Characterization of fatty acid composition of healthy and diseased coral mucus released from host and/or endosymbiont for their possible role in coral health

Phartade Vilas Bhagwat^{a,b}, Chinnarajan Ravindran^{a*}, Keerthana, V.^a, and Mamatha, S.S^c.

^aBiological Oceanography Division, CSIR-National Institute of Oceanography, Dona Paula, Goa 403004, India.
^bDepartment of Biotechnology, Goa University, Taleigao Plateau, Goa- 403206, India.
^cCSIR-Central Food Technological Food Research Institute, Mysuru- 570020, India
*Corresponding author's email id: cravindran@nio.org

Abstract

Coral mucus serves as a barrier, protecting corals from a variety of biotic and abiotic stresses. Coral mucus contains all essential nutrients including carbohydrates, amino acids and fatty acids (FA). Although fatty acids, an essential nutrient, have been extensively studied in coral tissue and endosymbionts, their presence in coral mucus has been poorly understood. Besides, there is no clear study on the coral tissue or endosymbiont as a key contributor to their release of fatty acids into the mucus, and their potential function in disease and healthy states. Thus, the present study examined the fatty acid profiles of diseased and healthy *Acropora* sp. and *Porites* sp. mucus. The study reveals that mucus contains FAs, and Palmitic acid, a precursor fatty acid, was the most common mucus fatty acid found in both corals. However, there were also differences in FAs between *Porites* sp. and *Acropora* sp., and as well as healthy and diseased mucus, indicating that any biotic or abiotic stress affects the diversity and occurrence of mucus fatty acids. Furthermore, the FAs in mucus was similar to that of heterotrophic coral tissue FAs rather than endosymbionts. As a result, our study not only contributes to a better understanding of mucus FAs potential role as indicators of coral health and symbiotic association, but it also indicates the probable source of these FAs.

Keywords: Coral, mucus, fatty acids, biomarker, dinoflagellate, polyps

1. Introduction

Coral mucus is a complex mixture of protein, carbohydrates, and lipids produced by mucocytes in the epidermal layer and used by the coral as an adaptation mechanism against different stress conditions (Brown and Bythell 2005; Bhagwat et al. 2024). Therefore, coral surface tissue that secretes the surface mucus layer (SML) serves as a barrier between animal tissue and environmental stresses, functioning as both a first line of defense and a physiological and physiochemical barrier (Brown and Bythell 2005; Bakshani et al., 2018). Among the complex mixtures of mucus, fatty acids (FAs) have not been clearly studied. FAs synthesis occurs both in the algal endosymbiont of the family Symbiodiniaceae and host corals (Oku et al. 2003). Endosymbiont produces fatty acids in algal plastids thylakoid membranes (Tchernov et al. 2004), while host coral synthesizes phospholipids in the endoplasmic reticulum (ER) (Chen et al. 2021). However, corals cannot produce all essential fatty acids (EFAs), because they cannot synthesize precursor fatty acids linolenic (18:3n-3) and linoleic (18:2n-6) acids (Latyshev et al. 1991; Bell et al. 2007). Hence, corals acquire the essential FA through heterotrophic feedings on phytoplankton such as dinoflagellates and diatoms, which are the sources of many polyunsaturated fatty acids (PUFAs), especially cervonic acid (C22:6n-3), timnodonic acid/eicosapentaenoic acid (C20:5n-3), monounsaturated FA (MUFAs), and short-chain saturated FAs (SAFAs) (Sebens 1987, McAuley 1994, Rocker et al. 2019). Besides, corals also acquire FAs endogenously through photosynthetic endosymbionts (Kellogg and Patton 1983; Patton and Burris 1983; Patton et al. 1983; Harland et al. 1993). FAs synthesis within the thylakoid membrane of Symbiodiniaceae includes the stepwise assembly of long-chain acetyl-CoA to form the 16-carbon palmitic acid (Kellogg and Patton 1983) and gets translocated to host tissues as 'lipid droplets' which contain glycolipids, phospholipids, and neutral lipids (Patton and Burris 1983; Kellogg and Patton 1983; Patton et al. 1983; Harland et al. 1993). When the essential FAs, linoleic acid (C18:2n-6) and αlinolenic acid (C18:3n-3) are obtained, the host coral modifies them using different sets of desaturases (D6 and D5) to produce arachidonic acid (C20:4n-6) and eicosapentaenoic acid (EPA), then EPA generates cervonic acid (Sargent et al. 1990).

Photosynthetic impairment due to bleaching may affect FAs metabolism, particularly the FAs synthesis pathway, leading to a change in FAs content and a decrease in lipogenesis (Bachok et al. 2006). This can compromise the energy reserves needed to support coral metabolism (Grottoli et al. 2004), and this energy store depletion also causes an increase in mortality and disease susceptibility (Meesters and Bak 1993; Mascarelli and Bunkley-Williams 1999). Since corals derive most PUFAs from endosymbiont algae (Papina et al. 2003), loss in algae is directly related to the reduction in PUFAs levels, specifically dinoflagellate markers FAs such as stearidonic acid (C18:4n-3),

docosapentaenoic acid (C22:5n-3), and cervonic acid (C22: 6n-3) (Graeve et al. 1994; Zhukova and Aizdaicher 1995). Thus, various factors alter the FAs composition of corals including food availability around the corals and their fatty acid content, morphology, polyps and Symbiodiniaceae concentration, nematocytes types, light availability, depth and seawater temperature (Rocker et al. 2019). The free FAs of the host and endosymbiont secreted into the mucus are important for various metabolic functions in coral reef ecosystem including energy carrier, particle trap, nutrient source for microbial communities, fuel for food chains (Binienda et al. 2013; Ravindran et al., 2023; Bhagwat et al. 2024). The coral also benefitted by secretion of mucus as defense responses against various biotic and abiotic stress factors (Bhagwat et al., 2023; Ravindran et al., 2023; Bhagwat et al. 2024). However, the role of FAs of coral mucus was poorly studied (Ravindran et al., 2020). Since corals depend on adequate energy storage and metabolic precursors for survival, growth and reproduction, examining healthy and stressed coral mucus FAs composition is a potential diagnostic indicator of coral health. Besides, the presence of certain fatty acids (FAs) from endosymbionts in the host coral (Papina et al. 2003; Treignier et al. 2008), along with the transfer of polyunsaturated fatty acids (PUFAs) from the host coral to its endosymbionts (Imbs et al. 2014), indicates a dynamic exchange of FAs between the host and its endosymbionts for growth and development. This interaction underscores the importance of understanding the complex biochemical relationships within coral holobionts. Thus, the present study aims to first determine and compare the FAs profile of healthy and diseased coral mucus of Porites and Acropora species, in order to understand the role of FAs under stress conditions. The second aim is to identify the potential source of FAs in coral mucus (whether host or endosymbiont).

2. Materials and methods

2.1. Sample collections

Acropora sp. and Porites sp. mucus of different healthy, bleached and diseased corals have been collected (Table 1) from three islands in the Gulf of Mannar, India for the analysis of associated fatty acid composition. Munnaikad island (SL1) is located at latitude: N 09°17' 36.2", longitude: E 079°08'12.6", depth at ~1 meter, pH-7.8, Temperature 28 °C at 7:30 AM, Salinity-3.6 %. Big Manali island (SL2) is located at latitude: N 09°13'15.6", longitude: E 079°08'38.5", pH-7.8, depth at 1-1.5meter, Temperature 29 °C at 9:00 AM, salinity-3.6 %. T. Nagar (SL3) is located at latitude: N 09°17'45.6", longitude: N 09°17'45.6", pH-7.8, depth at 3-4-meter, Temperature 28 °C at 07:43 AM, salinity-3.6 %. Mucus was collected with a sterile syringe without a needle from the surface of the corals. Diseased mucus samples such as white patch *Acropora* disease and *Porites* pink line syndrome were collected along the disease margins. Healthy mucus was collected as described by Ravindran et al. (2020). Similarly, diseased mucus samples such as white patch *Acropora* sp. *Porites* pink spot, and pink line syndrome (Fig. 1) were collected in triplicate from every colony. Bleached coral mucus was collected from completely bleached *Porites* sp. tissues to compare how its fatty acid composition changes when endosymbiotic zooxanthellae are expelled under bleached conditions, relative to healthy corals (with zooxanthellae) and diseased corals (Fig. 1). The samples were collected in triplicate from a single individual colony at each location. The sampling was conducted at three different islands, each representing a distinct coral health condition: SL1, dominated by diseased coral colonies; SL2, characterized by bleached coral colonies; and SL3, consisting of healthy coral colonies. Thus, for each health status category (healthy, bleached, diseased), samples were obtained from a different colony, corresponding to the specific coral health condition at each location. The decision trees and identification guides provided by the Coral Disease and Health Consortium and the National Oceanic and Atmospheric Administration (NOAA) were used in identifying coral diseases. Thus, all collected coral mucus was brought to the laboratory within 24 hours with cold ice packs, kept in cold conditions, and processed immediately.

