



# International Journal of Applied Sciences and Biotechnology

**ISSN 2091-2609**

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**CODEN (Chemical Abstract Services, USA): IJASKD**

**Vol-3(3) September, 2015**

**Available online at:**

<http://www.ijasbt.org>

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**Impact factor\*:** 1.422

**Scientific Journal Impact factor#:** 3.419

**Index Copernicus Value:** 6.02

**IBI Factor 2015\*\*:** 4.19

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Research Article

## A METAGENOMICS ANALYSIS ON B-CAROTENE SYNTHESIS IN *NEUROSPORA CRASSA*

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### Abstract

We have studied *insilico* on evolutionary uniqueness of phytoene synthase, which is one of the regulatory enzymes of  $\beta$ -carotene synthesis in *Neurospora crassa*. This study reveals multiple sequence alignments showed high sequences with similarity within a species of bacteria, fungi and higher plants. This results designate interestingly between species of bacteria-fungi, fungi-plant, and among the species of bacteria-fungi-plant, showed tremendously less sequence with similarity, except bacteria-plant (high sequence with similarity) respectively. In Phylogenetics tree analysis showed within species of bacteria, fungi and plant 91%, 92% and 99% homology. Whereas in between species of bacteria-fungi, bacteria-plant, fungi-plant, and among the species bacteria-fungi-plant showed 99%, 96%, 100%, and 91%-99% homology respectively. *N. crassa* phytoene synthase enzyme encode (Isoprenoid Biosynthesis enzymes, Class 1) protein size 610aa, Cyanobacteria phytoene encode (Isoprenoid Biosynthesis enzymes, Class 1) protein size 310aa, and *Oryza sativa Indica* phytoene synthase 1 (chloroplast), (Isoprenoid Biosynthesis enzymes, Class 1) encode protein size 421aa (*e*- value 0.0, 0.0 and 0.0; identity 100%, 100% and 100%; Max.score:1238, 644 and 870) respectively. We studied *insilico* on basis of an evolutionary Endosymbiotic theory; a bacterium is the ancestors to eukaryotes.

**Keywords:** Beta-Carotene; Phytoene synthase (psy); Carotenoids; *Neurospora crassa*; *Oryza sativa Indica*; Cyanobacteria.

### Introduction

Carotenoids are yellow, orange, and red pigments; which are widely distributed in nature (Goodwin, 1988) and extensively accumulate in some flowers and fruits (Suzuki et al., 2007; Tanaka et al., 2008). Carotenoids are 40 carbon isoprenoids units, and polyene chains containing conjugated double bonds. Carotenoids play crucial roles in structure and function of the photosynthetic apparatus of bacteria, algae, fungi and higher plants (Demmig-Adams and Adams, 1993, Goodwin, 1980). The most abundant Carotenoids are  $\beta$ -carotene, which accumulates as the end product in carotenogenic species of the Zygomycotina, Ascomycotina and Basidiomycotina (Goodwin, 1980). The Carotenoids biosynthesis has been studied extensively in the filamentous fungus, *Neurospora crassa* (Harding et al., 1969; Harding and Shropshire, 1980; Lauter and Russo, 1991; Morelli et al., 1993; Sandmann, 1993; Eberle and Russo, 1994; Li and Schmidhauser, 1995), Four genes are known to be involved in Carotenoids biosynthesis in *N. crassa*; three albino genes (*al-1*, *al-2*, *al-3*) and yellow-1 gene (*yl-1*) (Goldie and Subden, 1973; Harding and Turner, 1981; Schmidhauser et al., 1994). The *al-2* gene encodes protein product, phytoene synthase, which catalyzes the condensation of two 20-carbon prephytoene pyrophosphate molecules (Harding and Turner, 1981;

Schmidhauser et al., 1994; Perkins et al., 2001) and possibly cyclization of lycopene to  $\beta$ -carotene and torulene (Verdoes et al., 1999). In Plants, Carotenoids are one such group of compounds that are synthesized in the plastids, chloroplasts and chromoplasts (Hirschberg, 2001) respectively. These compounds are important in nature as they are involved in light-harvesting, photo protection, and pollinator attraction in plants (Tracewell et al., 2001; Szabo et al., 2005; Dong et al., 2007).

The availability and analysis of *N. crassa* pigment mutants helped to establish the carotenogenic pathway in the fungus (Goldie and Subden, 1973). The first committed step is the condensation of two molecules of geranyl geranyl pyrophosphate (GGPP) to form phytoene, catalyses by the enzyme phytoene synthase (PSY), a transferase enzyme; it needs  $Mn^{+2}$  for its activity. In bacteria, the desaturation of phytoene is by one enzyme, carotene desaturase (CrtI) but in plants required two enzymes, phytoene desaturase (PDS) and zeta-carotene desaturase (ZDS) (Cunningham et al., 1994; Hirschberg, 2001, Bartley et al., 1999). The Carotenoids pathway branches at the cyclization of lycopene, which is acted upon phytoene synthase (PSY), lycopene cyclase (LCY), and bacteria CRTI genes into rice produced a  $\beta$ -carotene rich variety (Ye et al., 2000; Beyer et al., 2002). Similarly, the co-expression of the daffodil PSY

and bacteria CRTI genes increased  $\beta$ -carotene rather than lycopene in rice endosperm (Ye et al., 2000; Al-Babili et al., 2006). Carotenoids biosynthetic pathways can be organized in a tree-like hierarchy (Fig. 8, supplementary). It is playing important biological roles as accessory light-harvesting components of photosynthetic systems, photo-protecting antioxidants and regulators of membrane fluidity (Bartley et al., 1990; Baima et al., 1991; Schmidhauser et al., 1994).

Industrially applications of Carotenoids pigments,  $\beta$ -carotene and astaxanthin are of increasing demand and a wide variety of market as a food coloring agents, e.g. margarine, soft drinks, baked goods, precursors of Vitamin A (pro-Vitamin A) in food and animal feed, additives to cosmetics, multivitamin preparations, antioxidants to reduce cellular or tissue damage. As antioxidants they are purported to have roles in protecting animals against cardiovascular disease and cancer (Al-Babili et al., 2001; Paine et al., 2005; DellaPenna and Pogson, 2006; Aluru et al., 2008). In addition, Carotenoids content in fruits and vegetables depends on several factors such as, genetic variety, maturity, post-harvest storage, processing and preparation.

Carotenoids play crucial roles in structure and function of the photosynthetic apparatus of bacteria, algae, fungi and higher plants. On this basis, we worked on an evolutionary analysis on  $\beta$ -carotene synthesis in *N. crassa*. An Endosymbiotic theory, a bacterium is the ancestors to eukaryotes (Margulis L, 1970; Margulis L and Sagan D, 1986; Sagan L, 1967); how could this  $\beta$ -carotene evolved in evolutionary perspectives in species of bacteria, algae, fungi and higher plants? This study reveals the conserve domain of Phytoene synthase (PYS), which is one of the regulator enzymes for  $\beta$ -carotene synthesis. The results showed interestingly high sequence similarity between species of bacteria-plant (Fig.4b) instead of bacteria- fungi and fungi-plant (Fig.5b; Fig.6b).

## Materials and Methods

*N. crassa* (Galagan et al., 2003) and several other fungi genome sequences have been provided a scope for comparative analysis; we got from Broad Institute (<http://www.broadinstitute.org/annotation/genome/neurospora>). Amino acid sequence of phytoene synthase in Bacteria, Fungi and Plant obtained from NCBI (<http://www.ncbi.nlm.nih.gov/protein>). BLASTN, TBLASTN, and BLASTP we got from the NCBI (<http://blast.ncbi.nlm.nih.gov/Blast>). *N. crassa*, *Cyanobacteria* and *Oryza sativa Indica* species of phytoene synthase (*psy*) genes encode protein sequence were selected based on consideration of E values (0.0-2e), 100-90 % identities and Maxi. score. The Conserved Domain

Database were obtained from NCBI (<http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml>), and multiple sequence alignments with ClustalX2 (Thompson et al., 1997) and followed by the Gene Doc (<http://en.bio-soft.net/format/GeneDoc.html>).

