Original Article

A PURPLE NON-SULFUR BACTERIUM PRODUCING POLYHYDROXYBUTYRATE AND THE CONSERVED REGION OF PHA SYNTHASE GENE

BACTÉRIA PÚRPURA NÃO SULFUROSA PRODUTORA DE POLIHIDROXIBUTIRATO E A REGIÃO CONSERVADA DO GENE PHA SINTASE

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ABSTRACT: This study aimed to screen purple non-sulfur bacteria capable of accumulating granules or polyhydroxybutyrate (PHB) inside the cells, identify the potent strain, assay the enzyme or PHA synthase, and compare the PHB synthase gene with that of related strains. A total of 58 strains of purple non-sulfur bacteria were isolated from 108 samples of chicken feces in the chicken-egg farm of the Department of Animal Science, Faculty of Natural Resources at Prince of Songkla University, Hat Yai, Thailand. After cultivating the bacteria in glutamate malate (GM) medium without added glutamic acid under light (3,000 Lux) at 35^{o}C for 5 days, the intracellular biopolymer granules of the bacteria were observed by using a Confocal Laser Scanning Microscope (CLSM) with excitation and emission wavelength of 530 and 605 nm, respectively. Gas chromatography (GC) was carried out for quantitative analysis of PHB. There were five strains, CH12, CH52, CH72, CH90 and CH92, showed biopolymer granules under CLSM, and accumulated PHB 5, 1.7, 1.5, 1.4 and 1.8% (w w^{-1}) of the cell dry weight (CDW), respectively. The 16S rDNA sequence analysis of CH12 strain showed a high homology of 100% correlation to that of *Rhodopseudomonas palustris* strain NCIB8288. Regarding the taxonomic characteristics and 16S rDNA sequence analysis, CH12 strain was identified as *Rps. palustris* NCIB8288. The PHA synthase activity of the crude extract from CH12 strain was 25 units/mL. The conserved regions could be aligned and selected among 5 strains of *Rhodopseudomonas palustris* (strains BisA53, TIE-1, CGA009, HaA2 and BisB18). The purified PCR product was obtained for further studies.

KEYWORDS: Chicken feces. *Rhodopseudomonas palustris*. Bioplastic. Polyhydroxybutyrate. *pha*C gene. Conserved region.

INTRODUCTION

Plastics are not environmentally friendly materials to the circumstances due to the long-term degradation. Although polyhydroxyalkanoate (PHA) is one of candidates for biodegradable plastics such as polylactides, aliphatic polyesters, polysaccharides, and the copolymers and/ or blends of these, it has attracted much interest because of its biodegradability and similar properties to conventional plastics (BYROM, 1994). There are about 150 kinds of hydroxyalkanoic acids as monomers of PHAs (STEINBüCHEL; VALENTIN, 1995) like inclusions or granules in the cell (DUNLOP; ROBARDS, 1973). The first PHA is the homopolymer poly(3-hydroxybutyrate) (PHB) found in 1925 by Maurice Lemoigne (JACKSON; SRIENC, 1994). PHAs are found in various kinds of microorganisms as energy and carbon reserve (SALEHIZADEH; LOOSDRECHT, 2004) under aerobic condition (GOBI; VADIVELU, 2014). They can be found in natural environments , for example, lake (ESTEVE et al., 1996), river (LOPEZ et al., 1995), knots of plant rhizoids (NDOYE et al., 1994), deep sea under high temperature (GUEZENNEC et al., 1998), and activated sludge (MINO et al., 1998). They can be observed in bacteria under unusual growth conditions or depletion of an essential nutrient other than the carbon source (ALDERETE et al., 1993).

Purple non-sulfur bacteria is a group of purple bacteria. They are heterotrophs using organic substrates, e.g. acetate, pyruvate and dicarboxylic acids. They are able to grow under aerobic, anaerobic and microaerobic conditions with light or without light. Nevertheless, they grow well under anaerobic to microaerobic condition with light. They also grow well in polluted environments (KOBAYASHI et al., 1978) which is well known for using them in wastewater treatments (KOBAYASHI; KOBAYASHI, 1995). The purple non-sulfur bacteria, for instance, *Rhodobacter sphaeroides* (KHATIPOV et al., 1998; BRANDL et al., 1991) and *Rhodospirillum rubrum* (BRANDL et al. 1991) are capable of producing PHBs. Thus, it is an expedient to get PHBs in purple non-sulfur bacteria using low-cost medium with environmental advantages. The aim of this study was to screen the

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potent strain which could produce PHBs from chicken feces, the PHA synthase assay and information of the conserved region of PHA synthase gene and primer design for the gene amplification. Further study on genetic manipulation to increase the PHB yield will have been carried out.

