Research Article

Polyhydroxyalkanoates Biopolymer Production by Moderately Halophilic *Paracoccus onubensis* Strain E3: Extraction, Characterization and Synergistic Activity with Sorafenib Drug against Hepatocellular Carcinoma through Molecular Docking Approach

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Abstract | Polyhydroxyalkanoates (PHAs) are biodegradable, low-cost, and ecofriendly polymers produced by various bacteria in the environment. The aim of this study was to investigate the use of moderately halophilic *Paracoccus onubensis* strain E3 as a promising PHA-producing bacteria isolated from a hypersaline environment in Egypt. The optimum conditions for PHAs production were explored and the recorded maximum yield of PHAs was 54.77 mg/ l after 72 h of incubation at 37 °C, pH 7, and 4 % NaCl (w/v), using constant carbon and nitrogen sources. PHAs were extracted and then subjected to Fourier transform infrared spectroscopy (FT-IR), where the spectrum showed a strong band at 1649.72 cm^{-1} for the carbon (C=O) stretching of the ester group, which is a common feature in PHAs structures, and Gas chromatography–mass spectrometry (GC-MS) analyses. The GC-MS identified twelve different biodegradable PHA compounds, including hexadecenoic acid and octadecanoic acid. The molecular docking study displayed high potential of the extracted PHA ingredients as drugs used against liver hepatocellular carcinoma (HCC); with binding affinity ranging between -4.0 to -6.5 kcal/ mol. This is in addition to the prediction of a synergistic effect of PHAs ingredients and sorafenib drug against HCC, showing a promising binding affinity ranging from 20.2 to 23.2 kcal/ mol. Our findings indicate that *Paracoccus onubensis* strain E3 is a promising candidate for producing PHAs, which could be used as a drug delivery system for HCC treatment with sorafenib drug.

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Introduction

Polyhydroxyalkanoates (PHAs) are biopolyesters mainly synthesized by prokaryotic microorganisms such as bacteria, which are 0.2–0.5 mm water-insoluble particles found in the bacterial cells or their cytoplasm (Aljuraifani *et al.*, 2019). PHAs accumulate intracellularly in

microorganisms as energy storage granules with physicochemical characteristics that resemble those of petrochemical polymers (Mukherjee and Koller, 2023). Polyhydroxybutyrate is the most prevalent type of PHAs, noted for its exceptional optical properties and significant UV resistance, which contribute to its strong market presence (Kumar *et al.*, 2023). This biopolymer possesses several properties, including high crystallinity, biodegradability, and resistance to ultraviolet radiation. Due to these characteristics, this material is utilized in the packaging of food and pharmaceuticals (Grgurević *et al.*, 2024). Various bacterial strains, spanning more than 90 taxa, including *Bacillus*, *Burkholderia*, *Cupriavidus*, and *Halomonas* sp. have been systematically investigated for their capacity to synthesize short-chain-length polyhydroxyalkanoates (scl-PHAs) (Yoo *et al.*, 2024). Among them, the *Halomonas mediterranei* is a notable bacterial species for its significant production of bioplastics, because of its quick development, adaptability in metabolism, genetic stability, and effective transformation mechanisms (Martínez *et al.*, 2022; Yoo *et al.*, 2024). Furthermore, agricultural food waste serves as a substitute substrate for biopolymers production, utilizing *Haloferax mediterranei*, a well-studied strain recognized for its capacity to produce polyhydroxybutyrate (PHB). This strain can prosper and produce bioplastics in high-salinity environments without the need for sterilization (Longo *et al.*, 2024). Synthesis of polyhydroxyalkanoates (PHAs) by halophilic bacteria especially those that can tolerate harsh salt conditions, has several distinct benefits. These include the need for high salinity, which inherently lowers the danger of microbial contamination, capacity to use a wide variety of inexpensive substrates, and an increased intracellular osmotic pressure, which facilitates cell lysis for PHAs extraction (Możejko-Ciesielska *et al.*, 2023). Halophiles possess distinctive adaptive characteristics that enable their survival under highly salt conditions; they have attracted considerable attention due to their remarkable adaptability and potential applications in biotechnology such as enzymes and bioplastics production (Gallo and Aulitto, 2024). But for industrial purposes, the microbial strains should satisfy the following requirements: (i) grow rapidly in cheap carbon sources, (ii) accumulate high PHBs content inside their cells with large size to ease of separation, (iii) have high efficiency for substrate transformation, and (iv) be harmless to animals, humans, and the environment (Chaudhary *et al.*, 2024). Therefore, there is still an ongoing need to identify a variety of halophilic microorganisms with unique characteristics that can produce high-yield PHA's biopolymers with an ease of extraction. These unique and distinctive polymers have been used in several fields, including medical (*i.e.*, medical materials and drug carriers), agriculture, and packaging (Das *et al.*, 2023; Satchanska *et al.*, 2024). Moreover, these polymers exhibit exceptional physicochemical features, making them highly favorable for drug delivery systems. HCC is a major type of primary liver cancer, responsible for approximately 75–85 % of mortality cases (Massarweh and El-Serag, 2017). Traditional cancer treatments such as surgery, chemotherapy, and radiation are effective but frequently result in non-specific side effects (Fahmy *et al.*, 2024). Sorafenib (SFB) serves as a first-line targeted therapy for patients with advanced HCC, expressing both anti-angiogenic and anti-proliferative effects on the tumor cells. The efficacy of SFB is limited by its off-target distribution, rapid metabolism, and multi-drug resistance. Recent studies have shown that tiny molecules composed of various materials can improve the targeting and therapeutic effectiveness of SFB in its treatment of HCC (Fan *et al.*, 2024). The current state of clinically viable therapy options for liver illnesses is suboptimal, highlighting the urgent need to investigate innovative medications and potential therapeutic approaches to address the existing limitations (Zhu *et al.*, 2023). Due to limitations of the single-drug chemotherapy, organizations have focused on developing drug delivery systems that simultaneously administer various combinations of therapeutic agents (Sari *et al.*, 2024). Combination of these considerations renders the halophilic bacteria as a highly attractive cellular facility for the synthesis of PHAs, therefore providing an environmentally sustainable and economically viable approach to PHAs production (Yoo *et al.*, 2024). Here, the objective of the current study was to identify the promising PHAs-producing moderate halophilic bacterial strain isolated from North Sinai solar saltern, achieving the industrial factors for microbial satisfaction. This study reports the highest yield of PHAs-biopolymer produced under optimized conditions, using cheap carbon and nitrogen sources, and was extracted easily from the moderate halophilic bacterial cells. Moreover, the chemical structures of PHAs indicate their potential against HCC, suggesting their utility as a promising drug delivery system capable of targeting specific sites within the body. Furthermore, an *in-silico* method suggested that

PHA components could lessen the sorafenib's adverse effects while increasing its anticancer effectiveness.

Materials and Methods

Sampling and site description

Three saline soil samples were collected from a solar saltern in North Sinai, Egypt, at about 10 cm of surface soil layer following a prior study conducted by Shokr *et al.* (2023). These samples were placed in sterile plastic pages and stored at -4 °C till further use.

Isolation of Polyhydroxyalkanoates producing bacteria and growth conditions

Fifty bacterial isolates were obtained *via* an isolation process. Briefly, 10 g of a soil sample were mixed with 100 ml of sterile seawater, followed by streak plating 0.1 ml onto Nutrient agar (NA) medium supplemented with 4 % NaCl and enriched with 1 % glucose as a carbon source (Hawas *et al.*, 2016). The plates were incubated at 37 ˚C for 48 h. Sudan Black B (0.05 %) was applied for 30 min. to all culture plates before being rinsed with ethanol. The promising results were represented by the development of plates that were black or dark blue in color, in reference to Hawas *et al.* (2016); Mustafa *et al.* (2020). For successful confirmation, the isolates that exhibited a positive result with Sudan Black B stain were re-stained with alcoholic Nile Blue A stain (1 %), and then the plates were exposed to 365 wavelengths of ultraviolet light (UV). Plates that displayed blue fluorescence expressed a positive result (Sohail *et al.*, 2020).

Biochemical and physiological characterization of the promising PHAs producing bacterial strain

For preliminary identification, the biochemical and physiological characteristics involving several *in vitro* assays (Pardamean *et al.*, 2021), including Gram stain, shape, endospore formation, motility, catalase production, oxidase, starch, indole acetic acid production, citrate, urease production, glucose fermentation, lactose fermentation, optimum pH, and DNA (G+C) content of the promising bacterial strain that can accumulate PHA granules, were determined according to Pardamean *et al.* (2021).