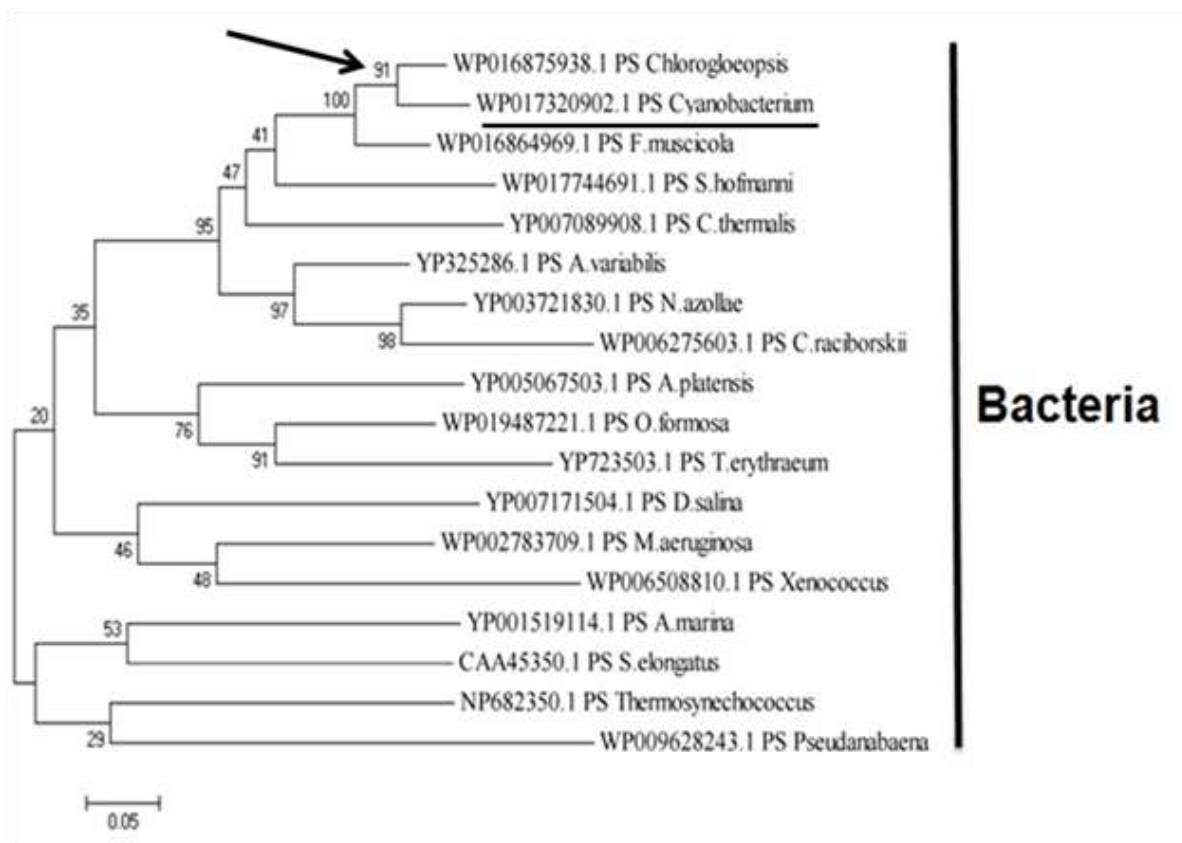
Phylogenetics analysis was done by using MEGA5.05 software (<http://www.megasoftware.net/>), using Maximum likelihood method, with 500 bootstrap replications.

## Results and Discussion

We found that the evaluated results of Phytoene synthase (*psy*) encode proteins in *N. crassa*, *Cyanobacteria* and *Oryza sativa indica*, showed high sequence similarity within of species bacteria, fungi and plant (Fig.1b, Fig.2b and Fig.3b). Fungi showed comparatively less sequence similarity than bacteria and Plants (Fig.2b). This results showed sequence similarity increasing order from bacteria to higher plants; within species of plants showed extremely high sequence similarity than bacteria and fungi (plant>bacteria>fungi) (Fig.1b, Fig.2b, Fig.3b; Table 2). Whereas in between two category of species bacteria- fungi, fungi-plant and bacteria-plant, this results showed interestingly, between bacteria-plant species showed high sequence similarity instead of bacteria-fungi and fungi-plant (Fig.4b, Fig.5b and Fig.6b). These results indicate sequence similarity increasing order between two category of species bacteria-plant high sequence similarity than fungi-plant and bacteria-fungi. The multiple sequence analysis of fungi-plant and bacteria-fungi, between these two cases showed sequence similarity extremely very less (bacteria-plant>fungi-plant>bacteria-fungi) (Fig.4a, Fig.5b and Fig.6b, Table2); in addition to evaluation of among three categories of species bacteria-fungi-plants showed tremendously very less sequence similarity (Fig.7b, Table2).

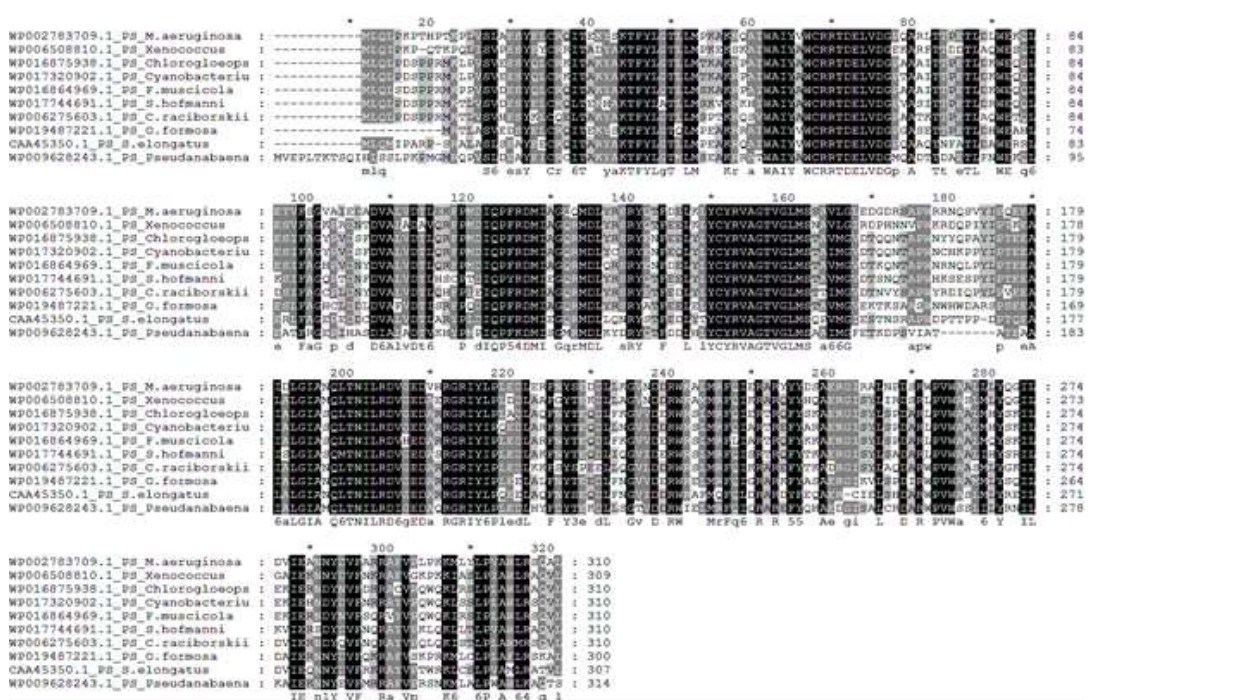
The evaluated results showed in Phylogenetics analysis of Phytoene synthase (*psy*) encode protein (PSY), homology within species of bacteria, fungi and plant 91%, 92% and 99 % (Fig.1a, Fig.2a and Fig.3a, Table1). Whereas in between two category species of bacteria-fungi, bacteria-plant, and fungi-plant showed results interestingly 99%, 96 % and 100% homology (Fig.4a, Fig.5a and Fig.6a, Table1), in addition to among the three categories of species bacteria-fungi-plants showed 99% homology (Fig.7a, Table1) respectively. The order increased from lower categories to higher categories of species from bacteria to higher plants, the homology increased (Fig.4a, Fig.5a and Fig.6a, Table1), the reason might be phytoene synthase encodes conserve domain unit Isoprenoid Biosynthesis enzymes, Class 1 widespread in *N. crassa*, *Cyanobacteria* and *Oryza sativa Indica*(bacteria, fungi and plant) respectively.