MATERIAL AND METHODS

A total of 108 samples of chicken feces in the chicken-egg farm of the Department of Animal Science, Faculty of Natural Resources at Prince of Songkla University, Hat Yai were used for isolation of purple non-sulfur bacteria. Glutamate-malate (GM) medium was prepared for growing the purple non-sulfur bacteria. 12.5 mL of GM medium without added glutamic acid in a 15-mL screw-cap tube used for cultivation of the bacteria. The starter used was 5% (v v^{-1}) and incubated under micro-aerobic condition with tungsten light intensity of 3,000 Lux at 35°C for 48 h. It was then centrifuged at 12,000 rpm for 5 min. The cell pellet was washed with 0.85% saline solution and freeze-dried for further study.

The 5-day incubation time of the strain was smeared and heat fixed on a glass slide. 1% aqueous solution of Nile blue A was used as a staining of PHB granules at 55°C for 10 min. It was then washed with tap water and 8% acetic acid solution for 1 min, respectively. It was washed with distilled water and blotted dry. The slide was remoistened with distilled water and covered with glass cover slip before observing by Confocal Laser Scanning Microscope (CLSM) with excitation and emission wavelength of 530 and 605 nm, respectively. The procedure was followed using the Holt and Ostle method (HOLT; OSTLE, 1982). *Azotobacter* sp. TISTR 1094 was used as a reference strain.

The method of esterification of PHB for GC analysis was followed according to the protocol described by Riis and Mai (RIIS; MAI, 1988). 20-40 mg of freeze dried bacterial cells was weighed and put into a 10 mL tightly sealable vial. It was added with 2 mL1, 2-dichloroethane (DCE), 2 mL n-propanol containing hydrochloric acid (HCl) (1 volume of concentrated HCl mixed with 4 volumes of n-propanol) and 200 µL internal standard (two gram of benzoic acid in 50 mL n-propanol). The mixture was shaken intermittently in water bath at 85° C for 4 h. It was then cooled to room temperature, added with 4 mL distilled water, and shaken for 20-30 sec, respectively. To determine the PHB content of the cells, the heavier phase or the DCE-propanol was collected and injected into the GC. The ratio of PHB to cell dry weight was defined as the PHB content.

The column used for determination of PHB by GC was HP-1 (HP 19091Z-413E, Crosslinked Methyl Siloxane, Hewlett Packard, USA) capillary column, 30 m in length with 0.32 mm inner diameter. The injection port and the flame ionization detector (FID) port was operated at 250° C. The following temperature profile used was 5 min at 80°C, followed by 7° C/min rise to reach the final temperature of 200° C. Nitrogen with the flow rate of 5 mL/min was used as the carrier gas. Injection volume was 1.0 µL and injection mode was splitless. A double flame ionization detector (FID) combined with a computer controlling gas chromatography (HP 6850) was used for analysis. To calibrate the GC, 200 mg of PHB was dissolved with a small volume of DCE by heating in a calibrated 10-mL flask. It was then cooled to room temperature and made up to 10 mL by distilled water. The solution was esterified as mentioned above, and injected to GC with the volume of 200; 400; 600; 800 and 1000 µL.

CH12 strain was identified based on the taxonomic characteristics according to the Bergey's Manual of Systematic Bacteriology (PFENNIG; TRUPER, 1989), and 16S rDNA sequence analysis. According to the standard method (SAMBROOK et al., 1989), the genomic DNA was extracted after cultivating the strain in GM medium under micro-aerobic condition with light (2,500 Lux) at 35° C for 12 hr. Universal primers, position 170 for forward and 705 for reverse, were used to amplify the gene using GeneAmp PCR System 9600. The length of PCR product was 537 bp. It was sequenced using API 377 DNA sequencer.