Scanning electron microscopy (SEM)

The moderate halophilic bacterial strain was cultured in nutrient broth at 37 ºC on a rotary shaker set to 180 rpm for 48 h to prepare them for scanning electron microscopy (SEM). The obtained bacterial culture was filtered through nucleopore filters, treated with 2.5 % glutaraldehyde for fixation, and rinsed with phosphate buffer. The bacterial culture was sequentially dehydrated using acetone solutions with various concentrations (30 %, 50 %, 70 %, 90 %, and 100 %) and then dried using critical point drying. Ultimately, the samples were affixed to metal stubs and covered with a 96 nm coating of gold. Photos were captured using a Hitachi S-570 scanning electron microscope (Hitachi Ltd., Tokyo, Japan) (Jeyanthi and Velusamy, 2016).

Molecular studies

DNA extraction, amplification, and 16S rRNA gene sequencing of the promising polyhydroxyalkanoates-producing bacterial strain: During the exponential growth phase of the moderate halophilic bacterial isolate, genomic DNA was obtained, according to the manufacturer's guidelines for Applied Biotechnology Co. Ltd., Egypt. Using the ABT DNA micro-extraction kit, a 16S rRNA gene was extracted utilizing a pair of bacterial universal primers (Invitrogen, USA), mainly forward: 27F (5-AGA GTT TGA TCC TGG CTC AG-3), reverse: 1492R (5-GGT TAC CTT GTT ACG ACT T-3) (Shokr *et al.*, 2023). The polymerase chain reaction (PCR) was performed under specified conditions utilizing a 50 µl reaction volume, including 30 cycles consisting of a 5 min. pre-denaturation at 95 ˚C, 1 min. denaturation at 94 ˚C, 1 min. annealing at 60 ˚C, 1 min. and 30 sec of extension at 72 ˚C, a final extension at 72 ˚C for 10 min., and a final hold at 4 ˚C for 10 min. The samples were prepared following the instructions provided by Macro Gen Company (South Korea) with a concentration of 50 ng/ l for each PCR product.

To conduct the phylogenetic analysis, similar 16S rRNA gene sequences were retrieved from the NCBI database. The multiple sequence alignment tool CLUSTAL-W was utilized to align a partial sequence. The MEGA X version 11 software package was then used to build a neighbor-joining tree (Tamura *et al.*, 2021).

Optimization of the growth conditions for the polyhydroxyalkanoates-producing bacterial strain

Approximately 100 µl of bacterial culture in the exponential growth phase were added to 100 ml of mineral salt medium (MSM) broth, composed

of the following components per liter of dist. water: K_2HPO_4 1.73 g, KH_2PO_4 0.68 g, $MgSO_4 \cdot 7H_2O$ 0.1 g, $FeSO_4 \cdot 7H_2O$ 0.03 g, NH_4NO_3 1.0 g, $CaCl_2 \cdot 2H_2O$ 0.02 g, and NaCl 4.0 g, with a final pH of 7.0 (Schlegel *et al*., 1961). The broth was supplemented with 20 g/ l glucose as a carbon source, as suggested by Aramvash *et al*. (2015). Afterward, the broth culture was incubated on a rotary shaker (37 ˚C/ 150 rpm). The effect of various growth factors on bacterial PHA production was tested. Accordingly, different pH ranges (6.5–7.5) in constant glucose and peptone as carbon and nitrogen sources, respectively, for varying periods (48 h - 96 h), were investigated to choose the optimum conditions for bacterial growth and production of PHAs. Cellular growth was measured using a Spectrophotometer (JENWAY 6300, United Kingdom) set to 600 nm. All assays were performed in triplicate.

Extraction of polyhydroxyalkanoates biopolymer from moderately halophilic Paracoccus onubensis strain E3

To extract the polyhydroxyalkanoates polymer, 10 ml of a bacterial incubated broth culture (at the optimum growth condition) were centrifuged at 10,000 rpm for 15 min. The supernatant was disposed of and the pellet was dissolved in 10 ml sodium hypochlorite, followed by incubation at 30 ˚C. After 2 h of incubation, the produced mixture was centrifuged at 5000 rpm for 15 min., and then washed with equal volumes of dist. water, acetone, and methanol, respectively. To obtain dry weight of the extracted polymer, the pellets were dissolved in 5 ml of hot chloroform, followed by evaporation through transferring the fluid onto a sterilized glass pan at 4 ˚C and then weighed. For dry-cell bio-mass measurement, 10 ml of the culture were centrifuged at 10,000 rpm for 15 min., and the obtained pellets were dried in an oven at 55 ˚C to a constant weight. Residual biomass (g/ l) was calculated as the difference between dry cell weight and dry weight of extracted PHBs (Hawas *et al*., 2016).

Characterization of polyhydroxyalkanoates biopolymer

Fourier transform infrared spectroscopy (FTIR) analysis: The PHAs product was characterized by using the FTIR spectrometer (Thermo Scientific Nicolet iS50, US) to identify its functional groups. Briefly, 1 mg of PHAs was finely mixed with 10 mg of spectral pure anhydrous potassium bromide crystal, and the powder was turned into a pellet. The transmitted light intensity was measured using the FT-IR spectrophotometer in relation to the absorption wavelength of 4000-400 cm^{-1} (Getachew and Woldesenbet, 2016).

Gas chromatography-mass spectrometry (GC-MS) analysis: Gas chromatography-mass spectrometry (GC-MS) analysis was performed on the extracted PHAs polymer following the technique provided by Ojha and Das (2017). The concentration of PHAs in each sample was quantified using a Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) that was fitted with a direct capillary column TG-5MS (30 mm × 0.25 mm × 0.25 µm film thickness). The column was placed in an oven originally established at a temperature of 50 ˚C and subsequently elevated at a rate of 5 ˚C/ min. until it attained a target temperature of 230 ˚C. The column was maintained at this temperature for 2 min. and then increased to a final temperature of 290 ˚C at a speed of 30 ˚C/ min. The final temperature was maintained for 2 min. The injector and MS transfer lines were kept at 250 °C and 260 °C, respectively. The carrier gas used was helium, which was maintained at a constant flow rate of 1 ml/ min. Approximately 3 min. solvent delay was implemented, and 1 µl diluted sample was automatically injected using an usingan AS1300 auto sampler that was connected to the gas chromatography (GC) in a split mode. The mass spectra *via* electron ionization (EI) were obtained using an ionization voltage of 70 eV. The spectra were collected in the range of 40–1000 m/z in a full-scanned mode, and the ion source temperature was maintained at 200 ˚C. The components were identified by comparing retention time and mass spectrum with WILEY 09 and NIST 11 mass spectrum databases.

Forward reaction prediction of the synergistic combination between polyhydroxyalkanoates and sorafenib drug against hepatocellular carcinoma (HCC)

A method for predicting the forward reactions was employed to investigate the various combined reactions between the extracted PHA compounds and the sorafenib drug. The ASKCOS MIT system (Sankaranarayanan and Jensen, 2023) was used to predict all potential subsequent reactions at https://askcos.mit.edu/ accessed on 27 November 2024, and the procedure utilized all the previously generated files in SDF format for the reaction.

Molecular docking of polyhydroxyalkanoates ingredients and predicted compounds against hepatocellular carcinoma (HCC)

The crystalline structures of Ornithine Am receptors

(1S,3S)-3-Amino-4-(hexafluoropropan-2-ylidenyl)-cyclopentane-1-carboxylic acid (BCF 3) were obtained from the protein data bank (PDB) with ID 7JX9 (Butrin *et al.*, 2020). Protein receptors were prepared into the auto dock tool 1.5.7 for prediction of the missing atoms, removal of water molecules, protonation and charge addition, and active site and pocket prediction. Subsequently, ten PHA ligand ingredients were obtained from the PubChem database and the predicted compound resulting from the synergistic combination between PHAs and sorafenib drug was prepared for docking analysis using Avogadro 1.2.0 software. The 2D structure of ligand complexes was modeled using BIOVIA Discovery Studio 2021. Molecular docking was conducted using AutoDockTools_1.5.7_vina docking approach. The drug-likeness of PHAs compounds was then assessed using Lipinski's rule of five (Elshafey *et al.*, 2025).

Statistical analysis

Statistical analysis of the obtained data was performed using the SPSS software (IBM Corporation, New York, USA). A One-Way Analysis of Variance (ANOVA) test was applied to analyze the data statistically, following the mathematical principles described by Snedecor and Cochran (1967), followed by post hoc comparisons using Duncan's multiple-range tests (Duncan, 1955).