2.2.Lipid extraction and fatty acid analysis of coral mucus

Lipid extraction from various healthy and diseased samples of Porites sp. and Acropora sp. mucus was carried out according to the Folch method (Folch et al. 1956; Ravindran et al. 2020). Coral mucus of about 0.2 ml was added to 5 ml of methanol: chloroform in the ratio of 1:2 and kept for ultrasonication for up to 30 minutes at 40 °C. Then the samples were transferred to the separating funnel. 2 ml of potassium hydroxide (KOH) was added (5 grams in 50 ml of methanol) to the sample for saponification and mixed well and allowed for separation till the separate layer formed. The upper layer was removed using a Pasteur pipette and transferred to a pear shape flask. The transesterification of the fatty acid components of the entire lipid was carried out following the protocol designed by Carreau and Dubacq (Crreau and Dubacq 1978). Fatty acid methyl esters (FAME) have been analyzed on a Gas Chromatography mass spectrometer (GC-MS) device (QP-2010, Shimadzu, Japan) prepared with a DB-23 capillary column (60 m x 0.25 um ID, 0.25 um film thickness) as earlier described by Ravindran et al. (2020). Methyl nanodecanoate (Supelco) was used as the spiked-in control and fatty acids were identified by comparing their methyl esters with standard fatty acids and mass spectra. To determine the exact detection limit for our specific analytical conditions, we ran a series of standards with known concentrations. We identified the lowest concentration at which the fatty acid could be reliably detected, which was 1 ng on-column, with a signal-to-noise ratio of at least 3:1.

The relative percentage of each fatty acid = (Area of each FAME peak/total area of all FAME peaks) X 100.

In this study, we investigated mucus compositions and compared our findings with data from previous studies that focused on endosymbiont and tissue samples. All studies, including ours, adhered to the analytical protocol established by Folch (1957) for lipid extraction and analysis. This standardized method ensures that the analytical techniques used across studies are consistent, facilitating a meaningful comparison of results. The Folch (1957) protocol involves a solvent extraction procedure that isolates lipids from biological samples, providing a reliable and reproducible means of analyzing lipid compositions. By employing this well-established method, we maintain comparability with prior research, which similarly relied on Folch's approach for lipid extraction from different sample types. Given that the same analytical methods were utilized across studies, the results are expected to be comparable in terms of the types and quantities of lipids identified.

2.3 Statistical analysis

One-way ANOVA, standard deviation, and correlation analyses were carried out using PAST software version 3.23 (Carpio et al., 2020). *Porites* sp. mucus FAs were compared for healthy, diseased and bleached using Tukey's HSD post-hoc tests. FAs values were measured in triplicates, and normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) were confirmed. For multiple comparisons, we applied the Benjamini-Hochberg FDR correction, considering only significant adjusted p-values. Key results, including degrees of freedom, F-values, unadjusted and adjusted p-values were obtained with post-hoc test results highlighted in a heatmap using asterisks for significant differences. Heatmap analyses were performed using the R package Phyloseq version 1.22.3 (McMurdie & Holmes, 2013). For data visualization, the R package ggplot2 was employed, with all analyses executed within R Studio version 4.3.2.

Results and discussions

3.1. Healthy and diseased coral mucus FA profile and their role under stress conditions.

The FAs profile of total lipid of healthy and diseased coral (Fig.1) mucus (Fig. 2a and 2b) consisted saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids (Table 2 and 3). Besides, FAs were observed with significant variations (p < 0.05) (Tables 2 and 3) reflecting the stress conditions. Among the eight detected SFAs, palmitic acid (C16:0) was observed predominantly in healthy mucus of *Porites* and *Acropora* sp., and it was 52 % enriched in *Acropora* sp. disease mucus (Table 2) and depleted about 95 % in both the disease *Porites* sp. of pink spot (PS) and pink line syndrome (PLS) mucus (Table 3). Although, the next abundant stearic acid (C18:0) was observed in both the healthy mucus of corals but was depleted in diseased mucus (Table 2 and 3). Lauric acid (C12:0) was found enriched with *Acropora* sp. diseased mucus (Table 2), and margaric

