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Effects of Halophilic Bacteria's Polyunsaturated Fatty Acids (PUFAs) on Human Health

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ABSTRACT
The need for polyunsaturated fatty acid (PUFA) generation from fish oil and plants has increased with the rise in health consciousness. PUFAs are extremely advantageous for human health and have a positive influence on heart and brain function. Humans cannot generate PUFAs on their own because they are essential fatty acids, so they must get them from their food. PUFAs are long-chain hydrocarbons with multiple double bonds that have methyl and carboxyl groups at opposite ends. The focus is mostly on halophiles due to the desire for PUFAs that can be produced at low cost, practically, and without risk, as well as the problem of contaminated fish oil and the need to prevent the exploitation of plants and marine life. High-salinity environments are ideal for halophilic bacteria. They are capable of withstanding salt concentrations of up to 30% and 1.7% (0.3 m) (5.1 m). Halophiles are effective at producing PUFAs on a large scale. In addition to resolving the problem of fish-derived PUFAs, which is a problem for the majority of vegans, switching from fish-derived PUFAs to microbial PUFAs has the potential to be a revolutionary and entirely sustainable solution. Eicosapentaenoic acids (EPA) and docosahexaenoic acids (DHA), which are obtained from halophilic bacteria, will be produced, characterized, and discussed in this article along with their comparison to PUFAs derived from fish and plants.

Keywords: polyunsaturated, production, human health, fish, halophilic bacteria, plants

I. INTRODUCTION

The long-chain hydrocarbons known as polyunsaturated fatty acids (PUFAs) have one or more double bonds. PUFAs often fall under the category of "essential fatty acids," meaning that people require them as a dietary supply. EPA and DHA are the two primary Omega-3 fatty acids that are extensively ingested around the world. From this point forward, the extended names will be explored; the abbreviated forms are already covered in the abstract. As they can prevent cardiovascular disease, diabetes, breast cancer, brain hemorrhage, Alzheimer's disease, intestinal inflammation, and allergy illnesses, EPA and DHA are extremely useful for human health. The primary sources of EPA and DHA intake worldwide are fish oil and plant derivatives. But this is where the issue with vegans comes up. Omega-3 products produced from fish frequently cause complications for vegans when consuming them. Similar environmental concerns exist now with the use of plant materials in the manufacture of PUFAs. Halophiles are a special type of marine life that nature has given us as a solution to this dilemma. As the name implies, halophiles are microorganisms that live in highly salinized environments. Halophilic microorganisms are classified according to the amount of salt they require and given the designation "halotolerant" since they can withstand salt concentrations of 1.7% to 30%. These are categorized as "light," "medium," and "heavy" halophiles. Academics and business leaders are increasingly identifying the production of PUFAs by halophiles as a key area of study.

EPA and DHA have numerous uses and advantages for health. They are extensively utilized in home fish farming and feeding, cattle and chicken feeding, effective ingredients in the pharmaceutical and cosmetic industries, vegetable oil production, and biofuel production, among other uses. The development of the retina and neurological system depends on DHA, but studies have shown that people with hypertriglyceridemia can efficiently lower their triglyceride levels by consuming pure EPA. These two advantageous PUFAs serve as building blocks for a variety of lipid hormones, including eicosanoids and prostaglandins. In this article, PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are extracted from halophiles, will be produced, characterized, used, and compared with PUFAs derived from fish and plants.