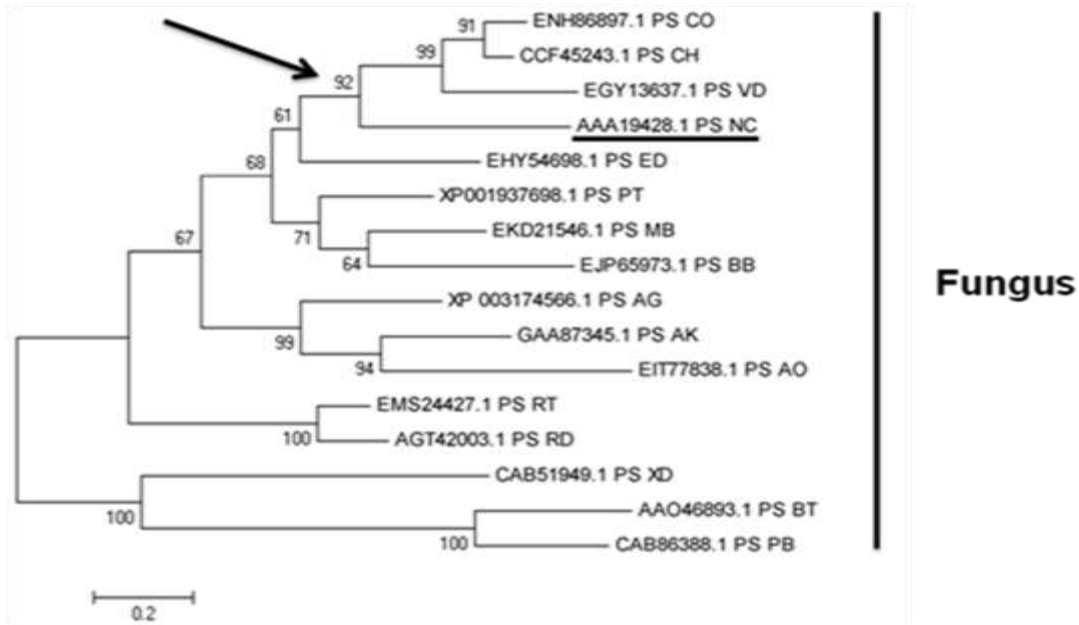


**Fig. 1a:** Phylogenetics tree of phytoene synthase (psy), PSY protein analysis, using Maximum likelihood method, with 500 bootstrap replications, and the software MEGA5.05; it showed 91% homology with in Bacterial species

[*Chlorogloeopsis*; *Cyanobacterium*; *F. muscicola*, *Fischerella muscicola*; *S.hofmanni* *Scytonema hofmanni*; *C.thermalis* *Chroococcidiopsis thermalis*; *A. variabilis* *Anabaena variabilis*; *N. azollae* *Nostoc azollae*; *C.raciborskii*, *Cylindrospermopsis raciborskii*; *A.platensis* *Arthrospira platensis*; *O. Formosa*, *Oscillatoria formosa*; *T. erythraeum*, *Trichodesmium erythraeum*; *D. salina* *Dactylococcopsis salina*; *M.aeruginosa* *Microcystis aeruginosa*; *Xenococcus*; *A.marina* *Acaryochloris marina*; *S.elongatus* *Synechococcus elongatus*; *T.elongatus* *Thermosynechococcus elongatus*; *Pseudanabaena*. ]

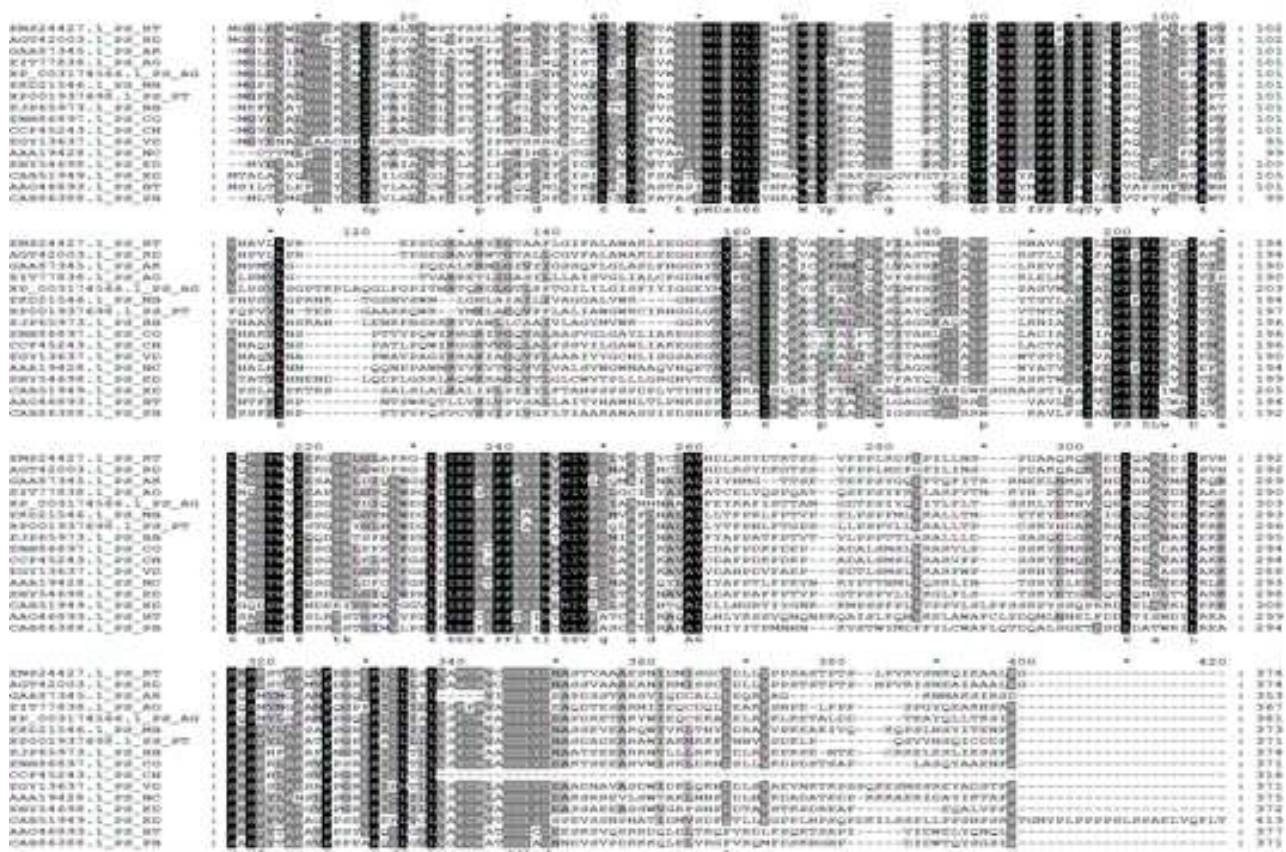


**Fig 1b:** Phytoene synthase (psy), PSY protein multiple sequence alignment showed high similarity (black color indicate ~100% similarity, grey color indicate ~80% similarity), within Bacterial species.