acid (C17:0) was enriched in all the mucus of bleached, PLS and PS of Porites sp. (Table 3). Myristic acid (C14:0) and pentadecyclic acid (C15:0) was enriched in PS mucus, while the arachidic acid (C20:0) was observed enriched in bleached Porites sp. mucus (Table 3). Palmitic acid and stearic acid were reported for their bactericidal activity (Ivanova et al. 2017), were enriched in diseased Acropora sp. mucus (Table 2 and 3), suggesting that mucus FAs have the potential of inhibiting coral pathogens. Besides, invertebrates associated with photosynthetic algae convert palmitic acid (C16:00), into longchain PUFAs α-linolenic acid and linoleic acid with the help of desaturase enzymes (Kellogg and Patton 1983; Dalsgaard et al. 2003). Six MUFAs were detected from the mucus, among which, Cis-7hexadecenoic acid (C16:1n-9), Palmitoleic acid (C16:1n-7), Oleic acid (C18:1n-9), were observed enriched in the Acropora sp. diseased mucus (Table 2), but the C16:1n-9 and C18:1n-9 was found enriched in bleached, PLS and PS mucus of Porites sp. (Table 3). The other MUFAs such as cisvaccenic acid (C18:1n-7), 9-eicosenoic acid or gadoleic acid (C20:1n-9), erucic acid (C22:1n-9) were observed depleted (Table 2 and 3) except C20:1n-9 enriched with PS mucus (Table 3). The detected n3 PUFAs such as alpha linoleic acid (C18:3n-3), stearidonic acid (C18:4n-3), eicosapentaenoic acid (C20:5n-3, EPA), docosapentaenoic acid (DPA) (C22:5n-3), docosahexaenoic acid (DHA) (C22:6n-3) were observed depleted with both the coral mucus (Table 2 and 3). EPA secreted into mucus may act to inhibit pathogens such as bacteria, protozoa, viruses, fungi, and cyanobacteria due to its potential antimicrobial activity (Desbois 2013). DHA plays a crucial role in maintaining membrane fluidity (Dalsgaard et al. 2003; Guil-Guerrero 2007; Russo 2009). DHA was observed in both endosymbionts and hosts. However, higher levels in healthy *Porites* sp. mucus, with a mean of $1.7 \% \pm 0.17 \%$, and a 35 % increase when compared with PLS mucus (1.1 % \pm 0.1 %) and 70 % enriched compared to PS mucus $(0.5 \% \pm 0.05 \%)$ (p < 0.05) (Table 2 and 3). Among the seven n6 PUFAs such as linoleic acid (C18:2n-6), gamma linoleic acid (C18:3n-6), Eicosadienoic acid (EDA) (C20:2n-6), dihomo-ylinolenic acid (DGLA) (C20:3n-6), arachidonic acid (C20:4n-6 (ARA), adrenic acid (ADA) (C22:4n-6), docosapentaenoic acid (DPA) (C22:5n-6) only linoleic acid (C18:2n-6) (Table 2 and 3) was found enriched in all the bleached, PLS and PS mucus of Porites sp. (Table 3). ARA is absent in diseased Acropora sp. mucus and observed 8.7 % in healthy Acropora sp. $(3.4\% \pm 0.37\%)$ (Table 2) and 60 % and 70 % enrichment in healthy Porites sp. mucus (3.4 $\% \pm 0.37$ %) (p < 0.05) (Table 3) suggesting that ARA is mostly reflected in healthy mucus as it has immune-related properties. EDA is well known for its metabolite function and act as a precursor for the synthesis of DGLA, which has antiinflammatory and anti-allergic properties (Kawashima et al. 2009; Huang et al. 2011). EPA, DHA, and ARA have been described for endosymbiont population maintenance, immune response, metabolism and respiration (Yamashiro et al. 1999; Hulbert 2003). Linoleic acid and y-linolenic acid are essential for proper physiological functioning, metabolism, and stress resilience (Hazel and Williams 1990), which supports our present study of enriched y-linolenic acid in bleached Porites sp. mucus (15 %) $(11.82 \% \pm 1.01 \%)$, PS (70 %) (3.85 % \pm 0.35 %) and PLS mucus (72 %) (4.28 % \pm 0.22 %) (Table 3). The *cis*-9-octadecenoic acid indicated the sensitivity of coral to thermal stress (Tchernov et al. 2004) supports our results of FAs enrichment in bleached and absent in healthy Porites sp. mucus (Table 3). Besides, PUFAs particularly ARA and eicosatetraenoic acid (C22:4n-6) in mucus represent a healthy association of endosymbiont with the polyps (Treignier et al. 2008). The n-3 and n-6 PUFAs higher ratio provides nutrients (Clarke and Jump 1993; Nomura et al. 2013) which supports our results with depleted observation in diseased Porites and Acropora sp. mucus, and enriched in healthy mucus (Table 2 and 3). Additionally, PUFAs were found to be 15 % lower in the PLS mucus (mean 11.9 % \pm 0.62 %) and 3.6 % lower in PS mucus (mean 13.5 % \pm 0.5 %) (Table 3). These reductions in PUFAs and dinoflagellate biomarkers C22:5n-3, and C22:6n-3 may be due to possible loss of endosymbionts (Table 3). The PUFA n-3 ratio was observed enriched in endosymbiont of Acropora sp. (supplementary table 1) and 6.7 % healthy Acropora sp. mucus (Table 2). Similarly, the n-3 PUFAs C18:2n-6 (LA) was enriched in bleached, PLS and PS of Porites mucus (Table 3) suggesting n-3 FAs for the possible role in coral survival and resistance to biotic and abiotic stress (Romdhane et al. 1995).-The results of occurrence of enriched PUFAs of healthy mucus of the present study is similar with that of earlier report of PUFAs representing the healthy corals, that is higher the ratio, the healthier the coral (r = 1)(Dalsgaard et al., 2003). The n-6 PUFA can help under increased thermal stress to maintain membrane fluidity (Nettleton 1995) was found enriched in bleached Porites sp. mucus of the present observation (Table 3). Thus, the fatty acid profiles in coral mucus, reflect the health status and stress conditions of corals, with notable significant variations between healthy and diseased states in Acropora sp. and Porites sp. (Figs. 2a and 2b). The results infer that the expressed diseased mucus FAs may play a role in defense response including antimicrobial property, immune response, thermal tolerance, etc. supporting coral recovery from biotic and abiotic stress.

3.2. Possible sources of FAs that secreted into the mucus

The secretion of FAs in the mucus of *Acropora* sp. and *Porites* sp. have not been well understood for their various sources such as endosymbionts, polyp tissues, and heterotrophic feeding (bacteria or other microorganisms). Mucus FAs of *Acropora* sp. (Fig. 3) and *Porites* sp. (Fig. 4) were characterized for their biomarkers such as SFA, MUFA and PUFA and compared with individual endosymbiont and host coral fatty acid profiles (Supplementary table 1 and 2). The presence of endosymbiont markers C16:3n-4, C16:4n-1 (Imbs and Yakovleva 2012),18:3n-6, C18:4n-3, 20:5n-3, and 22:6n-3 (Graeve et al. 1994; Zhukova and Aizdaicher 1995; Imbs et al. 2010) and polyp tissue markers C20:4n-6, 22:5n-3 and C22:4n-6 (Chen et al., 2017) in healthy, bleached and diseased mucus of the present study

suggests that the endosymbionts and polyps act as a source in secreting FAs into the mucus (Fig 3 and 4). Endosymbiont biomarker FAs were enriched with 19 % in healthy Porites sp. mucus (15.7 % \pm 0.05 %) compared to bleached *Porites* mucus (12.72 % \pm 0.07 %), 65 % than PLS mucus (5.38 % \pm 0.46 %), and a 67 % than PS mucus (5.15 % \pm 0.05 %) (Table 2 and 3). Polyp tissue markers (host biomarker) were relatively enriched (16.42 ± 1) in healthy Acropora sp. mucus but absent in Acropora sp. disease mucus (Fig 3). Similarly, polyp tissue marker FAs were also significantly higher in healthy *Porites* sp. mucus (7.7 $\% \pm 0.05$ %) and depleted by 66 % in PLS (2.6 $\% \pm 0.03$ %), 72 % in bleached mucus (2.1 $\% \pm 0.05$ %), and 68 % in PS mucus (2.4 $\% \pm 0.05$ %) (Table 3 and Fig. 4). Bacterial biomarker FAs C15:00, C17:00, C16:1n-9, C16:1n-7, C18:1-7, etc (Volkman et al. 1980; Jeffries 1972; Bachok et al. 2006) were observed 273 % enriched (p < 0.05) in bleached *Porites* sp. mucus (10.42 % \pm 0.07 %) (Fig. 3 and 4; Table 2 and 3) similar with that of the earlier report suggesting that bacterial abundance is high in bleached corals (Bachok et al. 2006). Pentadecylic acid occurrence in PS mucus (Table 3) may act as a bacterial biomarker representing the bacterial load in PS disease. As sulfated carbohydrates and polysaccharides of mucus glycoproteins were reported in A. formosa mucus (Meikle et al. 1987) we found sulfate-reducing bacterial markers palmitoleic acid and heptadecanoic acid (Findlay and Dobbs 2018) in both Acropora sp. and Porites sp. mucus (Table 3 and 4). The presence of specific FAs markers in the mucus of Acropora sp. and Porites sp. reveal the diverse FAs contributions from endosymbionts, host tissues, and bacteria, reflecting the complex biochemical interactions between coral and its associated organisms. Besides, the coral disease and bleaching impacts the relative contribution of different holobiont members to fatty acids in the mucus.

The FAs profiles of *Acropora* sp. and *Porites* sp. mucus also reveal differences in coral feeding strategies such as heterotrophic feeding during stress or in the absence of endosymbiont. Cis-gondoic acid (CGA) heterotrophic marker was more in hosts than their endosymbiont which suggests they have come in mucus by heterotrophic feed and from coral polyps (Mies et al. 2018) (Supplementary Table 1 and 2). In PLS mucus CGA was absent whereas it was found 0.7 % enriched in PS mucus (Table 3). This is may be due to heterotrophic feeding preference by *Porites* sp. when it is affected by PLS disease condition (Table 3). Similarly, CGA was absent in *Acropora* sp. diseased mucus as well (Table 2). C22:5n-3, a marker of heterotrophic feed (Imbs et al. 2010; Figueiredo et al. 2017) was found 70 % enrichment with healthy *Porites* sp. mucus (4.3 % \pm 0.26 %) and host tissue reveals higher heterotrophy by the *Porites* sp. suggesting the transfer/secretion of C22:5n-3 from polyp tissue into mucus (Table 3). The higher proportion of C20:5n-3 to C22:6n-3 (EPA: DHA) in healthy *Acropora* sp. mucus indicates comparatively autotrophic, whereas a lower value indicates comparatively heterotrophic feeding (Rocker et al. 2019) (Table 2). Higher PUFA: SFA the planktivores feed markers

