Research Article

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Palmitic acid and long-chain polyunsaturated fatty acids dominate in mycelia of mangrove *Halophytophthora* and *Salispina* species in Taiwan

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Abstract: Marine oomycetous species produce, among other fatty acids, omega-6 arachidonic acid (ARA) and omega-3 eicosapentaenoic acid (EPA), with implications for the industrial potential of this group of organisms and the need to find an isolate with high production. This study screened 14 isolates of marine oomycetous species: *Halophytophthora avicenniae*, *H. batemanensis*, *H. exoprolifera*, *H. polymorphica* and *Salispina spinosa* cultured from fallen mangrove leaves in Taiwan for 24 saturated and unsaturated fatty acids in their mycelia. This paper is the first to report C18:1n-7 vaccenic acid, C20:1 eicosenoic acid, C24:1 nervonic acid, C20:2n-6 eicosadienoic acid, C22:4n-6 adrenic acid, C20:4n-3 eicosatetraenoic acid and C22:5n-3 docosapentaenoic acid in mycelia of *Halophytophthora* and *Salispina* species, and the

fatty acid profiles of *H. batemanensis* and *H. exoprolifera*. Five fatty acids were dominant in the mycelia of the isolates, i.e. C16:0 palmitic acid, C18:1n-9 oleic acid, C18:2n-6 linoleic acid, C20:4n-6 arachidonic acid and C20:5n-3 eicosapentaenoic acid. For the essential fatty acids, *S. spinosa* produced the highest level of arachidonic acid (27–31% of total fatty acid (TFA), 141–188 mg l⁻¹ yield) while *H. avicenniae* IMB212 produced the highest percentage of EPA (15% of TFA) while *H. polymorphica* IMB227 produced the highest yield (96 mg l⁻¹). Different species and isolates of the same species produced different fatty acid profiles, and further research effort may yield a high production isolate of industrial significance and also important fatty acids from the marine environment.

Keywords: biofuel; degrader community; docosahexaenoic acid; essential fatty acids; marine fungi.

1 Introduction

Halophytophthora is an aquatic oomycetous genus, and includes both freshwater and marine species: *H. avicenniae*, *H. batemanensis*, *H. exoprolifera*, *H. fluviatilis*, *H. insularis*, *H. masteri*, *H. polymorphica*, *H. porrigovesica*, *H. souzae* and *H. vesicula* (Jesus et al. 2019; Marano et al. 2014; Yang and Hong 2014). *Salispina*, is a related genus and was established to accommodate two subspecies of *Halophytophthora spinosa* (var. *spinosa* and var. *lobata*) (Li et al. 2016). *Salispina* currently includes *S. hoi*, *S. intermedia*, *S. lobata* and *S. spinosa* (Bennet and Thines 2018; Li et al. 2016). The life cycle of *Halophytophthora/Salispina* is characterized by a vegetative mycelial phase and a reproductive phase producing sympodial or irregular branching sporangiophores, papillate/non-papillate zoosporangia with smooth wall or spines, with or without an operculum, vesicle and plug (Nakagiri 2002). *Halophytophthora/Salispina* species generally are leaf degraders in tropical/subtropical mangrove environments but

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have also been isolated from leaf litter and organic debris in temperate locations (Man 2019; Nigrelli et al. 2013).

A fatty acid is a carboxylic acid with a long aliphatic chain, and can be categorized as saturated or unsaturated. The aliphatic chains in saturated fatty acids are linked by single bonds while those in unsaturated fatty acids are linked by one (monounsaturated fatty acid, MUFA) or more (polyunsaturated fatty acid, PUFA) double bonds. Docosahexaenoic acid (DHA, 22:6 (n-3)), eicosapentaenoic acid (EPA, 22:5 (n-3)), and arachidonic acid (ARA, 20:4 (n-6)) are examples of commonly known PUFAs that are beneficial to human health (Zárate et al. 2017). Many microorganisms are known to produce these essential PUFAs including fungi, Stramenopiles (thraustochytrids), bacteria, Cryptophyta, Haptophyceae and Alveolata (Kothri et al. 2020; Pang et al. 2016).

Pang et al. (2016) were the first to show that *Halophytophthora* and *Salispina* species (*H. avicenniae*, *H. polymorphica*, *H. vesicula*, *Salispina spinosa*) produced 14 different types of saturated (C10:0 capric acid, C12:0 lauric acid, C14:0 myristic acid, C15:0 pentadecanoic acid, C16:0 palmitic acid, C17:0 heptadecanoic acid, C18:0 stearic acid) and unsaturated (C16:1 palmitoleic acid, C16:2 hexadecadienoic acid, C18:1n-9c oleic acid, C18:1n-9t elaidic acid, C18:2n-6 linoleic acid, C20:4n-6 arachidonic acid, C20:5n-3 eicosapentaenoic acid) fatty acids in their mycelia, and different species showed dissimilar profiles in terms of type and quantity. For example, a high level of arachidonic acid (ARA), but no eicosapentaenoic acid (EPA), was produced by *Halophytophthora spinosa* (Pang et al. 2016), and this characteristic was used to justify the transfer of *H. spinosa* to the new genus *Salispina* (Li et al. 2016). EPA was also absent in isolates of *S. spinosa* and *S. hoi* from the Philippines (Caguimbal et al. 2019; Devanadera et al. 2019). Devanadera et al. (2019) further found C20:0 arachidic acid, C22:0 behenic acid, C23:0 tricosanoic acid, C24:0 lignoceric acid, C22:1 erucic acid, C18:3n-6 γ -linolenic acid, C20:3n-6 dihomogamma-linolenic acid and C18:3n-3 α -linolenic acid in mycelia of two unidentified *Halophytophthora* isolates and *S. hoi*.

Our research was proposed because: (1) unsaturated fatty acids, such as DHA and EPA, are beneficial to human health with fish oil as their main source; (2) there is a shortfall in the supply of DHA and EPA for human consumption based on the human population and the recommended daily intake of these essential fatty acids (Sprague et al. 2016); (3) an alternative source of DHA and EPA is required to meet demand for these fatty acids; (4) *Halophytophthora* and *Salispina* have been shown to produce a range of fatty acids including EPA (Pang et al. 2016). In this study, 24 saturated and unsaturated fatty acids in mycelia of 14 isolates of *Halophytophthora* species (*H. avicenniae*, *H. batemanensis*, *H. exoprolifera*, *H. polymorphica*) and *Salispina spinosa* cultured from mangrove leaves in Taiwan were screened.

This study is the first to screen *Halophytophthora* and *Salispina* species for the fatty acids C18:1n-7 vaccenic acid, C20:1 eicosenoic acid, C24:1 nervonic acid, C20:2n-6 eicosadienoic acid, C22:4n-6 adrenic acid, C20:4n-3 eicosatetraenoic acid and C22:5n-3 docosapentaenoic acid. The fatty acid profiles of *Halophytophthora batemanensis* and *H. exoprolifera* are screened for the first time.

2 Materials and methods

2.1 Isolation

Fallen mangrove leaves were collected from mangroves at Hsin-fong (Hsinchu County), Chu-nan (Miaoli County), Pu-zi (Chiayi County), Fang-yuan (Zhanghua County) and Sih-caio (Tainan County), all located at the western side of the main Taiwan island. In the laboratory, the leaves were briefly washed with tap water and rinsed twice with sterile seawater (30 ‰). Washed leaf pieces (~1 cm² square) were submerged-inoculated in 15 ml sterile seawater (3‰) supplemented with 0.5 g l⁻¹ each of penicillin G sodium salt (Bioshop, Burlington, Canada) and streptomycin sulfate (Bioshop, Burlington, Canada) in Petri dishes and incubated at 25 °C in the dark for 2 days. Under a stereomicroscope, individual zoosporeangia of different morphologies were picked with a flame-sterilised forceps and subcultured onto PYGS (peptone-yeast extract-glucose seawater) agar plates (4 g l⁻¹ peptone (Oxoid, Basingstoke, UK), 4 g l⁻¹ yeast extract (Oxoid, Basingstoke, UK), 4 g l⁻¹ glucose (Bioshop, Burlington, Canada), 14 g l⁻¹ agar (Bioshop, Burlington, Canada), 30 g l⁻¹ artificial sea salt) supplemented with antibiotics (0.5 g l⁻¹ penicillin G sodium salt (Bioshop, Burlington, Canada), 0.5 g l⁻¹ streptomycin sulfate (Bioshop, Burlington, Canada)). A total of 14 isolates were obtained.

2.2 Identification

For identification, sequence analysis of the 18S and internal transcribed spacer (ITS) regions of the rDNA were used. Mycelia were scraped from the surface of PYGS plates and ground into fine powder in liquid nitrogen using a mortar and a pestle. Genomic DNA was extracted using the DNeasy Plant DNA Extraction Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The nuclear rRNA genes were amplified using primers NS1/NS4 or NS6 (18S) and ITS1 or ITS5/ITS4 (ITS) (White et al. 1990). PCR reactions were performed in a 25 µl volume containing 0.5 µl of the extracted DNA, 1 µl each of the two primers (10 µM), 12.5 µl Taq premix (BIOMAN, Taipei), 10 µl PCR water. The amplification cycle consisted of an initial denaturation step of 95 °C for 5 min followed by 35 cycles of (a) denaturation (95 °C for 30s), (b) annealing (55 °C for 30s) and (c) elongation (72 °C for 30 s) and a final 5-min elongation step at 72 °C. The PCR products were analyzed by agarose gel electrophoresis and sent to Genomics, Taipei, Taiwan for sequencing using the same primers. The sequences obtained were checked for ambiguity and assembled in MEGA7 (Kumar et al. 2016) and submitted to the National Center for Biotechnology Information (NCBI) for a nucleotide BLAST search.