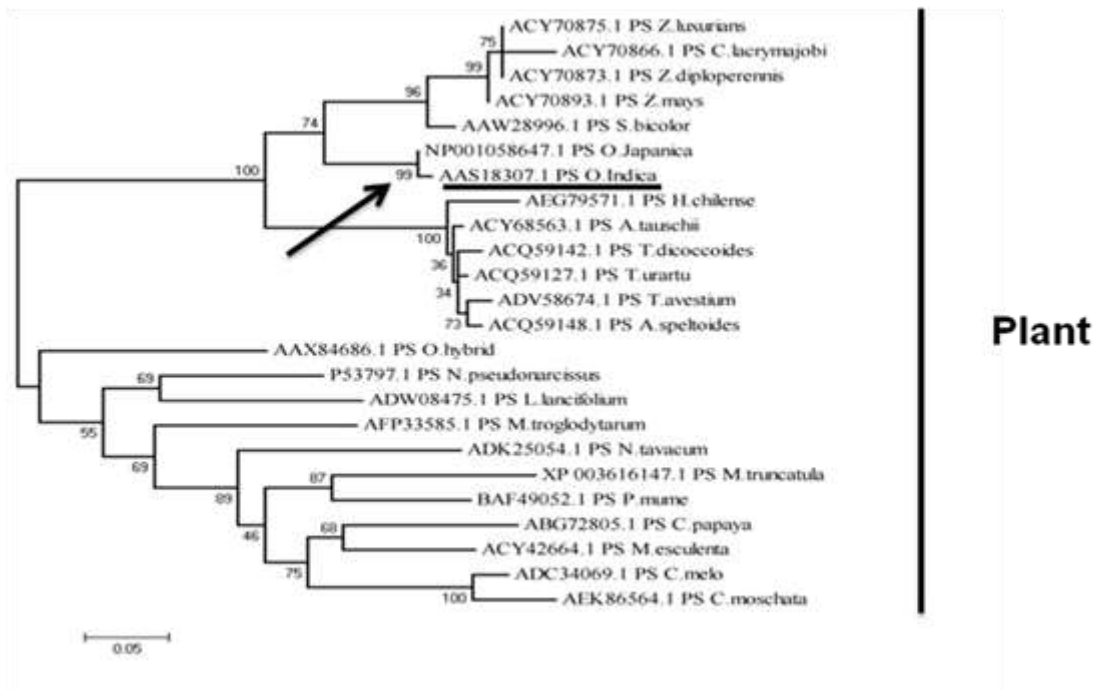
**Fig. 2a:** Phylogenetics tree of phytoene synthase (psy), PSY protein analysis, using Maximum likelihood method, with 500 bootstrap replications, and the software MEGA5.05;

[It showed 92% homology with in fungal species.CO *Colletotrichum orbiculare*; CH *Colletotrichum higginsianum*; VD *Verticillium dahlia*;NC *Neurospora crassa*; ED *Exophiala dermatitidis*; PT *Pyrenophora tritici*;MB *Marssonina brunnea*; BB *Beauveria bassiana*; AG *Arthroderma gypseum*; AK *Aspergillus kawachii*;AO *Aspergillus oryzae*; RT *Rhodosporidium toruloides*; RD *Rhodosporidium diobovatum*;XD *Xanthophyllomyces dendrorhous*; BT *Blakeslea trispora*; PB *Phycomyces blakesleeanus*.]



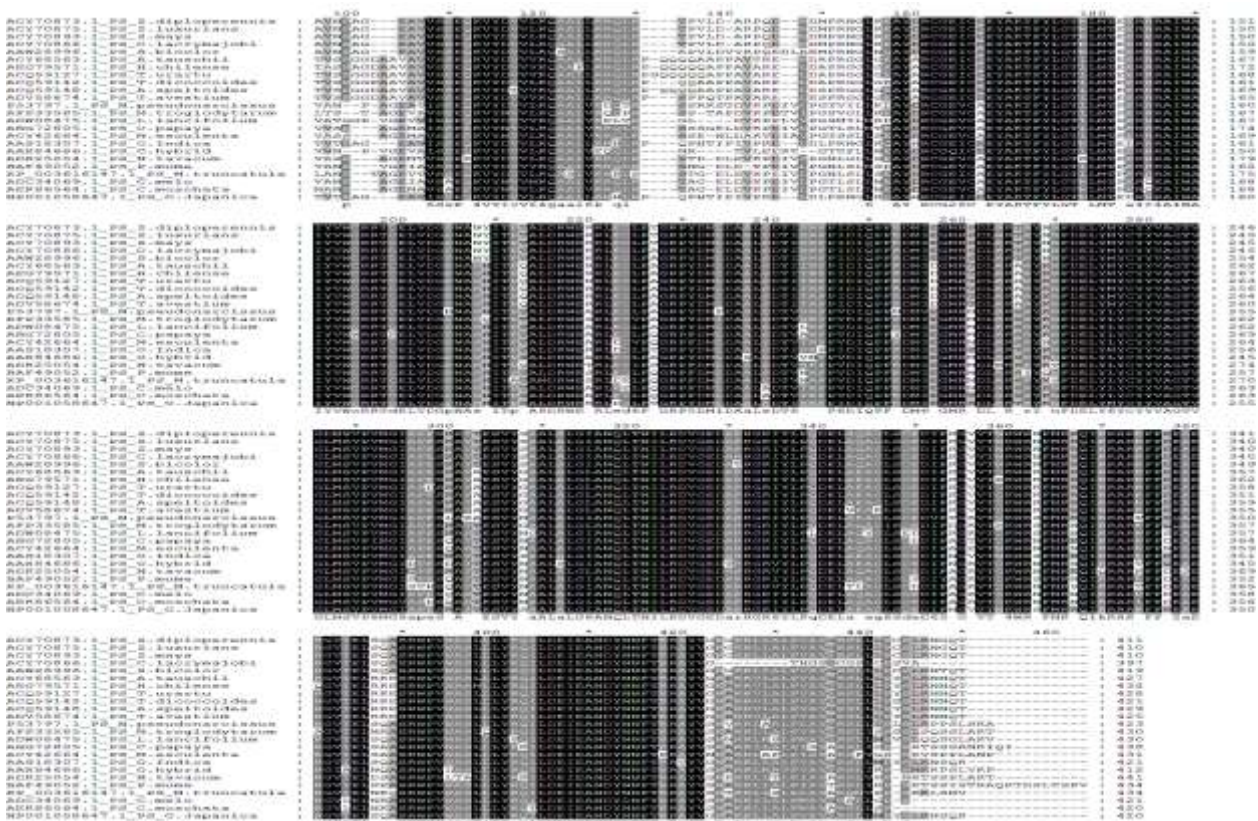
**Fig. 2b:** Phytoene synthase (psy), PSY protein multiple sequence alignment showed less similarity (black color indicate ~100% similarity, grey color indicate ~80% similarity), with in Fungi species.