that found in endosymbiont may be transferred from planktivorous feed preference of host coral (Rocker et al. 2019) (Supplementary Table 2). Besides, the PUFA to SFA ratio was 533 % enriched in bleached *Porites* sp. mucus, 333 % (1.32 % \pm 0.05 %) PLS (0.78 % \pm 0.03 %), and 283 % PS (0.69 % \pm 0.03 %) (Table 3). This suggests that under stress conditions, *Porites* sp. polyps prefer relatively planktivorous heterotrophic feed for their survival. Thus, the existence of heterotrophic FAs in mucus was observed, indicating that they were transferred from the tissue polyp as a source. Endosymbiont produces PUFAs such as <u> α -linolenic acid</u> (C18:3n-3) and linolenic acid and transfers them to the host (Bachok et al. 2006; Teece et al. 2011). However, host corals can synthesize n-3 PUFA (Kabeya et al. 2018) and acquire n-3, n-6, and n-9 FAs through heterotrophic feeding (Hinrichs et al. 2013; Seemann et al. 2013). Overall, FAs content and source in corals depend on multiple factors such as coral morphology, the abundance of polyps and endosymbionts, type of nematocytes (Teece et al. 2011) light, depth, temperature and other environmental factors (Oku et al. 2003; Harland et al. 1992).

4. Conclusion

The FAs profiles of *Acropora* sp. and *Porites* sp. mucus reveal significant differences between healthy and diseased states reveals that the expressed diseased FAs may play a vital role in coral recovery from biotic and abiotic stress. Further, endosymbiotic algae and polyps both contribute to the mucus FAs components, however, the individual percent concentration of mucus FAs was more resembled by the host FAs profile than the endosymbiont. Besides, biomarker FAs of mucus were also found to be more similar to coral polyps compared to endosymbionts. In addition, there was not much difference observed among diseased mucus fatty acid percentages of both coral species except palmitic acid, but there was a difference in bleached fatty acid profile than diseased as it lost endosymbiont. There were also differences in FAs between PLS and PS *Porites* sp., as well as *Acropora* sp. healthy and white patch diseased mucus, indicating that coral stress affects the diversity and occurrence of mucus fatty acids. Thus, the characterization of mucus FAs aid in understanding the complex interactions between coral hosts, endosymbionts, and feeding heterotrophic organisms and their possible role in coral health and stress response.

Acknowledgements

The authors thank the Director, of CSIR-National Institute of Oceanography for the facilities. Thanks, Ministry of Environment, Forest and Climate Change (MoEFCC), Govt. of India, New Delhi and Forest Department, Principal Chief Conservator of Forests (PCCF) and Chief Wildlife Warden, Chennai-15, Tamil Nadu and Wildlife Warden, Gulf of Mannar Marine National Park, Tamil Nadu, India, for the entry and as well as for collecting the samples. VBP was supported by CSIR (UGC) (Grant No.20/12/2015(ii)EU-V). This is NIO contribution #...

Author contributions CR Conceptualization, supervision, fund acquisition, writing and editing the original draft, performed data analysis. PVB and CR wrote the manuscript. PVB, KV and MSS performed the experiments. All authors read and approved the final manuscript.

Funding This study was supported by the Department of Biotechnology (DBT), (Grant No. BT/PR15162/AAQ/3/752/2015) India to Dr. C.R. PVB was supported by CSIR (UGC) (Grant No.20/12/2015(ii) EU-V).

Data availability All data analysis of the present study will be provided based on request.

Declarations

Conflict of interest The authors have no conflict of interests.

Ethical standards Not applicable.

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Figure legend

Fig. 1- Healthy, diseased and bleached corals a. *Porites* sp. healthy, b. *Porites* sp. with pink spot disease, c. bleached *Porites* sp, d. *Porites* sp. with pink line syndrome, g. *Acropora* sp. healthy, h. *Acropora* sp. white patch disease.

Fig. 2- Relative abundance of FAs in healthy, diseased and/or bleached mucus of corals 2**a**. *Acropora* sp. 2b. *Porites* sp. (all are in three replicates). 2b. *Porites* sp. heatmap shows the data distribution with annotations indicating significant p-values. The significance levels are represented by asterisks: *** (p < 0.001), ** (p < 0.01), and * (p < 0.05). Non-significant values ($p \ge 0.05$) are left unannotated. Color intensity represents the magnitude of the underlying data values, with darker shades indicating higher values.

Fig. 3- Comparison of the relative concentrations of fatty acid classes (% of total fatty acids) among healthy anddiseased *Acropora* mucus of the present study with earlier described tissue (*A. samoensis, A. intermedia, A. muricata*) and endosymbionts FAs. SAFAs- saturated fatty acids, MUFAs-monounsaturated fatty acids, PUFAs - polyunsaturated fatty acids, endosymbiont biomarker FAs, Coral host biomarker FAs and bacterial biomarker FAs.

Fig. 4- Comparison of the relative concentrations of fatty acid classes (% of total fatty acids) among healthy, white patch, pink line syndrome and pink spot diseased *Porites* mucus of the present study with earlier described endosymbiont and host FAs. SAFAs-saturated fatty acids, MUFAs-monounsaturated fatty acids, PUFAs-polyunsaturated fatty acids, endosymbiont biomarker FAs, Coral host biomarker FAs and bacterial biomarker FA.

















Figure 4

Table 1- Method of sampling mucus and characterization of healthy, diseased and bleached corals of Acropora and Porites sp.

Coral mucus	Healthy, diseased and bleached corals characterized and followed for the sample
	collection (CDHC and NOAA)
Healthy Acropora sp.	Smooth, intact tissue with no signs of sloughing, lesions, or discoloration. Clear, white growth
	tips.
White patch Acropora sp.	Lesions are focal to multifocal, coalescing spots, entirely encircled by living tissue. They
	begin on an unaffected branch surface or at the edge of an old lesion. These lesions have a
	sharply defined leading edge of tissue loss; remnants of tissue may be present, and corallites
	can be broken. Lesions range from 1 to 80 cm in diameter.
Healthy Porites sp	Smooth intact tissue with no signs of lesions bleaching, or tissue loss Bumpy or nodular
ficatily i offices sp.	surface trained of Derites could without one signs of ensaier on showmal mouths
	surface, typical of Porties corais, without any signs of erosion or abnormal growths.
Bleached <i>Porites</i> sp.	They appear pale or white due to the loss of their symbiotic algae or the reduction in the
	algae's pigment.
Pink line syndrome Porites	Distinct pink line or band around the edge of the lesion. Affected areas show tissue loss and
sp.	are typically surrounded by living tissue. The lesions are usually sharply demarcated,
	indicating a clear boundary between healthy and affected tissue.
Pink spot <i>Porites</i> sp.	These spots are small, localized areas of pink pigmentation on the coral surface. Pink spots
	can vary in size but are generally small and isolated. They can appear singly or in clusters
	across the coral's surface.

