Evaluating the Possibility of Using Polyhydroxyalkanoate Aquatic Byproducts of Microbial Strains Isolated From the Sea Of Vietnam

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Abstract

As plastic waste is currently an exponentially urgent issue, the usage of bioplastics to replace synthetic plastics would help to resolve the existing pollution. In addition to providing the nutrients and minerals required for the culture medium, the waste sources and byproducts of the seafood processing industry also include a wide variety of nutrients. The fat produced during the processing of the byproducts would also be a viable, affordable supply of carbon for microbial strains that can make bioplastics in the water. Particularly in this study, 191 strains were selected for growth and use of aquatic byproducts from marine habitats. The fluorescence microscopy analysis of these strains' capacity to collect PHA revealed that 35 of the strains treated with Nile blue A displayed bright orange hue. The three strains GB300, GB505, and GB515 with the best density and accumulated plastic particle size were gathered using transmission electron microscopy pictures. These three strains are known to belong to three genera based on morphological traits, culture traits, and 16S rRNA gene sequences: two genera are actinomyces (Marmoricola sp. and Piscicocus sp.), and one strain belongs to the genus Labrenzia. Three marine microbial strains' 16S rRNA gene sequences were stored in the Genbank under the accession numbers MN519525, MN519529, and MN525591, respectively corresponding to GB505, GB300, and GB515.

Keywords*: Bioplastics, PHA, PHB, Aquatic Organism, Actinomyces.*

INTRODUCTION

Plastic is among the most used materials nowadays all around the world. They are essential ingredients in the manufacture of tools, packaging, home appliances, medical equipment, and many other items. Despite the fact that plastics' technological advantages are widely acknowledged, they are undoubtedly the major contributor to environmental pollution and have a detrimental effect on the ecology of the planet since it is difficult for plastic waste to decompose in the natural environment. Polyhydroxyalkanoates (PHA) and poly-hydroxybutyrates (PHB) are two of the most frequently mentioned biodegradable polymers in recent years as they share several similar characteristics like material properties to synthetic polymers (plasticity, hydrophobicity, etc.), biocompatibility, and complete biodegradability after treatment (Braunegg et al., 1998). Bioplastics can be used in a variety of industries, including agriculture, construction, car interiors, electrical equipment, containers, packaging, and medical products (such as bone screws, membranes, and sewers) (Meng et al., 2014; Sugappriya et al., 2019; Chen, et al 2010).

When the culture medium is out of balance owing to a lack of oxygen, nitrogen, phosphorus, sulphur, or magnesium and an excess of carbon sources, polyhydroxyalkanoates (PHA), represented as poly-hydroxybutyrates (PHB), accumulate in many bacteria as a reserve material. When resources like nitrogen, phosphate, or sulphate become scarce, or when oxygen levels are low, or when there is a high C:N ratio, PHA and PHB will be accumulated by many bacteria, PHA accumulation by microorganisms can be promoted (Amrita et al., 2014; Madison and Huisman, 1999). Several bacteria such as *Azotobacter, Bacillus, Archaebacteria, Methylobacteria, Pseudomonas* have been found to synthesize PHA to varying degrees. *Ralstonia eutropha* (formerly *Alcaligenes eitrophus*) has been the subject of many published researches since it can accumulate PHA up to 80% of cell dry weight (Lee, 1996).

A diversified environment with a vast diversity of aquatic products, creatures, plants, and marine microbes in Vietnam is seen to have significant potential. The growth of fishing, aquaculture, and seafood processing has been linked to coastal communities and has grown to be a significant economic sector in socioeconomic development, fostering economic restructuring. However, there are still a lot of challenges that need to be addressed about the usage and management of seafood processing products. Since this specific source of raw materials has little economic value and is very nutrient-dense, it may be utilised as a cheap supply of nutrients to enable the growth of microbes that can harvest PHA at a lower cost, opening up new application possibilities. future for this product will make it more well-liked. In this article, we concentrate on learning about bacteria from marine environments that have the capacity to create plastic and may use byproducts and waste from the preparation of seafood as a source of nutrients for development to provide the groundwork for the manufacture of biodegradable plastics from this substance.

MATERIALS AND METHODOLOGY

Subject of the study

191 strains of microorganisms isolated from the seas of Vietnam are kept in the seed collection of the Institute of Marine Biochemistry, Vietnam Academy of Science and Technology. The strain was activated and cultured on a research-friendly medium. – *Isolated Medium Marine Agar 2216 (g/l):*Peptone 5,0; Yeast 1,0; Citrate Iron 0,1; NaCl 19,45; $MgCl_2$ 8,8; Na_2SO_4 3,24; $CaCl_2$ 1,8; KCl 0,55; $NaHCO_3$ 16; KBr 0,08; Agar 20,0. Types of microelements (mg/l): $SrCl_2$ 34,0; H_3BO_3 22,0; Na_2SiO_3. 4,0; NaF 2,4; NH_4NO_3 1,6; Na_2HPO_4 8,0.