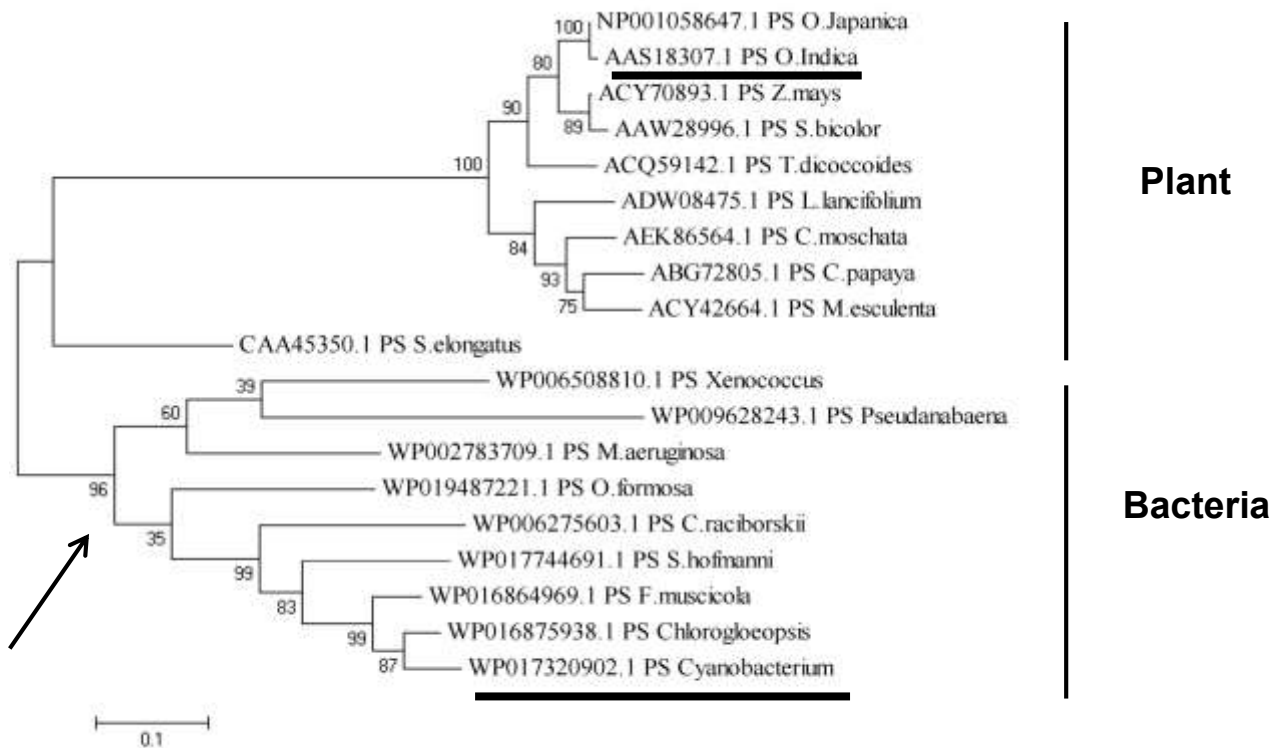


**Fig. 3a:** Phylogenetics tree of phytoene synthase (psy), PSY protein analysis, using Maximum likelihood method, with 500 bootstrap replications, and the software MEGA5.05;

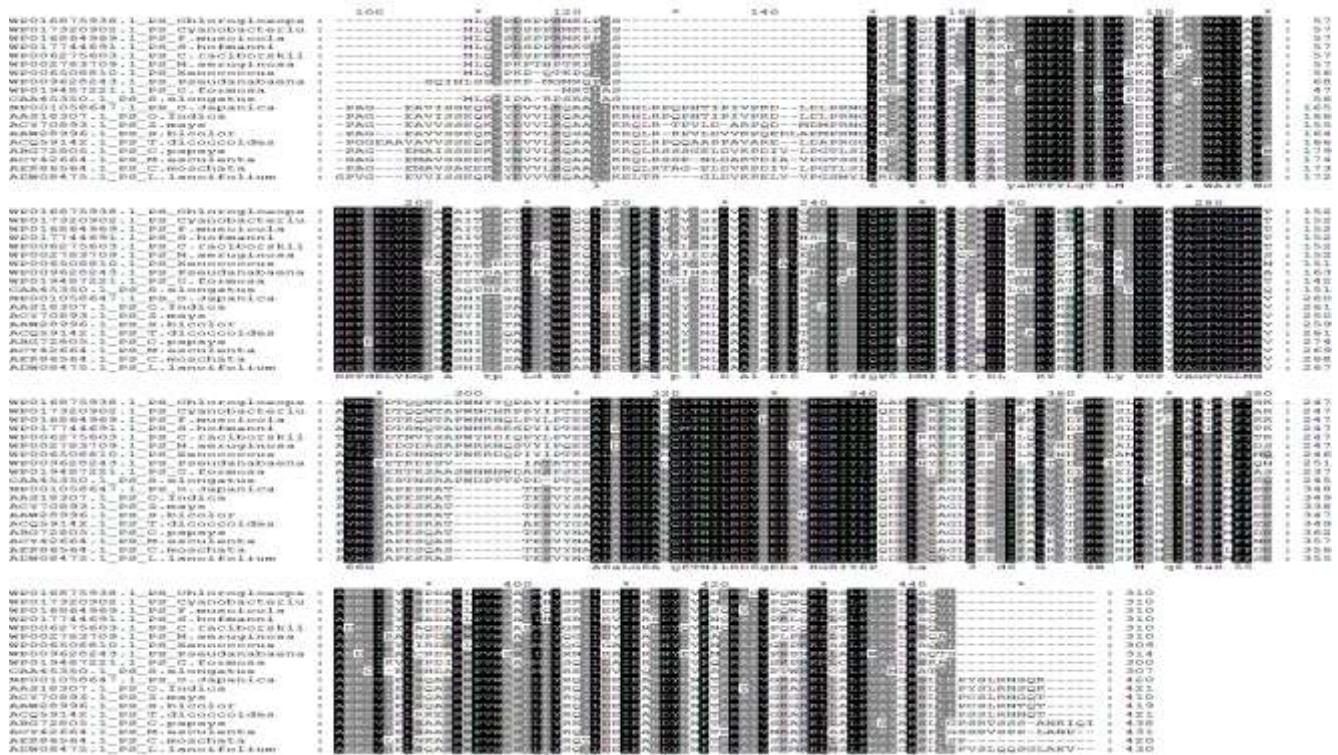
[It showed 91% homology with in Plant species. *Zea luxurians*; *Coix lacrymajobi*; *Zea diploperennis*; *Zea mays*; *Sorghum bicolor* ; *Oryza sativa* Japanica; *Oryza sativa* Indica ; *Hordeum chilense*; *Aegilops tauschii*; *Triticum dicoccoides* ;*Triticum urartu*; *Triticum aestivum*; *Aegilops speltoides*; *Oncidium hybrid* ; *Narcissus pseudonarcissus*; *Lilium lancifolium*; *Musa troglodytarum*; *Nicotina tavaicum*; *Medicago truncatula*; *Prunus mume*; *Carica papaya*; *Manihot esculenta*; *Cucumis melo*.]



**Figure 3b:** Phytoene synthase (psy), PSY protein multiple sequence alignment showed very high similarity (black color indicate ~100% similarity, grey color indicate ~80% similarity), with in Plant species. **Figure: 4a** Phylogenetics tree of phytoene synthase (psy), PSY protein analysis, using Maximum likelihood method, with 500 bootstrap replications, and the software MEGA5.05; it showed 96% homology among bacteria-plant species.