2.3 Fatty acid analysis

Fourteen isolates were grown on PYGS agar plates for a week at 25 °C in the dark. Two agar disks (6 mm in diameter) were cut from the colony

margin with a sterile Pasteur pipette and inoculated into Erlenmeyer flasks containing 20 ml medium with 4 g l⁻¹ glucose (Bioshop, Burlington, Canada), 4 g l⁻¹ yeast extract (Oxoid, Basingstoke, UK), 4 g l⁻¹ peptone (Oxoid, Basingstoke, UK) and 30 g l⁻¹ artificial sea salt. Triplicate flasks were grown for each isolate and incubated at 25 °C in an orbital shaker at 100 rpm for one week. Mycelial biomass in the flasks was filtered, freeze-dried, weighed and ground.

Folch's method was used for lipid extraction (Folch et al. 1957). Distilled water was added to 30 mg of freeze-dried mycelia, vortexed and allowed to stand for 30 min. Chloroform/methanol (2:1, v/v; 20 ml) was added for extraction at 4 °C overnight. Sodium chloride solution (0.9%, w/v; 4 ml) was added and the extracted lipids in chloroform were evaporated under a stream of nitrogen. The concentration of the lipids was adjusted to 2 mg ml⁻¹ with chloroform. For fatty acid methyl ester (FAME) analysis, 0.1 ml of the total lipid-chloroform solution fraction was mixed with 0.1 ml of triheptadecanoin (0.16 mg ml⁻¹; internal standard), evaporated under a stream of nitrogen, and reacted with 14% (w/v) boron trifluoride methanol complex for 20 min at 95 °C (Morrison and Smith 1964). Identification of FAME was performed by extraction with hexane, and separation and quantification using an Agilent 6890 gas chromatograph (GC) equipped with a flame-ionization detector and a fused-silica capillary column (Omegawax; 30 m × 0.32 mm, i.d., film thickness 0.25 µm, Supelco, Bellefonte, Pa., USA). The carrier gas was helium. The injector temperature was set at 205 °C, and the detector temperature at 240 °C. The temperature of the oven was initially 140 °C, and raised to 205 °C in increments of 6 °C min⁻¹ and held for 20 min. The fatty acid peaks were identified by comparing their retention times to a mixture of FAME standards (mixture RL-461, Nu-Chek-Prep, Inc., Elysian, Minn., USA) and quantified according to their peak area using the Agilent ChemStation (Agilent Technologies, Palo Alto, Calif., U.S.A.) and the technique of internal standardization with triheptadecanoin serving as a standard (Sigma, St. Louis, Mo., USA).

The 24 fatty acids detected are listed in Table 1. The individual fatty acids produced by *Halophytophthora* spp. and *Salispina spinosa* were expressed as percentage of total fatty acid and yield (mg l⁻¹ culture medium). Statistics was run in R (version 4.0.2). Data were compared using Kruskal-Wallis test, followed by Dunn's multiple comparisons test. Multidimensional scaling (MDS) plot, based on Bray-Curtis distances, was performed on the fatty acid profiles of the isolates.

3 Results

3.1 Identification of *Halophytophthora*/ *Salispina* isolates

A total of 14 isolates were cultured from the fallen mangrove leaves. Based on the BLASTn results of 18S and ITS rDNA, 11 isolates were identified to belong to four *Halophytophthora* species (*H. avicenniae*, *H. batemanensis*, *H. exoprolifera* and *H. polymorphica*; Table 2). The BLASTn results of the ITS sequences suggest that IMB222, IMB224 and IMB226 belonged to the genus *Phytophthium* but the query coverage was very low (17–18%). These three isolates were identified as *Salispina spinosa* based on the partial 18S rDNA sequences (100% query coverage, 99% sequence similarity).

Table 1: Fatty acids analysed in mycelia of species of *Halophytophthora* and *Salispina* in this study.

Lipid number	Common name
C12:0	Lauric acid
C14:0	Myristic acid
C15:0	Pentadecanoic acid
C16:0	Palmitic acid
C18:0	Stearic acid
C20:0	Arachidic acid
C22:0	Behenic acid
C24:0	Lignoceric acid
C16:1	Palmitoleic acid
C18:1n-9	Oleic acid
C18:1n-7	Vaccenic acid
C20:1	Eicosenoic acid
C22:1	Erucic acid
C24:1	Nervonic acid
C18:2n-6	Linoleic acid
C18:3n-6	γ-Linolenic acid
C20:2n-6	Eicosadienoic acid
C20:3n-6	Dihomo-gamma-linolenic acid
C20:4n-6	Arachidonic acid
C22:4n-6	Adrenic acid
C18:3n-3	α-Linolenic acid
C20:4n-3	Eicosatetraenoic acid
C20:5n-3	Eicosapentaenoic acid
C22:5n-3	Docosapentaenoic acid

3.2 Fatty acid profile

Fatty acid profiles of the 14 isolates examined in this study are presented in Tables 3 and 4, showing the median percentage of TFA for the 24 different saturated and unsaturated fatty acids, and the yield (mg l⁻¹), respectively. Mean percentage of TFA is given in Table 5. Species of *Halophytophthora* and *Salispina* produced a wide range of saturated and unsaturated fatty acids in varying quantities. Based on percentage of TFA (Table 3), five fatty acids were dominant (median >10%), including C16:0 palmitic acid (20–36%), C18:1n-9 oleic acid (7–20%), C18:2n-6 linoleic acid (13–25%), C20:4n-6 arachidonic acid (4–31%) and C20:5n-3 eicosapentaenoic acid (0.01–15%). Concerning yield (Table 4), these five fatty acids were also dominant: C16:0 palmitic acid (64–227 mg l⁻¹), C18:1n-9 oleic acid (36–121 mg l⁻¹), C18:2n-6 linoleic acid (43–155 mg l⁻¹), C20:4n-6 arachidonic acid (18–188 mg l⁻¹) and C20:5n-3 eicosapentaenoic acid (0.05–96 mg l⁻¹). Eleven fatty acids were produced in low percentages of TFA (median <3%) and yield (median <12 mg l⁻¹): 12:0 lauric acid, 15:0 pentadecanoic acid, 20:0 arachidic acid, 22:0 behenic acid, 24:0 lignoceric acid, C18:1n-7 vaccenic acid, C20:1 eicosenoic acid, C24:1 nervonic acid, C20:2n-6 eicosadienoic acid, C18:3n-3 α-linolenic acid and C20:4n-3 eicosatetraenoic acid.

Table 2: Results of nucleotide BLAST search in NCBI of the internal transcribed spacers (ITS) and 18S rDNA sequences of the 14 isolates of *Halophytophthora* [*H. avicenniae* (Gerr.-Corn. et J.A. Simpson) H.H. Ho et S.C. Jong, *H. batemanensis* (Gerr.-Corn. et J.A. Simpson) H.H. Ho et S.C. Jong, *H. exoprolifera* H.H. Ho, Nakagiri et S.Y. Newell, *H. polymorphica* (Gerr.-Corn. et J.A. Simpson) H.H. Ho et S.C. Jong) and *Salispina* [*S. spinosa* (Fell et Master) Marano, A.L. Jesus et Pires-Zottar.] examined in this study.

Isolate no.	Locality	ITS rDNA						
		Sequence length (bp)	Max score	Query coverage (%)	Sequence similarity (%)	BLAST results (highest score)	Accession no.	Proposed taxon
IMB212	Xin-fong, Hsinchu, Taiwan	903	1611	96	100	<i>Halophytophthora avicenniae</i>	KM205198	<i>Halophytophthora avicenniae</i>
IMB213	Xin-fong, Hsinchu, Taiwan	871	1543	95	100	<i>Halophytophthora avicenniae</i>	KM205202	<i>Halophytophthora avicenniae</i>
IMB215	Chu-nan, Miaoli, Taiwan	882	1541	97	99	<i>Halophytophthora avicenniae</i>	AY598668	<i>Halophytophthora avicenniae</i>
IMB219	Sih-caio, Tainan, Taiwan	870	1543	95	100	<i>Halophytophthora avicenniae</i>	KM205204	<i>Halophytophthora avicenniae</i>
IMB225	Pu-zi, Chiayi, Taiwan	849	1537	98	99	<i>Halophytophthora avicenniae</i>	KM205202	<i>Halophytophthora avicenniae</i>
IMB216	Fang-yuan, Zhan-ghua, Taiwan	925	1631	97	99	<i>Halophytophthora batemanensis</i>	GU258916	<i>Halophytophthora batemanensis</i>
IMB217	Sih-caio, Tainan, Taiwan	932	1482	98	96	<i>Halophytophthora batemanensis</i>	GU258910	<i>Halophytophthora batemanensis</i>
IMB220	Pu-zi, Chiayi, Taiwan	908	1696	99	99	<i>Halophytophthora batemanensis</i>	GU258916	<i>Halophytophthora batemanensis</i>
IMB221	Pu-zi, Chiayi, Taiwan	929	1633	97	99	<i>Halophytophthora batemanensis</i>	GU258916	<i>Halophytophthora batemanensis</i>
IMB214	Xin-fong, Hsinchu, Taiwan	865	1502	95	99	<i>Halophytophthora exoprolifera</i>	KT455402	<i>Halophytophthora exoprolifera</i>
IMB227	Sih-caio, Tainan, Taiwan	891	1240	98	92	<i>Halophytophthora polymorphica</i>	KT455403	<i>Halophytophthora polymorphica</i>
IMB222	Pu-zi, Chiayi, Taiwan	879	271	18	96	<i>Phytopythium sp.</i>	KM061666	Not proposed
			271	18	96	Uncultured stramenopile clone	HQ191349	
IMB224	Pu-zi, Chiayi, Taiwan	888	271	17	96	<i>Phytopythium sp.</i>	KM061666	Not proposed
			271	18	96	Uncultured stramenopile clone	HQ191349	
IMB226	Pu-zi, Chiayi, Taiwan	888	271	17	96	<i>Phytopythium sp.</i>	KM061666	Not proposed
			271	18	96	Uncultured stramenopile clone	HQ191349	
IMB212	Xin-fong, Hsinchu, Taiwan	1050	1923	100%	99%	<i>Halophytophthora avicenniae</i>	HQ161104	<i>Halophytophthora avicenniae</i>
IMB213	Xin-fong, Hsinchu, Taiwan	1050	1940	100%	100%	<i>Halophytophthora avicenniae</i>	HQ161104	<i>Halophytophthora avicenniae</i>
IMB215	Chu-nan, Miaoli, Taiwan	1317	2433	100%	100%	<i>Halophytophthora avicenniae</i>	HQ161104	<i>Halophytophthora avicenniae</i>
IMB219	Sih-caio, Tainan, Taiwan	1318	2423	100%	100%	<i>Halophytophthora avicenniae</i>	HQ161104	<i>Halophytophthora avicenniae</i>
IMB225	Pu-zi, Chiayi, Taiwan	1050	1940	100%	100%	<i>Halophytophthora avicenniae</i>	HQ161104	<i>Halophytophthora avicenniae</i>
IMB216	Fang-yuan, Zhan-ghua, Taiwan	1318	2435	100%	100%	<i>Halophytophthora batemanensis</i>	GU994179	<i>Halophytophthora batemanensis</i>
IMB217	Sih-caio, Tainan, Taiwan	1317	2427	100%	99%	<i>Halophytophthora batemanensis</i>	GU994179	<i>Halophytophthora batemanensis</i>
IMB220	Pu-zi, Chiayi, Taiwan	1318	2427	99%	99%	<i>Halophytophthora batemanensis</i>	GU994179	<i>Halophytophthora batemanensis</i>
IMB221	Pu-zi, Chiayi, Taiwan	1327	2451	100%	100%	<i>Halophytophthora batemanensis</i>	GU994179	<i>Halophytophthora batemanensis</i>