Culture medium

NSH1 medium tested the ability of PHA-producing strains isolated from the sea:

K_2HPO_4 7g/l, KH_2PO_4 2g/l, $Na_3C_6H_5O_7$ 0.5g/l, $MgSO_4$ 0.1g/l, $(NH_4)_2SO_4$ 1g/l, pH-7.0±0.2; Dextrose : 10g/l; Sea salt 30g/l;

Media and culture conditions for PHA strains

According to Rodriguez-Contreras et al. (2013), during phase 12, culture medium was a basic mineral medium for strain development for fermentation, with the following changes to the content (%): $Na_2HPO_4.2H_2O$ 0,4; KH_2PO_4: 0,15; $MgSO_4.7H_2O$ 0,02; $(NH_4)_2SO_4$ 0,2; $CaCl_2.2H_2O$ 0,002; NH_4Fe(III) Citrate 0,005; peptone yeast 0,5; peptone 0,5; glucose 1; Sea salt 30g/l; 1ml of trace element was added after the medium had been sterilized.

Phase 2: The culture medium in this phase was the medium that stimulated the bioplastic production process (%): $Na_2HPO_4.2H_2O$ 0,45; KH_2PO_4 0,15; $MgSO_4.7H_2O$ 0,02; $(NH_4)_2SO_4$ 0,2; NH_4Fe(III) Citrate 0,005; acid citric 0,1; Glucose 1; Peptone yeast 0,05; micronutrient (2 ml), Sea salt 30g/l. Ingredients included in micronutrient solution (mg/l): $ZnSO_4.7H_2O$ 10; $MnCl_2.4H_2O$ 3; H_3BO_3 30; $CoCl_2.6H_2O$ 20; $CuCl_2.2H_2O$ 1; $NiCl_2.6H_2O$ 2; $Na_2MoO_4.2H_2O$ 3.

Components of the second cultivated phase of mineral medium: Fish fat was used as a medium to speed up the production of mineral bioplastic 2 and added at a rate of 2%; 0,3% arabic gum ; micronutrients (2 ml); yeast extract was replaced by fish extract (0,5%).

Fish waste was used in the water-based media (hearts, heads, fins, etc.). 0,5 µg/ml of Nile Blue A reagent (mixed in DMSO) is added to the medium.

Methods of pretreatment of aquatic by-products

Methods of processing by-product samples: 300 g of aquatic byproducts were weighed and ground with 300 ml of distilled water, then incubated at 50^{o}C for two hours. After that, collect the fat phase, separate the remaining impurities, and then allow the solution to cool at ambient temperature. Up until usage, samples had been kept at -20°C. This suspension would be added to the bacterial cell biomass grown in phase 1 (growth phase) culture medium as an additional source of nutrients (Mohapatra et al., 2017).

Prior to filtering, the by-products-containing slurry should be treated with a 0.6% lipase:protease combination in a 1:1 ratio. After incubating for 2 hours at 37^{o}C on a magnetic stirrer at a stirring speed of 200 rpm, the by-products were pre-filtered to collect the solution and remove any leftover by-product debris. During the fat phase, 0.3% lipase would be specifically incubated. After 30 minutes of incubation at 100

degrees Celsius, the enzyme was rendered inactive. The post-incubation solution should be stored at -20^{o}C for subsequent investigation.

Total DNA extraction method

The ChargeSwitch® gDNA Mini Bacteria Kit was used to extract the whole DNA of bacterial strains capable of synthesising bioplastic (Thermo Fisher Scientific).

16S rRNA gene amplification PCR method

To perform this reaction, two 16SFP/RP primers (Sequence design of primer pairs: Forward primer 16SFP: 5'-TCCTACGGGAGGCAGCAGTG-3'; Reverse primer 16SRP: 5'-TACGGTTACCTTGTTACGACTT-3') would be used to run PCR with PCR reaction components: 16SFP: 1 µL, 16SRP: µL Master Mix 2X: 12.5 µL Temp: 1 µL H_2O: 9.5 µL, total reaction volume: 25 µL; Heat cycle used: 94^{o}C – 3 minutes, (94^{o}C – 30 seconds, 50^{o}C – 45 seconds, 72^{o}C – 2 minutes) x 32 cycles, 72^{o}C – 8 minutes, samples kept at 4^{o}C. PCR products should be checked by electrophoresis on 1% agarose gel to detect polymorphisms of specific DNA fragments.

16S rRNA gene sequence analysis

The 16S rRNA sequences from the investigated strain were evaluated and compared using BLAST online software to assess how well the study strain matched the information supplied on the gene bank at www.ncbi.nlm.nih.gov/BLAST. The produced sequences were corroborated using MEGA v6.0 software, the Neighbor-joining tree (NJ) technique, and the pairwise comparison distance model. The Bootstrap 1000 index was used to examine branching reliability on NJ pedigree trees.

Methods of measuring the capacity of Nile Blue A-containing culture media to accumulate PHA

The method of observing the PHA accumulation capability of marine strains was employed with certain modifications to meet the strains used in this experiment. Nile blue augmented NHS1 medium Sea strains were infected with a solution at a final concentration of 0.5 µg/ml. To perform this approach, Nile blue A was diluted in dimethylsulfoxide (DMSO) at a concentration of 0.25 mg/ml. A large petri dish containing the previously prepared selection medium and the study strain was infected, divided, and cultured for 72-96 hours at 30°C to 35°C. It was removed in order to investigate the accumulating PHAs in the cells using fluorescence microscopy or UV light at 312 nm. PHA-collecting cells would look vivid orange when stained with Nile Blue A (Yamaguchi et al., 2019).