II. PRODUCTION OF EPA AND DHA

Gammaproteobacteria have a significant role in PUFA synthesis. Along with species of Flexibacter and Psychroserpenes, this halophilic class also contains several Shewanella, Photobacterium, Moritella, and other related family members. (2018) Moi et al. Deep research has been done on the PUFA production by halophiles, and some scientists are curious as to why PUFAs are necessary for marine ecosystems. According to Yoshida et al. (2016), halophilic bacteria that are found between 1000 and 2000 kilometers deep in the ocean, where the temperature is extremely low and the pressure is quite high, frequently require PUFA in their cell membrane. The high-density fatty acids in their cell membrane act as insulators against pressure and cold shocks. EPA has been listed in several sources as a requirement for survival in cold and high-pressure environments; however, some have disputed this. For instance, the strain Shewanella livingstonensis Ac10 produces a lot of EPA at 4 °C; it has been noted that at this temperature, EPA makes up 5% of the total fatty acids in their cell membrane. A mutant strain of Ac10 and its wild-type strain were grown at 4 °C to demonstrate the requirement for EPA. In contrast to the wild-type Ac10, it was demonstrated that the mutant strain was unable to survive at this temperature (Kawamoto et al., 2009). Similar evidence that EPA-deficient strains cannot develop between 0.1 MPa and 20 MPa between 4° and 20°C was shown by Shewanella piezotolerans WP3, an isolate of a psychotolerant and piezotolerant bacterium from the Pacific Ocean (Wang et al., 2004). (Usui et al., 2012). However, as was already mentioned, other articles disputed the notion that EPA is a necessary condition for marine microorganisms. Deep marine vents can support the growth of the mutant Shewanella marinintesina IK-1 strain, which lacks EPA (Yoshida et al., 2016). Similar to this, the replacement of MUFA in the Photobacterium profundum SS9 mutant strain allows it to grow at lower temperatures.

Table 1: The top halophiles in terms of EPA production are listed.

Bacteria	Type of PUFA	PUFA %	Origin
Shewanella pneumatohori SCRC- 2738	EPA	36.6	Pacific mackerel
hewanella hanedai ATCC 33224	EPA	22.2	Arctic Ocean
Shewanella benthica ATCC 43992	EPA	16.0	Intestine, holothurians
Shewanella gelidimarina ACAM 456	EPA	16.0	Antarctica
Vibrio sp. strain 29-1	EPA	19.7	Deep Sea Sediment
Vibrio sp. strain 814-4	EPA	17.9	Deep Sea Sediment
Photobacterium profundum SAMA2	EPA	15.0	Tidal Flat sediment at Wadden
Photobacterium Profundum DSJ4	EPA	13.0	Deep sea sediment
Vibrio sp. strain 5710	DHA	22.7	Deep sea sediment
Vibrio sp. strain 5705	DHA	21.5	Deep sea sediment
Vibrio sp. strain 5703	DHA	18.6	Deep sea sediment
Moritella marina MP-1	DHA	12	Deep Sea
Colwellia psychrerythraea ACAM 550	DHA	8.0	Antarctica

III. HALOPHILES' PUFA BIOSYNTHESIS AND GENES REQUIRED

Polyketide synthase (PKS), a critical enzyme in halophiles, is required for PUFA production. For the formation of PUFAs, the PKS biosynthetic pathway contains four significant recurring stages. Condensation comes first, then reduction, dehydration, activation, and then reduction once more (Gong et al., 2014). 2004 (Ratledge). EPA and DHA are created by numerous bioprocessing stages. Fig. 1 depicts the PKS biosynthetic pathway for the synthesis of EPA and DHA. The desaturase/elongase route is distinct from the PKS pathway. Desaturation by desaturase enzymes of oleic acid happens, followed by elongation by the elongase enzyme of stearidonic acid, which is followed by multiple desaturations and elongations to generate EPA and DHA in the desaturase/elongase pathway. (2013) Sakuradani et al. there are five PUFA-coding genes in all, numbered PfaA through PfaE, which have been shown to be present in numerous halophilic bacteria. Four

different bacteria, Photobacterium profundum SS9, Shewanella pneumatophori SSRC-2738, Moritella marina MP-1, and Pseudoalteromonas sp. DS-12, had their genes compared. These five genes have a lot of similar protein expressions, but they vary in the number of domains. These genes produce the phosphopantetheinyl acyl carrier protein (ACP), acetyl transferase (AT), dehydratase (DH), ketoacyl reductase (KR), and ketoacyl synthase (KR) (PT). The following table 2 displays the various numbers of domains for PUFA-producing gene clusters in these four halophilic bacteria. (2006) (Orikasa et al.) Yoshida et al., 2016 (Shulse & Allen, 2011) (Shulse & Allen, 2011) (2016) Yoshida et al. 2002, Allen & Bartlett 2014's Gong et al. (2012) Cao & Cao (1999; Tanaka et al.) [19] (1996, Yazawa).