After cultivating *Rps. palustris* CH12 for 3 days, the grown cells were harvested (0.62 g of wet cells from 100-mL culture). It was then suspended in 5.0 mL of 10 mM Tris-HCl buffer pH 7.5, and centrifuged at 10,000 rpm, 4° C for 5 min. The pellet of cells was put on ice and added 1.5 mL of extraction buffer (10 mM Tris-HCl buffer pH 7.5 containing 10 mM EDTA, and 1 mM DTT). It was mixed well by vortex mixer, and then sonicated for 5 min, and paused every 15 sec for 5 sec. The sonicated mixture was centrifuged at 10,000 rpm, 4° C for 5 min. The supernatant was kept at 4° C for PHA synthase assay.

PHA synthase was assayed by a modified method of Dai and Reusch (DAI; REUSCH, 2008). The substrate, 0.88 mM DL-β-hydroxybutyryl coenzyme A was dissolved in 10 mM Tris-HCl, pH 7.5. The reaction mixture contained 0.088 mM DL-β-hydroxybutyryl coenzyme A, 100 mM KCl, and

100 µL supernatant of sonicated cells. It was incubated at 37° C for 30 sec. The reaction mixture was stopped by adding 20 µL of 0.44 M TCA. It was then centrifuged at 12,000 rpm for 5 min. A 102 µL-supernatant was pipetted into an eppendorf tube, and added with 573 µl of 1 mM 5,5'-dithiobis-(2-nitrobenzoic acid). The coenzyme A concentration was measured spectrophotometrically at 412 nm. Duplicate assays and a blank reaction were carried out. For determination of enzymatic activity, standard curve was performed at the same conditions. One unit of enzyme is defined as an amount of enzyme which catalyze 1 µmol DL-β-hydroxybutyryl coenzyme A into coenzyme A per min under the assay conditions.

In order to get multiple sequence alignment, the Clustal X program was used.

A highly conserved region was selected and used for primer design. The forward and reverse primers were approved by Vector NTI program. The PHA synthase gene was amplified 35 cycles with a 2-sec, 55° C denaturation step, a 30-sec, 52.5° C annealing step, a 2-sec, and 72° C extension step. The PCR product was purified by PureLinkTM Quick Gel Extraction Kit (Invitrogen) and analyzed on 1% agarose gel electrophoresis.

RESULTS AND DISCUSSION

A total of 58 strains of purple non-sulfur bacteria could be isolated from 108 samples of chicken feces under micro-aerobic conditions with light. After cultivating 58 strains of purple non-sulfur bacteria in GM medium without added glutamic acid under micro-aerobic conditions under light (3,000 Lux) at 35° C for 5 days, only five strains: CH12, CH52, CH72, CH90 and CH92, showed biopolymer granules under fluorescence microscopy and accumulated PHB 5; 1.7; 1.5; 1.4 and 1.8% (w w^{-1}) of the CDW, respectively, analysed by using GC. The results do not correlate to those of other purple non-sulfur bacteria, for example, *Rhodospirillum rubrum* and *Rhodobacter sphaeroides* (*Rba. sphaeroides*) are able to accumulate PHA under nitrogen-limited source conditions 45% and 60-70%, respectively (BRANDL et al. 1989; BRANDL et al. 1991), and *Rba. sphaeroides* accumulate PHB higher in lactate-glutamate than in lactate-ammonium medium (KHATIPOV et al. 1998). However, PHB accumulation of the five strains was higher than that of the type strain, *Azotobacter* sp. TISTR 1094, 1.1% (w w^{-1}) of the CDW. The CH12 strain was selected as the most potent strain for further study.

The CH12 strain was identified as *Rhodopseudomonas palustris* (*Rps. palustris*) strain NCIB8288 based on the taxonomic characteristics (Table 1) and 16S rDNA sequence analysis (Figure 1).