Method to observe the ability to accumulate PHA by staining with Nile blue

Collect the biomass of the study strain that has grown on mineral medium 2 48 hours after adding a drop of sterile water to a clean slide. Dissolve the inoculum then immediately dry the mixture over an alcohol lamp. The slide should be evenly covered with 20 µl of Nile blue A (diluted in DMSO at 0.25 mg/ml) and incubated for 10 minutes at 55°C. Following the collection of the sample, 8% acetic acid was drip-applied, left to rest for about a minute, and then washed twice with gently running water. Dry the samples before utilising 460 nm fluorescence microscopy on them (Ostle and Holt, 1982). When examined with a CKX53 olympus - Japan fluorescence microscope via a glass filter to detect the blue excitation wavelengths, the bioplastic particles generated in the sample will appear bright orange (The experiment was conducted out at the Vietnam Academy of Science and Technology's Institute of Marine Biochemistry).

Method to determine bioplastic accumulation capacity by transmission electron microscopy (TEM)

It is still feasible to guarantee the microscopic structure of bacteria for the purpose of examining it using a transmission electron microscope (TEM). This procedure involved fixing the sample, adding 3% glutaraldehyde, and then washing it twice for ten minutes each time with cacodylate buffer; fix the sample for two hours in 1% osmic acid in cocadylate buffer; rewash the sample twice for ten minutes each time with cacodylate buffer; Dehydrate the samples by washing twice for a total of 15 minutes at increasing alcohol concentrations of 50°, 70°, 80°, 90°, 95°, and 100°; De-alcoholization with propylene oxide 3 times at 10 minute intervals; Transfer the sample for 2-3 hours via a 1:1 combination of epon and propylene oxide; Sample would be added to the epon mixture, which was kept at 4 °C for 12 to 20 hours. Samples were impregnated and cast in an epon mixture, then incubated at 37 °C for 24 hours and 60 °C for 48 hours. Cut a 50 nm thick slide; Pick up the copper grid slice; Use lead citrate and uranyl acetate to stain ultrathin slides. After processing, the material would be examined under a microscope using Jeol, Japan's JEM-1400 HVDTTQ glass.

Extraction method to determine bioplastic structure from selected strains

To extract the wort, the biomass fermentation broth on appropriate medium should be centrifuged. The resulting biomass was soaked in acetone for 24 hours at room temperature at a mass-to-volume ratio of 1:10 (mass:volume). After filtering the biomass, immerse it in 1:10 (mass: volume) chloroform for another 15 hours or more. Add cold methanol (0°C–4°C) in a ratio of 1:2.5 by volume to the filtrate of the chloroform solution after removing the solvent from it by roughly 1/5 of the volume (while pouring and stirring the cold solvent container). After 15 minutes at 0°C, filter the white precipitate and dry the solution.

Analysis method of bioplastic structure obtained from bacteria

NMR spectra were captured using a Bruker AM500 FT-NMR Spectrometer with a TMS internal standard and $CDCl_3$ as the measurement solvent.

The *IR* spectrum was obtained using the KBr pelletizing technique using Shimazdu's FTIR-Affinity-1S. At 27°C, infrared spectra between 4000 and 400 cm^{-1} were captured (Analytical work was carried out at the Medicine Research and Development Center, Institute of Marine Biochemistry and Institute of Chemistry, under the Vietnam Academy of Science and Technology.)

RESULTS AND DISCUSSION

Preliminary screening of microbial strains isolated from the sea with the ability to accumulate bioplastics

Through an initial screening process using NSH1 medium supplemented with Nile Blue A reagent and 191 marine bacterial strains, we identified 35 strains that produce a bright orange hue under UV light at 312 nm and are capable of producing bioplastics. From 35 strains that were screened, cells were stained using the Nile Blue A technique under 1000x magnification using fluorescent electron microscopy (CKX53, Olympus, Japan). Figure 1 displays the outcomes of testing the capacity of marine microbial strains to accumulate PHA under fluorescence microscopy. Cell morphological properties are shown in Table 1 and Figure 2.

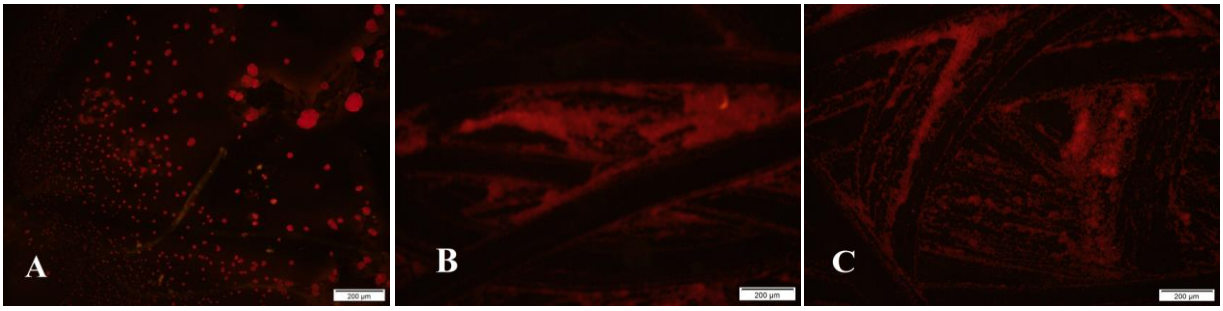

Figure 1: Image results in bright orange streaks and spots of 05 selected strains on CKX53 olympus fluorescence electron microscope by Nile Blue A cell staining method. (A): GB300 strain, (B): Strain 505. (C): Strain 515.