Table 2: (continued)

Isolate no.	Locality	ITS rDNA						
		Sequence length (bp)	Max score	Query coverage (%)	Sequence similarity (%)	BLAST results (highest score)	Accession no.	Proposed taxon
IMB214	Xin-fong, Hsinchu, Taiwan	1053	1945	100%	100%	<i>Halophytophthora exoprolifera</i>	GU994167	<i>Halophytophthora exoprolifera</i>
IMB227	Sih-caio, Tainan, Taiwan	1049	1921	100%	99%	<i>Halophytophthora polymorphica</i>	GU994176	<i>Halophytophthora polymorphica</i>
IMB222	Pu-zi, Chiayi, Taiwan	1039	1919	100%	99%	<i>Salispina spinosa</i>	HQ161101	<i>Salispina spinosa</i>
IMB224	Pu-zi, Chiayi, Taiwan	1039	1908	100%	99%	<i>Salispina spinosa</i>	HQ161101	<i>Salispina spinosa</i>
IMB226	Pu-zi, Chiayi, Taiwan	1061	1943	100%	99%	<i>Salispina spinosa</i>	HQ161101	<i>Salispina spinosa</i>

Concerning the essential arachidonic (ARA) and eicosapentaenoic (EPA) acids, *S. spinosa* isolates produced the highest amount of ARA (26–31% of TFA, 141–188 mg l⁻¹ yield) of all the isolates, but produced only low levels of EPA (0.01–0.03% of TFA, 0.05–1.19 mg l⁻¹ yield; Figure 1). *Halophytophthora* spp. generally produced slightly higher quantities of EPA (8–15% of TFA, 27–96 mg l⁻¹) than ARA (4–12% of TFA, 18–93 mg l⁻¹). For EPA, *H. avicenniae* IMB212 produced the highest percentage of TFA (15%) while *H. polymorphica* IMB227 produced the highest yield (96 mg l⁻¹) of EPA.

The cluster analysis based on the fatty acid profile is shown in Figure 2. The fatty acid profiles of *H. avicenniae*, *H. batemanensis* and *H. polymorphica* were similar, while *H. exoprolifera* and *S. spinosa* formed different clusters based on percentage of TFA (Figure 2a). However, the clusters were more dispersed based on fatty acid yield (Figure 2b); *H. exoprolifera*, *S. spinosa*, *H. avicenniae* (IMB213, IMB225) and *H. polymorphica* formed a separate cluster from the main cluster consisting of *H. avicenniae* (IMB212, IMB215, IMB219) and *H. batemanensis* (IMB216, IMB217, IMB220, IMB221).

4 Discussion

DHA and EPA have been suggested to benefit fetal development, cardiovascular function, and Alzheimer's disease in humans (Swanson et al. 2012). The marine Labyrinthulomycetes are prolific producers of DHA, but poor producers of EPA (Ou et al. 2016). Oomycota and Mucoromycota, on the other hand, produce EPA, in particular, species of *Mortierella* with high yields of EPA (Cheng et al. 1999; Jang et al. 2005). *Halophytophthora* species, in comparison, produce a lower yield of EPA but they produce comparatively high levels of palmitic, oleic and linoleic acids, which may be of industrial value (Table 5).

The results of the fatty acid analysis confirm that C16:0 palmitic acid, C18:1n-9 oleic acid, C18:2n-6 linoleic acid, C20:4n-6 arachidonic acid and C20:5n-3 eicosapentaenoic acid were dominant fatty acids in mycelia of the studied *Halophytophthora* and *Salispina* species. Some of these fatty acids are beneficial to human health; oleic acids in lowering LDL-cholesterol levels which can lead to cardiovascular diseases (Pérez-Jiménez et al. 2007), linoleic acid as a structural component of cell membranes, an energy source (Whelan and Fritsche 2013) and a precursor of longer chain PUFAs, ARA and EPA in the prevention of coronary heart diseases, obesity and hypertension (Ye and Ghosh 2018). This highlights the potential of *Halophytophthora* and *Salispina* species as alternative sources of this fatty acids.

This study is the first to analyse C18:1n-7 vaccenic acid, C20:1 eicosenoic acid, C24:1 nervonic acid, C20:2n-6 eicosadienoic acid, C22:4n-6 adrenic acid, C20:4n-3 eicosatetraenoic acid and C22:5n-3 docosapentaenoic acid in mycelia of selected *Halophytophthora* species and *Salispina spinosa*. Yields of these fatty acids were low (<1.28% of TFA, <9.82 mg l⁻¹ yield), but some might be beneficial to human health, for example, vaccenic acid in suppressing tumor growth and coronary heart disease (Field et al. 2009), and nervonic acid in improving oligodendrocyte function (Lewkowicz et al. 2019).

Table 5 summarises the fatty acid profiles of *Halophytophthora* and *Salispina* species based on results of this study and those from the literature (Caguimbal et al. 2019; Devanadera et al. 2019; Pang et al. 2016). This is also the first study to screen the fatty acid profiles of *H. batemanensis* and *H. exoprolifera*. In particular, *H. exoprolifera* produced a different fatty acid profile from other *Halophytophthora* spp. and *S. spinosa*. The proportion of fatty acids, i.e. percentage of total fatty acid, produced by the different *Halophytophthora* and *Salispina* species and

Table 3: Fatty acid profile (as percentage of total fatty acids) in mycelia of 14 isolates of *Halophytophthora* spp. (*H. avicenniae*: IMB212, IMB213, IMB215, IMB219, IMB225; *H. batemanensis*: IMB216, IMB217, IMB220, IMB221; *H. exoprolifera*: IMB214, *H. polymorphica*: IMB227) and *Salispina spinosa* (IMB222, IMB224, IMB226).