Fatty acid	Healthy mucus	Diseased mucus	% Change	Enriched/Depleted	Potential Origin*				
C12:00	0±0	2.69±0.6	100	Enriched	Bacterial Feeding				
C14:00	1.39±0.5	0±0	NA	Depleted	Bacterial Feeding				
C15:00	0.41±0.1	0±0	NA	Depleted	Bacterial Feeding				
C16:00	28.85±1.9	60.43±2	52	Enriched	Host and Endosymbiont				
C17:00	0.43±0.11	0±0	NA	Depleted	Bacterial Feeding				
C18:00	35.99±4	28.26±2.7	-27	Depleted	Host and Endosymbiont				
C20:00	0.48±0.09	0±0	NA	Depleted	Bacterial Feeding				
C22:00	0±0	0±0	NA	NA	NC				
Σ SFA	67.56±1.5	91.38±3.6	26	Enriched	MS				
C16:1n-9	$0.48{\pm}0.08$	0.9±0.2	46	Enriched	Host and Bacterial				
C16:1n-7	0.71±0.17	2.8±0.27	74	Enriched	Bacterial Feeding				
C18:1n-9	2.98±0.31	4.92±0.12	39	Enriched	Host and Bacterial				
C18:1n-7	0±0	0±0	NA	NA	NC				
C20:1n-9(CGA)	1.5±0.18	0±0	NA	Depleted	Host (Heterotrophic)				
C22:1n-9	0±0	0±0	NA	NA	NC				
Σ MUFA	5.67±0.54	8.62±0.55	34	Enriched	MS				
C18:3n-3 (ALA)	2.97±0.28	0±0	NA	Depleted	Endosymbiont				
C18:4n-3 (SDA)	0±0	0±0	NA	NA	NC				
C18:5n-3	0±0	0±0	NA	NA	NC				
C20:5n-3 (EPA)	3.78±0.29	0±0	NA	Depleted	Endosymbiont				
C22:5n-3 (DPA)	0±0	0±0	NA	NA	NC				
C22:6n-3 DHA)	0±0	$0{\pm}0$	NA	NA	NC				

Table 2- Overview of Acropora sp. mucus fatty acids which were significantly enriched/depleted in diseased compared to healthy coral mucus (Values
are mean \pm SD (n=3)).

Σ n-3 PUFA	6.75±0.28	0±0	NA	Depleted	-
C18:2n-6 (LA)	2.4±0.38	0±0	NA	Depleted	Endosymbiont
C18:3n-6 (GLA)	0±0	0±0	NA	NA	NC
C20:2n-6 (EDA)	0.51±0.13	0±0	NA	Depleted	Endosymbiont
C20:3n-6 (DGLA)	0.69±0.13	0±0	NA	Depleted	Endosymbiont
C20:4n-6 (ARA)	8.7±0.66	0±0	NA	Depleted	Host
C22:4n-6 (ADA)	7.72±0.46	0±0	NA	Depleted	Endosymbiont
C22:5n-6	0±0	0±0	NA	NA	NC
Σ n-6 PUFA	20.02±1	0±0	NA	Depleted	MS
C16:2n-7	$0{\pm}0$	0±0	NA	NA	NC
C18:2n-7	$0{\pm}0$	0±0	NA	NA	NA
Σ PUFA	26.77±0.84	0±0	NA	Depleted	MS
n3: n6 PUFA	0.34±0.11	0±0	NA	Depleted	Endosymbiont
PUFA: SFA	$0.4{\pm}0.07$	0±0	NA	Depleted	-
EPA: ARA	0.4±0.12	0±0	NA	Depleted	-
EPA: DHA	$0{\pm}0$	0±0	NA	NA	NC
n-3: n-6	1.22±0.15	0.9±0.02	NA	Depleted	-
Bacterial biomarker	16.42±1	0±0	-35	Depleted	Bacterial Feeding
Host biomarker	0±0	0±0	NA	NA	Host
Symbiodiniaceae biomarker	0±0	2.69±0.6	NA	NA	Endosymbiont

* "NA= not applicable as particular fatty acid is absent in one or many mucus", "Host- coral polyps", "Endosymbiont- Corals endosymbiotic *Symbiodiniaceae*"," Host (Heterotrophic)-Heterotrophic feeding of corals from Planktons and other organisms", "Bacterial- from bacteria", "NC- source is not clear", "MS-multiple sources and not clear", "– is ratio of FAs."

Fatty acids	Healthy	Bleached	% Change	Enriched/ Depleted	Pink line syndrome	% Change between	Enriched/ Depleted	Pink spot disease	% Change	Enriched/ Depleted	Potential Origin*	
			between	Depicteu	disease	healthy &	Depreteu	uiseuse	between	Depicteu	o ng	
			healthy			pink line			healthy			
			&			syndrome			& pink			
C12 00			bleached			0	NT A		spot	NT A		
C12:00	3.01±0.29	0±0	0	NA	0±0	0	NA	0±0	0	NA	Bacterial Feeding	
C14:00	6.13±0.29	0±0	-100	Depleted	3.76±0.52	-38	Depleted	6.89±0.22	12	Enriched	Bacterial Feeding	
C15:00	1.31±0.4	0.9±0.1	-31	Depleted	0±0	-100	Depleted	1.77±0.32	35	Enriched	Bacterial Feeding	
C16:00	40.0+0.02	0.10	-100	Depleted	2 01 10 45	-95	Depleted	1.02+0.21	-95	Depleted	Host and	
C17:00	40.8±2.03	0±0	100	Enrichad	2.01±0.45	100	Enrichad	1.92±0.21	100	Enrichad	Endosymbiont Destarial Faading	
C17:00	0±0	9.52±0.55	100	Enriched	5.795±0.27	100	Enriched	6.87±0.58	100	Enriched	Bacterial reeding	
C18:00	28.06+1	0+0	-100	Depleted	3 66+0 23	-8 /	Depleted	2 7+0 49	-90	Depleted	Host and Endosymbiont	
C20:00	28.00±1	6 68+0 48	100	Enriched	0+0	0	NA	0+0	0	NA	Bacterial Feeding	
C22:00	0±0	0.08±0.48	0	NA	0±0	0	NA	0±0	0	NA	NA	
ΣSFA	79 31+2 3	17 1+0 8	-78	Depleted	15 2+0 42	-81	Depleted	20 15+0 39	-74	Depleted	MS	
C16:1n-9	77.51-2.5	17.1±0.0	837	Enriched	13.2-0.42	984	Enriched	20.13-0.37	842	Enriched	Host and	
	4.18±0.3	39.2±0.9	,		45.33±1.52			39.4±3.4			Bacterial	
C16:1n-7	1.97±0.11	0±0	-100	Depleted	1.25±0.3	-36	Depleted	1.25±0.39	-36	Depleted	Bacterial Feeding	
C18:1n-9			100	Enriched		100	Enriched		100	Enriched	Host and	
	0±0	23.91±1			26.25±2.28			24.19±1.3			Bacterial	
C18:1n-7	0±0	0±0	0	NA	0±0	0	NA	0±0	0	NA	NA	
C20:1n-9(CGA)			0	NA		0	NA		100	Enriched	Host	
G00 1 0	0±0	0±0	0		0±0			0.77 ± 0.03	0		(Heterotrophic)	
C22:1n-9	0±0	0±0	0	NA	0±0	0	NA	0±0	0	NA	NA	
Σ MUFA	6.15±0.2	1.7±0	925	Enriched	72.835±2.9	1084	Enriched	65.61±4.7	967	Enriched	MS	
C18:3n-3 (ALA)	0±0	0±0	0	NA	0±0	0	NA	0±0	0	NA	Endosymbiont	
C18:4n-3 (SDA)	0±0	0±0	0	NA	0±0	0	NA	0±0	0	NA	NA	
C18:5n-3	0±0	0±0	0	NA	± 0	0	NA	0±0	0	NA	NA	
C20:5n-3 (EPA)	0±0	0±0	0	NA	0±0	0	NA	0±0	0	NA	Endosymbiont	
C22:5n-3 (DPA)	4.3±0.26	1.2±0	-72	Depleted	1.3±0.2	-69	Depleted	1.4±0.3	-67	Depleted	NA	

Table 3- Overview of *Porites* sp. mucus fatty acids which were significantly enriched/depleted in diseased compared to healthy coral mucus (Values are
mean \pm SD (n =3)).

