Inactivation of the \textit{ptsP} or \textit{ptsO} gene reduced PHB accumulation, whereas the \textit{ptsN} inactivation doubled PHB synthesis (Segura and Espin, 1998; Noguez et al., 2008). In the case of \textit{ptsP} or \textit{ptsO} inactivation, the terminal phosphoryl acceptor enzyme IIA\textsuperscript{Ntr} is not phosphorylated (Noguez et al., 2008). Further inactivation of \textit{ptsN} in these mutants removes the non-phosphorylated EIIA\textsuperscript{Ntr}, which completely or partially restores PHB accumulation. Intriguingly, these \textit{pts} mutations affect the transcription levels of the \textit{phbR} and \textit{phbBAC} genes. The \textit{phbR} mRNA levels and subsequent transcription of \textit{phbBAC} genes in the \textit{\Delta ptsP} mutant and \textit{\Delta ptsO} mutant are reduced, whereas they increase in the \textit{\Delta ptsN} mutant (Noguez et al., 2008). It suggests that the non-phosphorylated EIIA\textsuperscript{Ntr} represses PHB synthesis by downregulating \textit{phbR} and \textit{phbBAC} expression in \textit{A. vinelandii}. It is noteworthy that the \textit{phbR} expression is regulated by the sigma factor RpoS (Hernandez-Eligio et al., 2011). RpoS serves as an activator in the PHB synthesis process as its inactivation decreased the \textit{phbR} and \textit{phbB} transcript levels and subsequently, reduced PHB accumulation (Fig. 3). A deeper insight into the regulatory role of PTS system reveal that non-phosphorylated EIIA\textsuperscript{Ntr} induces proteolytic degradation of RpoS by ClpAP complex (composed of hexameric ATPase/protein-unfoldase, ClpA and the tetradecameric proteolytic component, ClpP) in \textit{A. vinelandii} (Fig. 3) (Muriel-Millán et al., 2017). RpoS degradation lowers the expression level of \textit{phbR}, the transcriptional activator of \textit{phbBAC}, thereby, diminishing the transcription of the biosynthetic operon. The \textit{phbR} expression is also post-transcriptionally regulated by a small RNA named ArrF (Azotobacter regulatory RNA involving Fe) (Muriel-Millán et al., 2014) (Fig. 3). ArrF links iron concentration in medium and PHA synthesis in \textit{A. vinelandii}. In the condition of iron excess, ferric uptake repressor (Fur) protein, a regulator of iron acquisition, complexes with Fe\textsuperscript{2+} and represses
the transcription of arrF gene. During iron limitation, this repression is released and arrF is transcribed (Jung and Kwon, 2008). Notably, the phbR expression is upregulated and PHB synthesis increased during iron-limiting conditions (Muriel-Millán et al., 2014). The arrF inactivation reduced the phbR and phbB transcripts levels, and PHB accumulation, indicating ArrF sRNA is a positive regulator of phbR expression during iron limitation.

GacS/GacA system composed of the sensor kinase (GacS) and the response regulator (GacA) is a two-component system present in several Gram negative bacteria including A. vinelandii. In the presence of appropriate extracellular signal, GacS protein autophosphorylates (Heeb and Haas, 2001). Eventually, GacA is phosphorylated and then acts as a transcriptional activator of several small RNA (sRNA), also known as Rsm (repressor of secondary metabolites) sRNA. These sRNAs further complex with RsmA protein which is a mRNA binding negative translational regulator (Manzo et al., 2011). The rsm sRNA-RsmA complex post-transcriptionally regulates the translation of the target mRNAs and controls protein synthesis. In A. vinelandii, Gac/Rsm system regulates the expression of pha genes by controlling the post-transcriptional expression of PhbR (Fig. 3). The phosphorylated GacA activates the transcription of one rsmY, and seven rsmZ1–rsmZ7 genes (Manzo et al., 2011). These sRNAs bind to the RsmA protein and counteracts its repressor effect. Deletion of gacS gene resulted in reduced PHB accumulation, indicating that the GacS/GacA cascade plays a positive role in the PHB synthesis (Castaneda et al., 2000). While rsmA deletion increased the PHB production by 25%, indicating that RsmA play a negative role in PHB synthesis (Hernandez-Eligio et al., 2012). Notably, the transcript levels of phbR and phbB was reduced in ΔgacA mutant, whereas they increased in ΔrsmA mutant. This suggests that GacS/GacA system counteracts the RsmA repressor and promotes phbR translation which further activates transcription of phbBAC. On contrary, deletion of gacA reduces the rsm sRNA transcription level, which is insufficient to counteract the repressor effect of RsmA protein. Therefore, binding of the free RsmA protein to 5′ leader region of phbR transcripts leads to degradation of the latter and hence reduces PhbR protein expression, and subsequent PHB production (Hernandez-Eligio et al., 2012).