Fatty acid	Isolate														
	IMB212	IMB213	IMB214	IMB215	IMB216	IMB217	IMB219	IMB220	IMB221	IMB222	IMB224	IMB225	IMB226	IMB227	
C12:0	0.06 ^a (0.06–	0.02 ^a (0.02–	0.03 ^a (0.03–	0.03 ^a (0.03–	0.03 ^a (0.03–	0.03 ^a (0.03–	0.01 ^a (0.01–	0.03 ^a (0.03–	0.06 ^a (0.05–	0.03 ^a (0.02–	0.02 ^a (0.02–	0.03 ^a (0.03–	0.02 ^a (0.02–	0.02 ^a (0.02–	
C14:0	0.07) 8.76 ^{ab} (8.64–8.8)	0.16) 7.44 ^{abc} (7.2–	0.03) 6.49 ^{abc} (6.24–6.5)	0.04) 6.03 ^{abc} (6.02–	0.05) 7.78 ^{abc} (7.68–	0.03) 8.11 ^{abc} (7.66–	0.02) 5.58 ^{abc} (5.03–5.6)	0.04) 9.03 ^a (8.9–9.53)	0.08) 10.22 ^a (9.38–	0.08) 10.62) 10.62) 0.16 ^{abc} (0.15–	0.05) 0.54 ^c (0.52–	0.02) 0.55 ^{bc} (0.53–	0.04) 6.39 ^{abc} (6.28–6.7)	0.02) 0.51 ^c (0.5–0.52)	0.02) 4.67 ^{abc} (4.18–
C15:0	0.57 ^{abc} (0.49–	0.38 ^{abc} (0.27–	0.30 ^{abc} (0.27–	1.11 ^{abc} (0.6–1.12)	0.25 ^{abc} (0.24–	0.13 ^{abc} (0.13–	0.73 ^{ab} (0.72–	0.15 ^{abc} (0.14–	0.16 ^{abc} (0.15–	0.08 ^c (0.08–	0.08 ^{ac} (0.07–	0.70 ^b (0.69–	0.08 ^c (0.08–0.1)	0.38–	
C16:0	0.58) 25.16 ^{ab} (25–	0.39) 26.00 ^{ab} (23.18–	0.33) 35.72 ^a (34.17–	27.33 ^{ab} (23.79–	0.28) 19.97 ^b (19.55–	0.15) 24.40 ^{ab} (22.94–	0.75) 22.03 ^{ab} (21.25–	0.17) 22.20 ^{ab} (22.05–	0.17) 22.67 ^{ab} (21.73–	0.09) 27.61 ^a (27.3–	0.09) 26.90 ^{ab} (26.89–	0.73) 27.17 ^{ab} (25.3–	0.42) 28.01 ^a (27.67–	0.42) 21.35 ^{ab} (21.32–	
C18:0	25.54) 1.88 ^b (1.8–2.01)	26.49) 2.58 ^{ab} (2.48–	36.72) 1.70 ^{ab} (1.45–	27.37) 3.04 ^{ab} (2.69–	20.08) 2.94 ^{ab} (2.76–	24.47) 3.50 ^{ab} (3.22–	22.45) 1.67 ^b (1.53–	22.41) 4.14 ^{ab} (3.96–	23.01) 5.25 ^a (4.73–5.3)	28.65) 2.30 ^{ab} (2.24–	26.95) 2.57 ^{ab} (2.46–	27.33) 2.18 ^{ab} (2.15–	28.52) 2.64 ^{ab} (2.38–	22.33) 3.90 ^{ab} (3.35–	
C20:0	4.54) 0.12 ^c (0.11–	3.33) 0.25 ^{abc} (0.25–	2.84) 0.13 ^{bc} (0.11–	3.66) 0.22 ^{abc} (0.2–0.27)	3.29) 0.34 ^{abc} (0.28–	3.61) 0.29 ^{abc} (0.27–0.3)	1.74) 0.19 ^{abc} (0.17–	4.17) 0.46 ^{ab} (0.43–	0.58 ^a (0.49–0.6)	3.12) 0.17 ^{abc} (0.16–	2.64) 0.19 ^{abc} (0.19–	2.36) 0.18 ^{abc} (0.18–	2.66) 0.21 ^{abc} (0.19–	4.54) 0.32 ^{abc} (0.26–	
C22:0	0.26) 0.07 ^c (0.07–	0.26) 0.10 ^{abc} (0.1–0.14)	0.17) 0.21 ^{abc} (0.21–	0.08 ^{bc} (0.07–	0.36) 0.16 ^{abc} (0.16–	0.13 ^{abc} (0.13–	0.19) 0.12 ^{abc} (0.12–	0.47) 0.11 ^{abc} (0.11–	0.17 ^{abc} (0.17–	0.18) 1.22 ^{ab} (1.17–	0.23) 2.22 ^a (2.21–	0.19) 0.09 ^{bc} (0.08–	0.21) 1.80 ^{ab} (1.69–	0.33) 0.42 ^{abc} (0.35–	
C24:0	0.08) 0.06 ^a (0.06–	0.03 ^a (0.03–	0.32) 0.06 ^a (0.05–	0.13) 0.08 ^a (0.07–	0.17) 0.06 ^a (0.05–	0.15) 0.11 ^a (0.1–0.12)	0.13) 0.05 ^a (0.04–	0.14) 0.05 ^a (0.04–	0.25) 0.03 ^a (0.03–	1.64) 0.26 ^a (0.25–	2.28) 0.68 ^a (0.63–	0.11) 0.05 ^a (0.04–	1.84) 0.55 ^a (0.44–	0.42) 0.04 ^a (0.04–	
C16:1	0.04) 1.88 ^{bc} (1.81–	0.04) 2.99 ^{ab} (2.8–3.09)	0.07) 6.25 ^a (6.13–	0.09) 2.67 ^{abc} (2.65–	0.08) 1.54 ^{bc} (1.51–1.7)	0.08) 2.49 ^{abc} (2.34–	0.06) 2.74 ^{abc} (2.73–	0.06) 1.99 ^{abc} (1.89–	0.05) 2.57 ^{abc} (2.26–	0.44) 2.33 ^{abc} (2.3–2.33)	0.69) 2.54 ^{abc} (2.39–2.9)	0.06) 2.63 ^{abc} (2.55–2.7)	0.57) 2.80 ^{abc} (2.51–	0.05) 1.16 ^c (1.15–1.3)	
C18:1n-9	1.97) 12.03 ^{abcd} (11.7–	14.52 ^{abcd} (13.08–	7.41) 15.90 ^{ab} (15.66–	2.71) 14.03 ^{abcd} (13.71–	19.64 ^a (19.29–	12.71 ^{abcd} (12.35–	2.75) 15.88 ^{ab} (15.87–	2.21) 15.33 ^{abcd} (14.99–	2.66) 13.48 ^{abcd} (12.49–	7.15 ^d (7.03–	8.22 ^{bcd} (8.08–	15.45 ^{abc} (15.42–	7.34 ^{cd} (7.1–7.86)	11.17 ^{abcd} (10.67–	
C18:1n-7	12.17) 12.17) 0.23 ^{abcd} (0.23–	14.71) 14.71) 0.36 ^{abcd} (0.35–0.4)	17.7) 0.59 ^{abcd} (0.53–	14.3) 0.27 ^{abcd} (0.27–	20.41) 0.61 ^{ab} (0.59–	14.26) 0.48 ^{abc} (0.45–	17.05) 0.27 ^{abcd} (0.27–	15.47) 0.55 ^{abc} (0.55–	15.01) 0.89 ^a (0.75–	7.21) 0.04 ^d (0.04–	8.37) 0.07 ^{bcd} (0.06–	16.8) 0.24 ^{abcd} (0.23–	0.05 ^{cd} (0.05–	11.65) 0.48 ^{abcd} (0.4–0.48)	
C20:1	0.23) 0.60 ^{abcd} (0.54–	0.31 ^{abcd} (0.27–	0.61) 0.45 ^{abcd} (0.39–	0.44) 0.37 ^{abcd} (0.35–	0.68) 0.89 ^a (0.88–	0.78) 0.88 ^{ac} (0.8–1.14)	0.29) 0.31 ^{abcd} (0.3–0.34)	0.58) 0.81 ^{ac} (0.8–0.82)	0.91) 0.60 ^{abc} (0.58–	0.05) 0.07 ^d (0.07–	0.08) 0.10 ^{bd} (0.1–0.11)	0.26) 0.26 ^{bcd} (0.25–	0.06) 0.09 ^{bd} (0.09–	0.54 ^{abcd} (0.51–	
	0.62) (0.54–	0.33) (0.27–	0.48) (0.39–	0.38) (0.35–	0.91) (0.88–	1.14) (0.8–1.14)	0.34) (0.3–0.34)	0.82) (0.8–0.82)	0.61) (0.58–	0.08) (0.07–	0.08) (0.1–0.11)	0.28) (0.25–	0.09) (0.09–	0.55) (0.51–	

Table 3: (continued)