C22:6n-3 (DHA)	1.7±0.17	0.9±1	-47	Depleted	1.1±0.1	-35	Depleted	0.5 ± 0.05	-70	Depleted	NA
Σ n-3 PUFA	6±0.36	2.1±0.18	-65	Depleted	2.4±0.28	-60	Depleted	1.9±0.33	-68	Depleted	MS
C18:2n-6 (LA)	0±0	7.72±0.62	100	Enriched	7.61±0.35	100	Enriched	6.75±0.31	100	Enriched	Endosymbiont
C18:3n-6 (GLA)	14±1	11.82±1.01	-15	Depleted	4.285±0.22	-69	Depleted	3.85±0.35	-72	Depleted	NA
C20:2n-6 (EDA)	0±0	0±0	0	NA	0±0	0	NA	0±0	0	NA	Endosymbiont
C20:3n-6			0	NA		0	NA		0	NA	Endosymbiont
(DGLA)	0 ± 0	0 ± 0			0 ± 0			0 ± 0			
C20:4n-6 (ARA)	3.4±0.37	0.9±0.13	-73	Depleted	1.3±0.17	-61	Depleted	1±0.36	-70	Depleted	Host
C22:4n-6 (ADA)	0±0	0±0	0	NA	0±0	0	NA	0±0	0	NA	Endosymbiont
C22:5n-6	0±0	0±0	0	NA	0±0	0	NA	0±0	0	NA	NA
Σ n-6 PUFA	17.4±0.62	20.44±0.64	17	Enriched	13.2±0.55	-24	Depleted	11.6±0.6	-33	Depleted	MS
C16:2n-7	0±0	0±0	0	NA	0±0	0	NA	0±0	0	NA	NA
C18:2n-7	0±0	0±0	0	NA	0±0	0	NA	0±0	0	NA	NA
Σ ΡυξΑ	23.4±0.65	22.54±0.58	39	Enriched	11.9±0.62	-15	Depleted	13.5±0.5	-3	Depleted	MS
n3: n6 PUFA	0.34±0.06	0.1±0	-70	Depleted	0.18±0.02	-47	Depleted	0.16±0.03	-53	Depleted	Endosymbiont
PUFA: SFA	0.29±0.05	1.3±0.05	533	Enriched	0.78±0.03	333	Enriched	0.69±0.03	283	Enriched	Endosymbiont
EPA: ARA	0±0	0±0	0	NA	0±0	0	NA	0±0	0	NA	-
EPA: DHA	0±0	0±0	0	NA	0±0	0	NA	0±0	0	NA	-
n-3: n-6	2.8±0.03	10.42±0.07	-66	Depleted	7.05±0.05	-33	Depleted	9.89±0.14	-33	Depleted	-
Bacterial			273	Enriched		152	Enriched		254	Enriched	Bacterial Feeding
biomarker	7.7±0.05	2.1±0.05			2.6±0.23			$2.4{\pm}0.05$			
Host biomarker	15.7±0.05	12.72±0.07	-72	Depleted	5.4±0.46	-66	Depleted	5.15±0.05	-69	Depleted	Host
Symbiodiniaceae			-19	Depleted		-65	Depleted		-67	Depleted	Endosymbiont
biomarker	3.01±0.29	0 ± 0		-	0 ± 0		-	$0{\pm}0$			-

* "NA= not applicable as particular fatty acid is absent in one or many mucus", "Host- coral polyps", "Endosymbiont- Corals endosymbiotic *Symbiodiniaceae*"," Host (Heterotrophic)-Heterotrophic feeding of corals from Planktons and other organisms", "Bacterial- from bacteria", "NC- source is not clear", "MS-multiple sources and not clear", "– is ratio of FAs."

Supplementary table 1

				Acropora	sp. mucus	present st	udy					Acropora sp. (Ravindran et al. 2020)	Acropora sp. (Means and Sigleo 1986)	Acropora samo al. 20	ensis (Kim et 21)	Acropora inte (Imbs et al.	rmedia 2014)	Acropora mi (Imbs et al.	uricata . 2014)
Fatty Acid	Healthy Mucus 1	Healthy Mucus 2	Healthy Mucus 3 Mean	Diseased Mucus 1	Diseased Mucus 2	Diseased Mucus 3	Mean	Df	F- Values	p-values	Corrected p- values (FDR)	Healthy Mucus	Healthy Mucus	Endosymbiont	Host	Endosymbiont	Host	Endosymbiont	Host
C12:00	0) 0	0 0±0	2	2.95	3.12	2.69±0.6	4	59.58	0.002	0.00316667	1.93	-	-	-	-	-	-	-
C14:00	0.82	1.58	1.77 1.39±0.5	0	0	0	0±0	4	22.93	0.009	0.0106875	1.42	11.3	3.37	2.66	2.7	1.9	3.5	3.1
C15:00	0.31	0.39	0.53 0.41±0.1	0	0	0	0±0	4	40.64	0.003	0.00407143	0.38	-	-	-	-	-	-	-
C16:00	26.89	28.95	30.71 28.85±1.9	62.38	58.42	60.5	60.43±2	4	394.8	0	NA	29.36	37.8	22.25	31.06	18.6	20.1	16.3	30.1
C17:00	0.32	0.43	0.54 0.43±0.11	0	0	0	0 0±0		45.8	0.002	NA	0.33	-	-	-	-	-	-	-
C18:00	40.05	36	31.95 35.99±4	27.2	26.28	31.3	28.26±2.7	4	7.6	0.05	0.05	35.19	7.6	5.73	14.34	5.4	13.6	4.7	10.1
