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Fatty acid profile of the chlorophyta species from Chile's sub-Antarctic region

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ABSTRACT

The purpose of the present study was to determine the fatty acid (FAs) profiles of various Chlorophyta species collected from Chile's sub-Antarctic region. Lipids were extracted via the modified Bligh and Dyer method, and the fatty acid profiles were assessed using gas chromatography. Saturated fatty acids (SAFA) accounted for 55.85 to 70.64% of the total FAs, and palmitic acid (C16:0) was the most abundant SAFA. Monounsaturated FAs (MUFAs) accounted for 7.2 to 28.12% of the total FAs, and palmitoleic acid (C16:1) was the most abundant within this class. The polyunsaturated fatty acids (PUFAs) contents in the species ranged between 10.99 and 34.67% of the total FAs, with higher proportions of ω-3 PUFA than ω-6. Alpha-linolenic acid (18:3) was the predominant PUFA in all of the species (3.08 to 25.20% of the total FAs). From our results, we conclude that the Chlorophyta species from Chile's sub-Antarctic region would be an interesting, versatile biotechnological resource due to its nutritional value and health benefits following their demonstration as a valuable source of MUFAs and PUFAs, which are known to be beneficial for both humans and animals.

Key words: Fatty acid, gas chromatography, lipids, macroalgae, PUFA, seaweed, sub-Antarctic region.

Abbreviations: **ALA:** Alpha-linolenicacid, **DHA:**Docosahexaenoicacid (C22:6*n*-3), **EPA:** Eicosapentaenoicacid (C20:5*n*-3), **FA:** Fatty acids, **FAME:** Fatty acid methyl esters, **GC:** Gas Chromatography, **FID:** Flame Ionization Detector, **MUFA:** Monounsaturated fatty acids, **MS:** Mass Spectrometry, **PUFA:** Polyunsaturated fatty acid, **SFA:** Saturated fatty acid, ***n*-3:** Omega-3 polyunsaturated fatty acid, ***n*-6:** Omega-6 polyunsaturated fatty acid.

INTRODUCTION

Macroalgae have a complex and dynamic taxonomy and are classified into three main divisions: Rhodophyta (red algae), Ochrophyta (brown algae) and Chlorophyta (green algae), depending on their pigmentation, morphological and anatomical characters (Leal et al., 2013) and their reserve substance.

Macroalgae are renewable reservoirs of bioactive pharmaceutical and nutraceutical compounds with potential food applications. They are reported to be rich in proteins, vitamins, minerals, soluble dietary fibers and fatty acids (Mohamed et al., 2012). Fatty acids (FAs) are carboxylic acids with long aliphatic chains that may be straight or branched and saturated or unsaturated. Polyunsaturated fatty acids (PUFA) are of nutritional importance, as they cannot be synthesized by humans and thus must be obtained through diet.

Macroalgae have been extensively explored for PUFA, which represent 10-70% of total fatty acids, as these molecules are thought to display anti hypercholesterolemic, antihypertensive, anti-inflammatory, antidiabetic and anticancer effects (Giudetti and Cagnazzo, 2012; Kim et al., 2014; Leaf, 2007; Nobre et al., 2013; Wang et al., 2014). Furthermore, it was demonstrated that macroalgal lipids are a better alternative to fish oil, as they feature a greater resistance to oxidation and a higher bioavailability (Mendis et al., 2011).

The fatty acid content in algae varies considerably depending on several factors, such as species, geographical location, season, developmental stageand environmental conditions (Mansilla and Avila, 2011). Algae in their natural habitats experience severe

Table 1. Collection information and the species used in the experiments.

Species	Collection Data
Ulva sp	IslaAstrea (54º35'42,7" x 72º03'52,1") 29/01/ 2011
Ulva rigida C. Agardh	Caleta Hood (53º494x72º434) 20/04/2011
Monostromahariotii Gain 1912	Caleta Seal (53º462 x 72º476) 19/04/2011
Ulva intestinalis Linnaeus	Caleta Seal(53º462 x 72º476) 19/04/2011
Ulva ramulosa (J. E. Smith) Carmichael	Caleta Seal(53º462 x 72º476) 19/04/2011
Cladophora sp	Bahía Nash (53º42'36,6"x 72º21'13,2") 02/06/2011

variations in salinity, nutrients, temperature and light, and as a result of thriving in diverse and extreme environments, they produce bioactive compounds and fatty acids that are not generally present in terrestrial plants. Furthermore, the most commonly changing observation following adverse environmental conditions in algae is their ability to adjust to lipid production and to alter fatty acid unsaturation (Guschina and Harwood, 2006).