Fatty acid	Isolate													
	IMB212	IMB213	IMB214	IMB215	IMB216	IMB217	IMB219	IMB220	IMB221	IMB222	IMB224	IMB225	IMB226	IMB227
C22:1	2.60 ^{ab} (2.25–2.65)	2.46 ^{ab} (2.39–2.72)	2.17 ^{ab} (1.94–2.25)	3.09 ^{ab} (2.7–3.17)	3.22 ^{ab} (2.82–3.41)	2.47 ^{ab} (2.3–2.61)	2.96 ^{ab} (2.77–3.3)	3.04 ^{ab} (2.79–3.12)	2.63 ^{ab} (2.56–2.8)	0.26 ^b (0.25–0.29)	0.33 ^b (0.33–0.36)	2.54 ^{ab} (1.99–2.59)	0.31 ^b (0.3–0.34)	3.56 ^a (3.43–3.87)
C24:1	0.29 ^{ab} (0.25–0.31)	0.35 ^{ab} (0.3–0.45)	0.58 ^a (0.55–0.59)	0.38 ^{ab} (0.36–0.47)	0.24 ^{ab} (0.21–0.26)	0.38 ^{ab} (0.37–0.41)	0.33 ^{ab} (0.32–0.42)	0.29 ^{ab} (0.24–0.33)	0.28 ^{ab} (0.24–0.29)	0.02 ^b (0.02–0.04)	0.03 ^b (0.03–0.06)	0.46 ^{ab} (0.29–0.46)	0.03 ^b (0.03–0.05)	1.28 ^a (1.18–1.56)
C18:2n-6	20.00 ^{abc} (19.89–20.04)	17.27 ^{abc} (17.21–19.44)	13.29 ^c (12.68–14.06)	17.28 ^{abc} (16.9–19.82)	15.00 ^c (14.33–15.03)	15.73 ^{bc} (15.46–17.24)	19.72 ^{abc} (19.3–19.74)	16.09 ^{bc} (15.94–16.3)	17.94 ^{abc} (17.09–18.95)	23.54 ^{ab} (22.68–23.57)	24.78 ^a (24.47–25.02)	16.43 ^{bc} (16.04–16.47)	22.84 ^{ab} (22.39–22.89)	20.18 ^{abc} (20.01–21.18)
C18:3n-6	2.04 ^{abc} (2.03–2.14)	1.60 ^{abc} (1.58–2.17)	1.10 ^{bc} (1.09–1.19)	1.59 ^{abc} (1.55–2.28)	2.55 ^{ab} (2.4–2.73)	3.31 ^{ab} (2.91–3.32)	1.22 ^{bc} (1.05–1.26)	3.79 ^a (3.66–3.93)	3.80 ^a (3.62–4.26)	1.05 ^{bc} (1.05–1.19)	1.46 ^{abc} (1.38–1.48)	1.82 ^{abc} (1.75–1.88)	1.07 ^{bc} (1–1.16)	0.77 ^c (0.68–0.79)
C20:2n-6	0.20 ^a (0.2–0.21)	0.17 ^a (0.11–0.17)	0.13 ^a (0.12–0.17)	0.19 ^a (0.18–0.22)	0.17 ^a (0.15–0.18)	0.27 ^a (0.27–0.29)	0.16 ^a (0.16–0.17)	0.19 ^a (0.19–0.19)	0.22 ^a (0.21–0.26)	0.32 ^a (0.32–0.34)	0.27 ^a (0.27–0.35)	0.14 ^a (0.13–0.17)	0.38 ^a (0.35–0.39)	0.22 ^a (0.22–0.25)
C20:3n-6	1.26 ^{ab} (1.23–1.33)	2.36 ^{ab} (2.2–3.7)	0.27 ^{ab} (0.23–0.66)	1.87 ^{ab} (1.72–3.53)	0.99 ^b (0.78–1.02)	3.70 ^{ab} (2.96–3.77)	1.73 ^{ab} (1.32–1.96)	2.63 ^{ab} (2.48–2.78)	3.46 ^a (2.93–3.74)	2.05 ^{ab} (1.95–2.08)	2.16 ^{ab} (2.02–2.24)	1.90 ^{ab} (1.76–2.16)	1.87 ^{ab} (1.72–2.22)	3.31 ^{ab} (3.13–3.44)
C20:4n-6	8.42 ^{cde} (8.13–8.52)	7.16 ^{de} (6.33–7.8)	4.06 ^e (3.7–4.58)	8.56 ^{bcde} (7.31–8.98)	9.80 ^{abcde} (9.72–9.82)	12.35 ^{abcd} (11.75–13.17)	10.79 ^{abcde} (10.71–11.82)	9.51 ^{abcde} (9.12–9.78)	6.39 ^{de} (6.34–6.4)	30.98 ^{ab} (29.24–31.17)	26.43 ^{abc} (26.25–26.97)	9.74 ^{abcde} (9.62–9.89)	29.50 ^a (29.23–29.89)	12.12 ^{abcd} (11.43–13.58)
C22:4n-6	0.02 ^{ab} (0.02–0.02)	0.02 ^{ab} (0.02–0.02)	0.03 ^{ab} (0.03–0.04)	0.03 ^{ab} (0.02–0.04)	0.02 ^{ab} (0.02–0.03)	0.06 ^a (0.06–0.07)	0.03 ^{ab} (0.04–0.04)	0.02 ^{ab} (0.02–0.03)	0.02 ^{ab} (0.02–0.03)	0.01 ^b (0.01–0.02)	0.01 ^{ab} (0.01–0.02)	0.03 ^{ab} (0.03–0.03)	0.02 ^{ab} (0.02–0.02)	0.05 ^{ab} (0.04–0.05)
C18:3n-3	0.01 ^a (0.01–0.02)	0.01 ^a (0.01–0.01)	0.01 ^a (0.01–0.01)	0.02 ^a (0.02–0.04)	0.01 ^a (0.01–0.01)	0.04 ^a (0.04–0.04)	0.02 ^a (0.02–0.03)	0.00 ^a (0.00–0.01)	0.03 ^a (0.02–0.04)	0.01 ^a (0.01–0.02)	0.02 ^a (0.02–0.03)	0.02 ^a (0.02–0.02)	0.01 ^a (0.01–0.01)	0.05 ^a (0.04–0.05)
C20:4n-3	0.04 ^{ab} (0.04–0.05)	0.07 ^a (0.07–0.15)	0.03 ^{ab} (0.03–0.14)	0.05 ^{ab} (0.05–0.12)	0.01 ^b (0.01–0.02)	0.03 ^{ab} (0.03–0.05)	0.05 ^{ab} (0.04–0.05)	0.05 ^{ab} (0.05–0.05)	0.10 ^a (0.08–0.11)	0.01 ^{ab} (0.01–0.02)	0.01 ^{ab} (0.01–0.01)	0.05 ^{ab} (0.05–0.07)	0.01 ^{ab} (0.01–0.01)	0.09 ^{ab} (0.08–0.1)
C20:5n-3	14.51 ^a (13.79–14.54)	11.12 ^{abc} (10.67–11.85)	8.05 ^{abc} (7.73–8.44)	11.47 ^{abc} (10.62–11.63)	14.43 ^a (13.96–14.5)	7.94 ^{abc} (7.25–8.39)	13.04 ^{ab} (12.81–13.13)	9.65 ^{abc} (9.46–9.69)	9.47 ^{abc} (8.41–10.1)	0.03 ^{bc} (0.03–0.05)	0.01 ^c (0.01–0.02)	11.48 ^{abc} (11.4–12.44)	0.03 ^{bc} (0.03–0.03)	12.53 ^{abc} (11.75–13.01)
C22:5n-3	0.04 ^{abc} (0.04–0.05)	0.04 ^{abc} (0.04–0.05)	0.04 ^{abc} (0.04–0.05)	0.06 ^{ab} (0.06–0.07)	0.05 ^{abc} (0.04–0.05)	0.09 ^a (0.08–0.09)	0.03 ^{abc} (0.03–0.04)	0.03 ^{abc} (0.03–0.04)	0.03 ^{abc} (0.03–0.04)	0.02 ^c (0.02–0.02)	0.01 ^c (0.01–0.01)	0.04 ^{abc} (0.04–0.05)	0.02 ^{bc} (0.02–0.02)	0.04 ^{abc} (0.03–0.05)

Data are expressed as median (interquartile range). Values in the same row with the same superscript letter are not significantly different at p=0.05.

Table 4: Fatty acid yield (mg l^{-1}) in mycelia of 14 isolates of *Halophytophthora* spp. (*H. avicenniae*: IMB212, IMB213, IMB215, IMB219, IMB225; *H. batemansensis*: IMB216, IMB217, IMB220, IMB221; *H. exoprolifera*: IMB214, *H. polymorphica*: IMB222, IMB224, IMB226).