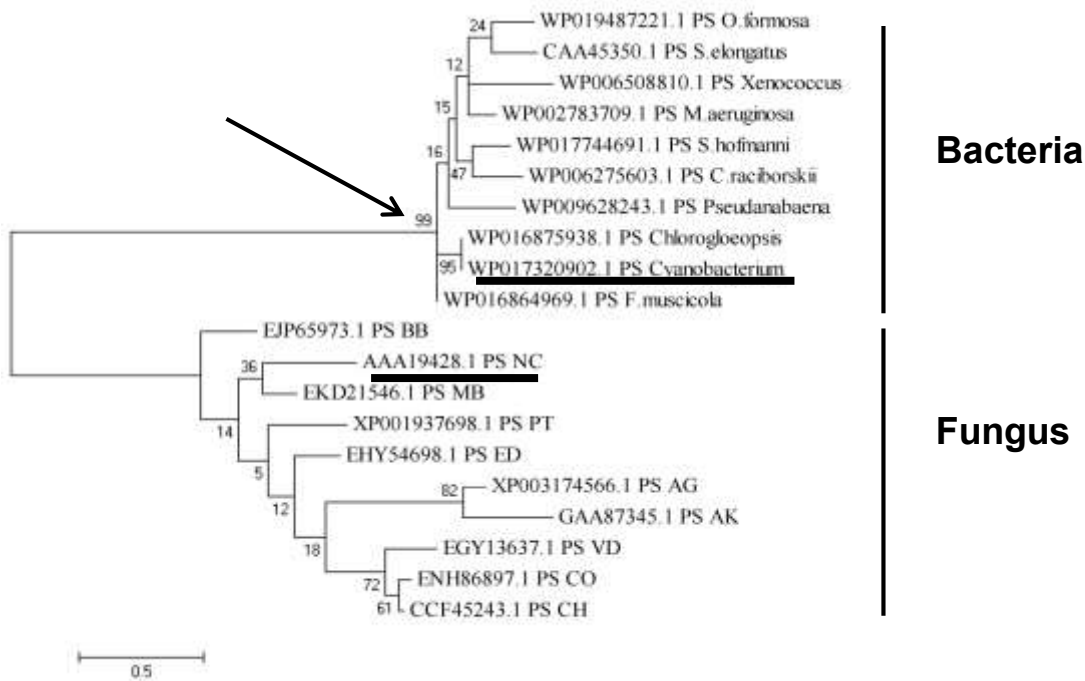


**Fig. 4a:** Phylogenetics tree of phytoene synthase (psy), PSY protein analysis, using Maximum likelihood method, with 500 bootstrap replications, and the software MEGA5.05; it showed 96% homology among bacteria-plant species.

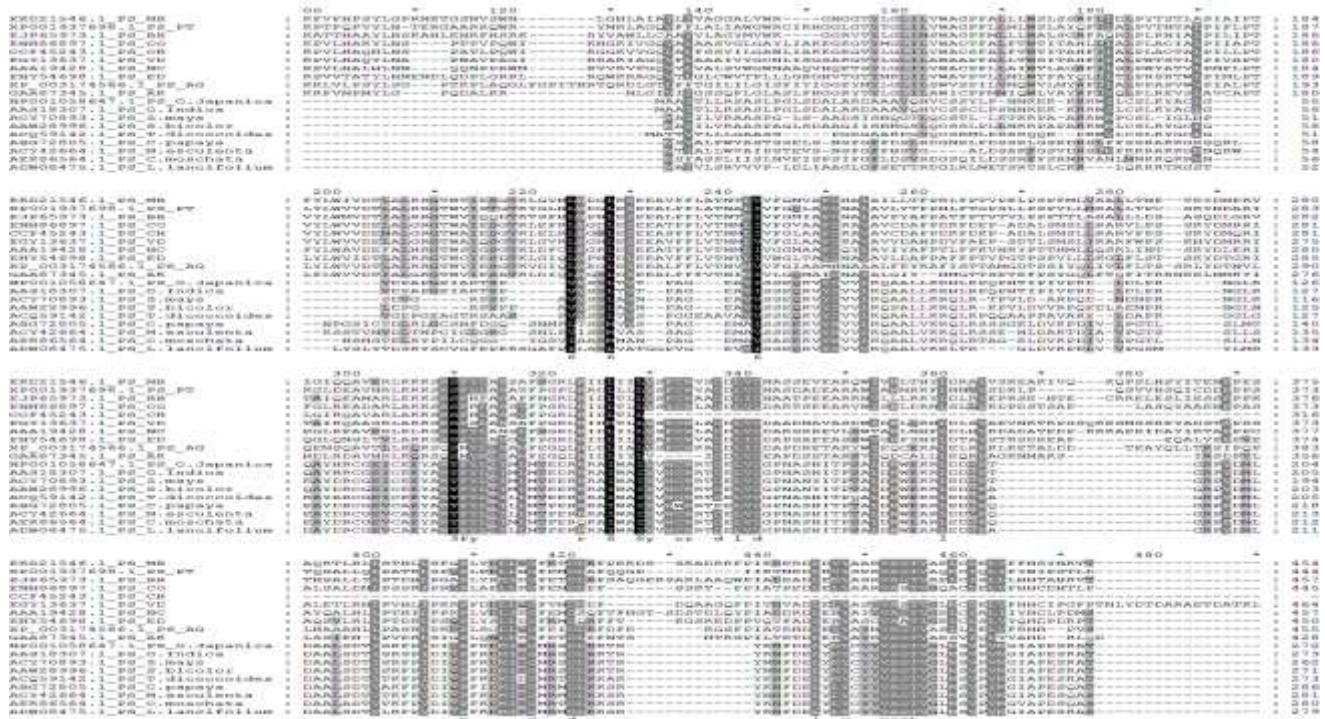


**Fig 4b:** Phytoene synthase (psy), PSY protein multiple sequence alignment showed high similarity (black color indicate ~100% similarity, grey color indicate ~80% similarity) between in Bacteria-Plant species.



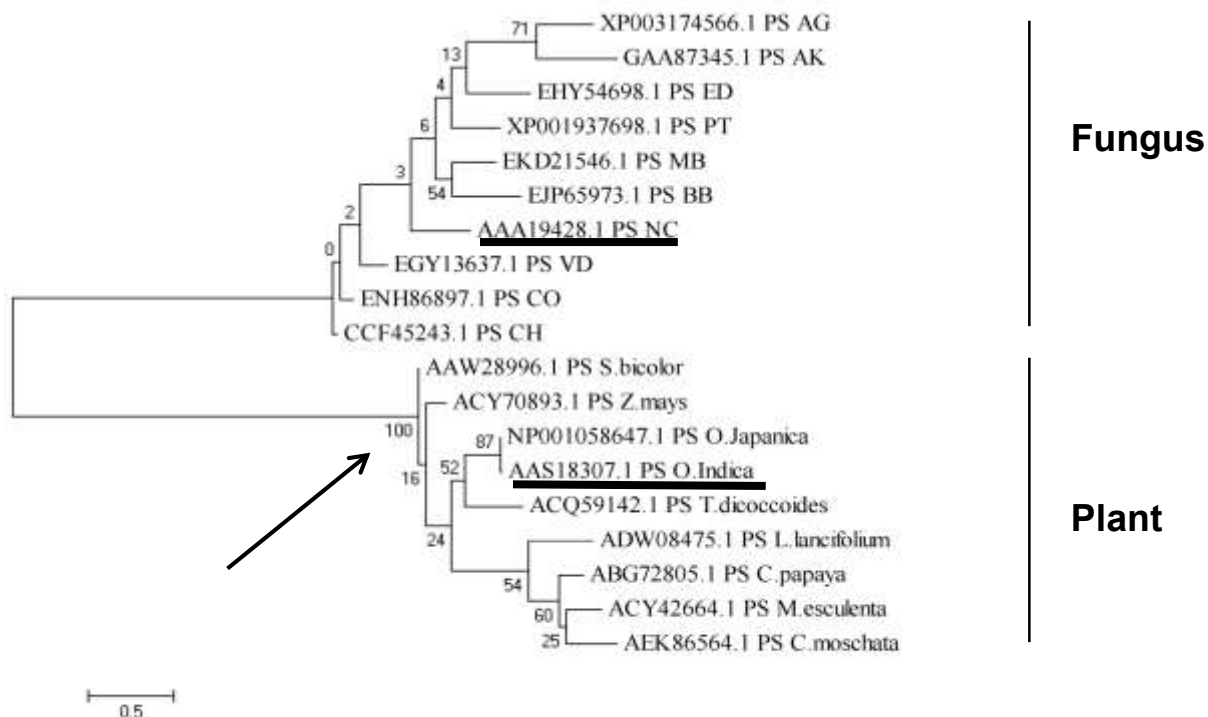


**Fig 5a:** Phylogenetics tree of phytoene synthase (psy), PSY protein analysis, using Maximum likelihood method, with 500 bootstrap replications, and the software MEGA5.05; it showed 99% homology among bacteria-fungal species.

