

# Polyhydroxyalkanoates (PHAs) Database: Genomics of Polyhydroxyalkanoates (PHAs) Biosynthesis

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## Abstract

Plastics are the most widely used synthetic polymers. Due to their non-degradative nature, synthetic polymers have become an environmental eyesore. Biodegradable plastics like Polyhydroxyalkanoates (PHAs) comprise a group of natural biodegradable polyesters that are synthesized by microorganisms. Polyhydroxyalkanoates were firstly discovered in prokaryotes as carbon and energy storage materials. Plastics are utilized in almost every sector. Plastics being xenobiotic are recalcitrant to microbial degradation. Polyhydroxyalkanoates have gained major importance due to their structural diversity and close analogy to plastics. Polyhydroxyalkanoates have promising properties such as high biodegradability in different environments. Polyhydroxyalkanoates can be degraded by many microorganisms using intracellular or extracellular PHA depolymerases. PHA depolymerases are very diverse in sequence and substrate specificity but share a common  $\alpha/\beta$ -hydrolase fold and a catalytic triad, which is also found in other  $\alpha/\beta$ -hydrolases. Polyhydroxyalkanoates, a biodegradable plastic, was produced in microorganisms and was first discovered by Lemoigne in 1925. It has a relatively high melting point and it gets crystallized rapidly. It has high melting temperature (175°C) and relatively high tensile strength (30-35 MPa).

Polyhydroxyalkanoates database is a single repository of genes and its genomic information is responsible for Polyhydroxyalkanoates to synthesize biodegradable plastics. It is based on genomic characterization of intermediates of Polyhydroxyalkanoates (CAB genes, responsible for biodegradable plastic synthesis) metabolic pathway.

**Keywords:** Polyhydroxyalkanoates; Database; Gensolution; PHA; Plastics; Biosynthesis; Biodegradable plastics; Biodegradable polymers; CAB genes

## Introduction

The PHB biosynthetic genes *phbA*, *phbB* and *phbC* are clustered and organized in one *phbCAB* operon but the similarity in the mechanisms of regulation of these divergent operons is as yet unknown. Structural studies will further improve our understanding of the mechanism of action of these enzymes and aid us in improving and selecting better candidates for increased production. Study on enzyme, PHA synthase, activity of extremely halophilic archaeon, *Haloarcula marismortui*, has suggested that PHA is constitutively expressed independent of nutrient rich or nutrient-limited conditions [1]. Polyhydroxyalkanoates (PHA) are produced in organisms under certain conditions with the help of the following enzymes: i)  $\beta$ -ketoacyl CoA thiolase (PhaA-EC 2.3.1.9), ii) NADPH dependent Acetoacetyl CoA reductase (PhaB-EC 1.1.1.36) and iii) PHA synthase (PhaC-EC 2.3.1.41). PHAs are gaining attention among biodegradable polymers due to their promising properties such as high biodegradability in different environments. At present, research is going on for high PHA producers. 253 sequenced genomes have been used for phylogenetic and statistical analyses of 3 genes which are involved in PHA biosynthesis. These have revealed around 24 organisms with an ability to acquire and adapt these genes from taxonomically distant relatives primarily through horizontal gene transfer (HGT) events. Microbes acquire or lose genetic material in an effort to encounter adverse environmental conditions, which threaten their existence. They undergo modification(s) of the existing regulatory mechanisms(s) or develop novel operons. These organisms may prove more amenable to genetic modifications using recombinant DNA technology. Certain among these organisms have the ability to use a wide range of industrial wastewater, degrade environmental pollutants, etc. This raised the intriguing possibility that they could be exploited to both breakdown wastes and produce PHAs.

PolyHydroxyAlkanoates Database supports query search on PHA biosynthetic genes by maintaining a comprehensive, non-redundant,

well-organized and freely available database. Presently the entries in this database are clustered in 23 different taxa. The Polyhydroxyalkanoates database is a genomic database of archaeal, bacterial and eukaryotic genomes having all or some of the PHA genes. The current version of the database includes statistical parameters as well as information about PHA genes, which deviate in certain parameters: G+C content, codon usage (Relative Synonymous Codon Usage, RSCU),  $\chi^2$  (ChiSquare) values. Under the hypothesis that genes from distantly related species have different nucleotide compositions and that these deviations in PHA genes may have been acquired by HGT.

## Features of the Polyhydroxyalkanoates Database

Author has created the database to facilitate compositional analysis and provide additional evidence for discussing the possible origin of PHA genes. The current version of the database contains organisms that are sorted alphabetically and classified taxonomically. It is the first database on genes of PHA metabolism, which could be extracted from the sequenced genomes. Purpose of developing such a database is to establish the database of a list of organisms as well as genes and its related physical and chemical properties of genes which are able to synthesize biodegradable plastic [2]. This database becomes helpful for the researchers who are working on such biodegradable plastic synthetic project. It provides researchers with this information on G+C

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content, codon usage (Relative Synonymous Codon Usage, RSCU),  $\chi^2$  (Chi-Square) values for each gene and its host genome for ready usage.