Observation (Figure 1) demonstrates that bioplastic molecules gathered in cells will produce bright orange streaks and patches when collected. Samples that do not generate PHA and controls will result in a black image. Based on this image, three strains of the 03 bacteria, strains GB300, GB505, and GB515, were chosen for further research as they had the most cells and the highest fluorescent staining capabilities. Table 1 below would demonstrate a summary of the findings from the characterisation of the cell shape and colonies of strain 03 bacterium.

Table 1: Morphological characteristics of 3 strains from the sea capable of selectively accumulating PHA

Sample	Characterization of colony morphology on NSH1 medium	Gram-positive/negative
GB300	Round, pinkish-white, glossy, detached from agar, slightly convex in the centre, and with a glossy surface. Diameter of the colony: 1.5 to 2 mm.	Gram-negative
GB505	Round, yellow-orange, dry, convex into large lumps on the agar surface and adhere to the agar bed. Dry surface. Diameter of the colony: 1 mm -1.5 mm.	Gram-positive

GB515	Round, yellow, dry, convex on the agar surface and adhere to the agar bed. Rough surface. Diameter of the colony: 1.5 mm – 2.0 mm.	Gram-positive

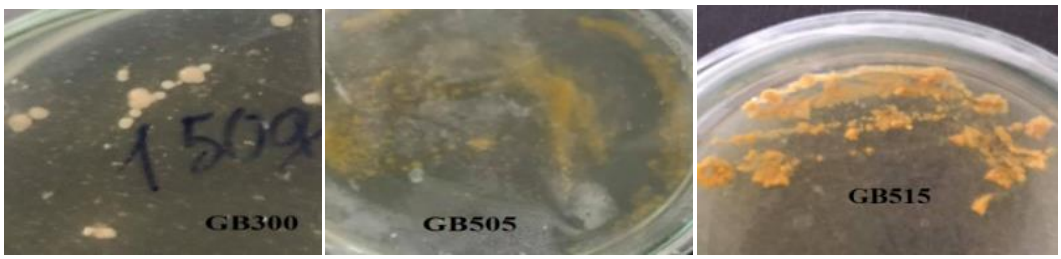

Figure 2: Image of colonies of selected strains on NHS1 medium

Quantification of bioplastic accumulation by transmission electron microscopy

PHA particles may be observed using phase inversion microscopy in cells stained with Sudan black B or Nile Blue A due to their refractive properties (Rehm, 2010). Transmission electron microscopy was utilised to develop all three strains on suitable conditions in order to capture the microbial strains' ability to accumulate resin. Figure 3 depicts the outcomes.

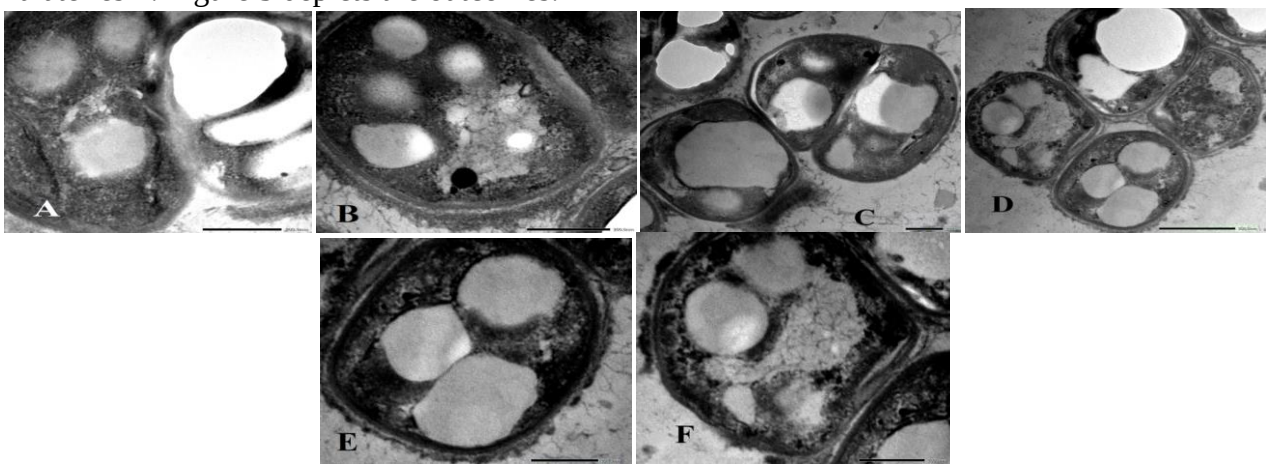

Figure 3: Cell image of bacterial strains by transmission electron microscopy (TEM). (A,B): Strain GB300; (C,D): Strain GB505; (E,F): Type GB515.