The Chile's sub-Antarctic region is characterized by strong seasonal light conditions and constant low temperatures. The region of Magallanes, located in the southwestern part of the South American continent (48°36' to 56°S; 66°25' to 75°40'W), is the world's largest representative sub-Antarctic environment, with a total of 391 species of macroalgae, of which 75 are Chlorophyta, 86 are Ochrophyta and 230 are Rhodophyta (Mansilla et al., 2012). Many of these species are commerciallyimportant for their alginates and carrageenans, and several species are currently being marketed for direct consumption by humans (Astorga-España and Mansilla, 2014; Mansilla et al., 2012).

Many studies have been performed worldwide to characterize algae species according to their chemical composition (Astorga-España and Mansilla, 2014; Gosch et al., 2012; Gressler et al., 2010;Kumari et al., 2010;Kumari et al. 2013). The detection, identification and precise quantification of lipid compounds are prerequisites for their potential utilization and exploration. Although considerable progress has been made in recent years, our current knowledge regarding the nutritional properties of macroalgae from Chile's sub-Antarctic region are not yet completely known, as studies concerning the chemical composition of sub-Antarctic macroalgae are lacking. Based on these considerations, the purpose of the present study was to use gas chromatography (GC) to determinethe fatty acid profiles of various Chlorophyta species collected from the region of Magallanes, in the southern-most part of Chile.

MATERIALS AND METHODS

Macroalgae samples

Macroalgae specimens were collected manually during low tide at depths between 30 cm and 1.20 m from the intertidal and subtidal zone at various locations at the region of Magallanes. Information about the macroalgae collected and used in this study is provided in Table 1.

The samples were transported in coolers filled with ice to laboratory refrigerators. In the laboratory, the samples were washed with Milli-Q deionized water to remove epiphytes, salt, and foreign matter. The samples were dried at room temperature (20°C) for 5 days.

Lipid extraction and fatty acid methyl ester preparation

The lipids of the lyophilized biomass were extracted via the modified Bligh and Dyer (1959) method. One gram of each macro algae was added to 30 ml chloroform/methanol (1:2, v/v) and 10 ml sodium sulfate (1.5 g/L) at 20°C under reflux. After 30 min, 10 ml chloroform and 10 ml sodium sulfate (1.5 g/L) were added. The seaweed extracts were centrifuged at 2500 rpm for 30 min, and the organic phase was separated. The solvent was removed from each extract under reduced pressure (Büchi Rotavapor) at 20 to 25°C.

The fatty acids extracted were converted to their respective methyl esters by esterification using 6 ml of a 2% (w/v) KOH methanolic solution during reflux for 8 min at 80°C while stirring. Seven milliliters of boron trifluoride methanol (Aldrich 14% BF_3 in methanol) and 5 ml of 20% (w/v) NaCl saturated water solution were added. The refluxed mixture was then transferred to a separatory funnel, and the reflux bottle was washed with 20 ml *n*-hexane. The hexane phase was dried with sodium sulphate, and the solvent was removed on a rotary evaporator (Büchi Rotavapor). The resultant mixture of methyl esters fatty acids (FAME) was diluted

Table 2. Fatty acid composition (% of total fatty acids) of Chlorophyta species from Chile's sub-Antarctic region.