Table 1. Comparison of the characteristics of *Rhodopseudomonas palustris* and CH12 strain

Characteristics	*Rhodopseudomonas palustris**	CH12 strain
Gram staining	negative	negative
Cell shape	rod	rod
Flagellation	1, polar	1, polar
Photoautotrophic growth	+	+
Carbon sources and photosynthetic electron donors		
Acetate	+	+
Butyrate	+	+
Citrate	±	+
Gluconate	ND	+
Glucose	±	-
Fructose	±	+
Sorbitol	+	+
Glycerol	+	+
Ethanol	±	+
Thiosulfate	+	-

Symbols: *, Imhoff; Truper, 1989

+, positive; -, negative; ±, positive in some strains but negative in other strains; ND, not determined.

aagtcagagg	tgaaagcctg	gagctcaact	ccagaactgc	ctttgatact
ggaagtcttg	agtatggcag	aggtgagtgg	aactgcgagt	gtagaggtga
aattcgtaga	tattcgcaag	aacaccagtg	gcgaaggcgg	ctcactgggc
cattactgac	gctgaggcac	gaaagcgtgg	ggagcaaaca	ggattagata
ccctggtagt	ccacgccgta	aacgatgaat	gccagccgtt	agtgggttta
ctcactagtg	gcgcagctaa	cgctttaagc	attccgcctg	gggagtacgg
tcgcaagatt	aaaactcaaa	ggaattgacg	ggggcccgca	caagcggtgg
agcatgtggt	ttaattcgac	gcaacgcgca	gaaccttacc	agcccttgac
atgtccagga	ccggtcgcag	agacgcgacc	ttctcttcgg	agcctggagc
acaggtgctg	catggctgtc	gtcagctcgt	gtcgtgagat	gttgggttaa
gtcccgcaac	gagcgcaacc	cccgtcctta	gttgcta	

Figure 1. 16S rDNA sequence of CH12 strain.

The evolutionary tree between *Rps. palustris* strain NCIB8288 and other bacteria showed that *Rps. palustris* strain NCIB8288 has close evolution to *Rps. palustris* strain KD1 (92.9%), *Rps. palustris* strain BIS3 (90.3%), *Rps. palustris* strain N1 (82.8%) and *Rps. palustris* strain TIE-1 (20.9%) (Fig. 2). Earlier reports on *Rps. palustris* producing PHB (de PHILLIPPIS et al. 1992; SAWAYAMA et al. 2000; SAWAYAMA et al. 2001; CARLOZZI; SACCHI, 2001; MUKHOPADHYAY et al., 2005) also point at the same results. The PHB accumulation of CH12 strain was low, 21% (w w^{-1}) of the CDW, compared to those of other bacteria such as *Methylobacterium extorquens* (46% (w w^{-1}) of the CDW) (BOURQUE et al., 1995), *Pseudomonas* sp. K (66% (w w^{-1}) of the CDW) (SUZUKI et al., 1986), *Alcaligenes eutrophus* (76% (w w^{-1}) of the CDW) (KIM et al., 1994). However, *Rps. palustris* has a number of advantages: (1) The CH12 strain was isolated from chicken feces, so it might grow in wastewater, especially wastewater containing organic matter. In addition, (2) CH12 strain could secrete proteinase outside the cell (data not shown). Thus, wastewater used as growth medium might be applied. *Rps. palustris* is capable of producing H_2 (CARLOZZI; SACCHI, 2001). (3) H_2 is proposed to be environmentally friendly fuel to the world in the future. Thus, all benefits mentioned above make CH12 strain an attractive model for the study of the wastewater treatment, H_2 production, PHB production, and/ or composition of general component of the cells.

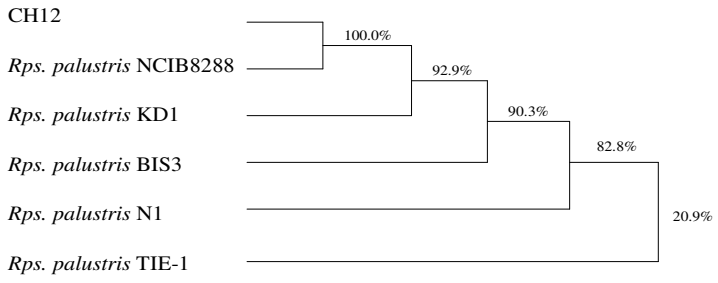

Figure 2. The evolutionary tree between CH12 and other *Rhodopseudomonas palustris* strains
The percentage shown in parentheses indicated the similarity of 16S rDNA sequence of CH12 to those of others.

To increase the expression of PHB synthesizing genes of CH12 strain, genetic manipulation need to be carried out for further study. Firstly the synthase was assayed and determined with standard curve of coenzyme A (CoA). The activity of PHA synthase of crude enzyme was 0. 111 (A_{412}) or 25.0 unit/mL. A standard curve of CoA versus A_{412} was shown in Figure 3.