Results

Screening and selection of the potent polyhydroxyalkanoates producing bacterial strain

Fifty bacterial isolates were obtained from a culture-based method of salty soil on NA with glucose as a carbon source. Sudan black-B and Nile blue-A dyeing approaches were used to screen for bacteria that produce PHAs. Results showed that *Paracoccus onubensis* strain E3 could accumulate PHAs by using Sudan Black-B dye, followed by a microscopic examination that showed an accumulation of black granules within the bacterial cells indicating positive synthesis of PHAs biopolymer. In addition, the plates stained with Nile blue-A were examined under UV light (365 wavelengths) to confirm the accumulation of PHAs.

Biochemical characterization and molecular identification of Paracoccus onubensis stain E3

Paracoccus onubensis stain E3 was Gram-negative, rod-shaped, non-motile, and had the ability to hydrolysis starch and glucose as examined in our previous study conducted by Shoker *et al.* (2023). Strain E3 exhibited 99.24 % similarity with *Paracoccus onubensis* and its gene sequence was deposited in GenBank under the accession number OL891886. A phylogenetic tree was generated demonstrating a correlation between moderate halophilic *P. onubensis* strain E and other bacterial species (Figure 1).

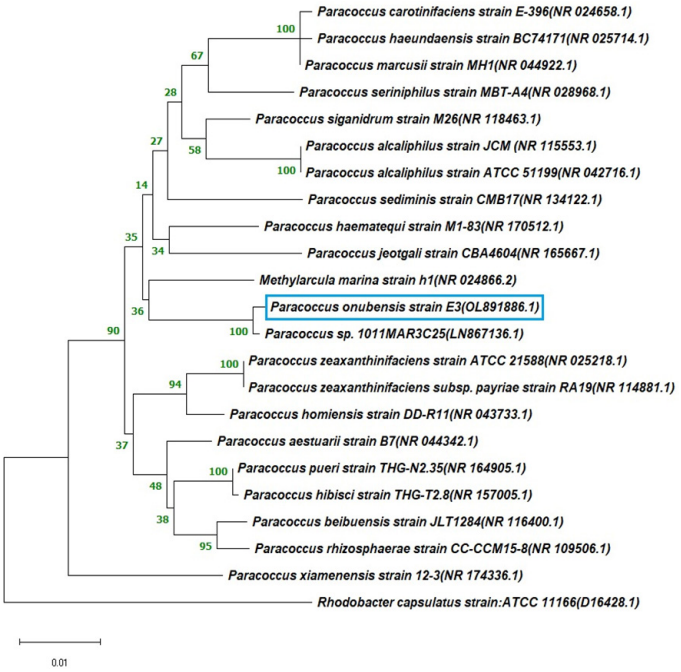

Figure 1: *Phylogenetic relationships of bacterial 16S rRNA gene sequences of Paracoccus onubensis strain E closely related to sequences of several strain retrieved from the GenBank database (Similarity > 90%).*

Scanning electron microscopy (SEM)

Scanning electron microscopy analysis confirmed the rod-shaped cells of *P. onubensis* that occurred singly or in pairs. The scale bars indicated the length of bacterial cell as 1µm (Figure 2).

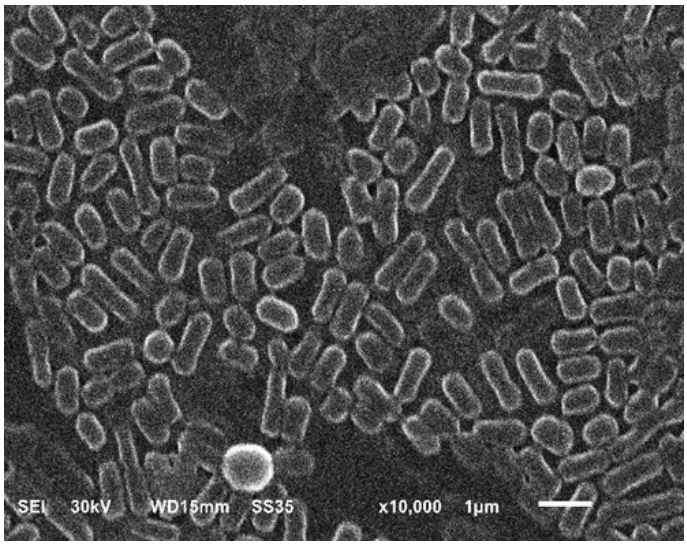

Figure 2: *Scanning electron microscope (SEM) of Paracoccus onubensis strain E3.*

Factors affecting Paracoccus onubensis strain E3 growth rate and polyhydroxyalkanoates production

Constantly, carbon sources such as glucose, nitrogen sources such as peptone, and varying pH and time factors were studied to evaluate the best growth rate of *P.s onubensis* strain E3 and the accumulation of PHAs. The results indicated a positive correlation between bacterial biomass and PHAs biosynthesis. After 72 h of bacterial incubation, the maximum growth rate and PHA production (54.77 mg/l) was attained (Figure 3a). At various tested pH levels, the highest PHAs accumulation (45.52 mg/ l) was obtained at pH 7, which was dramatically reduced after and before this pH value (Figure 3b).

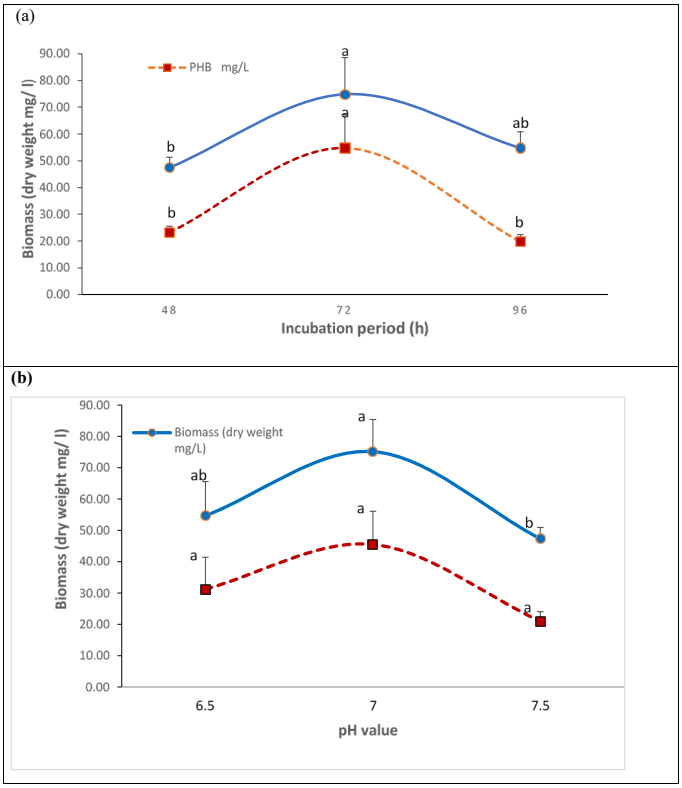

Figure 3: *The Factors affecting polyhydroxyalkanoates (PHAs) production (a): Incubation period, (b): pH value, at constant carbon and nitrogen source on growth rate and polyhydroxyalkanoates (PHAs) production by Paracoccus onubensis strain E. on the same line the values set by one-self letter(s) were not significantly different. Error bars represent the standard deviation (±SD).*

Chemical characterization of polyhydroxyalkanoate polymer

Fourier transform infrared spectroscopy (FTIR): The PHAs extracted from moderate halophilic *P. onubensis* strain E3 (Figure 4a) was analyzed *via* FTIR to determine the main functional categories present in it. The spectrum of the PHAs showed a strong band at 1649.72 cm^{-1} for the carbon (C=O) stretching of the ester group, which is a common feature in PHA's structures. The peak at 3434.05 cm^{-1} indicated a strong stretching H bond created by the terminal group (-OH). A band at 2923.55 cm^{-1} was assigned to (C–H) methylene and methyl groups, respectively. Peaks at 1494.58 and 1434.79 cm^{-1} were related to ($-CH_2$). The FTIR spectrum also peaked at 1230 cm^{-1} corresponding to the (C–O) group (Figure 4b).