Another possible regulatory protein involved in PHA metabolism of A. vinelandii is CydR (Fig. 3). It is an oxygen-dependent global regulatory protein. This FNR-like transcription factor necessary for the regulation of cytochrome bd expression in A. vinelandii (Wu et al., 1997). Interestingly, a putative CydR binding site is present upstream of phbB in this strain (Setubal et al., 2009). The ΔcydR mutant showed increased β-ketothiolase and acetoacetyl-CoA reductase activity, compared with the wild-type strain (Wu et al., 2001). PHB was accumulated in the ΔcydR mutant throughout the exponential phase, whereas wild-type accumulated PHB only during the stationary phase. Thus, deletion of cydR imparts a positive impact on the expression of PHA genes and PHA synthesis in A. vinelandii.

Specific regulator-mediated regulation in haloarchaea

The genomes of several haloarchaeal species including H. mediterranei, Haloarcula hispanica, Haloarcula marismortui, Halomicrobium mukohataei, Halorhabdus tiamatea, Haloterrigena turkmenica and Halopiger xanaduensis, harbour a conserved pha gene cluster, phaJ1phaRPEC (Cai et al., 2012). Among them, H. mediterranei is a natural PHBV producer (Koller et al., 2008) and its PHBV synthesis and regulation have been systematically characterized (Lu et al., 2008; Han et al., 2012; Chen et al., 2020). H. mediterranei possesses a set of novel enzymes/pathways for PHBV synthesis that are quite distinct from their bacterial counterparts. The precursors for PHBV monomers are acetyl-CoA and propionyl-CoA. Acetyl-CoA is produced via glycolysis and propionyl-CoA is supplied by four different pathways, citramalate/2-oxobutyrate pathway, the aspartate/2-oxobutryrate pathway, the methylnalonyl-CoA pathway and 3-hydroxypropionate pathway (Han et al., 2013). The two precursors are condensed by two novel β-ketothiolases enzymes (BktB and PhaA). BktB condenses two acetyl-CoA to acetoacetyl-CoA, whereas PhaA condenses acetyl-CoA and propionyl-CoA to form 3-ketovaleryl-CoA (Hou et al., 2013). Unlike the tetrmeric bacterial β-ketothiolases that consists of identical subunits, each BktB and PhaA is composed of two different subunits. The larger subunit (BktBα or PhaAα) is the catalytic subunit and determines the substrate specificity. The smaller subunit (BktBβ or PhaAβ) is essentially involved in the enzymatic activity. Moreover, the catalytic residues (Ser-His-His) in BktBα or PhaAα is distinct from that of the bacterial β-ketothiolases (Cys-His-Cys). Next, the acetoacetyl-CoA reductases (PhaB1 and PhaB2) reduce acetoacetyl-CoA and 3-ketovaleryl-CoA to 3-hydroxybutyryl-CoA (3HB-CoA) and 3-hydroxyvaleryl-CoA (3HV-CoA), respectively (Feng et al., 2010). Finally, the 3-hydroxyacyl-CoA (3HA-CoA) are polymerized into PHBV by a novel class III PHA synthase (PhaEC) composed of two subunits, PhaC and PhaE (Lu et al., 2008). Additionally, its genome contains three cryptic pha genes, phaC1, phaC2 and phaC3. PhaC1 and PhaC3 individually forms an active PHA synthase with PhaE and leads to PHBV synthesis in a PHA synthase gene deletion mutant, H. hispanica PHB-1 (Han et al., 2010). During carbon starvation, enoyl-CoA dehydratase (encoded by phaJ1) mediates degradation of the accumulated PHBV by catalysing the dehydration of (R)-3HA-CoA.
into enoyl-CoA which finally enters the β-oxidation pathway. So far, the regulatory proteins that are known to be involved in PHBV synthesis in *H. mediterranei* are PhaR and PPS (PEP synthetase)-like proteins (Fig. 4) (Cai et al., 2015; Chen et al., 2020).