According to the results of cytology of bacterial strains by transmission electron microscopy, all three of the marine bacterial strains collected plastic particles could produce white plastic particles. The particle size is not uniform; there are tiny particles and particles larger than 600 nm x 600 nm, which are distinct, highly dense, and take up the majority of the volume in the cell. Due to their refractive characteristics, bioplastic particles within cells could be seen using phase inversion microscopy. The bioplastic particles in the bacterial cell might show up as transparent, separate, and clearly demarcated from one another under transmission electron microscopy (TEM). A single phospholipid membrane surrounds a large number of polymer molecules that make up the PHA/PHB particle structure, and on this membrane, certain proteins like protein synthase, protein depolymerase, structural proteins, regulatory proteins or cytoplasmic protein (Rehm, 2010).

Results of identification of selected strains

To better understand the biological properties of the researched strains and to improve the fermentation conditions for next research and applications, the identification and building of phylogenetic trees of selected strains is necessary. Figure 4 displays the 16S rRNA gene PCR results of a few bacteria.

Figure 4. Electrophoresis of 16S rRNA gene PCR products of 3 selections: GB515 (1), GB505(2), BP300(3);

After being processed and compared with the similarity of the NCBI databank on Genbank, the 16S rRNA gene sequences of three chosen bacterial strains revealed that strain GB300 belongs to the genus *Labrenzia*, strain GB505 belongs to the genus *Marmoricola*, and strain GB515 belongs to the genus *Piscicoccus*. According to comparison findings of strain similarities, strain GB300 was classified as *Labrenzia aggregata* since its 16S rRNA sequence was 99.04% identical to that of *Labrenzia aggregata* A11D-311 code on Genbank MK493546.1. *Marmoricola aquaticus* GB505 is a strain that is 99.07% similar to *Marmoricola aquaticus* B374 code JN615437.2. *Piscicoccus intestinalis* GB515 is the strain identified by strain GB515's 16s rRNA sequence, which shares 99.51% similarity with *Piscicoccus intestinalis* NBRC 104926 Ngc37-23 with code NR_113144.1 on Genbank. After processing, the sequences were registered on Genbank under the strain numbers GB505, GB300, and GB515 with the codes MN519525, MN519529, and MN525591.

PHA generation ability of selected bacterial strains

On phase 1 culture medium and phase 1 culture media supplemented with fish, shrimp, and crab byproducts without and with enzyme treatment and under various circumstances, the three chosen strains were assessed for their capacity to proliferate. To test the capacity to biosynthesize PHA, microbial cells that had been grown for hours were collected and put to phase 2 fermentation media. The quantity of separated PHA/CDW was used to estimate the strains' PHA content. The study samples' capacity to produce PHA when cultivated on various media at the proper period and transferred to phase 2 medium was used to estimate their PHA content. According to the findings, various strains' effects on phase 1 culture also have an impact on phase 2 culture's ability to produce PHA. Two of the three chosen marine strains, GB 505 and GB300, are less able to produce PHA than GB515, the third strain.

Table 2. Results of assessment on the ability to create PHA strain DV01

Biomass of strains cultured in the field	Bioplastic content (g/g CDW) / Culture time on phase 2 (hours)			
	24	48	72	96
Phase 1 Medium	0.002	0.051	0.263	0.255
Phase 1 Medium + pangasius extract	0.001	0.057	0.275	0.253
Phase 1 Medium + shrimp/crab extract	0.001	0.049	0.260	0.256
Phase 1 Medium plus 1*	0.002	0.053	0.289	0.278
Phase 1 Medium plus 2**	0.001	0.045	0.264	0.256

*Phase 1 Medium plus 1 + enzymatically treated pangasius extract

**Phase 1 Medium plus 2 + enzymatically treated shrimp/crab extract

Table 3. Results of assessment on the ability to create PHA strain GB300

Medium	Bioplastic content (mg/g CDW) / Culture time on phase 2 (hours)			
	44h	64h	72h	96h
Phase 1 Medium	0.77	2. 09	2.27	2.49
Phase 1 Medium + pangasius extract	0.81	1.72	2.35	2.01
Phase 1 Medium + shrimp/crab extract	0.75	1.39	1.90	1.81
Phase 1 Medium plus 1	0.77	2.03	2.69	2.63
Phase 1 Medium plus 2	0.68	1.45	1.94	1.69

Table 4. Results of assessment on the ability to create PHA strain GB515

Medium	Bioplastic content (mg/g CDW) / Culture time on phase 2 (hours)			
	120h	144h	168h	192h
Phase 1 Medium	4.80	13. 59	41.13	40.04
Phase 1 Medium + pangasius extract	4.93	22.62	42.05	41.05
Phase 1 Medium + shrimp/crab extract	4.36	16.17	29.86	28.64
Phase 1 Medium plus 1	4.77	22.03	51.50	50.91
Phase 1 Medium plus 2	3.98	21.45	39.69	39.47

The two strains of GB505 had maximal PHA contents which were 5.41 mg/g of dry biomass. At the same time, strain GB300 took 72 hours to attain its maximum resin concentration of 2.69 mg/g of dry biomass taken from the strain culture solution. The GB515 marine strain's PHA concentration peaked at 51.05 mg/g of dry biomass of culture fluid after 168 hours when biomass was taken from phase 1 medium supplemented with enzyme-treated pangasius extract, according to research using the crotonic technique.