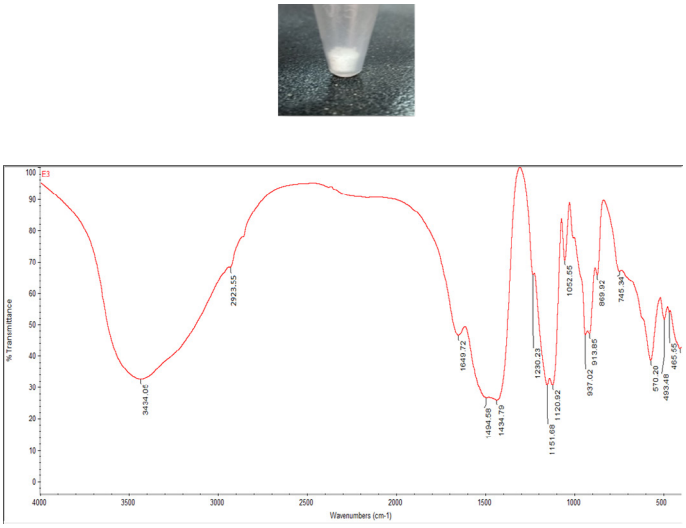

Figure 4: *(a): Extracted polyhydroxyalkanoates (PHAs), (b): Fourier Transform Infrared (FTIR) spectrum of polyhydroxyalkanoates (PHAs) extracted from moderate halophilic Paracoccus onubensis strain E3.*

Gas chromatography-mass spectrometry (GC–MS): The PHAs polymer produced from moderate halophilic *P. onubensisstrain* E3 was analyzed using GC-MS to determine its composition. The PHAs was transformed into volatile hydroxycarboxylic acid methyl esters using acidic methanolysis. The distinct peaks identified in the mass spectra aided in determining the carbonyl and hydroxyl terminations of the corresponding PHAs (Table 1).

Molecular docking analysis of polyhydroxyalkanoates against hepatocellular carcinoma (HCC)

Polyhydroxyalkanoates compound extracted from moderate halophilic *P. onubensis* strain E showed that all its ten ingredients could adhere to Lipinski's rule, although the six compounds chosen for molecular docking research against the HCC protein were the most effective according to their binding affinity with protein (Figure 5). The six compounds binding affinity scores to the HCC protein ranged from -4.0 to -6.5 kcal/ mol (Table 2). Several types of chemical ligand-receptor interactions can be studied in Discovery Studio binding to receptors. By analyzing these interactions, researchers can gain insight into the mechanisms of drugs action and design new drugs with improved potency and specificity (Figure 5).

Table 1: *Chemical composition of the biodegradable polyhydroxyalkanoates biopolymer produced by moderate halophilic Paracoccus onubensis strain E3 using GC-MS analysis.*

RT	Compound	Area %	Chemical formula	Molecular weight
13.17	Triacetin	1.93	$C_9H_{14}O_6$	218
17.31	2,4-di-tert-butylphenol	11.19	$C_{14}H_{22}O$	206
18.82	Spathulenol	2.32	$C_{15}H_{24}O$	220
24.24	2-pentyl6-(4-pentylphenyl)2,6-naphthalendicapoxylate	0.93	$C_{28}H_{32}O_4$	432
26.27	Hexadecenoic acid, methylester	4.38	$C_{17}H_{34}O_2$	270
29.41	9,12-Octadecadienoic acid-methyl ester	2.72	$C_{19}H_{34}O_2$	294
30.06	Heptadecanoic acid, 16-methyl-, methyl ester	3.43	$C_{19}H_{38}O_2$	298
32.21	Isopropyl linoleate	6.84	$C_{21}H_{38}O_2$	322
35.01	9,12-Octadecadienoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester	0.94	$C_{21}H_{38}O_4$	354
36.73	Isopropyl linoleate	1.46	$C_{21}H_{38}O_2$	322

Where; RT: Retention time of the compounds based on GC–MS peaks. The compounds are listed in order of their elution from a TG5MS column.

Table 2: *Molecular docking analysis of polyhydroxyalkanoates biopolymer ingredients from Paracoccus onubensis strain E3 against hepatocellular carcinoma (HCC) protein.*

No.	Compound name	Chemical formula	Binding-energy (Kcal/ mol)
1	9,12-Octadecadienoic acid (z,z) methyl ester	$C_{19}H_{34}O_2$	-4.9
2	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	-4.0
3	Hexadecenoic, methyl ester	$C_{17}H_{34}O_2$	-4.7
4	Isopropyl linoleate	$C_{21}H_{38}O_2$	-4.7
5	Spathulenol	$C_{15}H_{24}O$	-6.5
6	Triacetin	$C_9H_{14}O_6$	-5.5
Drug	Sorafenib	$C_{21}H_{16}ClF_3N_4O_3$	-8.9

Table 3: *Molecular docking analysis of the predicted compounds resulting from the synergistic combination between extracted polyhydroxyalkanoates (PHAs) biopolymer and sorafenib drug.*

Forward reaction prediction of compounds	Target protien Hepatocellular carcinoma (HCC)	Binding energy (Kcal/ mol)
Predicted compound from 9,12-Octadecadienoic acid (z,z) methyl ester and sorafenib drug	Ornithine Am receptors (1S,3S)-3-Amino-4-(hexafluoro-propan-2-ylidenyl)-cyclopen-tane-1-carboxylic Acid (BCF3)	20.2
Predicted compound from Isopropyl linoleate and sorafenib drug		21.9
Predicted compound from-Spathulenol and sorafenib drug		23.2
Predicted compound between Triacetin and sorafenib drug interaction		-4.9

Forward reaction prediction of the synergistic combination between polyhydroxyalkanoates and sorafenib drug against hepatocellular carcinoma (HCC): In this investigation, *in silico* analysis of the predicted compounds derived from the synergistic combination between PHAs and sorafenib drug displayed the potential to inhibit HCC. The binding energies between predicted compounds and amino acids of receptor proteins linked to HCC are shown in Table 3, while predicted compounds resulting from combination between 9,12-Octadecadienoic acid (z,z) methyl ester and sorafenib drug showed high binding energy with receptor protein 20.2 Kcal/ mol. This study identified conventional hydrogen bonds with GLY A:325, CYS A:330, and ASN A:326 residues, indicating that unfavorable interactions may play a significant role in HCC treatment, involving various amino acids such as META A:139, TYR A:323, THR A:109, TYR A:299, and ASN A:140 (Figure 6a).

Additionally, alkyl and pi-alkyl bonds were observed with PRO A:138, ALA A:333, TYR A:123, VAL A:301, LEU A:110, and LEU A:328 (Figure 6a).

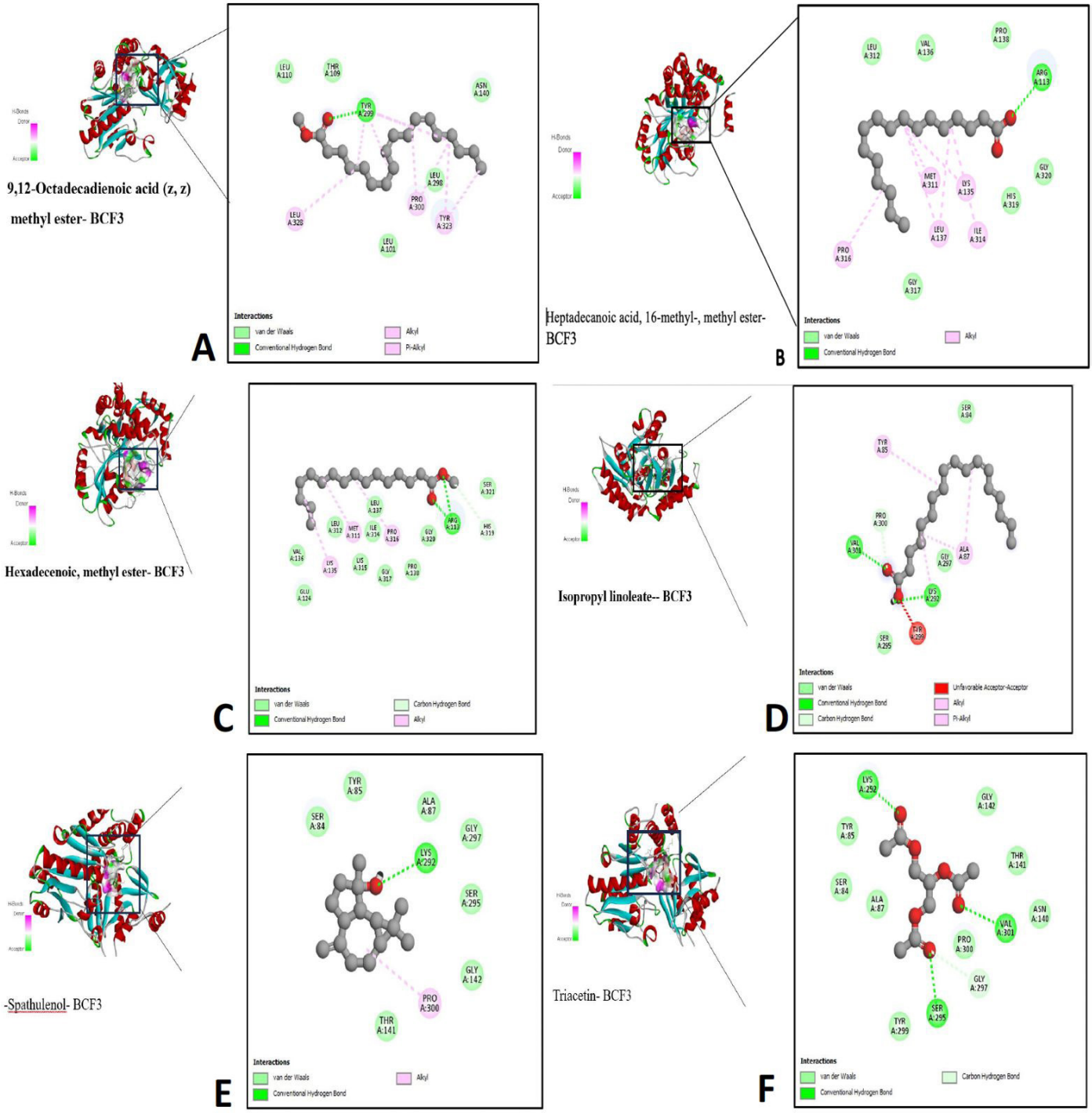

Figure 5: *Interaction of polyhydroxyalkanoates (PHAs) compounds in active site with (1 S ,3 S)-3-Amino-4-(hexafluoropropan-2-ylidenyl)-cyclopentane-1-carboxylic acid (BCF3).*