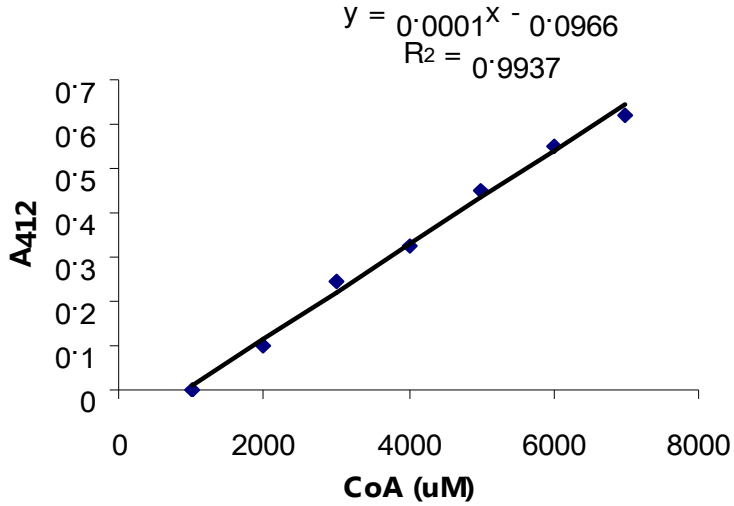

Figure 3. Standard curve of coenzyme A (CoA)

According to the multiple sequence alignment using Clustal X program, there were 5 groups giving a well-conserved sequence (Fig. 4). They were as follows: hydroxyalkanoic acid synthase, class I (*Rhodopseudomonas palustris* BisA53), Poly(R)-hydroxyalkanoic acid synthase, class I (*Rhodopseudomonas palustris* BisB18), hydroxyalkanoic acid synthase, class I (*Rhodopseudomonas palustris* TIE-1), phbC Poly(R)-hydroxyalkanoic acid synthase, class I (*Rhodopseudomonas palustris* CGA009), and Poly(R)-hydroxyalkanoic acid synthase, class I (*Rhodopseudomonas palustris* HaA2).