The *phaP* gene located upstream of the *phaEC* encodes the major phasin protein in *H. mediterranei*. Knockout of the *phaP* gene significantly reduced PHBV accumulation and affected the number and size of PHA granules in *H. mediterranei* (Cai et al., 2012). However, the *H. mediterranei* PhaP shares no sequence similarity with bacterial phasins and its homologs are only distributed among haloarchaea. Probably, the PhaP protein of *H. mediterranei* represents a novel haloarchaeal type phasin family. The *phaP* gene in this strain is co-transcribed with the *phaR* gene. The promoter region of *phaRP* operon consists of four tandemly repeated sequences (Cai et al., 2015). Most likely, this negative cis element acts as the binding site for the PhaR protein. The haloarchaeal PhaR homologs constitute of an AbrB-like domain in the C-terminus, which is critical for the repressor function of PhaR and supposedly acts as the DNA-binding motif. The PhaR protein acts as a negative transcriptional regulator of *phaRP* operon by specifically interacting with its promoter. Importantly, in haloarchaea, PhaR regulates the transcription of *phaR* and *phaP* by binding to their common promoter. However, in bacteria, *phaP* and *phaR* possesses independent promoters and PhaR interacts to the respective promoters to regulate their expression (Maehara et al., 2002; Pötter et al., 2002). Strikingly, PhaR promotes PHBV synthesis in *H. mediterranei* in a PhaP-independent pathway as phaR complementation in ΔphaRP mutant completely restored PHBV accumulation (Cai et al., 2015). Such kind of phenomenon is similar to *C. necator* (York et al., 2002). The ΔphaRP cells produced only one or two medium-sized PHA granules, compared with the multiple moderate-sized PHA granules in the wild-type strain (Cai et al., 2015). Complementation of only phaR in ΔphaRP led to the synthesis of one or two large-sized PHA granules. On the other hand, only phaP complementation produced several irregularly shaped small- or medium-sized PHA granules. A similar granule morphology was also observed when *phaP* was overexpressed using a strong promoter in ΔphaRP. Thus, it was suggested that PhaR controlled the *phaP* expression that might be necessary for synthesis of PHA granules with regular morphology in *H. mediterranei*.

The PEP/pyruvate interconversion in *H. mediterranei* is mediated by *pyk* and *pps* genes (Chen et al., 2019). The *pyk* gene encoding pyruvate kinase catalyses the conversion of PEP to pyruvate whereas the reverse reaction is catalysed by PEP synthetase, encoded by *pps* gene. Deletion of the *pps* gene led to a 35.9% increase in PHBV production most likely by channelling more pyruvate towards PHBV synthesis. Intriguingly, *H. mediterranei* genome possesses a novel protein, PPS-like, that shows high homology with PPS protein. However, this protein is not involved in the PEP/pyruvate interconversion, instead, deletion of the *pps*-like gene led to a 70.46% increase in PHBV accumulation. It has been speculated that PPS-like protein evolved as a regulator protein from the PPS protein (Chen et al., 2020). Deletion of *pps*-like gene promoted the expression of the