Table 2: Shows the different groups of genes that make PUFAs in these four halophilic bacteria.

Genes Bacteria	PfaA	PfaB	PfaC	PfaD	PfaE
Phobacterium Profundum SS9	5ACP+KR+ KS+AT	AT	2KS+3DH	ER	Absent
Shewanella Pneumatophori SCRC-2738	5ACP+KR+ KS+AT	AT	2KS+3DH	ER	PT
Moritella marina MP-1	5ACP+KR+ KS+AT	KS+AT+DH	2KS+DH	ER	PT
Pseudoalteromonas sp. DS-12	5ACP+KR+ KS+AT	2KS	3DH+ PT	ER	PT

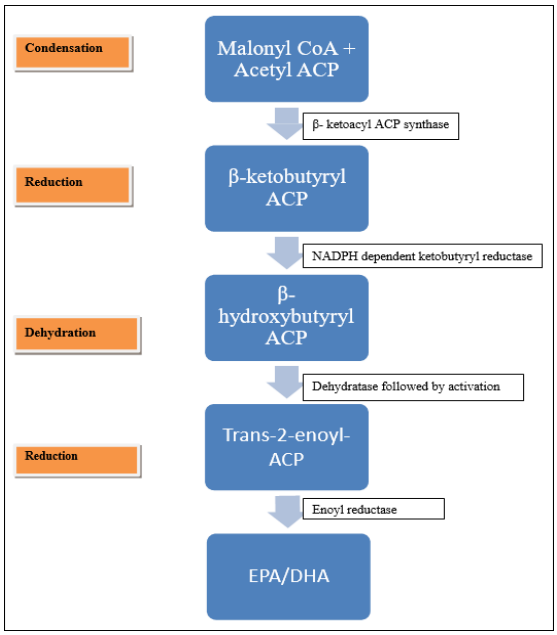

Figure 1: Depicts the PKS biosynthetic pathway for the synthesis of EPA and DHA

IV. CHARACTERIZATION OF PUFA

Direct viewing via the H2O2-plate experiment The use of an H2O2 test to determine H2O2 in a cell or other biological system is a simpler method for screening and isolating PUFA-producing bacteria. A crucial regulator for numerous oxidative stress-related states, hydrogen peroxide (H2O2) is a reactive oxygen metabolic by-product produced by both enzymatic and non-enzymatic mechanisms. The marine bacteria are grown in LB (Luria-Bertani) media (1% tryptone, 0.5% yeast extract, and 1% NaCl composition per liter) at 26 to 30 degrees Celsius for 24 hours at 180 revolutions per minute. In addition, 0.5% of NaCl is added to ensure appropriate bacterial development. Direct observation reveals that a key distinguishing factor between bacteria that express PUFAs (without a zone of inhibition) and bacteria that do not produce PUFAs is the oxidative balance of PUFAs in developing bacteria in response to increased H2O2 (a zone of inhibition). PUFAs are the most susceptible to reactive oxygen species and oxygen in general (ROS). Tilay and Annapure (2012).