Moreover, a predicted compound resulting from the combination between Isopropyl linoleate and sorafenib demonstrated a high binding affinity of 21.9 Kcal/ mol, exhibiting carbon-hydrogen and conventional hydrogen bonds with amino acids GLY A:329 and CYS A:330. Nevertheless, unfavorable interactions were noted with PRO A:300, ASN A:140, MET A:139, ASN A:326, TYR A:323, and TYR A:299, which may contribute to the treatment of the HCC (Figure 6b). Furthermore, the predicted compounds arising from combination between Spathulenol and sorafenib drug displayed higher binding affinity of 23.2 Kcal/ mol with HCC protein, due to the presence of unfavorable pump in amino acids MET A:139, VAL A:301, ASN A:326, and CYS A:330 (Figure 6c). Meanwhile, the predicted compounds arising from combination between Triacetin and sorafenib drug showed binding affinity of -4.9 Kcal/ mol with HCC protein, due to the presence of unfavorable pump in amino acids MET A:139 and conventional hydrogen bond with amino acids GLY:325, ASN A:326, and CYS A:330 (Figure 6d).

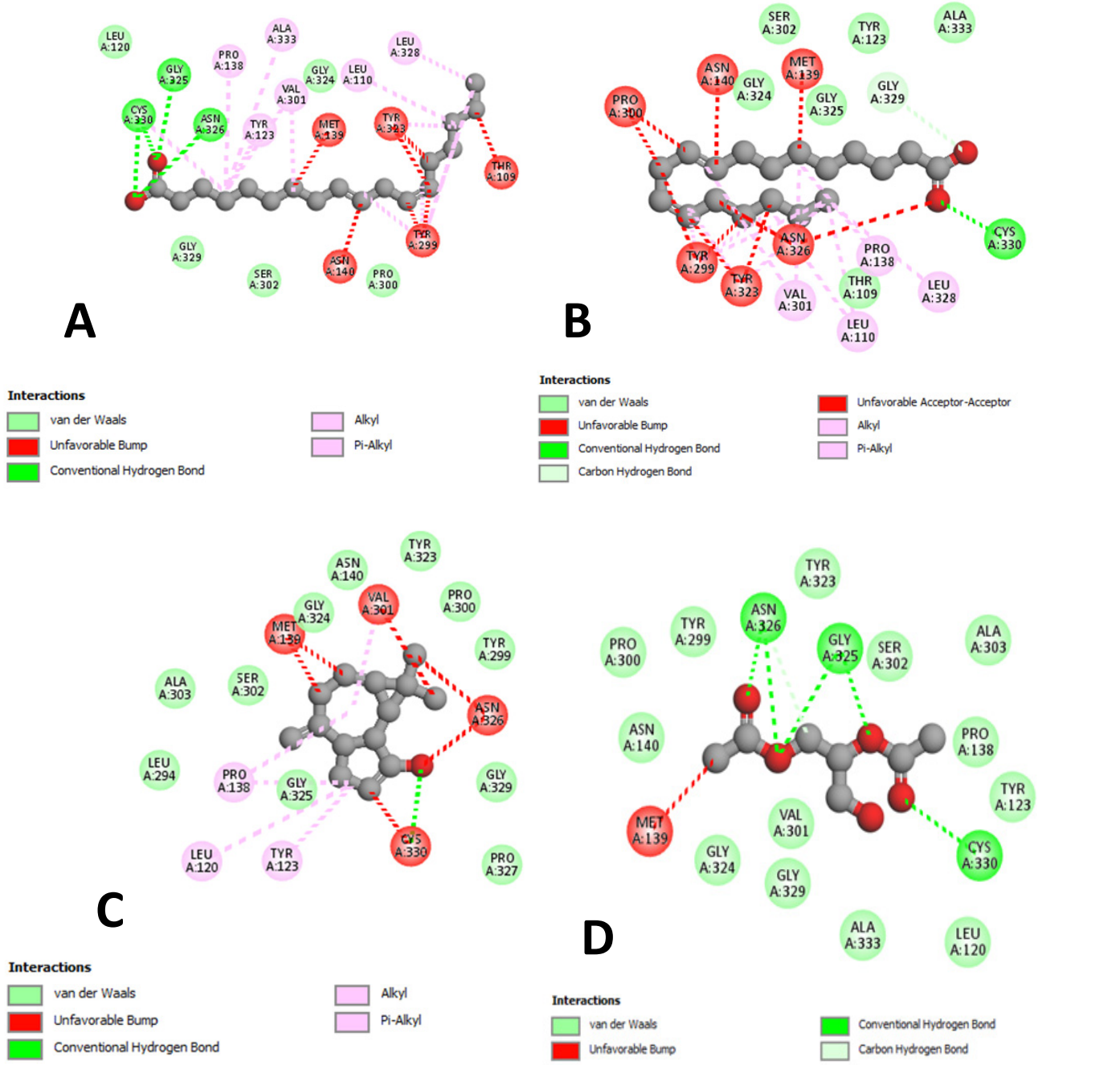

Figure 6: *Molecular docking interactions between predicted compounds from the synergistic combination between the most potent polyhydroxyalkanoates (PHAs) ingredinents and sorafenib drug against hepatocellular carcinoma (HCC) protien. (A): Predicted compound from 9,12-Octadecadienoic acid (z,z) methyl ester and sorafenib druginteraction, (B): Predicted compound from Isopropyl linoleate and sorafenib drug interaction, (C): Predicted compound between Spathulenol and sorafenib drug interaction. (D): Predicted compound between Triacetin and sorafenib drug interaction.*

Discussion

Biodegradable bio-based polymers sourced from various biomasses, including plant, animal, marine bacteria, or forestry materials demonstrate potential in substituting the traditional petrochemical polymers. Extensive research and development efforts have been undertaken for decades regarding potential biodegradable bio based polylactic acid (PLA), polyhydroxyalkanoates (PHAs), and succinate polymers (Jha *et al.*, 2024). In the current study, the promising bacterial strain was identified as *P. onubensis* strain E by analyzing the *16S RNA* gene, which displayed the formation of PHA's granules. This species is a moderately halophilic bacterium within the family *Rhodobacteraceae* that exists in marine habitats, encouraging the use of a variety of microorganisms that produce biopolymers and can synthesize specific genes and enzymes (Gutierrez-Patricio *et al.*, 2021).The obtained results of this study aligns with earlier researches conducted by Bordel *et al.* (2021), which studied the production

of PHB granules by *P. denitrificans* strain Pd1222 using laser scanning confocal microscopy and gas chromatography analysis. Optimal microbial growth conditions may improve and sustain the balanced metabolic response within bacterial cells; hence, microbial products and biomass might be augmented, as supported by Sasidharan *et al.* (2015); Mohandas *et al.* (2017). The current results showed that maximum production of PHAs by *P. onubensis* strain E3 was achieved after 72 h of incubation; however, extending the fermentation period reduced PHAs production. This may be attributed to consumption of the carbon source, resulting in exhaustion of PHAs or decaying of the enzyme activity responsible for PHAs biosynthesis in line with the results reported by Thapa *et al.* (2018). In addition, the obtained results revealed that the optimum pH for PHAs biosynthesis at natural conditions was pH 7 at 37 °c. in agreement with Sohail *et al.* (2020), who reported that the highest PHBs production and biomass of *Pseudodonghicola xiamenensis* was obtained at 35 °C, while the synthesis of PHB by *Streptomyces thermophilus* using molasses was (0.701 g/ l) at 50 °C (Sasidharan *et al.*, 2015). In this investigation, *P. onubensis* strain E3 displayed considerable growth adaptability in seawater (NaCl: 4 %) Similarly, Kawata and Aiba (2010) proved that *Halomonas* sp. accumulated PHA at NaCl requirements (3–15 %) for its optimal growth. Furthermore, Bordel *et al.* (2021) found that increasing the salt concentration to a higher degree than that of the optimal value decreased *P. denitrificans* growth and PHB accumulation due to high osmotic stress. Additionally, Yoo *et al.* (2023) showed that *Halomonas getboli* accumulates ions and solutes inside the cell to adapt to high salinity environments. Similarly, changes in *Halomonas* strains brought on by UV irradiation have been shown to promote intracellular solute accumulation (Wang *et al.*, 2023).