![Fig. 4. Regulation of PHBV synthesis in *H. mediterranei*. A. PhaR negatively regulates its own and *phaP* expression by binding to their common promoter. B. Negative regulation of *pha* genes by PPS-like protein. The red arrow indicates repression effect.](image-url)
PHA monomer supplying pathway and upregulated the expression of the phaEC, phaR and phaP genes in H. mediterranei. It activates the transcription of the three cryptic genes, especially phaC1 (Chen et al., 2020). Moreover, both PhaC1 and PhaC3 forms functional PHA synthase with PhaE and leads to PHBV synthesis in the Δpps-likeΔphaC mutant of H. mediterranei. Intriguingly, PPS-like protein effectively interacts with the promoter region of the phaC1 gene and forms protein-DNA complex. This indicates that PPS-like protein acts as a repressor of the phaC1 expression and hence, its gene deletion leads to an upregulation of the phaC1 expression. Such type of regulatory function of PPS-like in PHA synthesis is reported for the first time and thus requires further characterization.

Regulation in other bacteria
PhaQ, a transcriptional regulator in Bacillus megaterium. PhaQ is a transcriptional regulatory protein identified in Bacillus megaterium (Lee et al., 2004). PhaQ differs from the PhaR protein of C. necator in amino acid sequence, N-terminal portion, cis-acting element sequence, as well as size (Maehara et al., 2002; Pötter et al., 2002). It has high amino acid sequence similarity with hypothetical PhaQs from Bacillus anthracis and Bacillus cereus (Ivanova et al., 2003; Lee et al., 2004). Possibly, PhaQs constitute a separate class of transcriptional regulator of PHB synthesis in Bacillus species. PhaQ in B. megaterium negatively autoregulates its own expression by interacting with its own promoter region and interfering the binding of RNA polymerase with its promoter (Lee et al., 2004). The phaQ gene is located upstream of the phaP gene and they are co-transcribed (McCool and Cannon, 1999; Lee et al., 2004). The genome of this strain consists of another pha gene cluster, phaRBC, transcribed as a tricistronic operon where phaR and phaC encode heterodimeric PHB synthase subunits. Generally, PhaP proteins are abundant and the concentration of transcriptional regulatory proteins like PhaR or PhaQ is low in PHA-producing bacteria (Lee et al., 2004). In B. megaterium, the intergenic region of phaQ-phaP shows no existence of a promoter. Thus, the differential expression of PhaQ and PhaP is possibly because the cell selectively degrades the phaQ transcript in the phaQP cotranscript by its post-transcriptional machinery, leaving the phaP transcript intact (Lee et al., 2004). The expression level of PhaP protein was low in B. megaterium with phaQ overexpressed. This indicates that PhaQ negatively regulates the expression of phaP in B. megaterium. PhaQ interacts with DNA and artificial PHB granules in vitro. It is also a PHB-responsive repressor as it could sense the presence of PHB granules in vivo. Perhaps, PHB may act as an inducer for phaP expression in the PhaQ-mediated regulation.

Novel regulatory proteins in Synechocystis sp.. Synechocystis sp. PCC 6803 is a PHA-accumulating cyanobacterium. During nitrogen depletion, this strain undergoes chlorosis in which the cells first accumulate glycogen and later synthesize PHB by metabolizing the intracellular glycogen pool (Koch et al., 2019). Two novel proteins, Str0058 and PirC, are known to regulate PHB synthesis in this strain (Koch et al., 2020). Additionally, response regulator Rre37 and SigE also regulates PHB synthesis in this strain. Str0058 shows structural similarity with regulatory phasin PhaF from Pseudomonas spp. but lacks the DNA binding domain (Koch et al., 2020). The Δstr0058 mutant synthesizes PHB but strikingly, no visible PHB granules were detected during the vegetative growth; whereas, it had abnormally increased number and improperly aggregated PHB granules during chlorosis. Possibly, Str0058 is involved in initiation of PHB granule formation. During vegetative growth, Str0058 aggregates in foci and then disappears during chlorosis. In the absence of Str0058, the PHB granules aggregates in an uncontrolled manner. Thus, Str0058 is a novel regulatory protein involved in the controlled PHB synthesis and granule formation in Synechocystis sp. PCC 6803.