Fatty acid	Isolate													
	IMB212	IMB213	IMB214	IMB215	IMB216	IMB217	IMB219	IMB220	IMB221	IMB222	IMB224	IMB225	IMB226	IMB227
C12:0	0.29 ^a (0.29–0.32)	0.05 ^a (0.05–0.39)	0.19 ^a (0.19–0.19)	0.11 ^a (0.09–0.14)	0.11 ^a (0.09–0.18)	0.15 ^a (0.13–0.15)	0.05 ^a (0.05–0.08)	0.17 ^a (0.14–0.20)	0.25 ^a (0.21–0.33)	0.15 ^a (0.10–0.25)	0.11 ^a (0.08–0.11)	0.23 ^a (0.20–0.27)	0.13 ^a (0.13–0.13)	0.15 ^a (0.15–0.15)
C14:0	42.49 ^{ab} (41.88–42.66)	18.34 ^{ab} (17.73–24.77)	41.26 ^{ab} (39.67–41.32)	21.83 ^{ab} (21.78–26.25)	27.82 ^{ab} (27.46–28.3)	40.81 ^{ab} (38.52–43.67)	28.77 ^{ab} (25.91–28.85)	51.23 ^a (50.5–54.07)	42.24 ^{ab} (38.75–43.87)	2.73 ^b (2.63–3.64)	2.94 ^b (2.81–3.05)	50.02 ^a (49.16–52.45)	3.25 ^b (3.16–3.28)	35.81 ^{ab} (32.01–36.92)
C15:0	2.76 ^{ab} (2.38–2.81)	0.94 ^{ab} (0.67–0.95)	1.91 ^{ab} (1.72–2.07)	4.02 ^{ab} (2.17–4.05)	0.89 ^{ab} (0.86–0.98)	0.65 ^{ab} (0.63–0.73)	3.76 ^{ab} (3.69–3.84)	0.85 ^{ab} (0.79–0.96)	0.66 ^{ab} (0.60–0.68)	0.40 ^b (0.40–0.43)	0.43 ^b (0.35–0.45)	5.48 ^a (5.40–5.68)	0.51 ^{ab} (0.51–0.61)	3.14 ^{ab} (2.91–3.22)
C16:0	122.03 ^{abc} (121.25–123.84)	64.08 ^c (57.13–65.29)	227.08 ^a (217.19–233.4)	98.95 ^{bc} (86.11–99.09)	71.41 ^c (69.89–71.81)	122.77 ^{abc} (115.42–123.1)	113.59 ^{abc} (109.56–115.75)	125.95 ^{abc} (127.15)	93.69 ^{bc} (89.81–95.08)	139.63 ^{abc} (138.06–144.89)	143.74 ^{abc} (144.01)	212.69 ^a (198.01–213.94)	178.55 ^{ab} (176.38–181.77)	163.71 ^{ab} (163.44–171.23)
C18:0	9.12 ^{bd} (8.71–9.75)	6.36 ^d (6.11–8.2)	10.81 ^{abcd} (9.19–18.02)	11.01 ^{abcd} (9.72–13.25)	10.51 ^{abcd} (9.85–11.77)	17.61 ^{abcd} (16.18–18.16)	8.61 ^{cd} (7.89–8.97)	23.49 ^{ab} (22.44–23.66)	21.70 ^{abcd} (19.53–21.88)	11.63 ^{abcd} (11.3–15.75)	13.73 ^{abcd} (13.15–14.11)	17.07 ^{abcd} (16.83–18.43)	16.83 ^{abcd} (15.17–16.96)	29.91 ^{ac} (25.65–34.77)
C20:0	0.58 ^b (0.51–0.65)	0.62 ^b (0.6–0.64)	0.83 ^{ab} (0.67–1.05)	0.80 ^{ab} (0.71–0.96)	1.22 ^{ab} (0.98–1.27)	1.46 ^{ab} (1.33–1.48)	0.98 ^{ab} (0.85–0.98)	2.61 ^a (2.41–2.67)	2.40 ^a (2.03–2.46)	0.86 ^{ab} (0.81–0.91)	1.02 ^{ab} (0.99–1.20)	1.41 ^{ab} (1.41–1.45)	1.34 ^{ab} (1.21–1.34)	2.45 ^a (1.96–2.49)
C22:0	0.34 ^c (0.32–0.36)	0.25 ^c (0.23–0.33)	1.34 ^{abc} (1.30–2.00)	0.29 ^{bc} (0.25–0.47)	0.57 ^{abc} (0.57–0.61)	0.65 ^{abc} (0.63–0.75)	0.62 ^{abc} (0.59–0.67)	0.62 ^{abc} (0.62–0.79)	0.70 ^{abc} (0.68–1.03)	6.17 ^{ab} (5.92–8.27)	11.86 ^a (11.78–12.16)	0.70 ^{abc} (0.59–0.86)	11.47 ^a (10.77–11.70)	3.22 ^{abc} (2.65–3.22)
C24:0	0.29 ^{abc} (0.29–0.32)	0.07 ^c (0.06–0.09)	0.38 ^{abc} (0.32–0.41)	0.29 ^{abc} (0.24–0.33)	0.21 ^{abc} (0.18–0.27)	0.55 ^{ab} (0.50–0.58)	0.26 ^{abc} (0.18–0.28)	0.28 ^{abc} (0.20–0.31)	0.12 ^{bc} (0.12–0.19)	1.31 ^{ab} (1.26–2.23)	3.63 ^{ab} (3.34–3.66)	0.39 ^{abc} (0.31–0.43)	3.51 ^a (2.80–3.63)	0.31 ^{abc} (0.27–0.35)
C16:1	9.12 ^{bd} (8.78–9.53)	7.37 ^{cd} (6.90–7.60)	39.73 ^a (38.97–47.11)	9.67 ^{bcd} (9.58–9.79)	5.51 ^d (5.38–6.06)	12.53 ^{abcd} (11.75–12.65)	14.13 ^{abc} (14.08–14.18)	11.29 ^{abcd} (10.69–12.54)	10.62 ^{abcd} (9.34–10.97)	11.78 ^{abcd} (11.63–11.78)	13.57 ^{abcd} (12.77–15.50)	20.59 ^{bc} (19.93–21.14)	17.85 ^{bc} (15.97–18.29)	8.89 ^{bcd} (8.82–9.93)
C18:1n-9	58.35 ^{abc} (56.75–59.02)	35.79 ^c (32.24–36.24)	101.08 ^a (99.52–112.52)	50.79 ^{abc} (49.64–51.77)	70.23 ^{abc} (68.96–72.97)	63.95 ^{abc} (62.12–71.73)	81.88 ^{ab} (81.83–87.91)	86.98 ^{ab} (85.02–87.74)	55.71 ^{abc} (51.6–62.03)	36.16 ^c (35.53–36.46)	43.92 ^{bc} (43.15–44.73)	120.94 ^a (120.71–131.51)	46.79 ^{bc} (45.26–50.07)	85.65 ^{ab} (81.78–89.29)
C18:1n-7	1.12 ^{abc} (1.09–1.12)	0.89 ^{abc} (0.86–0.97)	3.75 ^a (3.34–3.88)	0.98 ^{abc} (0.96–1.57)	2.18 ^{abc} (2.09–2.43)	2.42 ^{ab} (2.26–3.92)	1.39 ^{abc} (1.37–1.50)	3.12 ^a (3.12–3.26)	3.68 ^a (3.10–3.76)	0.20 ^c (0.20–0.25)	0.37 ^{bc} (0.32–0.43)	1.88 ^{abc} (1.80–2.00)	0.32 ^{bc} (0.32–0.35)	3.68 ^a (3.07–3.68)
C20:1	2.91 ^{abcd} (2.62–3.01)	0.76 ^{bcd} (0.67–0.80)	2.86 ^{abcd} (2.48–3.02)	1.34 ^{abcd} (1.27–1.38)	3.18 ^{abc} (3.13–3.25)	4.43 ^a (4.00–5.71)	1.60 ^{abcd} (1.55–1.75)	4.60 ^a (4.54–4.65)	2.48 ^{abcd} (2.40–2.52)	0.35 ^d (0.33–0.38)	0.53 ^{cd} (0.51–0.59)	2.04 ^{abcd} (1.96–2.19)	0.57 ^{cd} (0.54–0.57)	4.14 ^{ab} (3.87–4.18)
C22:1	12.61 ^{abcd} (10.91–12.83)	6.06 ^{bcd} (5.89–6.70)	13.80 ^{abcd} (12.3–14.3)	11.19 ^{abcd} (9.78–11.48)	11.51 ^{abcd} (10.08–12.18)	12.43 ^{abcd} (11.57–13.13)	15.26 ^{abc} (14.28–17.01)	17.25 ^{ab} (15.83–17.7)	10.87 ^{abcd} (10.56–11.57)	1.31 ^b (1.26–1.44)	1.76 ^{cd} (1.74–1.92)	19.88 ^{abc} (15.58–20.24)	1.98 ^{bcd} (1.91–2.17)	27.30 ^a (26.26–29.64)
C24:1	1.41 ^{ab} (1.21–1.50)	0.86 ^{ab} (0.74–1.11)	3.69 ^a (3.50–3.75)	1.38 ^{ab} (1.30–1.68)	0.86 ^{ab} (0.75–0.93)	1.91 ^{ab} (1.86–2.06)	1.70 ^{ab} (1.65–2.14)	1.65 ^{ab} (1.36–1.84)	1.16 ^{ab} (0.99–1.20)	0.10 ^b (0.10–0.20)	0.16 ^b (0.16–0.32)	3.60 ^{ab} (2.23–3.60)	0.19 ^b (0.19–0.29)	9.82 ^a (9.05–11.92)
C18:	97.00 ^{abcd} (96.44–97.19)	42.57 ^d (42.41–47.91)	84.49 ^{abcd} (80.61–89.38)	62.56 ^{cd} (61.18–71.76)	53.64 ^d (51.23–53.73)	79.15 ^{abcd} (77.76–86.72)	101.68 ^{abcd} (99.51–101.78)	91.29 ^{abcd} (90.41–92.45)	74.14 ^{bcd} (70.63–78.32)	119.05 ^{abcd} (114.67–119.17)	132.41 ^{abc} (130.73–133.67)	128.61 ^{abc} (125.56–128.93)	145.59 ^{ab} (142.69–145.88)	154.74 ^a (153.44–162.37)
2n:6	9.89 ^{abc} (9.89–9.89)	3.94 ^a (3.94–3.94)	6.99 ^{abc} (6.99–6.99)	5.76 ^{abc} (5.76–5.76)	9.12 ^{abc} (9.12–9.12)	16.65 ^{abc} (16.65–16.65)	6.29 ^{ab} (6.29–6.29)	21.50 ^c (21.50–21.50)	15.70 ^{bc} (15.70–15.70)	5.31 ^{ab} (5.31–5.31)	7.80 ^{abc} (7.80–7.80)	14.25 ^{abc} (14.25–14.25)	6.82 ^{abc} (6.82–6.82)	5.90 ^{ab} (5.90–5.90)

Table 4: (continued)