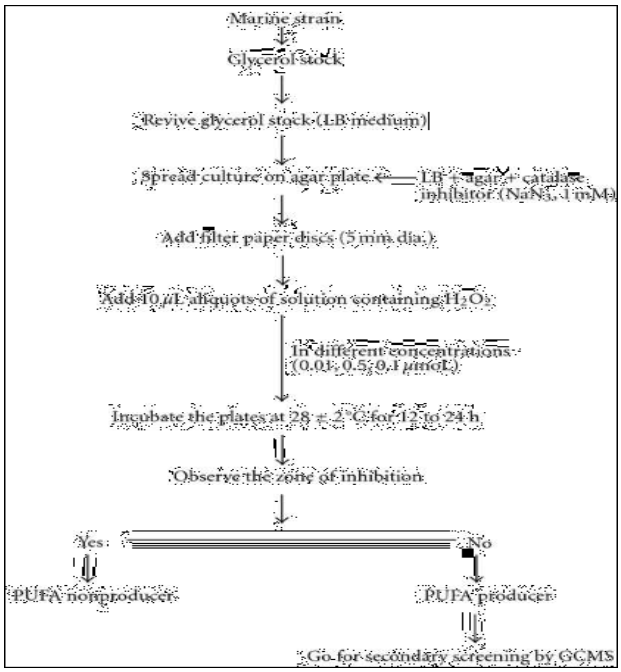

Figure 2: Primary marine isolate screening protocol (Tilay & Annapure, 2012)

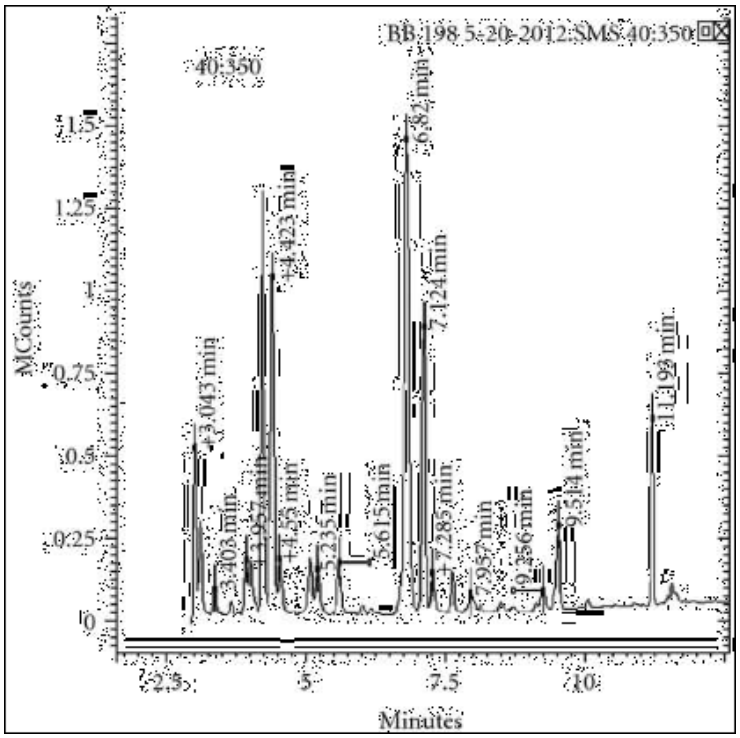

Figure 3: A gas chromatogram showing the fatty acid profile of a selected halophilic isolate was produced following first screening. (2012)

V. FATTY ACID METHYLESTER PREPARATION (FAMES)

The sample is cultivated in Marine Salt Medium (MSM) (81.0 g NaCl, 10.0 g yeast extract, 9.6 g MgSO4, 7.0 g MgCl2, 5.0 g proteose peptone no. 3, 2.0 g KCl, 1.0 g glucose, 0.36 g CaCl2, 0.06 g NaHCO3, and 0.026 g NaBr composition per liter, with pH 5-9) and it is then incubated for The cells are collected after 48 hours by centrifuging them for 12 minutes at 10,000 rpm. The resulting supernatant is then discarded, and the cell pellets are then suspended in 1.0% NaCl (w/v) and centrifuged once more. Each bacterial culture tube is kept at 4 °C. Then, after reweighing the bacterial cells, a fresh solution of the transesterification reaction mix (methanolic HCl (0.6 N) in 4 ml) was placed in the tubes. The solutions were then sealed securely, vortexed for 5–10 s, and heated in an 80°C±2°C water bath for 2 h. The tubes were promptly chilled with ice after that. By adding 2 volumes of hexane and then 1 volume of hexane and centrifuging the resulting FAMES at 5,000 rpm for 15 minutes, the resultant FAMES were extracted twice. The top phase of the hexane layer is used for gas chromatography analysis. In 2010, Jadhav et al.