To fetch the query result from database, two major independent modules are designed which are useful to analyze the CAB genes of organism. To access database, the web page is designed which is good to look at, user friendly and completely validated which provide easy to query search on database. The home page of Polyhydroxyalkanoates database provides facilities to query search by entering search key of organism's name or by clicking on alphabetic list of organisms/taxons. The Polyhydroxyalkanoates database has the following features:

### Non-redundancy

The database is non-redundant. The redundancy of the database is fully controlled for improving the performance of queries and for saving storage space on the database server.

### Classification

The entries of this database are grouped in 23 different taxons. The organisms having phaCAB gene(s) are linked with taxon from which it belongs taxonomically. The records of the gene in organism sub-group are identified by unique record ID. Each record ID has associative amino acid record and preceding and succeeding gene record with the respective sequence.

### Cross references

To optimize information retrieval, PHA records are cross-referenced to NCBI database. The enzymes located on reference pathway of Butanoate metabolism are also crossreferenced to the KEGG and Biocarta databases [3].

### Information retrieval

The database serves as a major information resource to support biodegradable intense researches in the area of plastic synthesis. Retrieval and knowledge discovery are facilitated by search options (prompting organism name, present alphabetic list of the organism and hierarchy of taxons). The alphabetic list makes it possible to rapidly retrieve information of organism of pha genes.

### Architecture model

The Polyhydroxyalkanoates database is based on Client/Server 3-Tier architecture computing model. The TP Monitor is used as a middle layer to establish the link between Client's request and Server's response.

### Database Content and Access

The Polyhydroxyalkanoates database is created in order to store all the sequence and statistical parameters of those genes which are involved in Polyhydroxyalkanoates metabolism pathway for bioplastics synthesis in a single place. This database also includes the preceding and succeeding genes of phaCAB genes present on genome map. The database also provides storage place of organism and taxon in which the genes are clustered.

Currently, there are 23 taxons, 233 organisms and 381 (*phaA*-111, *phaB*-199, *phaC*-71) genes entries in the database. Each gene has nucleotide sequence, amino acid sequence and the details of preceding and succeeding genes with associated parameters.

The Polyhydroxyalkanoates database home page of GenSolution website provides space to prompt the organism name. For easy access, home page provides alphabetic list of organism names. The taxon

link displays the hierarchy list of taxons and nested organisms. After entering the organism name or on clicking resultant organism name, user will get the result. The result page provides phaCAB (*phaA*, *phaB*, *phaC*) genes in separate column with all parameters in rows. The search results also provide further links to the nucleotide and amino acid sequence of individual genes and details of preceding and succeeding genes. On clicking GI Number (located amino acid, preceding and succeeding gene result page) user finally links with NCBI database for associated gene. Pathway Information hyperlink provides the reference pathway of Butanoate metabolism for PHA production.

### Database Organization

The Polyhydroxyalkanoates database which is available on GenSolution website is nonredundant. All the operations on the query search are basically performed on the organism name. First search the query search module; if found then find the genotype(s) of the respective organism ID (maintained by the database organization). Now it gives the detail information of the gene(s) -phaCAB. The cross-reference for individual enzymes of a particular phaCAB gene with NCBI database provides more details of gene and sequence. Each result page provides hyperlinked id for respective Amino acid sequence and the preceding and succeeding gene. All the organism records are maintained by super-group of referenced taxon.

Check for query program syntax. Data integrity and hyperlinked reference ID are run on all entries standardization rules are revised; such type of cross check is maintained by using reserved id of each entities.

Standardization rules and controlled vocabularies are applied for organism names, gene names, genetic information and all other fields. The used keywords to represent all information of the database are derived from many authoritative sources whenever possible. The GenSolution website provides the details of the used keywords [4].

### Data Extraction

The list of taxon and organisms name has been narrowed down from NCBI and KEGG genome databases. Two global databases (KEGG & Biocarta) have a collection of metabolic pathways which helps to identify the CAB genes responsible for Polyhydroxyalkanoates. The NCBI database resource helps to collect properties of all genes which are present in Polyhydroxyalkanoates database [5-7].

### Availability

The PolyHydroxyAlkanoates Database is freely available on GenSolution website which is accessible at [http://www.gensolution.org/pha\\_gene\\_db.asp](http://www.gensolution.org/pha_gene_db.asp) or [http://gensolution.azgroup.org/pha\\_gene\\_db.asp](http://gensolution.azgroup.org/pha_gene_db.asp). All comments, queries and correction should be sent by email to [gensolution@az-group.org](mailto:gensolution@az-group.org) or [dralamgeer@az-group.org](mailto:dralamgeer@az-group.org). For this, the website provides feedback and contacts link on home page of GenSolution website (<http://www.gensolution.org>).

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