Fatty acid	Isolate												
	IMB212	IMB213	IMB214	IMB215	IMB216	IMB217	IMB219	IMB220	IMB221	IMB222	IMB224	IMB225	IMB226
C18:	(9.85–10.38)	(3.89–5.35)	(6.93–7.57)	(5.59–8.24)	(8.58–9.74)	(14.64–16.70)	(5.39–6.47)	(20.77–22.27)	(14.96–17.59)	(5.31–6.02)	(7.37–7.91)	(13.66–14.72)	(6.34–7.36)
3n-6													
C20:	0.97 ^{abcd}	0.42 ^d	0.83 ^{abcd}	0.69 ^{bcd}	0.61 ^{cd}	1.36 ^{abcd}	0.82 ^{abcd}	1.08 ^{abcd}	0.91 ^{abcd}	1.62 ^{ab}	1.44 ^{abc}	1.10 ^{abcd}	2.42 ^c
2n-6	(0.97–0.99)	(0.27–0.42)	(0.76–1.08)	(0.63–0.78)	(0.54–0.63)	(1.33–1.46)	(0.80–0.88)	(1.05–1.08)	(0.85–1.05)	(1.59–1.69)	(1.42–1.84)	(1.02–1.29)	(2.23–2.45)
C20:3n-6	6.11 ^{ab}	5.82 ^{ab}	1.72 ^b	6.77 ^{ab}	3.54 ^b	18.62 ^{ab}	8.92 ^{ab}	14.92 ^{ab}	14.30 ^{ab}	10.37 ^{ab}	11.54 ^{ab}	14.87 ^{ab}	11.92 ^{ab}
	(5.97–6.45)	(5.42–9.11)	(1.46–4.20)	(6.21–12.76)	(2.77–3.65)	(14.87–18.94)	(6.81–10.08)	(14.04–15.74)	(12.11–15.44)	(9.84–10.49)	(10.79–11.97)	(13.74–16.87)	(10.96–14.15)
C20:4n-6	40.84 ^{abcd}	17.65 ^d	25.81 ^{cd}	30.99 ^{cd}	35.04 ^{bcd}	62.14 ^{abcd}	55.63 ^{abcd}	53.96 ^{abcd}	26.41 ^{cd}	156.67 ^{ab}	141.23 ^{ab}	76.24 ^{abc}	188.04 ^a
	(39.41–41.32)	(15.59–19.23)	(23.52–29.12)	(26.47–32.49)	(34.76–35.10)	(59.10–66.24)	(55.22–60.92)	(51.71–55.49)	(26.2–26.45)	(147.87–157.63)	(140.27–144.09)	(75.27–77.42)	(186.29–190.50)
C22:4n-6	0.10 ^{ab}	0.05 ^b	0.19 ^{ab}	0.11 ^{ab}	0.07 ^{ab}	0.30 ^a	0.15 ^{ab}	0.11 ^{ab}	0.08 ^{ab}	0.05 ^{ab}	0.05 ^{ab}	0.23 ^{ab}	0.13 ^{ab}
	(0.10–0.10)	(0.05–0.05)	(0.16–0.25)	(0.07–0.13)	(0.07–0.09)	(0.28–0.35)	(0.15–0.21)	(0.11–0.14)	(0.08–0.12)	(0.05–0.08)	(0.05–0.08)	(0.23–0.23)	(0.10–0.13)
C18:3n-3	0.05 ^a	0.02 ^a	0.06 ^a	0.07 ^a	0.04 ^a	0.20 ^a	0.10 ^a	0.00 ^a	0.12 ^a	0.05 ^a	0.11 ^a	0.16 ^a	0.06 ^a
	(0.02–0.07)	(0.01–0.11)	(0.06–0.86)	(0.05–0.14)	(0.04–0.04)	(0.18–2.04)	(0.08–0.13)	(0.00–0.03)	(0.08–0.17)	(0.05–0.10)	(0.11–0.16)	(0.12–0.16)	(0.06–0.10)
C20:4n-3	0.19 ^{ab}	0.17 ^{ab}	0.19 ^{ab}	0.18 ^{ab}	0.04 ^{ab}	0.15 ^{ab}	0.26 ^{ab}	0.28 ^{ab}	0.41 ^{ab}	0.05 ^b	0.05 ^{ab}	0.39 ^{ab}	0.06 ^{ab}
	(0.19–0.22)	(0.17–0.36)	(0.16–0.89)	(0.16–0.42)	(0.04–0.05)	(0.15–0.25)	(0.21–0.26)	(0.26–0.28)	(0.33–0.43)	(0.05–0.08)	(0.05–0.05)	(0.35–0.55)	(0.06–0.06)
C20:5n-3	70.37 ^{ab}	27.41 ^{bcd}	51.18 ^{abcd}	41.53 ^{abcd}	51.60 ^{abcd}	39.95 ^{abcd}	67.23 ^{abc}	54.75 ^{abcd}	39.14 ^{abcd}	0.15 ^{cd}	0.05 ^d	89.87 ^a	0.19 ^{cd}
	(66.86–70.49)	(26.29–29.21)	(49.14–53.65)	(38.43–42.09)	(49.90–51.85)	(36.48–42.19)	(66.05–67.70)	(53.64–54.98)	(34.76–41.74)	(0.15–0.25)	(0.05–0.08)	(89.20–97.38)	(0.16–0.19)
C22:5n-3	0.19 ^{abc}	0.10 ^{bc}	0.25 ^{ab}	0.22 ^{abc}	0.18 ^{abc}	0.45 ^a	0.15 ^{abc}	0.17 ^{abc}	0.12 ^{abc}	0.10 ^{bc}	0.05 ^c	0.31 ^a	0.13 ^{abc}
	(0.17–0.24)	(0.09–0.11)	(0.25–0.32)	(0.20–0.24)	(0.14–0.18)	(0.38–0.45)	(0.15–0.18)	(0.17–0.20)	(0.10–0.17)	(0.08–0.10)	(0.05–0.05)	(0.31–0.35)	(0.13–0.13)

Data are expressed as median (interquartile range). Values in the same row with the same superscript letter are not significantly different at p=0.05.

Species	<i>Halophyto tophthora avicenniae</i> IMB212	<i>Halophyto tophthora avicenniae</i> IMB213	<i>Halophyto tophthora avicenniae</i> IMB215	<i>Halophyto tophthora avicenniae</i> IMB219	<i>Halophyto tophthora avicenniae</i> IMB225	<i>Halophyto tophthora batemanensis</i> IMB216	<i>Halophyto tophthora batemanensis</i> IMB217	<i>Halophyto tophthora batemanensis</i> IMB220	<i>Halophyto phthoria exoprolifera</i> IMB214	<i>Halophyto tophthora polymor phica</i> IMB227	<i>Salispina spinosa</i> IMB222	<i>Salispina spinosa</i> IMB224	<i>Salispina spinosa</i> IMB226
Medium	4 g l ⁻¹ glucose, 4 g l ⁻¹ yeast extract, 4 g l ⁻¹ peptone, 3% artificial sea salt												
Fatty acid profile (% in total fatty acid)	C10:0 0.06	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	C12:0 8.70	0.11	0.03	0.01	0.03	0.04	0.03	0.03	0.03	0.02	0.04	0.02	0.02
	C14:0 5.02	9.02	6.83	5.22	6.52	7.80	8.19	9.28	6.33	4.44	0.65	0.55	0.50
	C15:0 0.52	0.31	0.78	0.73	0.71	0.26	0.14	0.16	0.30	0.40	0.08	0.07	0.09
	C16:0 25.30	24.45	24.99	21.79	26.03	19.76	23.47	22.24	35.35	21.98	28.10	26.92	28.12
	C17:0 NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	C18:0 1.91	3.01	3.22	1.62	2.28	3.05	3.38	4.04	2.29	3.95	2.80	2.54	2.48
	C20:0 0.12	0.25	0.23	0.17	0.18	0.31	0.28	0.44	0.14	0.28	0.17	0.21	0.20
	C22:0 0.07	0.12	0.11	0.12	0.09	0.17	0.14	0.13	0.28	0.37	1.46	2.25	1.75
	C23:0 NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	C24:0 0.06	0.03	0.08	0.04	0.05	0.06	0.11	0.04	0.06	0.04	0.37	0.65	0.49
	C16:1 1.89	2.93	2.68	2.74	2.62	1.62	2.40	2.07	6.94	1.24	2.31	2.68	2.65
	C16:2 NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	C18: 11.90	13.68	14.00	16.65	16.33	19.91	13.50	15.19	16.94	11.15	7.11	8.22	7.52
	1n-9c NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	1n-9t NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	C18: 0.23	0.38	0.38	0.28	0.24	0.64	0.66	0.57	0.56	0.43	0.05	0.07	0.05
	1n-7 NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	C20:1 0.57	0.29	0.36	0.32	0.27	0.89	0.99	0.81	0.43	0.52	0.07	0.10	0.09
	C22:1 2.40	2.59	2.88	3.06	2.20	3.08	2.45	2.93	2.07	3.67	0.27	0.35	0.32
	C24:1 0.28	0.38	0.42	0.38	0.34	0.23	0.39	0.28	0.57	1.40	0.03	0.05	0.04
	C18: 19.95	18.67	18.72	19.45	16.20	14.57	16.55	16.12	13.40	20.73	22.98	24.73	22.57
	2n-6 NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	C18: 2.10	1.97	2.02	1.13	1.81	2.57	3.05	3.79	1.15	0.72	1.14	1.42	1.08
	3n-6 NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	C20: 0.20	0.13	0.20	0.16	0.15	0.16	0.28	0.19	0.15	0.24	0.33	0.32	0.36
	2n-6 NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	C20: 1.29	3.14	2.87	1.61	1.97	0.87	3.25	2.62	0.50	3.27	2.00	2.12	2.00
	3n-6 NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	C20: 8.29	7.03	8.00	11.42	9.76	9.76	12.49	9.43	4.17	12.63	29.95	26	