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A53
GAGCTGATGCAGCTCATCCAGTATCACCCGACCACCGAAACCGTGCTGCGCACGCCACTG 771
BisB18
GAATTGATGCAGCTGATCCAATATCAGCCGACCACCGAGAACGTGCTGCGCACGCCGCTG 774
TIE-1
GAAATCATGCAGCTCATCCAGTATGAGCCCGCCACCGCGACGGTGCAGCGCACGCCGCTG 774
CGA009
GAGATCATGCAGCTCATCCAGTATGAGCCCGCCACCGCGACGGTGCAGCGCACGCCGCTG 774
HaA2
GAGATCATGCAACTGATCCAGTACACGCCCGCGACCGAGACGGTGCTGCGCACGCCTCTG 774
          ** * ***** ** ***** **   ** * **** * **** ********* ***
A53
CTGATCGTGCCGCCGTGGATCAACAAATACTACATCCTCGATTTGAAGCCGGAAAAATCC 831
BisB18
TTGATCGTGCCGCCGTGGATCAACAAGTATTACATTCTCGACCTCAAGCCGGAAAAATCC 834
TIE-1
CTGATCGTGCCGCCGTGGATCAACAAGTACTACATTCTCGATCTGAAGCCCGAGAAGTCG 834
CGA009
CTGATCGTGCCGCCGTGGATCAACAAGTACTACATTCTCGACCTGAAGCCCGAGAAGTCG 834
HaA2
CTGATCGTGCCGCCATGGATCAACAAGTTCTACATTCTCGATCTCAAGCCCGAGAAGTCG 834
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A53
TTCATCAAATGGTGCGTCGACCAGGGGCTCACCGTGTTCGTGATCTCCTGGGTCAATCCG 891
BisB18
TTCGTCAAATGGTGCGTCGACCAGGGCGTCACGGTGTTCGTGATCTCCTGGGTCAATCCC 894
TIE-1
TTCATCAAATGGTGCGTCGACCAGGGCCTCACCGTGTTCGTGATCTCCTGGGTCAACCCG 894
CGA009
TTCATCAAATGGTGCGTCGACCAGGGCCTCACCGTGTTCGTGATCTCCTGGGTCAACCCG 894
HaA2
TTCATCAAATACTGCGTCGACCAGGGTCTCACCGTGTTCGTGATCTCCTGGGTCAATCCC 894
          *** ******  **********  **** ********************** **
A53
GACGAGGCGCTGCGGCACAAATCCTTCGACGACTACATGAAGCAAGGCCCGCTGACCGCG 951
BisB18
GACAAGAGCCTCGGCCACAAGACCTTCGACGACTACATGAAACAGGGTCCGCTGACCGCG 954
TIE-1
GACAAGAGCCTCGCCGACAAGGACTTCGCCGACTACATGAAGCTCGGCCCGCTGACCGCG 954
CGA009
GACAAGAGCCTCGCCGACAAGGATTTCGCCGACTACATGAAGCTCGGCCCGCTGACCGCG 954
HaA2
GACAAGCGCCTCGCCGACAAGAGCTTCGCCGACTACATGAAGCTCGGGCCGCTGACCGCG 954
          *** **  **   ****  **** ********** * ** **********
A53
ATGGATGTGATCGAAACGATCACCGGCGAGATGAAGGTGCACACGCTCGGCTATTGCGTC 1011
BisB18
ATGGACGTCATCGAACAGGTCACCGGCGAGATGAAGGTGCACACCATCGGCTACTGCGTC 1014
TIE-1
ATGGACGTCGTCGAGAAGGTCACCGGCGAGATGAAGGTCCACACGCTGGGCTACTGCGTC 1014
CGA009
ATGGACGTCGTCGAGAAGGTCACCGGCGAGATGAAGGTCCACACGCTGGGCTACTGCGTC 1014
HaA2
ATGGACGTGATCGAGAAGGTCACCGGCGAGCTGAAGGTGCACACCATCGGCTATTGCGTC 1014
          ***** **  ****  * ********** ******* *****  * ***** ******
A53
GGCGGCACCCTGCTGGCAACGACGCTGGCGTGGCTGGCCGACAAGCGCCGGGTGCGGGTC 1071
BisB18
GGCGGCACCCTGCTCGCCGCGACGCTGGCCTGGCTCGCCGAAAAGCGCCGGGTCCGCGTC 1074
TIE-1
GGCGGCACCCTGCTCGCCTCGACGCTGGCCTGGCTCGCCGAGCGCCGCCGGGTCCGCGTT 1074
CGA009