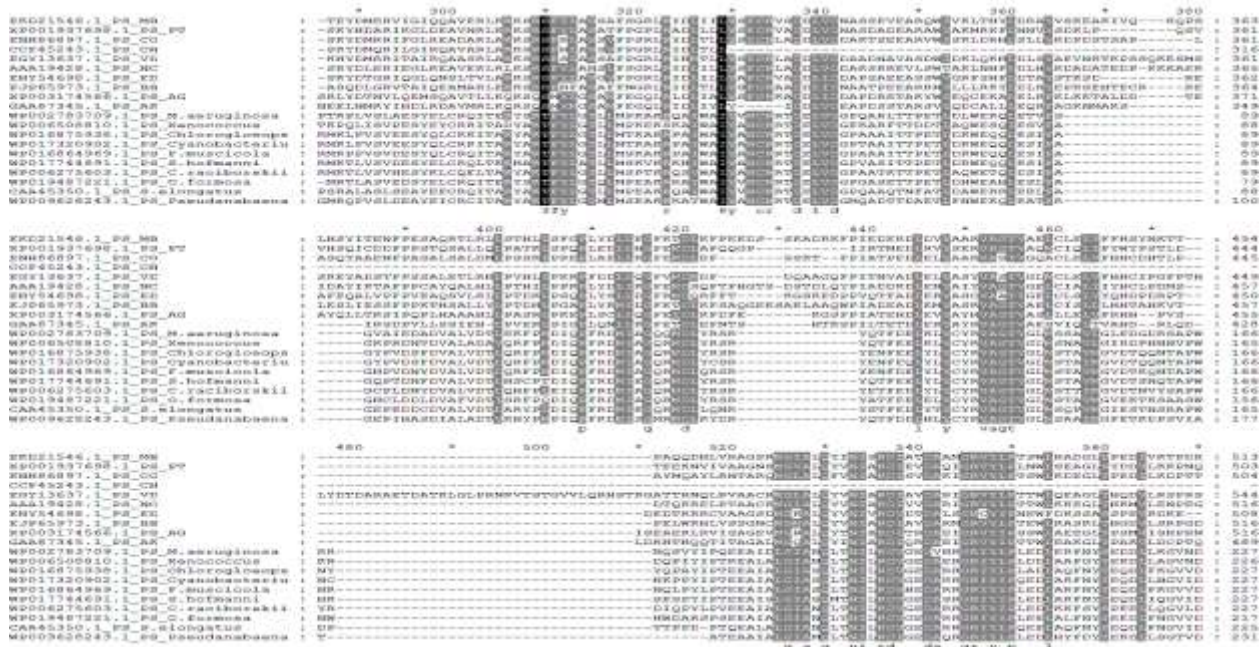


**Fig 5b:** Phytoene synthase (psy), PSY protein multiple sequence alignment showed extremely very less similarity (black color ~ 100% similarities, grey color ~80% similarity) between in Fungi-Plant species.

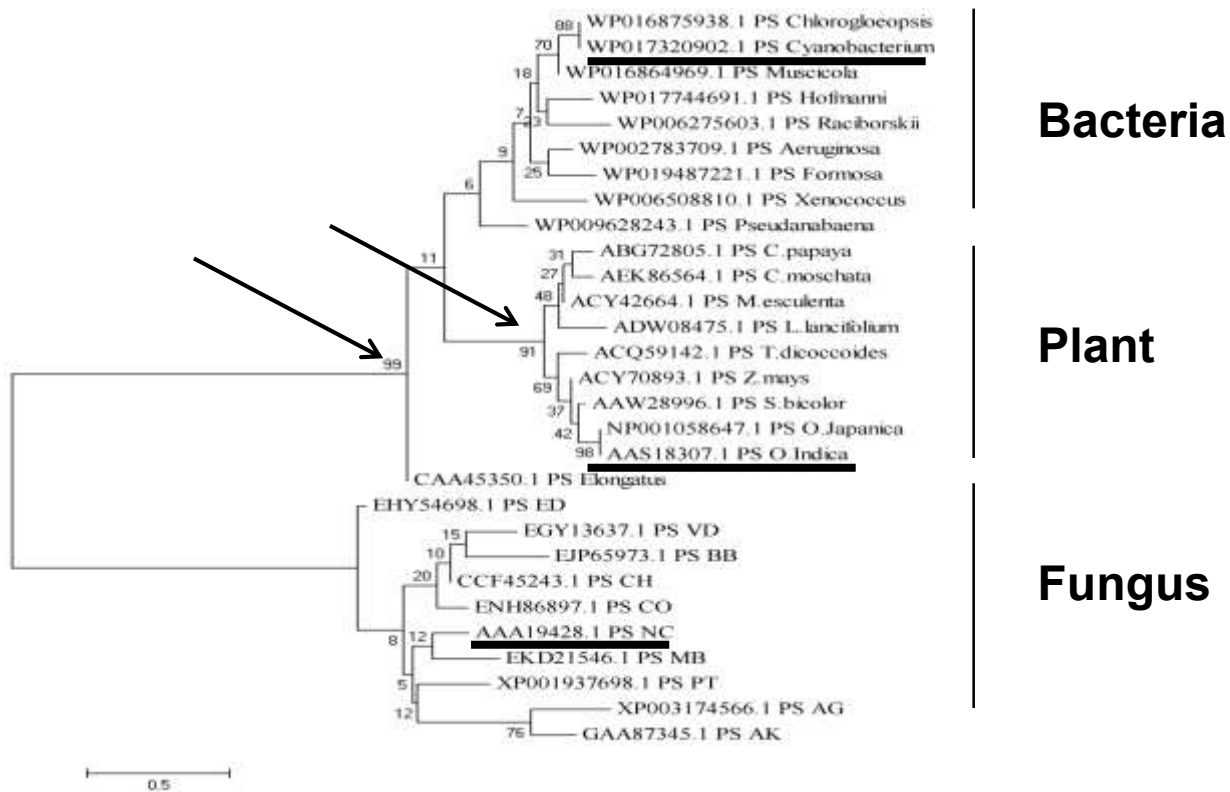




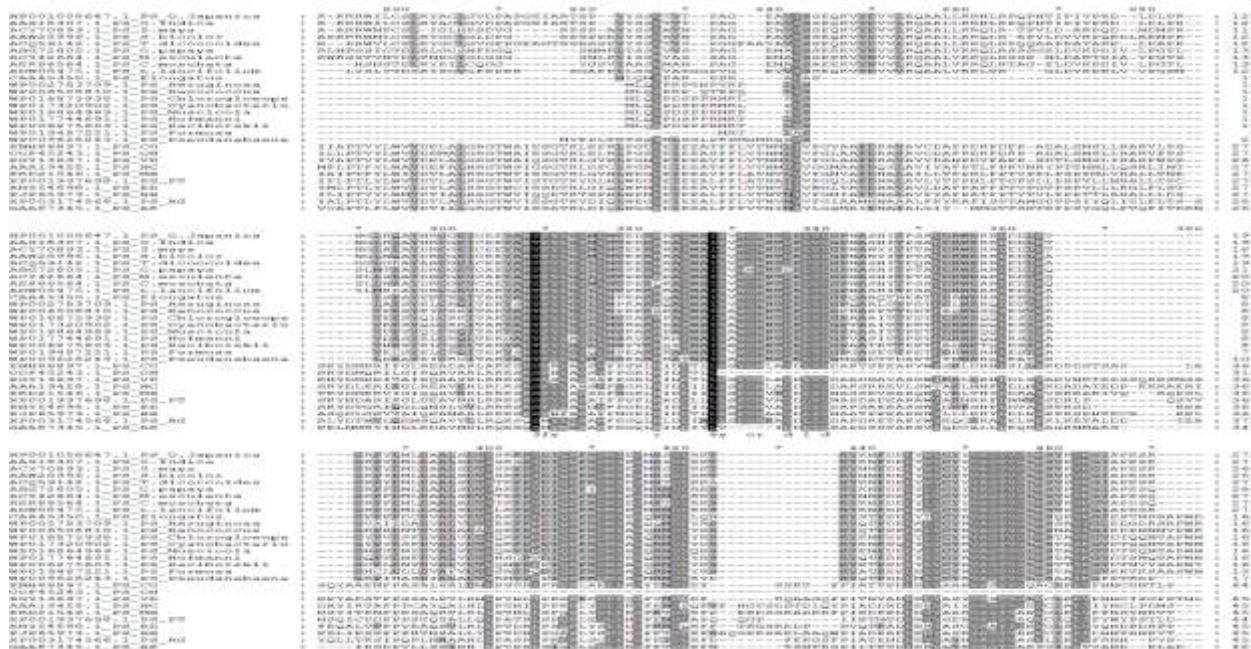
**Fig: 6a** Phylogenetics tree of phytoene synthase (psy), PSY protein analysis, using Maximum likelihood method, with 500 bootstrap replications, and the software MEGA5.05; it showed 100% homology among fungal-plant species.