C20:00	0.58	0.39	0.47 0.48±0.09	0	0	0	0±0	4	75.9	0.001	0.0019	0.88	-	0.13	0.59	0.3	2.2	0.2	1
C22:00	0) 0	0 0±0	0	0	0	0±0	4	A NA	NA	NA	-	-	0.17	0.15	-	-	-	-
Σ SFA	68.97	67.74	65.97 67.56±1.5	91.58	87.65	94.92	91.38±3.6	4	109.7	0.0005	0.00152	69.49	56.7	31.65	48.8	27	37.8	24.7	44.3
C16:1n-9	0.41	0.57	0.46 0.48±0.08	1.15	0.73	0.82	0.9±0.2	4	9.51	0.03	0.03166667	0.51	20.9	2.31	2.29	1.5	1.2	0.5	1.1
C16:1n-7	0.51	0.78	0.84 0.71±0.17	3.08	2.54	2.78	2.8±0.27	4	125.8	0.0004	0.00152	0.65	20.9	0	0	1.9	1.1	1.1	2.6
C18:1n-9	2.68	2.95	3.31 2.98±0.31	5.06	4.85	4.85	4.92±0.12	4	98.5	0.0006	0.00162857	3.04	9.7	2.35	2.66	2.9	3	3.3	5.1
C18:1n-7	0) 0	0 0±0	0	0	0	0±0	4	A NA	NA	NA	-	-			0.6	0.3	0.2	0.4
C20:1n-9 (CGA)	1.68	1.5	1.32 1.5±0.18	0	0	0	0±0	4	208.3	0.0001	0.00063333	1.47	-	0.48	2.88	1.3	2.8	0.6	1.7
C22:1n-9	0	0 0	0 0±0	0	0	0	0±0	4	NA NA	NA	NA	-	-	0.13	0.22	-	-	-	-
Σ MUFA	5.28	5.8	5.93 5.67±0.54	9.16	8.05	8.65	8.62±0.55	4	61.1	0.003	0.00407143	5.67	51.5	5.27	8.05	8.2	8.4	5.7	10.9
C18:3n-3 (ALA)	2.67	3	3.24 2.97±0.28	0	0	0	0±0	4	223	0.0001	0.00063333	3.08	0.8	2.35	11.98	-	-	-	-
C18:4n-3 (SDA)	0	0 0	0 0±0	0	0	0	0±0	4	NA NA	NA	NA	-	-	10.39	1.26	13	0.9	13.1	0.9
C18:5n-3	0	0 0	0 0±0	0	0	0	0±0	4	NA NA	NA	NA	-	-	-	-	2.8	0.2	3.9	0.2
C20:5n-3 (EPA)	3.82	4.05	3.47 3.78±0.29	0	0	0	0±0	4	502.4	0	NA	3.67	-	13.72	5.69	14.7	8.7	7.7	4.4
C22:5n-3 (DPA)	0	0 0	0 0±0	0	0	0	0±0	4	NA NA	NA	NA	1.2	-	0.4	0.96	2.1	4.3	1.9	2.4
C22:6n-3 (DHA)	0) 0	0 0±0	0	0	0	0±0	4	NA NA	NA	NA	7.4	-	19.59	6.66	8.4	5	9.2	3.6
Σ n-3 PUFA	6.49	7.05	6.71 6.75±0.28	0	0	0	0±0	4	1717.6	0	NA	15.35	0.8	46.45	26.55	42	19.1	35.8	11.5
C18:2n-6 (LA)	1.98	2.5	2.72 2.4±0.38	0	0	0	0±0	4	119.6	0.0004	0.00152	2.38	11.8	0.93	0.81	1	0.6	0.8	1.1
C18:3n-6 (GLA)	0	0 0	0 0±0	0	0	0	0±0	4	NA	NA	NA	-	-	13.55	5.47	8.2	2.7	8.2	2.8
C20:2n-6	0.42	0.66	0.45 0.51±0.13	0	0	0	0±0	4	45.6	0.002	0.00316667	0.6	-	0	0	2	0.4	1.2	0.5
C20:3n-6 (DGLA)	0.65	0.58	0.84 0.69±0.13	0	0	0	0±0	4	78.9	0.0009	0.0019	0.62	-	0.35	1.04	0.4	1	-	1.8
C20:4n-6 (ARA)	8.47	9.45	8.18 8.7±0.66	0	0	0	0±0	4	512.7	0	NA	9.74	-	1.28	7.17	5.3	17.2	8	10.2
C22:4n-6 (AdA)	8.17	7.74	7.25 7.72±0.46	0	0	0	0±0	4	843.8	0	NA	6.68	-	0.48	2	2.3	8.1	3.4	10.7
C22:5n-6	0	0 0	0 0±0	0	0	0	0±0	4	NA	NA	NA	-	-	-	-	-	-	-	0.1
Σ n-6 PUFA	19.69	20.93	19.44 20.02±1	0	0	0	0±0	4	1888	0	NA	20.02	11.8	16.59	16.49	11.676	30	21.6	27.2
C16:2n-7	0	0 0	0 0±0	0	0	0	0±0	4	NA NA	NA	NA	-	-	-	-	-	0.2	1.6	0.2
C18:2n-7	0	0 0	0 0±0	0	0	0	0±0	4	NA	NA	NA	-	-	-	-	0.1	-	0.3	0.1
Σ PUFA	25.94	26.75	27.62 26.77±0.84	0	0	0	0±0	4	3045	0	NA	35.37	12.6	63.04	43.04	53.776	49.3	59.3	39
n-3: n-6 PUFA	0.34	0.23	0.45 0.34±0.11	0	0	0	0±0	4	28.6	0.006	NA	0.77	0.07	2.8	1.61	3.6	0.64	1.66	0.42
PUFA: SFA	0.37	0.39	0.418 0.4±0.07	0	0	0	0±0	4	796	0.0007	0.0016625	0.82	0.91	1.99	0.9	1.99	1.3	2.4	0.9
EPA: ARA	0.45	0.43	0.422 0.4±0.12	0	0	0	0±0	4	2725	0.004	NA	3.38	-	10.8	0.8	2.8	0.5	0.96	0.43
EPA: DHA	0	0 0	0 0±0	0	0	0	0±0	4	A NA	NA	NA	0.5	-	0.7	0.8	1.75	1.74	0.84	1.22
Bacterial biomarker	1.12	1.4	1.14 1.22±0.15	0.9	0.88	0.92	0.9±0.02	4	12.4	0.02	0.02235294	1.48	1.8	2.31	2.29	2.5	1.4	1.3	3
Host biomarker	15.4	16.44	17.42 16.42±1	0	0	0	0±0	4	792.7	0	NA	21.26	17.42	2.16	10.13	9.7	29.6	13.3	23.3
a 11 P 1 11			0.0.0	0	0		0.0									a	4.0.0	00.4	0.7