Table 5. Results of assessment on the ability to create PHA strain GB505

Medium	Bioplastic content (mg/g CDW) / Culture time on phase 2 (hours)			
	120h	144h	168h	192h
Phase 1 Medium	2.37	4. 49	5.11	5.33
Phase 1 Medium + pangasius extract	2.25	4.02	5.23	5.25
Phase 1 Medium + shrimp/crab extract	2.71	3.09	4.82	4.86
Phase 1 Medium plus 1	2.54	4.05	5.41	5.36
Phase 1 Medium plus 2	2.13	3.75	5.07	4.65

According to Ciesielski's published findings from 2006 and 2013, the bacteria *Labrenzia* sp. belongs to the group *Proteobacteria*: The majority of the bacteria that can biosynthesize PHA that have been identified so far are largely Gram-negative and belong to the *Proteobacteria* phylum. This group is split up into several subgroups, each having a large number of member genera (Ciesielski, 2006; Ciesielski, 2013). In their 2011 study, Na Xiao and Nianzhi Jiao indicated that *Labrenzia alexandrii* DFL 11T is capable of producing polyhydroxyalkanoate (PHA) in cells by using evidence from differential scanning calorimetry, Fourier transform infrared spectroscopy, and an analysis of the 1H nuclear magnetic resonance spectrum (Xiao and Jiao, 2011).

Numerous studies have shown that, under harsh circumstances, a wide variety of marine microbe species store PHA particles intracellularly utilising carbon and energy stored inside their cells. PHB is produced by conditions like an excess carbon supply and a nitrogen constraint that stimulate the body. PHB accumulation is negatively impacted by high NaCl concentrations in hot and cold temperatures as well as deficits in potassium, calcium, and magnesium (Panda, 2007). It has been discovered that some *haloarchaeal* species from genera including *Haloferax, Haloarcula, Natrialba, Haloterrigena, Halococcus, Haloquadratum, Halorubrum, Natronobacterium, Natronococcus,* and *Halobacterium* are effective producers of PHB (Poli et al., 2011). For the first time, it was discovered that bacteria from the genera

Beneckea and *Vibrio*, which were isolated from marine sediments, were excellent at producing PHA (Lopez-Cortes et al., 2008). PHBs with lower molecular weight (about 14,000 Da) were also found in *Bacillus subtilis* and *Streptomyces* from the sea (Reusch et al., 1999).

Actinomycete-group bacteria have been identified as the source of bioplastic manufacture over time, including the species *Nocardia*, *Rhodococcus*, *Kineosphaera limosa*, *Nocardia*, *Rodococcus*, and *Streptomyces*. Transmission electron microscope images show that filamentous rays and spores accumulate bioplastic (Matias F et al., 2009). Recently, marine microorganisms have been considered as viable candidates for the creation of bioplastics. The ability to use seawater as a growth medium, the possibility for plastic manufacture, and the capability to avoid contamination are all advantages of employing marine bacteria for bioplastic biosynthesis. Extracellular biology will play a role in the large-scale industrial manufacturing of bioplastics in a variety of ways (Numata, Doi, 2012).

Determination of polymer structure from strain GB505

On the IR spectrum in Figure 5A, the strong absorption bands at 1728 cm^{-1} and 1743 cm^{-1} are typical for the valence vibration of the C=O bond. The absorption band at 1039 cm^{-1} characterizes the valence vibration of the C-O bond. The absorption bands at 2974, 2947 and 2883 cm^{-1} characterize the valence vibrations of the C-H bond in the methyl (CH_3-), methylene (-CH_2-) and methine (>CH-) groups.