VI. FAME ANALYSIS BY GCMS

Based on their respective retention durations, the Varian 220-MS and Varian 450-GC were used to evaluate the analyte FAME, and the known standards were used as a point of comparison. A capillary column manufactured of CP-SIL 88 silica fused with methyl silicone with an internal diameter of 25 mm by 0.25 mm and an outside diameter of 0.39 mm was included in the connected set of equipment (Jadhav et al., 2010). The column oven was set to go from 160 to 220°C in seven minutes, then stay at 220°C for ten minutes, while the injector was maintained at 250°C. The carrier gas employed was helium, and the flow rate was held constant at 1 mL/min. According to Tilay and Annapure (2012), the GCMS had a mass range of 40–350 atomic mass units, a 70 eV ionization voltage, and a 220°C trap temperature.

Molecular biology methods like PCR (Polymerize Chain Reaction), RAPD (Random Amplified of Polymorphic DNA), and sequencing of 16S ribosomal polymer genes can be used to reliably explore a wide range of bacteria. Random amplification of polymorphic DNA (RAPD) analysis, which uses nine random primers to measure genetic linkage, is the easiest, fastest, and least expensive way to do this.

The following steps are involved in the Fatty Acid Methyl Esterification (FAME) trans esterification process:

1. Correctly harvest cells and store the cell pellet.
2. Pulverize the dried cell pellet to a fine powder, and then add 10–20 mg to a 1.8 mL salt-glass sample ampule with a PTFE- lined cover.
3. More or less add 0.5 mL of 5% H2SO4 in wood spirit to the ampule's dry biomass.
4. After tightly sealing the ampule, incubate it at 90 °C for 90 minutes, and then let it cool.
5. Flip the mixture over and stir in 0.4 of the dissolvent.
6. Mix 0.8 mL of 100 percent NaCl with water well.
7. Permit the mixture to separate into two layers.
8. After moving the top dissolvent layer to a new ampule, does the dissolvent extraction on the liquid layer that is still there and mix the dissolvent phases.
9. As required, carry out GC-MS or GC-FID analysis. (2002) Allen & Bartlett

Table 3: Displaying the percentage of EPA and DHA production sourced from fish

Fish Resources	% of EPA production	% of DHA production
Sardine	6.6	19.0
Herring	8.5	8.3
Shad	0.12	0.43
Atlantic Salmon	6.2	5.8
Pink Salmon	1.7	3.3
Brown trout	0.9	3.5
Surf smelt	3.6	5.7
Alaska pollock	1.0	2.4
Sudan catfish	0.1	0.7
Garfish	0.01	0.15

Table 4: Displays the source percentage for EPA and DHA-producing plants.

Plant source	% of EPA production	% of DHA production
Chia seeds	0	0
Flax seeds	0	0
Walnuts	0	0
Brusells sprouts	0	0
Hemp seeds	0	0
Perilla oil	0	0

VII. COMPARING PUFA OBTAINED FROM FISH AND PLANTS

However, plants are unable to create EPA and DHA, which are necessary ingestible polyunsaturated fatty acids that are mostly consumed by humans. Instead, plants synthesize PUFA as linoleic acid (ALA) and linoleic acid (LA), which are also forms of omega-3 fatty acids. Therefore, we can classify them as minimal producers of EPA and DHA. They can, however, be a useful source for other applications that use ALA and LA. Because of this, humans must rely on the production of PUFA by microalgae, macroalgae, and other halophilic organisms. Therefore, it is evident from the above tables that plants cannot be highlighted as an efficient producer of EPA and DHA and that they should be given less priority than halophilic bacteria, which are capable of producing large quantities of PUFAs, notably EPA and DHA. Additionally, the removal of trees and the rise in global warming levels are causing a lack of plants all over the world. It is our responsibility to reduce their consumption as much as we can, and halophiles are a great replacement because they are abundant everywhere and have a high capacity to produce PUFA.