The other protein PirC negatively regulates the activity of 2,3-phosphoglycerate-independent phosphoglycerate mutase (PGAM) catalysing the conversion of 3-phosphoglycerate to 2-phosphoglycerate in lower glycolysis leading to acetyl-CoA production (Orthwein et al., 2021). Under sufficient nitrogen concentration, PirC protein complexes with a signal processor protein, PII. However, during nitrogen limitation, PirC is released from the complex and it inhibits the PGAM activity. Thus, the carbon flux is re-directed towards glycogen synthesis instead of acetyl-CoA formation. Interestingly, deletion of pirC led to increased glycogen catabolism, leading to acetyl-CoA production and hence, over-accumulation of PHB under nitrogen limitation in this strain. Further expression of the phaA and phaB genes from C. necator, under the control of a strong promoter, in the pirC-deleted mutant of Synechocystis sp. PCC 6803 significantly improved PHB production (Koch et al., 2020). During nitrogen/phosphorus limitation and in the presence of acetate as carbon source, this ΔpirC mutant accumulated up to 81% (wt) PHB compared with only 32% (wt) by wild type under alternating light/dark regime. Thus, it is evident that cultivating regulatory mutants in optimized culture conditions is a promising method to maximize PHA production.

SigE, a group 2 RNA polymerase sigma factor, is reported to regulate PHB biosynthesis in Synechocystis
Regulation of polyhydroxyalkanoates synthesis

Overexpression of sigE increased PHB production under nitrogen depletion. Moreover, the enzymes involved in glycogen catabolism and oxidative pentose phosphate pathway were upregulated. Thus, engineering of sigE modified the metabolic pathway from glycogen to PHB biosynthesis during nitrogen depletion. Another regulator that regulates carbon storage distribution in this strain is Rre37. This OmpR-type response regulator is induced by nitrogen depletion. Overexpression of re37 decreased the glycogen level as it accelerated the catabolism of glycogen and enhanced PHB accumulation (Osanai et al., 2014). Overexpression of sigE and re37 further enhanced PHB accumulation. Rre37 preferentially activated phaA and phaB, whereas SigE activated phaC and phaE expressions.

**PHB synthesis regulation in Herbaspirillum seropedicae SmR1.** NtrB/NtrC system is a two-component sensor-activator regulatory system comprising of a signal transduction protein (P_II and/or P_2), effector protein, sensor (NtrB) and regulatory (NtrC) protein. It is the key regulatory system for nitrogen metabolism in bacteria. During nitrogen limitation, the nitrogen sensing protein, uridylyl transferase/uridylyl-removing enzyme uridylylates P_II or P_2 protein (Persuhn et al., 2000). The uridylylated P_II or P_2 protein enables autophosphorylated NtrB to transfer a phosphoryl group to NtrC. NtrC acts as a transcriptional regulator that controls the expression of nitrogen-dependent genes. In the presence of excess nitrogen, P_II or P_2 protein is non-uridylylated that inhibits the kinase activity of NtrB. Moreover, it stimulates the phosphatase activity of NtrB that results in non-phosphorylation of NtrC, thereby inactivating the regulatory function of NtrC (Hervás et al., 2010). Herbaspirillum seropedicae SmR1 is a PHA-accumulating nitrogen-fixing diazotrophic bacterium belonging to β-Proteobacteria. This strain possesses the components of the NTR system. Deletion of the ntrC gene increased the PHB accumulation, irrespective of the nitrogen status (Sacomboio et al., 2017). A deeper insight shows that ntrC deletion upregulated the expression of zwf gene, which encodes G6PDH (glucose-6-phosphate dehydrogenase). In the ΔntrC mutant, the G6PDH activity and NADPH/NADP⁺ ratio increased by 2.8-fold and 2.1-fold, respectively. The increased NADPH favours PHB biosynthesis as it is a cofactor for PhaB. Moreover, it also creates a higher pool of reducing power and PHB generation acts as a redox sink. Thus, up-regulation of zwf gene is the key factor leading to higher PHB production in the ΔntrC mutant (Sacomboio et al., 2017).