GGCGGCACCCTGCTCGCCTCGACGCTGGCCTGGCTCGCCGAGCGCCGCCGGGTGCGCGTC 1074
HaA2
GGCGGCACCCTGCTCGCCTCGACGCTGGCCTGGCTGGCCGAGCGCCGCCGCCAGCGCGTC 1074
          ********** **  ********** ***** *****   *****   ** **
A53
ACCTCCGCGACCTTCCTGACCACGCAAGTCGACTTCACCCATGCCGGCGACTTGATGGTG 1131
BisB18
ACCTCGGCGACGCTGCTCACCACCCAGGTGGATTTCACCAATGCCGGCGATCTCTTGGTG 1134
TIE-1
ACCTCGGCGACCTTCCTGACCACGCAGGTCGACTTCACCCATGCCGGCGACCTGATGGTG 1134
CGA009
ACCTCGGCGACCTTCCTGACCACGCAGGTCGACTTCACCCATGCCGGCGACTTGATGGTG 1134
HaA2
ACCTCGGCGACCTTCCTGACCACCCAGGTCGATTTCACCCATGCCGGCGACCTCAGCGTG 1134
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          ***** *****  * ** ***** ** ** ** ****** **********  *   ***
A53
TTCGTCGACGAGGAGCAGATCGCGGCGCTGGAGCAGGAGATGAAGTCTGTCGGCGTGCTC 1191
BisB18
TTCGTCGACGAGGATCAGATCGCCGCGTTGGAGCGCGAGATGCAGGCCAGCGGCGTGCTG 1194
TIE-1
TTCGTCGACGAGGAGCAGATTTCCGCGGTCGAACGCGAGATGAAGGTCACCGGCGTGCTC 1194
CGA009
TTCGTCGACGAGGAGCAGATTTCCGCGGTCGAACGCGAGATGAAGGTCACCGGCGTGCTC 1194
HaA2
TTCGTCGACGAGGGCCAGATCTCGGCGCTGGAGCGCGACATGCAGACGACCGGCGTGCTC 1194
          *************  *****  * *** * ** *  ** *** **    *********
A53
GAGGGCTCCAAGATGGCGATGGCCTTCAACATGCTGCGCTCGAACGACCTGATCTGGTCC 1251
BisB18
GAAGGCTCGAAGATGGCGATGGCCTTCAACATGCTGCGCTCCAACGACCTGATCTGGTCC 1254
TIE-1
GAAGGCGCCAAGATGGCGATGGCCTTCAACATGCTGCGGCCGAACGATCTGATCTGGTCC 1254
CGA009
GAAGGCGCCAAGATGGCGATGGCCTTCAACATGCTGCGGCCGAACGATCTGATCTGGTCC 1254
HaA2
GAAGGCGCCAGGATGGCGATGGCGTTCAACATGCTGCGGTCGAACGACCTGATCTGGTCC 1254
          ** *** * * ************ **************  * ***** ************
A53
TACGTCGTCAATAACTACCTGAAGGGCAAGTCGCCCTCGCCCTTCGACCTGCTGCACTGG 1311
BisB18
TATGTGGTCAATAACTATCTGAAGGGCCAGCCGCCGTCGGCGTTCGACCTGTTGCACTGG 1314
TIE-1
TACGTCGTCAATAACTACCTGAAGGGCCAGCCGCCGCAGGCGTTCGACCTGCTGCACTGG 1314
CGA009
TACGTCGTCAACAACTACCTGAAGGGACAGCCGCCGCAGGCGTTCGACCTGCTGCACTGG 1314
HaA2
TATGTGGTCAGCAACTATCTGAAGGGCCAGCCGCCCGCCGCGTTCGACCTGCTGCACTGG 1314
          ** ** ****  ***** ********  ** ****    * ********** ********
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Figure 4. Multiple sequence alignment of *pha*C gene of *Rhodopseudomonas palustris* strains: *Rhodopseudomonas palustris* BisA53 (A53), *Rhodopseudomonas palustris* BisB18 (BisB18), *Rhodopseudomonas palustris* TIE-1 (TIE-1), *Rhodopseudomonas palustris* CGA009 (CGA009), and *Rhodopseudomonas palustris* HaA2 (HaA2). The star represents the highly conserved region shown in panel.