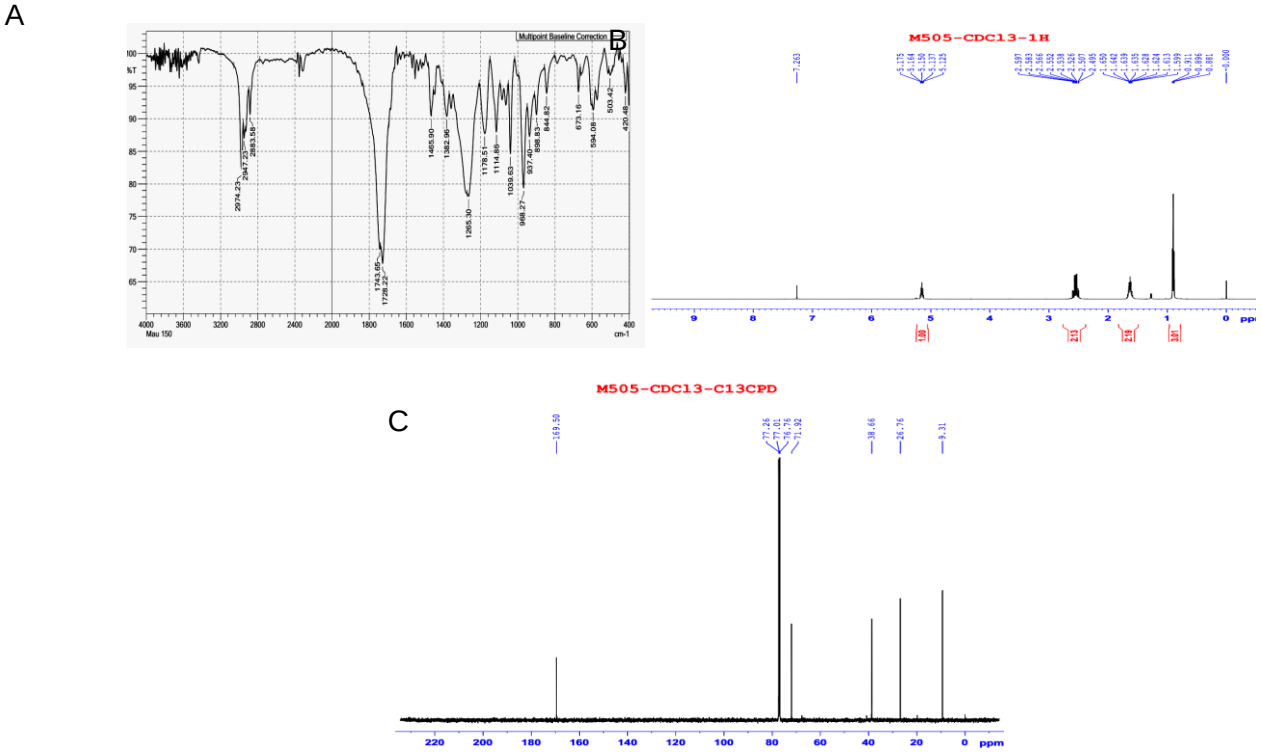

Figure 5. Infrared (IR) (A) and magnetic resonance spectroscopy, ^{13}C – NMR (C) of strain GB505

^1H spectral results of strain GB505 in Figure 5B showed that, on the ^1H NMR spectrum (measured in $CDCl_3$, 500 MHz), there is a resonance signal of an ethyl group (CH_3-CH_2-) with a signal of a methyl group (CH_3-) as a triplet at δ_H 0.89 (3H, t, J = 7.5 Hz) and a methylene group (-CH_2-) as a multiplet at δ_H1.62 (2H, m); one methine group (>CH-) at δ_H 5.15 (1H, qui, J = 6.0 Hz) and another methylene group at δ_H 2.50 (1H, dd, J = 6.0, 15.5 Hz) and 2.56 (1H, dd, J = 7.0, 15.5 Hz).

Figure 5C shows a ^{13}C NMR spectrum with 5 carbon resonance signals: 1 methyl group (δC 9.31), 2 methylene groups (δC 26.76 and 38.66), a methine group coupled to oxygen (δC 71.92), and an ester carbonyl group (δC 169.50). The IR and NMR data of strain GB505 demonstrate that this strain can produce a bioplastic known as Poly(3-hydroxyvalerate) or P(3HV).

Determination of polymer structure from strain GB515

For the results of strain GB515, on the ^1H NMR spectrum (measured in $CDCl_3$, 500 MHz), there are similar resonance signals from an ethyl group with a methyl group (CH_3-) shown as a triplet at δ_H 0.89 (3H,

t, J = 7.5 Hz) and a methylene group (-CH_2-) as a multiplet at δ_H 1.62 (2H, m); a methine group (>CH-) at δ_H 5.15 (1H, qui, J = 6.0 Hz) and another methylene group at δ_H 2.50 (1H, dd, J = 5.5, 15.5 Hz) and 2.56 (1H, dd, J = 7.5, 15.5 Hz). These ^{1}H NMR spectral data allow the identification of polymers from strain 515 that are structurally similar to polymers from strain 505 as Poly(3-hydroxyvalerate).

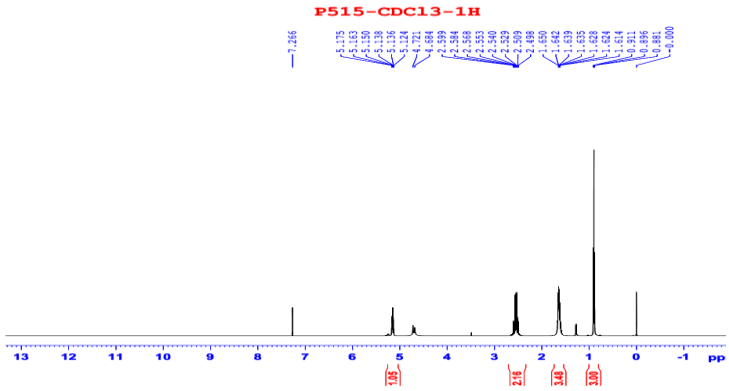

Figure 6. Spectrum ^{1}H NMR of strain GB515

Results of plastic analysis from bacteria strain GB300

The polymer from strain GB300 was identified as Poly(3-hydroxybutyrate or P(3HB) with the following NMR and IR spectral characteristics:

On the ^{1}H NMR spectrum (measured in $CDCl_3$, 500 MHz), there is a resonance signal of a doublet methyl group (CH_3-) at δ_H 1.26¬(3H, d, J = 6.0 Hz), a methylene group (-CH_2-) at δ_H 2.46 (1H, d, J = 5.5, 15.5 Hz) and 2.59 (1H, d, J = 7.5, 15.5 Hz), and a methine group (>CH-) at δ_H5.24 (1H, six, J= 6.0 Hz). On the ^{13}C NMR spectrum (measured in $CDCl_3$, 125 MHz) there is a resonance signal of 4 carbons including 1 methyl group (δ_C 19.76), 1 methylene group (δ_C 40.81), 1 oxygen-linked methine group (δ_C 67.62) and one methine ester carbonyl group (δ_C 169.14). The IR spectrum has a strong absorption band at 1724 cm^{-1} and 1056 cm^{-1}, which is typical for the valence vibrations of the C=O and C-O bonds. The absorption bands at 2978, 2952 and 2887 cm^{-1} characterize the valence vibrations of the C-H bond in the methyl (CH_3-), methylene (-CH_2-) and methine (>CH-) groups.

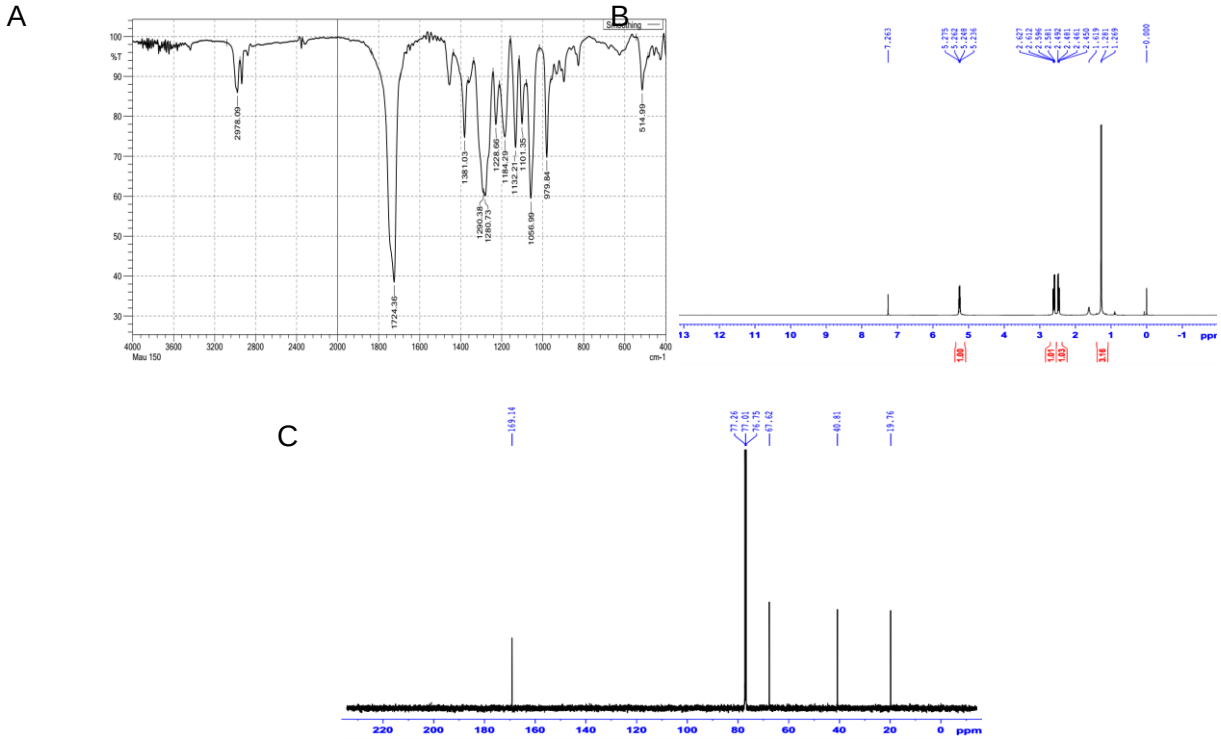

Figure 7. Infrared (IR) (A) and magnetic resonance spectroscopy ^{1}H – NMR (B), ^{13}C – NMR (C) of

strain GB300

CONCLUSION

From 191 strains of microorganisms isolated from Vietnamese seas, we have determined three strains with strong bioplastic synthesising potential. These strains comprise two actinomycetes (*Marmoricola* and *Piscicocus*), one bacterium strain, and 35 bacteria strains capable of bioplastic production (belonging to the genus *Labrenzia*).

According to the findings of this study, microorganisms discovered in Vietnamese waterways could have a potential for manufacturing biosynthetic bioplastics. However, there are still issues that need to be addressed, such as material recovery and more comprehensive application testing, in order to be able to commercialise bioplastic materials derived from marine microorganisms in the future.

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