There exists a cyclic dependency between PHB synthesis and redox-responsive transcriptional regulator Fnr, in *H. seropedicae* SmR1. Transcriptional activation of phaC genes by Fnr is required for proper PHB synthesis, whereas optimal PHB production is important for maintaining the Fnr activity (Batista et al., 2018). Deletion of the three Fnr encoding genes reduced PHB accumulation and the transcription levels of the three phaC genes (phaC1, phaC2 and phaC3). During oxygen limitation, the absence of PHB in the ΔphaC1 mutant disturbed the redox balance of the cell as the mutant was unable to maintain the NAD(P)H/NAD(P)⁺ ratios, resulting in increased sensitivity of the cells to oxidative stress. Notably, the redox balance of cell is Fnr-dependent because synthesis of cytochrome c proteins involved in electron transport chain is regulated by Fnr. Thus, redox balance and PHB biosynthesis is tightly controlled in *H. seropedicae* SmR1.

**Herbaspirillum seropedicae** SmR1 also has a special type of PHB synthesis regulation known as responsive backup circuit (RBC). The genome of this strain consists of three genes encoding putative phasins, phaP1, phaP2 and phaP3 (Alves et al., 2016). PhaP1 is the major phasin followed by PhaP2 in wild type, while PhaP3 is very less abundant and detectable only in the ΔphaP1 mutant. The expression of both the phaP1 and phaP2 genes are regulated by the PhaR regulator (Kadowaki et al., 2011). PhaR possesses 83% sequence identity with C. necator PhaR and is also capable of binding to DNA and PHB granules. It represses the expression strength of its own promoter and phaP1 and phaP2 promoters. In the wild-type strain, the transcription strength of phaP2 promoter was 8-fold lower compared with the phaP1 promoter (Alves et al., 2016). Strikingly, the expression of phaP2 increased by 6-fold in the ΔphaP1 mutant. The expression of phaP2 is not fully activated in wild type but it serves as a backup phasin gene to guarantee proper phasin expression and allows PHB synthesis to some extent in the ΔphaP1 mutant. Such a regulation type, known as RBC, increases robustness of the microorganisms under unfavourable conditions.

**Riboregulation in Sinorhizobium meliloti.** Small RNAs are known to regulate complex metabolic processes by modulating the expression of multiple target genes in response to various stimuli (Beisel and Storz, 2010). Sinorhizobium meliloti produces PHB under unbalanced growth conditions. The fate of the carbon source in such unbalanced conditions is strictly regulated by MmgR sRNA in this strain. MmgR (Makes more granules Regulator) is a negative regulator of PHB accumulation (Lagares et al., 2017). During growth, depletion of nitrogen source acts as a stimulus for inducing mmgR expression. Once MmgR sRNA is activated, it finely tunes the PHB synthesis. It limits the PHA accumulation to an optimum level, despite of the presence of excess carbon.
source in the medium. Clearly, PHB accumulation was enhanced and granules were irregularly shaped in the ΔmrrR mutant. This was accompanied by overexpression of the PhaP1 and PhaP2 proteins. Thus, MrrR sRNA mediated PHB accumulation in S. meliloti involves negative post-transcriptional regulation of PhaP expression.