Table 5: Displaying the source as a percentage of halophiles that produce EPA and DHA

Halophiles source	% of EPA production	% of DHA production
Shewanella pneumatohori SCRC-2738	36.6	12
Shewanella hanedai ATCC 33224	22.2	8.0
Shewanella benthica ATCC 43992	16.0	23.7
Shewanella gelidimarina ACAM 456	16.0	20.0
Vibrio sp. strain 29-1	19.7	14.8
Vibrio sp. strain 814-4	17.9	19.7
Photobacterium profundum SAMA2	15.0	23.3
Photobacterium Profundum DSJ4	13.0	17.4
Vibrio sp. strain 5710	13.0	22.7
Vibrio sp. strain 5705	13.8	21.5
Vibrio sp. strain 5703	12.4	18.6

VIII. GAINS FROM EPA AND DHA

As was already said, people can't make important polyunsaturated fatty acids on their own, like omega-3 fatty acids like eicosapentaenoic acid and docosahexaenoic acid.EPA and DHA have a number of health advantages, including supporting healthy brain development, preventing cardiovascular disease, having anti-inflammatory and antioxidant qualities, serving as cattle feed, and being more affordable to produce than biofuel. Compared to EPA, docosahexaenoic acid is more important for the development of the fetal brain. It has been demonstrated that a DHA shortage in pregnant rats results in reduced brain connectivity and decreased brain function in response to environmental stimuli. Pregnant women who consume DHA throughout pregnancy have children with improved problem-solving abilities, fewer allergic reactions, stronger immune systems, improved retinal development, superior eye-hand coordination, and even no longer run the danger of developing asthma (Sharma et al., 2020).

In addition to helping prevent myocardial infarction, sudden cardiac death, atherosclerosis, and coronary artery disease, EPA and DHA also increase the high-density lipoprotein to low-density lipoprotein ratio, lower triglyceride levels, and lower cholesterol levels. EPA and DHA are much healthier than arachidonic acid. The consumption of PUFA has tripled over the last six years, with consumption between 2013 and 2015 averaging between 120 and 140 thousand metric tons. According to Sharma et al. (2020), modern civilization has started ingesting EPA and DHA in amounts ranging from 0.2 to 4.0 g per day.

Eicosapentaenoic acid plays a significant role in preventing atherosclerotic plaques, decreasing macrophage aggregation and thereby preventing autoimmune disease, increasing the HDL to LDL ratio, increasing the content of collagen

and smooth muscle cells, and reducing internal inflammation by inhibiting the expression of adhesion molecules, monocyte chemoattractant protein-1, and matrix metalloproteinase, which are produced by macrophages. Additionally, it prevents the expression of cells like dendritic cells and tumor necrosis factors, which are produced when atherosclerosis is present. EPA has antioxidant properties since it also scavenges the secondary metabolites in the form of radicals formed under atherosclerotic circumstances (Sharma et al., 2020).

As a result, PUFA are good for human health. Like omega-3 fatty acids, EPA and DHA are essential for the development of the neurological and circulatory systems and should be consumed often. Halophile-derived dietary PUFA are the finest option for vegans worldwide to replace fish-derived dietary PUFA. PUFAs obtained from halophiles have no negative side effects, can be manufactured in larger quantities, and are commercially viable, do not harm marine life, and have no negative environmental consequences.