	Ulva sp	*Ulva rigida*	*Monostromahariotii*	*Ulva intestinalis*	*Ulva ramulosa*	*Cladophorasp*
C10:0	-	0.49	-	0.62	1.24	-
C12:0	-	0.12	0.11	1.64	1.53	0.20
C13:0	-	0.31	0.14	0.64	0.72	0.44
C14:0	3.01	2.30	1.99	1.93	3.76	7.10
C14:1	-	-	-	-	-	0.16
C15:0	0.24	1.75	2.01	1.05	3.04	0.16
C15:1	-	-	-	-	-	0.23
C16:0	50.66	44.80	38.25	43.11	46.52	44.56
C16:1	1.71	7.39	18.87	23.28	6.75	12.40
C17:0	0.11	0.38	1.05	0.64	0.95	0.53
C17:1	3.79	2.31	0.73	1.20	1.28	0.94
C18:0	1.01	3.11	2.56	6.92	6.52	1.85
C18:1n9c	1.17	3.10	6.65	2.05	5.82	5.02
C18:2n6c	4.96	2.35	2.54	2.13	3.69	4.93
C18:2n6t	-	0.43	-	-	-	-
C18:3n6	0.52	0.34	0.60	0.59	0.74	0.64
C18:3n3	25.20	7.90	4.90	4.91	3.08	3.53
C20:0	0.58	2.22	2.28	1.17	1.85	1.04
C20:1n9c	0.31	1.18	0.88	0.76	0.69	1.57
C20:2	0.24	-	-	0.38	0.65	0.88
C20:3n6	0.45	1.92	1.82	0.67	0.77	0.70
C21:0	-	0.93	0.85	0.56	0.51	-
C20:4n6	0.37	0.96	-	0.53	1.18	1.19
C20:3n3	0.24	-	1.53	0.58	0.68	1.15
C20:5n3	2.68	2.96	3.87	0.84	1.23	2.73
C22:0	1.60	3.23	3.99	1.23	2.43	1.44
C22:1n9c	0.11	1.78	1.77	0.40	0.60	1.03
C22:6n3	0.25	3.67	-	0.74	1.45	1.73
C24:0	0.67	-	2.62	1.01	1.57	1.61
C24:1n9	0.11	1.79	-	0.43	0.74	1.24
Σω-3	28.37	14.53	10.3	7.07	6.44	9.14
Σω-6	6.3	6.0	4.96	3.92	6.38	7.46
ω-6/ω-3	0.22	0.41	0.48	0.55	0.99	0.82
ω-3/ω-6	4.50	2.42	2.07	1.80	1.00	1.22

with hexane and subjected to GC flame ionization detection (FID) analysis.

Quantitative analysis

The quantitative GC analyses were performed according to the following conditions using a gas chromatograph GC/FID-2010 with an AOC-20i autosampler (Shimadzu Corporation, Kyoto, Japan) equipped with a fused-silica capillary column (Rtx-WAX, 30 m × 0.25 mm I.D. × 0.25 µm film thickness). Injections were performed at a 1:25 split ratio, and hydrogen was used as the carrier gas under constant flow mode at 1.2 ml/min. The injector was heated to 250°C in a flame ionization detector operated at 250°C. The initial programmed oven temperature was 100°C, which was increased by 7°C/min up to 200°C, increased by 5°C/min to 202.6°C, and held isothermally for 2 min at this temperature. It was then increased by 5°C/min to 222.9°C and held isothermally for 2 min and then increased by 5°C/min to 230°C and held isothermally for 10 min at 230°C. The internal standard solution containing non adecanoate methyl ester (C19:0 ≥ 99.0%; Sigma-Aldrich, St. Louis, Missouri, USA) was prepared at a concentration of 2 mg/ml by dissolving 20 mg methyl nonadecanoate in 10 ml *n*-hexane in a volumetric flask.

RESULTS

Table 2 reports the fatty acid profiles of the Chlorophyta species that were studied. The determination of FAs in algae was performed by comparing the retention time of the standard FAME 37-Mix (Supelco, Bellefonte, Pennsylvania, USA) via the GC/FID analyses (Figure 1). A typical chromatogram of fatty acid compositions in *Ulva sp, Ulva rigida, Monostroma hariotii, Ulva intestinalis, Ulva ramulosa,*and *Cladophora sp* is given in Figure 2.

Thirty fatty acids, included in the range C10:0-C24:1n9 and exceeding a minimum of 0.01% of the total FAs, were identified. Saturated fatty acids (SAFAs) accounted for 55.85% (*Monostromahariotii*) to 70.64% (*U. ramulosa)* of the total FAs. Palmitic acid (C16:0) was the most abundant SAFA, corresponding to 38.25 - 50.66% of the total FAs.

Monounsaturated fatty acids (MUFA) accounted for 7.2% (*Ulva sp)* to 28.12% (*U. intestinalis)* of the total FAs.