**PHA synthesis regulation exerted by Quorum sensing and other global regulators.** Quorum sensing (QS) is a global regulatory mechanism of gene expression in response to extracellular concentration of certain signal molecules called autoinducers (Bassler, 2002). The concentration of the autoinducers increase with bacterial cell density, and hence QS system allows bacteria to monitor their population density by altering various gene expressions (Venturi, 2006). Gram negative bacteria use acylated homoserine lactone (AHL) as autoinducer signal molecules. In *Pseudomonas aeruginosa*, QS system via LasI/LasR participates in regulating PHA biosynthesis (Xu et al., 2011). LasI, an AHL synthase, catalyses the production of 3-oxo-dodecanoyl homoserine lactone (3-O-C12-HSL) as a signal molecule which binds to the transcriptional regulator, LasR (Steindler et al., 2009). The 3-O-C12-HSL interacts with the N-terminal domain of the LasR protein and induces structural changes that allows the protein to bind to the DNA sequences of target genes and regulate their transcription (Kiratisin et al., 2002). Deletion of lasI or lasR or both in *P. aeruginosa* reduced the PHA content (Xu et al., 2011). Moreover, the mutants showed significantly reduced levels of phaC1 expression. Possibly, LasR mediated QS system downregulates phaC1 expression that reduces intracellular PHA biosynthesis in *P. aeruginosa*.

*Pseudomonas chlororaphis* PA23 has three distinct QS systems, Phzl/PhzR, CsaI/CsaR and AurI/AurR, among which Phzl/PhzR is known to affect PHA biosynthesis as its deletion led to a reduced PHA production (Nandi et al., 2016; Mohanan et al., 2019). This strain also possesses another global regulator ANR which is an oxygen-sensitive transcriptional factor (Sawers, 1991). ANR acts by binding to the conserved anr box present in the promoter of its target genes (Mohanan et al., 2019). In *P. chlororaphis* PA23, ANR and Phzl/PhzR are subjected to cross-regulation (Nandi et al., 2016). ANR positively regulates phzl and phzR, whereas PhzR negatively regulates anr transcription. Intriguingly, both the QS-deficient and Δanr mutant showed similar levels of reduced PHA accumulation. However, the pha gene expression profiles in the two mutants were different. The downregulation of the pha genes including phaC1, phaC2, phaZ, phaD, phaF and phal were more pronounced in QS-deficient strain than Δanr mutant. Notably, the promoter region of the anr gene possesses a phz box which suggests that it is recognized by the PhzR, activated by AHL. Thus, it is possible that the effect of ANR on PHA synthesis is mediated via QS system (Mohanan et al., 2019). Moreover, as ANR protein is a global regulator affecting multiple genes, it possibly has an overlapping effect on PHB production. It is revealed that anr deletion in *P. pseudomomas extremasulis* decreases the oxidative stress resistance and disturbs the redox balance maintenance under limited oxygen supply (Tribelli et al., 2013). Furthermore, recent studies show that ANR-controlled genes include those related to central carbon metabolism and regeneration of NADPH in *P. putida* (Tribelli et al., 2019). Taken together, all these factors probably have an added influence resulting in a reduced PHB accumulation in the Δanr mutant of *P. chlororaphis* PA23 (Mohanan et al., 2019).