In the present study, *P. onubensis* strain E3 utilized cheap and available carbon source such as glucose, although the carbon source is a significant cost component for the production of PHAs in consistency with Akkoyunlu *et al.* (2024), who reported that different carbon sources from economic materials such as fructose, formic acid, and carbon dioxide (CO_2) were used for industrial scale PHAs production process. On the other hand, *Halomonas* sp. KM-1 was reported to produce PHAs using glycerol as the sole carbon source (Hagagy *et al.*, 2021). The previous study conducted by Ben-Abdallah *et al.* (2024) reported the generation of PHBs by several types of halophilic bacteria, mainly *Haloarcula marismortui*, *H. tradensis*, and *H. quadrata* utilizing starch as a carbon source. The primary factor limiting PHAs production is the nitrogen source. According to Sharma *et al.* (2017), the bacterial strains need a high concentration of nitrogen, as the nitrogen supply decreases, the bacteria begin to producing the polymer as a survival strategy. Current results demonstrated that *P. onubensis* strain E3 had the maximum growth rate on using peptone as a nitrogen source. These results agree with the findings previously reported by Muigano *et al.* (2024), who reported that an alkaliphilic and moderately halophilic bacterium *H. alkalicola* has the ability to accumulate PHAs on using peptone, which was the best nitrogen source that enhanced the growth rate and the accumulation of PHAs within the bacterial cells.

The results of FTIR and GC-MASS analysis in this study agree with Muigano *et al.* (2024), who showed that the range of (C=O) as a carbonyl group is a characteristic feature shared by all PHA's structures. The presence of a strongly stretching (H) bond formed by the terminal (-OH) group resembles the results revealed by Muigano *et al.* (2024). In addition, GC-MS analysis was conducted in this study to detect the biodegradable components of the extracted PHAs biopolymer, where the molecular ion peak in the mass spectrum and the elemental analysis agreed well with the molecular formula of the PHAs biopolymer. Meanwhile, the PHAs biodegradable components such as hexadecenoic acid and octadecanoic acid were recorded in the GC-MS report, confirming the presence of PHAs. This result is in accordance with this reported by Zahra *et al.* (2025), revealing the appearance of other ethyl esters, including pentadecanoic acid, hexadecanoic acid, and octadecanoic acid ethyl ester, indicating the presence of medium-chain length PHAs. On the other hand, the human liver constitutes around 2.5 % of the average body mass, rendering it the most substantial organ in the body. It has a critical function in many essential processes such as the breakdown of proteins, lipids, and carbohydrates, and the removal of harmful substances (Sender *et al.*, 2023). The prevention and treatment of HCC continues to be a worldwide concern, due to the lack of reliable diagnostic biomarkers and therapeutic approaches. Additionally, HCC poses diagnostic challenges owing to the absence or ambiguity of its symptoms (Chan *et al.*, 2024).

Despite the use of several treatment strategies such as chemotherapy and radiotherapy, the survival rates of liver cancer patients do not experience substantial enhancements. According to Hai *et al.* (2021), a wide range of sulfur-containing substances with biological activities has been isolated from different marine environments. These substances include secondary metabolites from moderate and halophilic *Halomonas* sp., which have shown antibacterial and antitumor activity, in addition to antiviral, anti-inflammatory, and antibiotic properties. Thus, these metabolites become important in the fight against cancer and the development of new drugs to treat it (Mahrosh and Mustafa, 2021). On the other hand, finding the precise location of a ligand molecule within the binding or active site of a target or receptor protein is the primary goal of molecular docking techniques. These approaches are also employed to predict the binding patterns between the ligand and the receptor protein (Hassan *et al.*, 2024). Combination therapies have demonstrated greater efficacy compared to monotherapy in the treatment of various cancer diseases. This advantage has historically been attributed to the capacity of combinations to tackle tumor heterogeneity; however, synergistic interaction has emerged as a prevalent explanation and design criterion for the new combinations (Plana *et al.*, 2022). A significant number of studies have concentrated on the development of drug delivery systems that simultaneously deliver various combinations of therapeutic agents to address the limitations associated with the single-drug chemotherapy (Khafaga *et al.*, 2024; Ullah *et al.*, 2025). These findings support our study which indicated that sorafenib-loaded PHAs compound is more effective than either sorafenib or PHAs compound alone. In the current study, combination of sorafenib and PHAs compound as 9,12-Octadecadienoic acid (z,z) methyl ester, Isopropyl linoleate, Spathulenol, and Triacetin demonstrated the potential and was more effective inhibitor of Ornithine Am receptors in treatment of HCC. PHAs molecules have the potential to be used as a drug carrier during treatment of liver cancer, as demonstrated by the drug-like analysis of some compounds recorded in this study. Similarly, several compound features, including maximum number of hydrogen bond acceptors, maximum number of hydrogen bond donors, minimum molecular mass of 500 Daltons, and maximum number of violations, pose significant challenges in the process of medicine development (Hamedo *et al.*, 2023; Sarfraz *et al.*, 2024). In the future, the chosen compounds such as 9,12-Octadecadienoic acid (z,z) methyl ester, Isopropyl linoleate, Spathulenol, and Triacetin may express promising potential as drug carriers for the treatment of hepatocellular carcinoma (HCC).

Conclusions and Recommendations

The promising moderate halophilic bacterial strain *P. onubensisstrain* E3 (OL891886) was recovered in a pure culture from Solar Saltern, North Sinai, Egypt. This strain showed a high potential for using glucose and peptone as sole carbon and nitrogen sources, respectively, for growth and PHAs production. Maximum production of PHAs by this strain (54.77 mg/ l) was obtained after 72 h of incubation at 37 °C, pH 7, and 4 % NaCl (w/v). FTIR analysis pattern displayed sharp peaks formed around the absorbed bands at 3434.05 cm^{-1}, 2923.55 cm^{-1}, 1649.72 cm^{-1}, 1494.58 cm^{-1}, and 1230 cm^{-1} corresponding to OH, CH3, C=O, CH2, and C–O groups, respectively, related to PHAs. Future studies will prioritize optimization of PHAs production by utilizing alternative low-cost feed stocks, with the aim of enhancing both material quality and productivity. *P. onubensis* strain E3 is a promising producer of PHAs biopolymer that can be used as a drug carrier for treatment of liver cancer.

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None to declare.

Novelty Statement

The novelty is found in the biotechnological capacity of the P. onubensis strain E3 as a sustainable and efficient producer of PHA, especially regarding its prospective use as a drug carrier for liver cancer treatment, distinguishing it from traditional PHA-producing strains. Utilizing alternative low-cost feedstocks to improve material quality and productivity will be the main focus of future research.

Author's Contribution

Conceptualization: NE, AEMS and HAH.
Methodology: NE and HAH.
Software: NE.
Validation: NE, HAH and AEMS.
Formal analysis: NE.

Investigation: OTA and NE.
Resources: OTA and NE.
Data curation: NE.
Writing-original draft preparation: NE, HAH.
Writing review and editing: HAH and NE.
Visualization: AEMS and NE.
Supervision: NE, HAH.