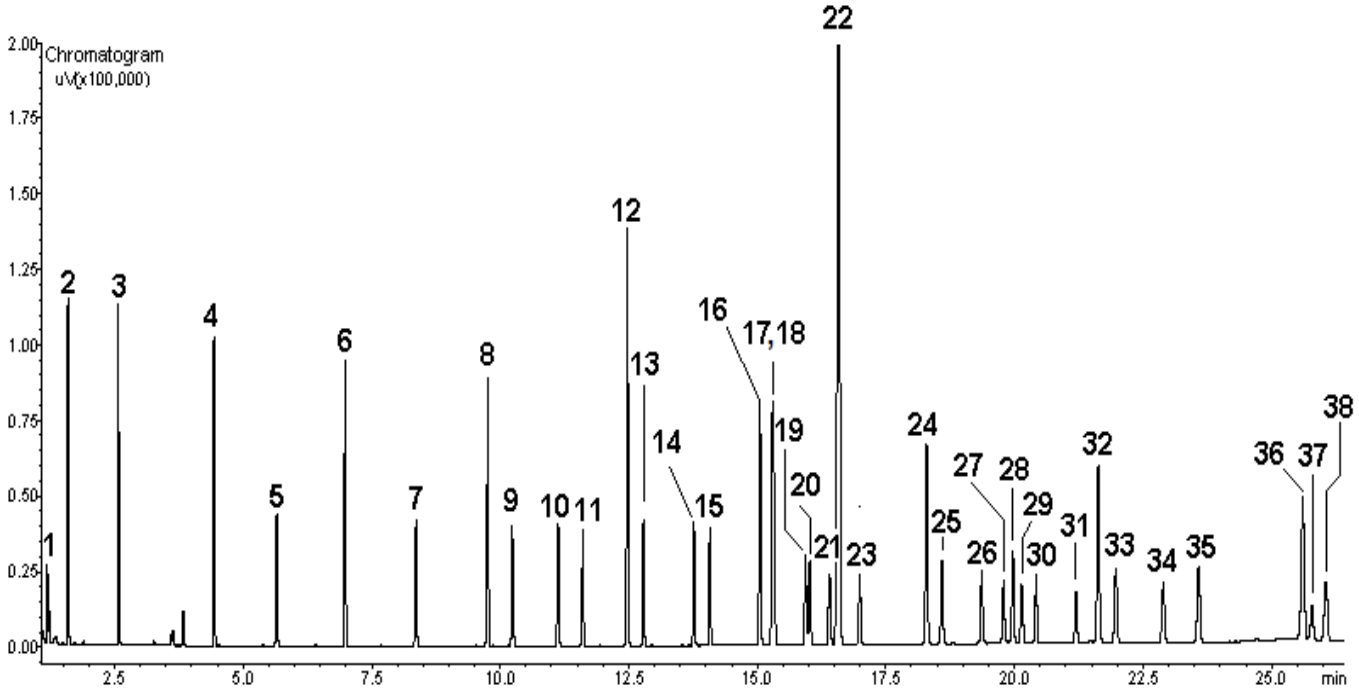

Figure 1. Chromatogram of fatty acids in standard FAME 37-Mix mixture (Supelco, Bellefonte, Pennsylvania, USA): 1 (4:0); 2 (6:0); 3 (8:0); 4 (10:0); 5 (11:0); 6 (12:0); 7 (13:0); 8 (14:0); 9 (14:1); 10 (15:0); 11 (15:1); 12 (16:0); 13 (16:1); 14 (17:0); 15 (17:1); 16 (18:0); 17 (18:1*n*9c); 18 (18:1*n*9t); 19 (18:2*n*-6c); 20 (18:2*n*-6t); 21 (18:3*n*-6); 22 (19:0) – internal standard; 23 (18:3*n*-3); 24 (20:0); 25 (20:1); 26 (20:2); 27 (20:3*n*-6); 28 (21:0); 29 (20:4*n*-6); 30 (20:3*n*-3); 31 (20:5*n*-3); 32 (22:0); 33 (22:1*n*-9); 34 (22:2); 35 (23:0); 36 (24:0); 37 (22:6*n*-3); 38 (24:1*n*-9).