This study

Reference	This study									
Species	<i>Halophyta-phthoravicenniae</i> IMB144	<i>Halophyta-tophthoravicenniae</i> IMB145	<i>Halophyta-tophthoravicenniae</i> IMB157	<i>Halophyta-tophthoravicenniae</i> IMB158	<i>Halophyta-tophthoravicenniae</i> IMB159	<i>Halophyta-phthoravicenniae</i> IMB160	<i>Halophyta-tophthoravicenniae</i> IMB164	<i>Halophyta-tophthorapoly-morphica</i> IMB146	<i>Halophyta-phthoravescula</i> IMB147	<i>Salispha-spinosa</i> IMB162
Medium	4 g l ⁻¹ glucose, 4 g l ⁻¹ yeast extract, 4 g l ⁻¹ peptone, 2.5 % seawater									
Fatty acid profile (% in total fatty acid)	C10:0 0.42	C10:0 0.38	C10:0 0.00	C05 0.05	C00 0.00	C00 0.00	C05 0.05	C13 0.13	C06 0.06	C00 0.00
	C12:0 1.08	C12:0 1.00	C12:0 0.17	C29 0.29	C19 0.19	C20 0.20	C32 0.32	C70 0.70	C06 0.06	C130 1.30
	C13:0 13.89	C13:0 13.75	C12:7 12.27	C15:20 15.20	C12:14 12.14	C12:88 12.88	C16:89 16.89	C15:19 19.51	C34 9.34	C11 0.11
	C15:0 0.67	C074 0.74	C040 0.40	C34 0.34	C89 0.89	C76 0.76	C120 1.20	C45 0.45	C79 0.79	C30:14 30.14
	C16:0 21.34	C19:97 21.97	C29:28 29.28	C26:63 26.63	C26:34 26.34	C26:26 26.26	C25:72 25.72	C13:40 13.40	C29:07 29.07	C138 1.38
	C054 0.54	C059 0.59	C052 0.52	C45 0.45	C85 0.85	C77 0.77	C90 0.90	C350 3.50	C114 1.14	C136 1.36
	C17:0 1.06	C110 1.10	C147 1.47	C173 1.73	C181 1.81	C141 1.41	C89 0.89	C172 1.72	C84 0.84	C136 1.36
	C20:0 NA	C20:0 NA	C147 NA	C173 NA	C181 NA	C141 NA	C89 NA	C172 NA	C84 NA	C136 NA
	C22:0 NA	C22:0 NA	C147 NA	C173 NA	C181 NA	C141 NA	C89 NA	C172 NA	C84 NA	C136 NA
	C23:0 NA	C23:0 NA	C147 NA	C173 NA	C181 NA	C141 NA	C89 NA	C172 NA	C84 NA	C136 NA
	C24:0 NA	C24:0 NA	C147 NA	C173 NA	C181 NA	C141 NA	C89 NA	C172 NA	C84 NA	C136 NA
	C16:1 2.43	C239 2.39	C279 2.79	C314 3.14	C308 3.08	C324 3.24	C321 3.21	C763 7.63	C225 2.25	C325 3.25
	C16:2 0.21	C023 0.23	C021 0.21	C017 0.17	C036 0.36	C037 0.37	C041 0.41	C090 0.90	C053 0.53	C004 0.04
	C18: 9.75	C10:11 10.11	C13:46 13.46	C14:68 14.68	C12:55 12.55	C12:74 12.74	C10:40 10.40	C6:61 6.61	C14:18 14.18	C4:11 4.11
	1n-9c									
	C18: 0.32	C034 0.34	C024 0.24	C027 0.27	C023 0.23	C027 0.27	C022 0.22	C121 1.21	C056 0.56	C002 0.02
	1n-9t									
	C18: NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA
	1n-7									
	C20:1 NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA
	C22:1 NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA
	C24:1 NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA
	C18: 18.87	C19:21 19.21	C16:63 16.63	C16:45 16.45	C16:82 16.82	C17:08 17.08	C17:82 17.82	C16:90 16.90	C13:21 13.21	C33:19 33.19
	2n-6									
	C18: NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA
	3n-6									
	C20: NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA
	2n-6									
	C20: NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA	CNA NA
	3n-6									
	C20: 11.00	C10:69 10.69	C7:53 7.53	C7:16 7.16	C10:84 10.84	C8:42 8.42	C7:47 7.47	C11:10 11.10	C14:72 14.72	C25:02

Table 5: (continued)

Reference	Pang et al. (2016)				
Species	<i>Halophytophthora vesicula</i> AK1YB2	<i>Halophytophthora vesicula</i> PQ1YB3	<i>Salispina spinosa</i> ST1YB3	<i>Halophytophthora vesicula</i> AK1YB2	<i>Halophytophthora vesicula</i> PQ1YB3
Medium	10 g l ⁻¹ glucose, 4 g l ⁻¹ yeast extract, 4 g l ⁻¹ peptone, 50% sterile seawater or 25 g l ⁻¹ marine salt	10 g l ⁻¹ glucose, 4 g l ⁻¹ yeast extract, 4 g l ⁻¹ peptone, 50% sterile seawater or 25 g l ⁻¹ marine salt	20% (v/v) V8 juice Campbell, 3 g l ⁻¹ calcium carbonate, 50% sterile seawater or 25 g l ⁻¹ marine salt	20% (v/v) V8 juice Campbell, 3 g l ⁻¹ calcium carbonate, 50% sterile seawater or 25 g l ⁻¹ marine salt	5% (v/v) clarified vegetable juice, 4 g l ⁻¹ glucose, 4 g l ⁻¹ yeast extract, 4 g l ⁻¹ peptone, 10 g l ⁻¹ marine salt
Fatty acid	NA	NA	NA	NA	–
C10:0	NA	NA	NA	NA	–
C12:0	NA	NA	NA	NA	0.81
C14:0	5.89	3.53	2.66	0.73	8.64
C15:0	NA	NA	NA	NA	0.79
C16:0	25.20	9.47	28.31	9.45	32.62
C17:0	NA	NA	NA	NA	30.00
C18:0	2.54	0.68	7.54	1.91	0.82
C20:0	NA	NA	NA	NA	0.56
C22:0	NA	NA	NA	NA	1.87
C23:0	NA	NA	NA	NA	2.20
C24:0	NA	NA	NA	NA	4.61
C16:1	3.56	3.87	3.06	1.30	–
C16:2	NA	NA	NA	NA	2.71
C18:	14.85	8.45	14.89	8.03	NA
1n-9c	NA	NA	NA	NA	15.56
C18:	NA	NA	NA	NA	32.12
1n-9t	NA	NA	NA	NA	NA
C18:	NA	NA	NA	NA	NA
1n-7	NA	NA	NA	NA	NA
C20:1	NA	NA	NA	NA	NA
C22:1	NA	NA	NA	NA	2.09
C24:1	NA	NA	NA	NA	NA
C18:	24.65	15.46	30.70	14.44	13.86
2n-6	NA	NA	NA	NA	16.12
C18:	NA	NA	NA	NA	1.98
3n-6	NA	NA	NA	NA	NA
C20:	NA	NA	NA	NA	NA
2n-6	NA	NA	NA	NA	3.25
C20:	NA	NA	NA	NA	–
3n-6	20.79	16.06	19.95	2.96	NA
C20:	NA	NA	NA	NA	NA
4n-6	NA	NA	NA	NA	NA
C22:	NA	NA	NA	NA	NA
4n-6	NA	NA	NA	NA	–
C18:	NA	NA	NA	NA	0.93
3n-3	NA	NA	NA	NA	NA
C20:	NA	NA	NA	NA	NA
4n-3	–	–	–	–	2.07
C20:	NA	NA	NA	NA	NA
5n-3	NA	NA	NA	NA	NA
C22:	NA	NA	NA	NA	NA
5n-3	NA	NA	NA	NA	NA
Reference	Cagujmbal et al. (2019)				
	Devanadera et al. (2019)				

NA, not available.

Figure 1: Production of arachidonic (ARA) and eicosapentaenoic (EPA) acids in mycelia of the 14 isolates of *Halophytophthora* spp. (*H. avicenniae*: IMB212, IMB213, IMB215, IMB219, IMB225; *H. batemanensis*: IMB216, IMB217, IMB220, IMB221; *H. exoprolifera* IMB214; *H. polymorphica* IMB227) and *Salispina spinosa* (IMB222, IMB224, IMB226): (a) percentage of total fatty acids based on median and interquartile range and (b) yield (mg l⁻¹).

isolates of the same species varied; 10%–17% oleic acid was produced by *H. avicenniae*, 8–15% by *H. vesicula* and 4–15% by *S. spinosa*. For linoleic acid, *H. avicenniae* produced 16–20%, *H. vesicula* 13–25% and *S. spinosa* 22–33%. ARA and EPA yields varied between *Halophytophthora* species, and within different isolates of *H. avicenniae* and *S. spinosa* analysed in this study and those in Pang et al. (2016). This may be attributed to the media (nutrient concentration, salinity) used in the different studies. Say et al. (2017) showed that nutrient composition, salinity and pH of the fermentation medium affected the type and quantity of fatty acids produced by a *Halophytophthora* isolate S13005YL1-3.1: 8, 10 and 13 fatty acids were produced in V8

SWD-A (pH 8, salinity 30), V8 SWD-B (pH 6, salinity 10) (V8 SWD: 20% clarified V8 juice, 80% sterile distilled water) and PYG (4 g l⁻¹ peptone, 4 g l⁻¹ yeast extract, 10 g l⁻¹ glucose, 50% sterile seawater or 25 g l⁻¹ sea salt) media, respectively. These variations in fatty acid profiles suggest that further studies are required to determine the best conditions and isolates for greater yields of these essential fatty acids.

In conclusion, *Halophytophthora* spp. and *S. spinosa* produced a wide range of saturated and unsaturated fatty acids in their mycelia and with varying yields. The dominant fatty acids were C16:0 palmitic acid, C18:1n-9 oleic acid, C18:2n-6 linoleic acid, C20:4n-6 arachidonic acid and

Figure 2: Multidimensional scaling (MDS) plot using Bray-Curtis distances based on fatty acid profile in mycelia of the 14 isolates of *Halophytophthora* spp. (*H. avicenniae*: IMB212, IMB213, IMB215, IMB219, IMB225; *H. batemanensis*: IMB216, IMB217, IMB220, IMB221; *H. exoprolifera* IMB214; *H. polymorphica* IMB227) and *Salispina spinosa* (IMB222, IMB224, IMB226): (a) percentage of total fatty acids and (b) yield (mg l^{-1}).

C20:5n-3 eicosapentaenoic acid, the latter two being essential fatty acids for human diet may be of great industrial value. In thraustochytrids, lipids were found to be possible energy sources for their motile cells (i.e. amoeboid cells) and during starvation (Jain et al. 2007), and a similar function may also apply to *Halophytophthora* and *Salispina* species. Marine thraustochytrids have been successfully used to replace dietary fish oil in feed of Atlantic salmon (Chang et al. 2020; Tibbetts et al. 2020). Thus, the results presented here show the potential for applications of *Halophytophthora* and *Salispina* in aquaculture feed, an area that requires further study and investment.

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