**Fig 6b:** Phytoene synthase (psy), PSY protein multiple sequence alignment showed extremely very less similarity (black color indicate ~100% similarity, grey color indicate ~80% similarity) between in Bacteria-Fungi species.



**Fig 7a:** Phylogenetics tree of phytoene synthase (psy), PSY protein analysis, using Maximum likelihood method, with 500 bootstrap replications, and the software MEGA5.05; it showed 99% homology among bacterial-plant-fungus species.



**Fig 7b:** Phytoene synthase (psy), PSY protein multiple sequence alignment showed extremely very less similarity (black color indicate ~100% similarity, grey color indicate ~80% similarity) among Bacteria-Fungi-Plant species.

## Discussion

According to an Endosymbiotic theory, a bacterium is the ancestors to eukaryotes (Margulis L, 1970; Margulis L and Sagan D, 1986; Sagan L, 1967). On the basis of Endosymbiotic theory, we evaluate conserve domain of

phytoene synthase (*psy*) encodes protein (PSY) sequence data for multiple sequence analysis and Phylogenetics tree within species, between species and among the species of (*N. crassa*, *Cyanobacteria* and *Oryza sativa Indica*) bacteria, fungi and plant. We consider conserve domain of *N. crassa* phytoene synthase enzyme encode protein size



275-575 aa (Isoprenoid Biosynthesis enzymes, Class 1), Cyanobacteria phytoene synthase encode protein size 10-270 aa (Isoprenoid Biosynthesis enzymes, Class 1), and *Oryza sativa* Indica phytoene synthase encode protein size 140-400 aa (chloroplast), (Isoprenoid Biosynthesis enzymes, Class 1) (*e*-value 0.0, 0.0 and 0.0; identity 100%, 100% and 100%; Max.score:1238, 644 and 870) respectively. The evaluated results showed between in species of bacteria-plant, fungi-plant and bacteria-fungi high sequence similarity, extremely very less similarity and extremely very less similarity (Fig.4b, Fig.5b and Fig.6b) respectively.

The Phylogenetics analysis results showed for Phytoene synthase (*psy*) encode protein domain (PSY) homology within bacteria, fungi and plant 91%, 92% and 99 % (supp.Fig.1a, Fig.2a, and Fig.3a Table1). Whereas in between two category species of bacteria-fungi, bacteria-plant, and fungi-plant Phylogenetics analysis result showed 99%, 96 %, and 100% homology (Fig.4a, Fig.5a, and Fig.6a, Table1), and among the species of bacteria-fungi-plant showed homology 99% (Fig.7a) respectively. The Phylogenetics tree analysis result indicates fungi -plant that has 100% homology (Fig.6a), whereas in between species of bacteria-fungi and bacteria-plant 96% and 99 % homology; because both of them are eukaryotes, and have comparatively more close relation with plants, they adopted the mechanism of photosynthesis, by using chlorophyll as an antenna for light capturing to conversion of light energy into chemical energy as a carbohydrates. The bacteria-fungi and bacteria-plant, both are the cases two different category of species prokaryotes and eukaryotes, as well phototrophic category species. But, they have distinct genomic characteristic features. The Phytoene synthase enzyme encode unit, Isoprenoid Biosynthesis enzymes, Class 1, Isoprenoid\_Biosyn\_C1, these residues mediate binding of prenyl phosphates via bridging  $Mg^{2+}$  ions is widespread in *N. crassa*, *Cyanobacteria* and *Oryza sativa Indica*(bacteria, fungi and plant) correspondingly.

**Table1:** Comparative homology in species

S.N.	within Species	Between Species	Among Species	Homology
1.	Bacteria			91%
2.	Fungi			92%
3.	Plants			99%
4.		Bacteria-Plant		96%
5.		Bacteria-Fungi		99%
6.		Fungi-Plant		100%
7.			Bacteria-Fungi-Plant	99%

**Table2:** Comparative multiple sequence alignment in species

S.N.	within Species	Between Species	Among Species	Similarity
1.	Bacteria			High
2.	Fungi			less
3.	Plants			Very High
4.		Bacteria-Plant		High
5.		Bacteria-Fungi		Extremely very less
6.		Fungi-Plant		Extremely very less
7.			Bacteria-Fungi-Plant	Extremely very less

## Conclusion

The phytoene synthase (*psy*) enzyme encodes protein (PSY) sequence of species in bacteria, fungi, and plant has taken from NCBI (*N. crassa* in fungi, *Oriza sativa indica* in plants, *cyanobacteria* in bacteria). We consider sequence of *e-values* 0.0, 0.0, 0.0, identity (100%, 100% and 100%); *N. crassa*, *Oriza sativa indica* and *cyanobacteria* accession id (AAA19428.1, AAS18307.1, WP017320902.1) (<http://www.ncbi.nlm.nih.gov>) respectively. A comparison of sequence in species has been done within species, between species and among the species by using Gene Doc and Clustal X2). Phylogenetics tree analysis was done within species, between species and among the species by using MEGA5.05. The evaluated results showed between species in bacteria-plant high sequence similarity than bacteria-fungi and fungi-plant; 100% homology between species of fungi-plant in Phylogenetics analysis, compared to bacteria-fungi and bacteria-plant (Fig.4a, Fig.4b, Fig.5a, Fig.5b, Fig.6a and Fig.6b). These results specifying in multiple sequence alignment and Phylogenetics analysis of phytoene synthase encode, Isoprenoid Biosynthesis enzymes, Class 1 conserve domain (PSY) widespread in *N. crassa*, *Cyanobacteria* and *Oryza sativa Indica*(bacteria, fungi and plant) respectively. Industrially applications of Carotenoids pigments,  $\beta$ -carotene and astaxanthin are of increasing demand and a wide variety of market as a food coloring agents. The  $\beta$ -Carotene is precursors of Vitamin A (pro-Vitamin A), available in plant food products used in multivitamin preparations, antioxidants to reduce cellular or tissue damage in many application. The result indicates computationally comparison of species of fungi-plant high sequence with similarity and 100% homology (Fig.6a, Fig.6b). *N. crassa*, we can grow easily in nutritional media in bioprocess units and isolate huge amounts of  $\beta$ -Carotene in lesser time when compared to plants. The  $\beta$ -Carotene in plants contenting fruits and vegetables production bit of time consuming process, and it depends on several factors such as genetic variety, maturity, post-harvest storage. We can study nutritional aspects of  $\beta$ -Carotene in *N.crassa* and

produce high amount of  $\beta$ -Carotene in lesser time by using bioprocess methodology and to provide global markets in less time and lesser price for human benefits as a multivitamin.

## Acknowledgement

I thank Fungal Genetics Stock Center (FGSC), University of Missouri, Kansas City, USA, Broad Institute and NCBI for providing sequences user friendly *N.crassa*, Cyanobacteria and *Oryza sativa* Indica. We thanked to RGUKT IIIT Nuzvid for partial financial support.

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