IX. DISCUSSIONS

This study offers information on the characteristics and advantages of PUFA (mostly EPA and DHA) produced by halophiles. In order to raise concerns about human health, we changed the concept of EPA and PUFA creation from fish and plant-derived PUFA to halophile-derived PUFA.The significance of halophiles is emphasized in this essay. Large factories called halophiles have the capacity to create more EPA and DHA than fish. They can take the place of plants since they only produce ALA and LA instead of EPA and DHA. Although microalgae are the main source of EPA and DHA, halophiles can also be a useful alternative. It is quite challenging to extract EPA and DHA and process them afterward. As previously stated, the class Gammaproteobacteria is important in the formation of PUFAs.The halophilic strains Shewanella pneumatophori SCRC-2738, Shewanella hanedai ATCC 3324, and Vibrio species strain 29-1 all produce significant amounts of EPA. Similar to DHA, Vibrio species strain 5710 produces the most, 22.7%, followed by Vibrio sp. strains 5705 and 5703, which produce 21.5% and 18.6% docosahexaenoic acid, respectively. According to Table 1,

Additionally, the PKS biosynthesis route, which is exclusive to halophiles, was mentioned. Condensation, reduction, dehydration, activation, and reduction are the steps in the route. These actions are repeated to create DHA and EPA. In halophiles, there are four genes called PfaA through PfaE that produce the proteins ACP, KR, KS, AT, DH, ER, and PT. These genes have different domains, so we have compared them in four different bacteria: Photobacterium profundum SS9, Shewanella pneumatophori SSRC-2738, Moritella marina MP-1, and Pseudoalteromonas sp. DS-12, based on the differences in the domains. As mentioned in Table 2,

The H2O2 test, a direct visualization technique for PUFA screening and separation, distinguishes between PUFA-producing organisms and non-PUFA-producing organisms based on their propensity to react with reactive oxygen species when a catalase inhibitor is present. It describes in detail how to prepare FAME for gas chromatography testing. Halophiles are cultivated in a marine salt medium, harvested by centrifugation, and then undergo a transesterification reaction in the presence of methanolic HCL 0.6N. Finally, the extracts were recovered using a twofold hexane treatment of two volumes and one volume for gas chromatography analysis.

Finally, we compared the PUFA produced by halophiles to those produced by fish and plants. We discovered that Shewanella pneumatohori SCRC-2738 produces the highest amounts of EPA and DHA, 36.6% and 12%, respectively. This is significantly more than that produced by fish species like sardines (6.6% EPA and 19.% DHA), herring (8.5% EPA and 8.3% DHA), Atlantic salmon (6.2% EPA and 5.8% DHA), Shewanella hanedai ATCC 33224, which produces 22.2% EPA and 8.0% DHA, Shewanella benthica ATCC 43992, which produces 16.0% EPA and 23.7% DHA, and so on, as indicated in Table 1.
These studies suggest that halophiles can be a reliable and potent source of dietary PUFA because they grow quickly and are easy to process afterward. This means that they should be encouraged for commercial production.

X. CONCLUSION

With the growing demand for healthy, beneficial lifestyles and naturally derived nutraceuticals, EPA and DHA play an important role in brain development, preventing cardiovascular disease and Alzheimer's disease, acting as anti-inflammatory, antioxidant, anti-allergic, and anti-cancerous products, and so on. Thus, as the population grows, it becomes increasingly difficult to synthesize omega-3 fatty acids on a large scale. Therefore, in terms of environmental concern and vegan ease of intake, PUFA derived from halophiles can be a superior option and a decent replacement for PUFA derived from fish. The advantages of halophiles-derived PUFA over microalgae-derived PUFA include non-toxicity, wide-scale production, elimination of the vegan consumption issue, cost-effective downstream processing, big factories for EPA and DHA production, etc. We can therefore draw the conclusion that PUFA derived from halophiles have great economic and research potential and can replace the need for and dependence on fish, while PUFA derived from plants are non-toxic, can be produced on a large scale, and can solve the problem of vegan consumption. They also have more cost-effective downstream processing than

PUFA derived from microalgae, large factories to produce EPA and DHA, etc. As a result, we can conclude that PUFA derived from halophiles has significant economic and research potential and has the potential to replace the requirement for and dependence on PUFA derived from fish and plants. It will inspire us to isolate new distinct genera of halophiles that can produce even more PUFA in the form of EPA and DHA if systematic screening, correct medium optimization, and optimal growth conditions are evaluated and maintained.

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