In *Burkholderia chlororaphis*, QS is a complex regulatory circuit. The scmR gene is a global regulator for synthesis of various secondary metabolites and also represents a key component of the QS system in this strain. Possibly, ScmR controls gene expression by regulating synthesis of AHL signalling molecule, or by directly binding to target genes, or by indirectly modulating gene expression via intermediate regulators (Le Guillouzer et al., 2020). *B. thailandensis* possesses three LuxI/LuxR-type QS systems, BtaI/BtaR1 (QS-1), BtaI2/BtaR2 (QS-2) and BtaI3/BtaR3 (QS-3). Among them, BtaI1, BtaI2 and BtaI3 are Lux-type synthases, and BtaR1, BtaR2 and BtaR3 are the LuxR-type transcriptional regulators (Le Guillouzer et al., 2017). In the ΔbtaR1 and ΔbtaR3 mutants, the transcription level of scmR gene was low, indicating that it is activated by QS-1 and QS-3 (Martinez et al., 2020). Interestingly, an interdependence between the QS-1 and QS-3 systems exists in this strain where BtaR1 possibly modulates QS-3 system and *vice versa* (Le Guillouzer et al., 2017). scmR deletion reduced phaC expression and led to 50% decrease in PHA production (Martinez et al., 2020). Complementation of the scmR gene restored the wild-type PHA production. Hence, ScmR positively affects PHA accumulation in *B. thailandensis*. Notably, the ΔbtaI3 or ΔbtaR3 mutant showed decreased PHA accumulation which might be due to the reduced expression of scmR gene. Thus, QS-3 positively controls the PHA synthesis probably by modulating the expression of scmR gene. Hence, QS-dependent regulation of the scmR gene contributes to the regulation of PHA synthesis in *B. thailandensis* (Martinez et al., 2020).

**Conclusions**

Bioplastics are an alternative solution to the increasing plastic pollution caused by over usage of synthetic...
Regulation of polyhydroxyalkanoates synthesis

plastics. PHA are promising bioplastics having potential application in various sectors including biomedical, and packaging industries. The main objectives of PHA research is to facilitate synthesis of PHA with novel characteristics and also with characteristics similar to the plastics so as to offer a greener alternative to the existing products. Moreover, to promote commercial PHA production for widespread applications. To achieve these, strain improvement is a basic requirement. Designing microbial strains having improved PHA-producing capabilities would reduce production cost of bioplastics. Most studies have showed that engineered strains for PHA will increase PHA biosynthesis and their competitiveness. However, genetic modification of organisms can have unpredictable consequences. Transferring gene from one microorganism to another, gene deletion or rearrangement of gene sequences can result into genetic instability or affect other physiological functions of microorganisms that can create obstacle for industrial applications. A single gene may be regulated by several regulatory factors and may control different physiological traits. Therefore, knowledge of regulatory mechanisms is important and might help to design improved strains with better genetic stability. Regulation is an integral part of PHA synthesis. Identifying the key players of PHA regulation and a deeper characterization of the regulatory pathway is necessary. In this review, different regulatory mechanisms involved in PHA synthesis from typical bacteria and haloarchaea have been discussed. Types of regulation in PHA metabolism is quite diverse, ranging from global regulators to specific regulators. Most organisms possessed multiple regulation models which affected the expression of pha gene cluster either positively or negatively. Notably, these regulators controlled PHA metabolism in different organisms at different levels. This review provided a simplified idea on how the regulatory models operated in some specific PHA-accumulating bacterial and haloarchaeal species. Such information on PHA regulation is crucial as it would direct the PHA researchers to develop improved PHA-producing microbial strains with the aid of metabolic engineering.

However, not all regulators involved in PHA synthesis have been identified till now. Hence, continued effort and further investigation is needed to elucidate the unknown regulatory circuits present in PHA metabolism. Moreover, the already characterized regulation models have been hardly applied for PHA production improvement. Thus, it is necessary to implement these regulation models in research work to achieve engineered strains with improved PHA production ability.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Fig. S1.** Natural pathways for SCL-PHA synthesis. Key enzymes involved are (1) β-ketothiolase, (2) 3-ketoacyl-CoA reductase, (3) Succinate semialdehyde dehydrogenase, (4) 4-hydroxybutyrate dehydrogenase, (5) 4HB-CoA transferase, (6) PHA synthase.

**Fig. S2.** Natural pathways for MCL-PHA synthesis. Key enzymes involved are (1) Enoyl-CoA hydratase (PhaJ), (2) β-ketoacyl-ACP reductase (FabG), (3) 3-hydroxyacyl-ACP: CoA transacylase (PhaG), (4) 3-hydroxyacyl-CoA ligase, (5) PHA synthase.