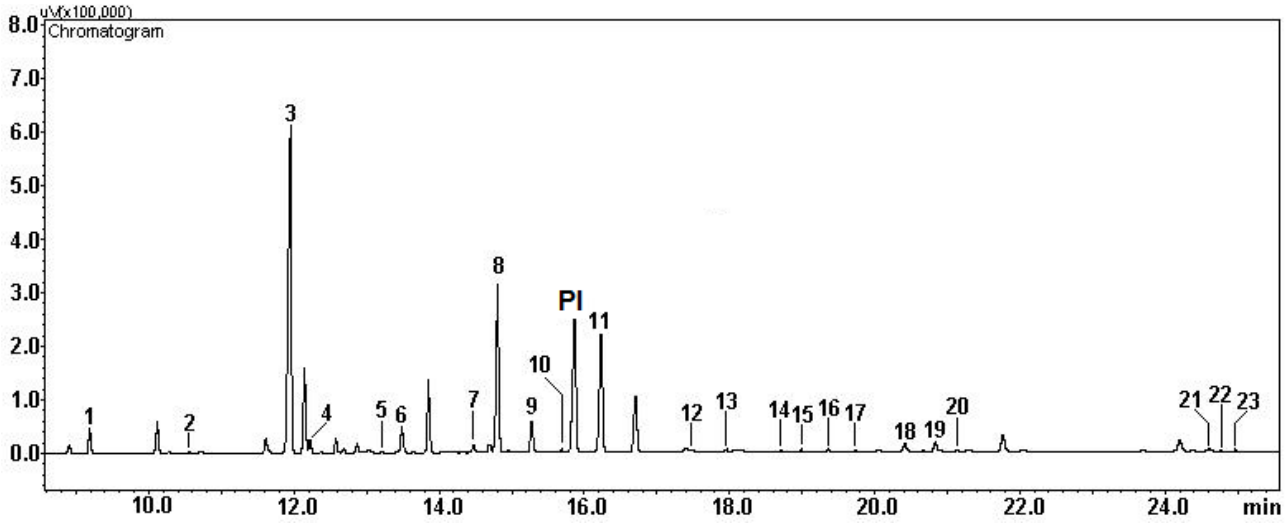

Figure 2. Chromatogram of fatty acids in *Ulva sp*: 1 (14:0); 2 (15:0); 3 (16:0); 4 (16:1); 5 (17:0); 6 (17:1); 7 (18:0); 8 (18:1*n*9c); 9 (18:2*n*6c); 10 (18:3*n*6); PI (19:0) internal standard; 11 (18:3*n*3); 12 (20:0); 13 (20:1); 14 (20:2); 15 (20:3*n*6); 16 (20:4*n*6); 17 (20:3*n*3); 18 (20:5*n*3); 19 (22:0); 20 (22:1*n*9); 21 (24:0); 22 (22:6*n*3); 23 (24:1*n*9).

Palmitoleic acid (C16:1) was the most abundant within this class, ranging from 1.71 to 23.28%. Oleic acid (C18:1, n-9, ranging from 1.17 to 6.65%) was the second most abundant MUFA in the studied species, except in *Ulva sp*, in which ginkgolic acid (C17:1) was higher than C16:1 and C18:1.

The PUFA contents in the species ranged between 10.99% (*U.intestinalis)* and 34.67% (*Ulva sp)* of total FAs. The studied species contained higher proportions of ω-3 PUFA than that of ω-6. Alpha-linolenic acid (18:3, n-3, ALA) was the predominant PUFA in all of the species (3.08 to 25.20% of the total FAs). This study further revealed that all of the species exhibited the characteristic profile of C18 > C20 PUFAs.

Ulvales members showed the highest levels of ω-3 FAs among all of the studied species. The ω-3/ ω-6 ratio

varied from 1.00 to 4.50, and therefore, the ω-6 / ω-3 FA ratio was lower than 1 for all of the species.

DISCUSSION

In the present work, the fatty acid profiles of Chlorophyta species from the Magellan region, Chile was analyzed. Palmitic acid was the most abundant SFA. However, the conversion of this FA into palmitoleic acid by the Δ9-desaturase enzyme (stearyl-CoA desaturase)results in the high levels of palmitic acid not being an aggravating factor for coronary problems.