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References

Akkoyunlu, B., Gabarre, C., Daly, S., Casey, E. and Syron, E., 2024. Process modeling for industrial scale polyhydroxybutyrate production using fructose, formic acid and CO_2: Assessing carbon sources and economic viability. Bioresour. Technol., 393: 130139. https://doi.org/10.1016/j.biortech.2023.130139

Aljuraifani, A.A., Berekaa, M.M. and Ghazwani, A.A., 2019. Bacterial biopolymer (polyhydroxyalkanoate) production from low-cost sustainable sources. Microbiol. Open, 8(6): e00755. https://doi.org/10.1002/mbo3.755

Aramvash, A., Akbari, S.Z., Dashti, A.S. and Ghafari, M.D., 2015. Statistical physical and nutrient optimization of bioplastic polyhydroxybutyrate production by *Cupriavidus necator*. Int. J. Environ. Sci. Technol., 12: 2307-2316. https://doi.org/10.1007/s13762-015-0768-3

Ben-Abdallah, M., Sayadi, S. and Karray, F., 2024. Overproduction of poly (3-hydroxybutyrate) by the extremely halophilic archaeon *Haloarcula* sp. CEJ40-10 using starchy substrate. Biomass Conver. Bioref., 14(15): 17837-17849. https://doi.org/10.1007/s13399-023-03909-3

Bordel, S., van Spanning, R.J.M. and Santos-Beneit, F., 2021. Imaging and modelling of poly (3-hydroxybutyrate) synthesis in *Paracoccus denitrificans*. AMB Express, 11: 113. https://doi.org/10.1186/s13568-021-01273-x

Butrin, A., Beaupre, B.A., Kadamandla, N., Zhao, P., Shen, S., Silverman, R.B., Liu, D. 2020. Structural and kinetic analyses reveal the dual inhibition modes of ornithine aminotransferase by (1 S, 3 S)-3-Amino-4-(hexafluoropropan-2-ylidenyl)-cyclopentane-1-carboxylic Acid (BCF3). ACS Chem. Biol., 16(1): 67-75. https://doi.org/10.1021/acschembio.0c00728

Chan, Y.T., Zhang, C., Wu, J., Lu, P., Xu, L., Yuan, H. Wang, N. 2024. Biomarkers for diagnosis and therapeutic options in hepatocellular carcinoma. Mol. Cancer, 23(1): 189. https://doi.org/10.1186/s12943-024-02101-z

Chaudhary, R., Nawaz, A., Fouillaud, M., Dufossé, L., Haq, I.U. and Mukhtar, H., 2024. Microbial cell factories: Biodiversity, pathway construction, robustness, and industrial applicability. Microbiol. Res., 15(1): 247-272. https://doi.org/10.3390/microbiolres15010018

Das, A., Ringu, T., Ghosh, S. and Pramanik, N., 2023. A comprehensive review on recent advances in preparation, physicochemical characterization, and bioengineering applications of biopolymers. Polymer. Bullet., 80(7): 7247-7312. https://doi.org/10.1007/s00289-022-04443-4

Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics, 11: 1–42. https://doi.org/10.2307/3001478

Elshafey, N., Hagagy, N., Awny, A. and Hamedo, H.A., 2025. Bioactive compounds from *Haloarcula* sp. strain NOS1: Antimicrobial, anti-inflammatory and anti-prostatic carcinoma integrated with molecular docking approach. Egypt. J. Bot., 65(1): 404-415. https://doi.org/10.21608/ejbo.2024.321028.3002

Fahmy, H.M., Shekewy, S., Elhusseiny, F.A. and Elmekawy, A., 2024. Enhanced biocompatibility by evaluating the cytotoxic and genotoxic effects of magnetic iron oxide nanoparticles and chitosan on hepatocellular carcinoma cells (HCC). Cell Biochem. Biophys., 82: 1027–1042. https://doi.org/10.1007/s12013-024-01256-2

Fan, Q.Q., Tian, H., Cheng, J.X., Zou, J.B., Luan, F., Qiao, J.X., Guo, D.Y. 2024. Research progress of sorafenib drug delivery system in the treatment of hepatocellular carcinoma: An update. Biomed. Pharmacother., 177: 117118. https://doi.org/10.1016/j.biopha.2024.117118

Gallo, G. and Aulitto, M., 2024. Advances in extremophile research: Biotechnological applications through isolation and identification techniques. Life, 14(9): 1205. https://doi.

org/10.3390/life14091205

Getachew, A. and Woldesenbet, F., 2016. Production of biodegradable plastic by polyhydroxybutyrate (PHB) accumulating bacteria using low-cost agricultural waste material. BMC Res. Notes, 9: 1–9. https://doi.org/10.1186/s13104-016-2321-y

Grgurević, K., Bramberger, D., Miloloža, M., Stublić, K., Ocelić Bulatović, V., Ranilović, J., Kučić Grgić, D. 2024. Producing and characterizing polyhydroxyalkanoates from starch and chickpea waste using mixed microbial cultures in solid-state fermentation. Polymers, 16(23): 3407. https://doi.org/10.3390/polym16233407

Gutierrez-Patricio, S., Gonzalez-Pimentel, J.L., Miller, A.Z., Hermosin, B., Saiz-Jimenez, C. and Jurado, V., 2021. *Paracoccus onubensis* sp. nov., a novel alpha-proteo bacterium isolated from the wall of a show cave. Int. J. Syst. Evol. Microbiol., 71(8): 004942. https://doi.org/10.1099/ijsem.0.004942

Hagagy, N., Saddiq, A.A.N., Tag, H.M., Abdelgawad, H. and Selim, S., 2021. Characterization of bioplastics produced by haloarchaeon *Haloarcula* sp. strain NRS20 using cost-effective carbon sources. Mater. Res. Express, 8(10): 105404. https://doi.org/10.1088/2053-1591/ac3166

Hai, Y., Wei, M.Y., Wang, C.Y., Gu, Y.C. and Shao, C.L., 2021. The intriguing chemistry and biology of sulfur-containing natural products from marine microorganisms (1987–2020). Mar. Life Sci. Technol., 3(4): 488-518. https://doi.org/10.1007/s42995-021-00101-2

Hamedo, H.A., Elkashef, A.A., Mansour, M.A.I. and Elshafey, N., 2023. *In vitro* and molecular docking studies on a novel *Brevibacillus borstelensis* NOB3 bioactive compounds as anticancer, anti-inflammatory, and antimicrobial activity. Egypt. J. Basic Appl. Sci., 10(1): 518-536. https://doi.org/10.1080/2314808X.2023.2236378

Hassan, M., Abdrabo, M.A., Hassan, S.W., Ibrahim, H.A., Awad, M.F., Abdul-Raouf, U.M. Song, Y. 2024. *In vitro* evaluation of the antimicrobial, antioxidant, and cytotoxicity potential coupled with molecular docking simulation of the dynamic fermentation characteristics of marine-derived bacterium *Halomonas saccharevitans*. Fermentation, 10(8): 433. https://doi.org/10.3390/fermentation10080433

Hawas, J.M.E.M., El-Banna, T.E.S., Belal, E.B.A. and El-Aziz, A.A., 2016. Production of bioplastic from some selected bacterial strains. Int. J. Curr. Microbiol. Appl. Sci., 5(1): 10-22.

Jeyanthi, V. and Velusamy, P., 2016. Anti-methicillin resistant *Staphylococcus aureus* compound isolation from halophilic *Bacillus amyloliquefaciens* MHB1 and determination of its mode of action using electron microscope and flow cytometry analysis. Indian J. Microbiol., 56: 148–157. https://doi.org/10.1007/s12088-016-0566-8

Jha, S., Akula, B., Enyioma, H., Novak, M., Amin, V. and Liang, H., 2024. Biodegradable biobased polymers: A review of the state of the art, challenges, and future directions. Polymers, 16(16): 2262. https://doi.org/10.3390/polym16162262

Kawata, Y. and Aiba, S., 2010. Poly (3-hydroxybutyrate) production by isolated *Halomonas* sp. KM-1 using waste glycerol. Biosci. Biotechnol. Biochem., 74(1): 175-177. https://doi.org/10.1271/bbb.90459

Khafaga, D.S., Eid, M.M., Mohamed, M.H., Abdelmaksoud, M.D., Afify, M., El-Khawaga, A.M. Abdelhakim, H.K. 2024. Enhanced anticancer activity of silver doped zinc oxide magnetic nanocarrier loaded with sorafenib for hepatocellular carcinoma treatment. Sci. Rep., 14(1): 15538. https://doi.org/10.1038/s41598-024-65235-6

Kumar, A., Mishra, R.K., Verma, K., Aldosari, S.M., Maity, C.K., Verma, S., Thakur, V. K. 2023. A comprehensive review of various biopolymer composites and their applications: from biocompatibility to self-healing. Mater. Today Sustain., 23: 100431. https://doi.org/10.1016/j.mtsust.2023.100431

Longo, A., Fanelli, F., Villano, M., Montemurro, M. and Rizzello, C.G., 2024. Bioplastic production from agri-food waste through the use of *Haloferax mediterranei*: A comprehensive initial overview. Microorganisms, 12(6): 1038. https://doi.org/10.3390/microorganisms12061038

Mahrosh, H.S. and Mustafa, G., 2021. An *in-silico* approach to target RNA-dependent RNA polymerase of COVID-19 with naturally occurring phytochemicals. Environ. Dev. Sustain., 23(11): 16674-16687. https://doi.org/10.1007/s10668-021-01373-5