The conserved regions in sequence alignments were used for designing primer. The forward and reverse primers were GGCAAGGTGATCTTCCAGAACG and CGCAGCATGTTGAASGCCATCG, respectively. Amplification of the conserved region between positions 766 and 1304 resulted in 538 bp products (Table 2). After purification of the PCR product and performed on 1.0% agarose gel electrophoresis, the 538 bp of purified PCR product was obtained. Thus, the designed primers had a good match the DNA template of CH12 strain. This result correlate to the report on phaC synthases play an important role in PHB production (AMARA; MOAWAD, 2011).

Table 2 Primer design of *pha*C gene

Primer Name	Primer Sequence	Tm (°C)	Length (bases)	Product Region	Product Length (bases)
Forward	GGCAAGGTGATCTTCCAGAACG	51.6	22	766 -1304	538
Reverse	CGCAGCATGTTGAASGCCATCG	53.4	22		

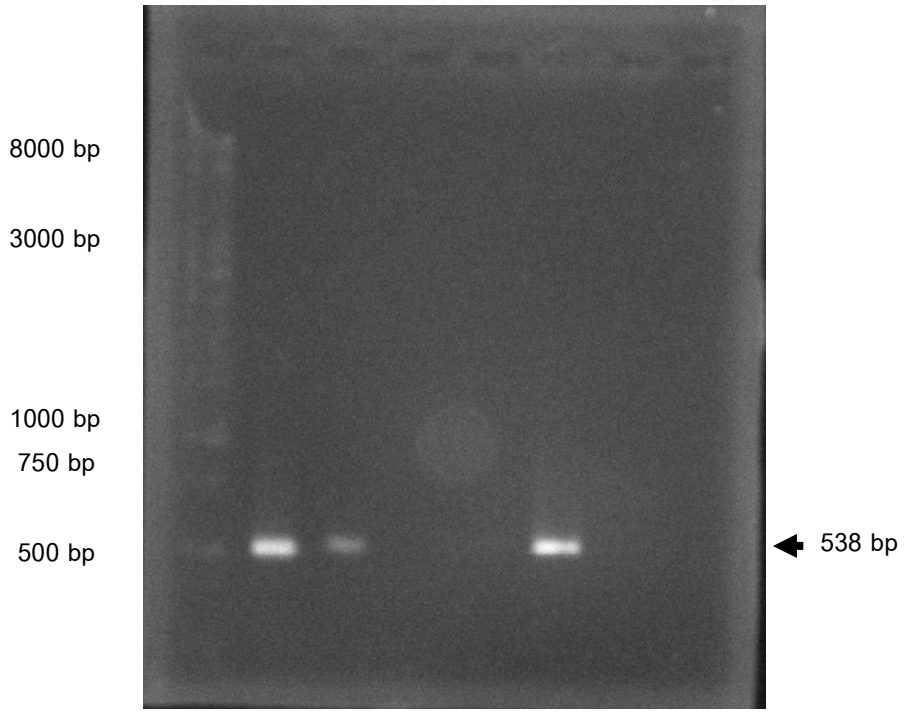

Figure 5. Amplified *phaC* gene after purification by PureLinkTM Quick Gel Extraction Kit (Invitrogen)

CONCLUSIONS

A purple non-sulfur bacterium capable of accumulating PHB inside the cells could be screened from chicken feces. It was named CH12 and identified as *Rhodopseudomonas palustris* based on taxonomic characteristics and 16S rDNA sequence analysis.

The CH12 strain had PHA synthase activity of 25.0 units/mL. The conserved region of *pha*C gene of CH12 strain could be aligned and selected among 5 related strains.

ACKNOWLEDGEMENT

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RESUMO: Este estudo teve como objetivo rastrear bactérias púrpuras não sulfurosas capazes de acumular grânulos ou polihidroxibutirato (PHB) dentro das células, identificar a estirpe potente, ensaiar a enzima ou PHA sintaxe, e comparar com o gene PHB sintase com aquele de estirpes relacionadas. Um total de 58 estirpes de bactérias púrpuras não sulfurosas foram isoladas a partir de 108 amostras de fezes de galinhas na granja produtora de ovos do Departamento de Ciência Animal, Faculdade de Recursos Naturais da Universidade Prince of Songkla, Hat Yai, Tailândia. Depois de cultivar as bactérias em um substrato de glutamato/malato (GM), sem ácido glutâmico adicionado, sob luz (3000 lux) a 35 ºC durante 5 dias, os grânulos de biopolímeros intracelulares das bactérias foram observados utilizando um microscópio confocal (do inglês *Confocal Laser Scanning Microscope* - CLSM) com comprimentos de onda de excitação e emissão de 530 e 605 nm, respectivamente. A cromatografia gasosa (do inglês *Gas chromatography* - GC) foi realizada para uma análise quantitativa de PHB. Havia 5 estirpes, CH12, CH52, CH72, CH90 e CH92, que mostraram grânulos biopoliméricos quando submetidos ao CLSM, e PHB-5 acumulado de 1.7, 1.5, 1.4 and 1.8% (w w-1) do peso celular seco (do inglês *cell dry weight* - CDW), respectivamente. A análise da sequência do rDNA 16S da estirpe CH12 demonstrou uma alta correlação de homologia de 100% para aquela da estirpe NCIB8288 da *Rhodopseudomonas palustris*. Em relação às características taxonômicas e da análise da sequência do rDNA 16S, a estirpe CH12 foi identificada como Rps. palustris NCIB8288. A atividade da PHA sintase do extrato bruto da estirpe CH12 foi de 25 unidades/mL. As regiões conservadas puderam ser alinhadas e selecionadas entre 5 estirpes de *Rhodopseudomonas palustris* (BisA53, TIE-1, CGA009, HaA2 e BisB18). O produto purificado da reação em cadeia da polimerase - PCR foi obtido para estudos futuros.

PALAVRAS-CHAVE: Fezes de galinha. Rhodopseudomonas palustris. Bioplástico. Polihidroxibutirato. Gene phaC. Região conservada.

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