Among MUFAs, palmitoleic acid and oleic acid were the predominant FA, which has been highlighted by other studies conducted on several green algae (Biandolino and Prato, 2006; Chakraborty and Santra, 2008; Kamenarska et al., 2004; Kumari et al., 2013; Matanjun et al., 2009; Pereira et al., 2012). Studies have suggested that palmitoleic acid helps increase insulin sensitivity, lowers inflammation and facilitates weight loss (Bernstein et al., 2014; Talbot et al., 2014; Yang et al., 2013).

Regarding PUFAs, the results revealed that all of the species exhibited the characteristic profile of C18 > C20 PUFAs, as previously demonstrated for other Chlorophyta species (Kumari et al., 2010; Kumari et al., 2013; Stabili et al., 2014). C18PUFAs are important part of both human and fish nutrition, although they are not able to be synthesized by vertebrates. Therefore, their considerably high presence in macroalgae from the region of Magallanes suggests the use of these algal species in the human diet or in the production of nutraceuticals and fish oil replacements (Kumari et al., 2013; Ortiz et al., 2009; Pereira et al., 2012; Stabili et al., 2014).

Alpha-linolenic acid was the dominant PUFA, similarly to other green macroalgae (Gosch et al., 2012; Stabili et al., 2014). ALA has been reported to have cardiovascular-protective, anti-cancer, neuro-protective, anti-osteoporotic, anti-inflammatory, and antioxidative effects (Deshpande et al., 2013; Kim et al., 2014, Zhao et al., 2004). Moreover, ALA is the precursor of the important longer ω-3 PUFAs: eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) (Brenna et al., 2009; Kim et al., 2014). The conversion of ALA to EPA involves sequential Δ6-desaturation, chain elongation and Δ5-desaturation. EPA is, in turn, converted to DPA. DHA is synthesized from DPA via further chain elongation, Δ6-desaturation and limited peroxisomal β-oxidation (Blanchard et al., 2013; Kim et al., 2014). EPA and DHA have vital roles in brain development, cardiovascular health and inflammatory responses (Das, 2006; Kim et al., 2014; Leaf, 2007; Wang et al., 2006). EPA and DHA can be obtained from the diet, and furthermore, as this FA is biosynthesized from ALA, the consumption of this essential precursor could be a complementary method to ensure sufficient n-3 PUFA bioavailability.

It is well known that very high ω-6 / ω-3 ratios can contribute to the development of several illnesses, particularly cardiovascular and various tumor diseases (Simopoulos, 2008). A ratio of ω-6 / ω-3 < 5:1 is recommended by the World Health Organization (WHO). In this study, the ω-6 / ω-3 ratio of FAs was lower than 1 for all of the studied species. Thus, Chlorophyta species from Chile's sub-Antarctic region could be considered both a good source of ω-3 and a possible tool for reducing the ω-6 / ω-3 ratio in the human diet. Moreover, it was demonstrated that lipid extracts from seaweeds can ameliorate health conditions of animals used in aquaculture (Cruz-Suarez et al., 2008), and therefore, the studied species could be employed as an enrichment in newly formulated feeds to be used in aquaculture.

The fatty acid profiles varied among seaweeds, and members of the same genus exhibited similar FA patterns but differed in their individual FA contents. In general, variations in the fatty acid contents may be attributed to both environmental factors (location, water temperature, light, concentrations of nitrogen and other compounds in water) and differences among species (Astorga-España and Mansilla, 2014). Our results may have been different if the species had been collected during other seasons, at other geographical location or at different developmental stages. Kumari et al. (2013) found stearidonic acid (C18:4, n-3; STA) in *Ulva* spp. varying from 3.6 to 20% of TFAs, although we did not identify this FA in our experiments.

Conclusion

From our results, we can conclude that Chlorophyta species from Chile's sub-Antarctic region can be considered an interesting, versatile biotechnological resource due to their nutritional value and health benefits. These species have been demonstrated as a valuable source of MUFAs and PUFAs, which are known to be beneficial for both humans and animals. Variations in the chemical composition of sub-Antarctic macroalgaecan be attributed to environmental, biological, and physiological differences among the species. More studies on fatty acids and other algal compounds are necessary topromote the sustainable exploitation of marine algae for the production of value-added seaweed products.

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