Martínez, G.M., Pire, C. and Martínez-Espinosa,

R.M., 2022. Hypersaline environments as natural sources of microbes with potential applications in biotechnology: The case of solar evaporation systems to produce salt in Alicante County (Spain). Curr. Res. Microbial Sci., 3: 100136. https://doi.org/10.1016/j.crmicr.2022.100136

Massarweh, N.N. and El-Serag, H.B., 2017. Epidemiology of hepatocellular carcinoma and intrahepatic cholangiocarcinoma. Cancer Contr., 24(3): 1–11. https://doi.org/10.1177/1073274817729245

Mohandas, S.P., Balan, L., Lekshmi, N., Cubelio, S.S., Philip, R. and Bright, S.I.S., 2017. Production and characterization of polyhydroxybutyrate from *Vibrio harveyi* MCCB 284 utilizing glycerol as carbon source. J. Appl. Microbiol., 122(3): 698-707. https://doi.org/10.1111/jam.13359

Możejko-Ciesielska, J., Ray, S. and Sankhyan, S., 2023. Recent challenges and trends of polyhydroxyalkanoate production by extremophilic bacteria using renewable feedstocks. Polymers, 15(22): 4385. https://doi.org/10.3390/polym15224385

Muigano, M.N., Anami, S.E., Onguso, J.M. and Mauti, G.O., 2024. Optimized poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) production by moderately haloalkaliphilic bacterium *Halomonas alkalicola* Ext. Int. J. Polymer Sci., 1: 6667843. https://doi.org/10.1155/2024/6667843

Mukherjee, A. and Koller, M., 2023. Microbial polyHydroxyAlkanoate (PHA) biopolymers intrinsically natural. Bioengineering, 10(7): 855. https://doi.org/10.3390/bioengineering10070855

Mustafa, G., Majid, M., Ghaffar, A., Yameen, M., Samad, H.A. and Mahrosh, H.S., 2020. Screening and molecular docking of selected phytochemicals against NS5B polymerase of hepatitis C virus. Pak. J. Pharma. Sci., 33(5): 2317-2322.

Ojha, N. and Das, N., 2017. Optimization and characterization of polyhydroxyalkanoates and its copolymers synthesized by isolated yeasts. Res.J.Pharma.Technol.,10(3):861-868.https://doi.org/10.5958/0974-360X.2017.00161.5

Pardamean, M.A., Islamy, R., Hasan, V.E.R.Y.L., Herawati, E.Y. and Mutmainnah, N., 2021. Identification and physiological characteristics of potential indigenous bacteria as bioremediation agent in the wastewater of sugar factory. Sains Malaysiana, 50(2): 279-286. https://doi.org/10.17576/jsm-2021-5002-01

Plana, D., Palmer, A.C. and Sorger, P.K., 2022. Independent drug action in combination therapy: Implications for precision oncology. Cancer Discovery, 12(3): 606-624. https://doi.org/10.1158/2159-8290.CD-21-0212

Sankaranarayanan, K. and Jensen, K.F., 2023. Computer-assisted multistep chemoenzymatic retrosynthesis using a chemical synthesis planner. Chem. Sci., 14(23): 6467-6475. https://doi.org/10.1039/D3SC01355C

Sarfraz, H., Khan, E. and Ahmad, I.Z., 2024. Unveiling the potential of bioactive compounds from *Linum usitatissimum* L. to target Hepatocellular carcinoma: an in silico-based approach. J. Biomol. Struct. Dynam., 9: 1-14. https://doi.org/10.1080/07391102.2024.2439039

Sari, L.M., Sari, D.K., Bustami, A., Gazali, A.D. and Auerkari, E.I., 2024. Cytotoxicity and apoptosis activities of passion fruit (*Passiflora edulis* Sims) seed extract on HSC-2, HSC-3, MCF-7, and HaCaT cell lines. J. Pharma. Pharmacogn. Res., 12(4): 659-672. https://doi.org/10.56499/jppres23.1798_12.4.659

Sasidharan, R.S., Bhat, S.G. and Chandrasekaran, M., 2015. Biocompatible polyhydroxybutyrate (PHB) production by marine *Vibrio azureus* BTKB33 under submerged fermentation. Annals Microbiol., 65: 455–465. https://doi.org/10.1007/s13213-014-0878-z

Satchanska, G., Davidova, S. and Petrov, P.D., 2024. Natural and synthetic polymers for biomedical and environmental applications. Polymers, 16(8): 1159. https://doi.org/10.3390/polym16081159

Schlegel, H.G., Kaltwasser, H. and Gottschalk, G., 1961. A submersion method for culture of hydrogen-oxidizing bacteria: Growth physiological studies. Archive Microbiol., 38: 209–222. https://doi.org/10.1007/BF00422356

Sender, R., Weiss, Y., Navon, Y., Milo, I., Azulay, N., Keren, L., Milo, R. 2023. The total mass, number, and distribution of immune cells in the human body. Proc. Natl. Acad. Sci., 120(44): e2308511120. https://doi.org/10.1073/pnas.2308511120

Sharma, P.K., Munir, R.I., Blunt, W., Dartiailh, C., Cheng, J., Charles, T.C., Levin, D. B. 2017. Synthesis and physical properties of polyhydroxyalkanoate polymers with different monomer compositions by recombinant Pseudomonas putida LS46 expressing a novel PHA synthase (PhaC116) enzyme. Appl. Sci., 7(3): 242. https://doi.org/10.3390/app7030242

Shokr, A., Abd-Elsalam, O., Hamedo, H. and Elshafey, N., 2023. Isolation and identification of Polyhydroxybutyrate (PHB)-producing microorganisms from various sources in North Sinai. Catrina Int. J. Environ. Sci., 27: 23–31. https://doi.org/10.21608/cat.2023.295260

Snedecor, G.W. and Cochran, W.G., 1967. Statistical methods. Iowa State University Press, Ames, Iowa. 240: 285.

Sohail, R., Jamil, N., Ali, I. and Munir, S., 2020. Animal fat and glycerol bioconversion to polyhydroxyalkanoate by produced water bacteria. e-Polymers, 20: 92–102. https://doi.org/10.1515/epoly-2020-0011

Tamura, K., Stecher, G. and Kumar, S., 2021. MEGA11: Molecular evolutionary genetics analysis version 11. Mol. Biol. Evol., 38(7): 3022-3027. https://doi.org/10.1093/molbev/msab120

Thapa, C., Shakya, P., Shrestha, R., Pal, S. and Manandhar, P., 2018. Isolation of polyhydroxybutyrate (PHB) producing bacteria, optimization of culture conditions for PHB production, extraction and characterization of PHB. Nepal J. Biotechnol., 6(1): 62-68. https://doi.org/10.3126/njb.v6i1.22339

Ullah, A., Khan, M., Zhang, Y., Shafiq, M., Ullah, M., Abbas, A., Diao, Y. 2025. Advancing therapeutic strategies with polymeric drug conjugates for nucleic acid delivery and treatment. Int. J. Nanomed., 20: 25-52. https://doi.org/10.2147/IJN.S429279

Wang, Z., Li, Y., Gao, X., Xing, J., Wang, R., Zhu, D., Shen, G. 2023. Comparative genomic analysis of *Halomonas campaniensis* wild-type and ultraviolet radiation-mutated strains reveal genomic differences associated with increased ectoine production. Int. Microbiol., 26(4): 1009-1020. https://doi.org/10.1007/s10123-023-00356-y

Yoo, Y., Kwon, D.Y., Jeon, M., Lee, J., Kwon, H., Lee, D., Criddle, C. S. 2024. Enhancing poly (3-hydroxybutyrate) production in halophilic bacteria through improved salt tolerance. Bioresour. Technol., 394: 130175. https://doi.org/10.1016/j.biortech.2023.130175

Yoo, Y., Lee, H., Lee, J., Khim, J.S. and Kim, J.J., 2023. Insights into saline adaptation strategies through a novel halophilic bacterium isolated from solar saltern of yellow sea. Front. Mar. Sci., 10: 1229444. https://doi.org/10.3389/fmars.2023.1229444

Zahra, I., Arshad, A., Betenbaugh, M., Shabbir, U., Nawaz, S., Liaqat, A., Shakoori, F. R. 2025. Biosynthesis of polyhydroxyalkanoates employing a newly isolated *Paramecium multimicronucleatum*. Int. J. Environ. Sci. Technol., 22: 365–374. https://doi.org/10.1007/s13762-024-05681-x

Zhu, L.R., Li, S.S., Ni, W.J. and Cai, M., 2023. Targeted modulation of gut microbiota by traditional Chinese medicine and natural products for liver disease therapy. Front. Immunol., 14: 1086078. https://doi.org/10.3389/fimmu.2023.1086078