	Supplementary table 2 Porites sp. current study												Porites sp. (Ravindran et al. 2020) cylindrica (Imbs et al. 2014)																		
Fatty Acids	Healthy 1	Healthy 2	Healthy 3	Mean	Bleached 1	Bleached 2	Bleached 3	Mean	Df	F- Values	Healthy and bleached P- value	Corrected p- values (FDR)	Pink Line Syndrome 1	Pink Line Syndrome 2	Pink Line Syndrome 3	Mean	Df	F- Values	Healthy and PLS diseased P- value	Corrected p- values (FDR)	Pink Spot Disease 1	Pink Spot Disease 2	Pink Spot Disease 3	Mean	Df	F- Values	Healthy and pink spot diseased P- value	Corrected p- values (FDR)	Healthy Mucus	Endosymbiont	Host
C12:00	2.67	3.13	3.23	3.01±0.29	0	0	0	0±0	4	304.6	0.0001	0.0001	0	0	0	0±0	4	304	0.001	0.0011	0	0	0	0±0	4	304	0.001	0.0011	3.66	0	0
C14:00	5.87	6.45	6.07	6.13±0.29	0	0	0	0±0	4	1298	0	0	3.18	4.2	3.9	3.76±0.52	4	46.6	0.002	0.0022	7.14	6.7	6.83 (6.89±0.22	4	12.6	0.02	0.021	5.48	2.2	1.8
C15:00	0.9	1.32	1.71	1.31±0.4	0.8	0.9	0.7	0.9±0.1	4	4.5	0.1	0.1025	0	0	0	0±0	4	31.4	0.005	0.0054	1.4	1.9	2.01	1.77±0.32	4	2.3	0.2	0.2	1.31	0	0
C16:00	39	43	40.4	40.8±2.03	0	0	0	0±0	4	1212	0	0	1.8	1.7	2.53	2.01±0.45	4	1043	0	0	2.1	1.68	1.98	1.92±0.21	4	1088	0	0	53.8	16	17.9
C17:00	0	0	0	0±0	10.15	9.25	9.16	9.52±0.55	4	907	0	0	6.1	5.6	5.67	6.795±0.2	4	1372	0	0	6.3	7.46	6.85 (6.87±0.58	4	420	0	0	2.6	0	0
C18:00	29.08	28.1	27	28.06±1	0	0	0	0±0	4	2181	0	0	3.65	3.9	3.43	3.66±0.23	4	1569	0	0	3.1	2.85	2.15	2.7±0.49	4	1455	0	0	23.46	3.5	18.2
C20:00	0	0	0	0±0	6.7	7.15	6.19	6.68±0.48	4	580	0	0	0	0	0	0±0	4	NA	0	0	0	0	0	0±0	4	NA	0	0	1	0.4	0.9
C22:00	0	0	0	0±0	0	0	0	0±0	4	NA	0	0	0	0	0	0±0	4	NA	0	0	0	0	0	0±0	4	NA	0	0	0	0	0
Σ SFA	77.52	82	78.41	79.31±2.3	17.65	17.3	16.05	17.1±0.8	4	1839	0	0	14.73	15.4	15.53	15.2±0.42	4	2121	0	0	20.04	20.59	19.82	0.15±0.3	4	1815	0	0	91.31	22.1	38.8
C16:1n-9	3.85	4.12	4.57	4.18±0.3	40.1	38.2	39.23	39.2±0.9	4	3543.6	0	0	47	44	45	15.33±1.52	4	2060	0	0	38.3	36	42.8	39.4±3.4	4	301	0.0001	0.0001	2.67	0.8	1.3
C16:1n-7	2.06	2	1.85	1.97±0.11	0	0	0	0±0	4	994.5	0	0	0.92	1.5	1.35	1.25±0.3	4	14.9	0.02	0.02	1.4	0.8	1.55	1.25±0.39	4	9.2	0.04	0.041	1.48	2.7	1.9
C18:1n-9	0	0	0	0±0	23.87	22.9	24.96	23.91±1	4	1614.7	0	0	26.2	24	28.56	26.25±2.2	4	397	0	0	23	24	25.57	24.19±1.3	4	1046	0	0	8.5	2.7	3.3
C18:1n-7	0	0	0	0±0	0	0	0	0±0	4	NA	0	0	0	0	0	0±0	4	NA	0	0	0	0	0	0±0	4	NA	0	0	0	0.3	0.4
C20:1n-9 (CGA)	0	0	0	0±0	0	0	0	0±0	4	NA	0	0	0	0	0	0±0	4	NA	0	0	0.75	0.8	0.75	0.77±0.03	4	2111	0	0	1	0.9	3.6
C22:1n-9	0	0	0	0±0	0	0	0	0±0	4	NA	0	0	0	0	0	0±0	4	NA	0	0	0	0	0	0±0	4	NA	0	0	0	0	0
2 MUFA	5.91	6.12	6.42	6.15±0.2	63.9/	61.1	64.19	1./±0	4	5201	0	0	74.12	69.5	74,91	12.835±2.9	4	1550	0	0	63.45	61.6	70.67	63.61±4.7	4	454	0	0	13.65	7.4	10.5
C18:3n-3 (ALA)	0	0	0	0±0	0	0	0	0±0	4	NA	0	0	0	0	0	0±0	4	NA	0	0	0	0	0	0±0	4	NA	0	0	4.5	0	0
C18:40-3 (SDA)	0	0	0	0±0	0	0	0	0±0	4	NA	0	0	0	0	0	0±0	4	NA	0	0	0	0	0	0±0	4	NA	0	0	0	14.1	1.5
(10:50-5 (20:5- 2 (ED.))	0	0	0	0±0	0	0	0	0±0	4	NA	0	0	0	0	0	±0	4	NA	0	0	0	0	0	0±0	4	NA	0	0	0	2.0	0.2
C20:50-3 (EPA)	0	0	0	0±0	0	0	0	0±0	4	NA 205.0	0.0001	0.0001	0	0	0	0±0	4	NA	0.0001	0 0001	1.4	0	0	0±0	4	NA 157	0 0002	0.0000	4.1	15.8	10.2
C22:50-5 (DPA)	4.2	4.1	4.0	4.3±0.20	1.3	0.9	1.4	1.2±0	4	205.8	0.0001	0.0001	1.0	1.4	1.1	1.3±0.2	4	200	0.0001	0.0001	1.4	1./	1.1	1.4±0.3	4	13/	0.0002	0.0002	0.9	1.2	4
V n 2 DUEA	1.5	1.8	1.8	1./±0.1/	0.8	0.9	215	0.9±1	4	48	0.002	0.0022	1.1	1.2	21	1.1±0.1	4	179	0.000	0.0003	0.45	0.55	0.5	0.0±0.00	4	212	0.0005	0.0005	4.54	45.1	3.0 10.2
211-3 FUFA	5./	0	0.4	0±0.30	2.1 8.2	7.05	2.15	2.1±0.18	4	280.9	0.0001	0.0001	2.0	2.0	2.1 7.91	2.4±0.28	4	1/0	0.0002	0.0002	1.00	2.25	6.65	1.9±0.33	4	1/02	0.0001	0.0001	61	40.1	00
C10:20F0 (LA)	15	12	14	1/4-1	0.2	1.90	12.76	11.92+1.01	4	4,04	0.05	0.054	1.2	1.02	/.01	1.01±0.55	4	270	0.0001	0.0001	2.5	/.1	2.95	2 95 0 25	4	275	0.0001	0.0001	0.1	1.1	0.9
C10:30-0 (GLA)	15	15	14	14±1 0±0	10.75	0	12.70	0±0	4	NA	0.05	0.034	4.5	4.00	4.5	1.20J±0.2	4	2/0 NA	0.0001	0.0001	0	4.2	0.05	0-01 010	4	215 NA	0.0001	0.0001	17	14.4	0.1
C20.200 C20.3n-6 (DCI A)	0	0	0	0±0	0	0	0	0±0	4	NA	0	0	0	0	0	0±0	4	NA	0	0	0	0	0	0±0	4	NA	0	0	0.84	0.4	0.4
C20.5IP0 (DOLA)	3	3.75	3.45	3 /1+0 27	0.75	0.05	1	0.0+0.13	4	117	0.0004	0.0004	11	1/	1/	1 3+0 17	4	767	0.000	0.0011	00	07	1/	1+0.36	4	63.4	0.001	0.0011	3.3	20	13.7
C20.41F0 (ARA)	0	0	0	0+0	0.15	0.55	0	0.7±0.15	4	NA	0.0004	0.0004	0	0	0	0+0	4	NA	0.0007	0.0011	0.7	0.7	0	0+0	4	NA NA	0.001	0.0011	56	12	81
C22:5n-6	0	0	0	0±0	0	0	0	0±0	4	NA	0	0	0	0	0	0+0	4	NA		0.0011	0	0	0	0±0	4	NA	0	0	0	1.2	81
Σn-6 PUFA	18	16.75	17.45	17 4+0 62	19.7	20.85	20.77	20 44+0 64	4	34.4	0.004	0 0044	12.6	13.28	1371	13 2+0 55	4	75	0.001	0	10.9	12	11.9	11 6+0 6	4	132	0.0003	0.0003	17.54	23.8	347
C16:2n-7	0	0	0	0+0	0	0	0	0+0	4	NA	0	0	0	0	0	0+0	4	NA	0	0	0	0	0	0+0	4	NA	0	0	0	0.9	01
C18:2n-7	0	0	0	0+0	0	0	0	0+0	4	NA	0	0	0	0	0	0+0	4	NA	0	0.0102	0	0	0	0+0	4	NA	0	0	0	0.1	0
Σ PUFA	23.7	22.65	23.85	23.4+0.65	21.8	22.65	22.92	22.54+0.58	4	3.5	0.1	0.1025	11.23	12.46	12	11.9+0.62	4	487	0	0	13.45	13.55	13.5	13.5+0.5	4	463	0	0	31.58	69.9	54.1
n3: n6 PUFA	0.316	0.44	0.35	0.34±0.06	0.1	0.1	0.1	0.1±0	4	52.7	0.2	0.2	0.21	0.195	0.15	0.18±0.02	4	20	0.01	0	0.17	0.1875	0.13445 (0.16±0.03	4	26	0.007	0.0075	0.8	1.89	0.56
PUFA: SFA	0.3	0.27	0.3	0.29±0.05	1.23	1.31	1.43	1.3±0.05	4	306.8		0	0.76	0.78	0.8	0.78±0.03	4	1030		0	0.68	0.69	0.69	0.69±0.03	4	1418		0	0.35	3.2	1.4
EPA: ARA	0	0	0	0±0	0	0	0	0±0	4	NA	0	0	0	0	0	0±0	4	NA	0	0	0	0	0	0±0	4	NA	0	0	1.24	5.45	0.74
EPA: DHA	0	0	0	0±0	0	0	0	0±0	4	NA	0	0	0	0	0	0±0	4	NA	0	0	0	0	0	0±0	4	NA	0	0	0.9	1.39	2.83
Bacterial biomarker	2.77	2.8	2.83	2.79±0.03	10.35	10.45	10.5	10.42±0.07	4	25946	0	0	7	7.1	7.05	7.05±0.05	4	15937	0	0	10.03	9.75	9.89	9.89±0.14	4	7356	0	0	2.18	3	2.3
Host biomarker	7.65	7.7	7.75	7.7±0.05	2.05	2.1	2.15	2.1±0.05	4	18816	0	0	2.55	2.85	2.4	2.6±0.23	2	6 1419	0	0	2.4	2.45	2.35	2.4±0.05	4	16854	0	0	6.8	5.3	25.8
Symbiodinium biomarke	15.65	15.75	15.7	15.7±0.05	12.65	12.8	12.7	12.72±0.07	4	3202	0	0	5.9	5.2	5.04	5.4±0.46	4	1509	0	0	5.15	5.2	5.1	5.15±0.05	4	66781	0	0	17.3	41.1	11.6

NA denotes values where statistical corrections were not applicable due to predefined significance thresholds or missing data." Title and authors - Characterization of fatty acid composition of healthy and diseased coral mucus released from host or/and endosymbiont for their possible role in coral health-Phartade Vilas Bhagwat, Chimarajan Ravindran, Keertha, V., Mamatua, S.S.