Proceedings of the Fourth International Coral Reef Symposium, Manila, 1981, Vol. 2

BIOCHEMICAL COMPOSITION OF SOFT AND HARD CORAL MUCUS ON A NEW CALEDONIAN LAGOONAL REEF

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ABSTRACT

A study of mucus and mucous aggregates emitted from hard and soft corals of the coral reef complex in front of Noumea (New Caledonia) was carried out to obtain information about the first stages of their transformation.

Mucus samples at the end of the low spring tide was lyophilized and biochemical compounds were determined.

An important mineral discharge, corresponding in part to salts coming from seawater, was included in the samples. The protein fraction confirms the relative abundance of dicarboxylic amino acids but a non-protein fraction (60 to 70% of total nitrogen) is present in polyp mucus. More than a third of dry tissue was represented by lipids: phospholipids constitute a large fraction of lipids in floating mucous aggregates but sterols are not abundant in the mucus present at the polyp surface. One of the principal results obtained in this study is the importance of monoacylglycerides in mucus-lipids.

The establishment of an agreement about sampling techniques is necessary to facilitate comparisons among different laboratory results.

INTRODUCTION

Since our work of 1977 (Daumas and Thomassin 1977), dealing with the composition of the mucus of *Fungia* and of *Palythoa* (a zoantharian), a few authors contributed new results on several points:

1. On the composition of mucus produced by other reef coelenterates (papers of Ducklow and Mitchell 1979a and Meyers 1977 on the fatty acids and hydrocarbons; Kokke et al. 1981 on sterol synthesis by cultured zooxanthellae and on sterol metabolism in Caribbean gorgonians; Meyers et al. 1978a and Meyers et al. 1978b on the fatty acid composition of Caribbean reef corals; Muscatine and D'Elia 1978 on the uptake, retention and release of ammonium);
2. On the importance and composition of the organic matter in the waters flowing over coral reefs (Meyers 1976, 1980) or in continental neritic waters (Alldredge 1979, Shanks and Trent 1979);
3. On the colonization of the mucus of soft corals, hard corals and zoantharia by bacterial populations (Mitchell and Chet 1975, Ducklow and Mitchell 1979b, Rublee et al. 1980, and Pascal and Vacelet 1981).

From a general point of view, all of these authors mention the structural changes of the mucus during its evolution, and its role as a particle trap (for example, the opacification of the mucus with the formation of nets as demonstrated by Ducklow and Mitchell 1979a). They also propound hypotheses on the possible chemical modifications associated with these transformations.

Our study on the mucus of reef corals and soft corals (genus *Lobophytum*) and mucous aggregates issued from the soft corals is still preliminary. It treats on the first stages of the transformations of the mucus naturally emitted on the reef flats.

In the coral reef complex in front of Noumea (New Caledonia), "Recif croissant", an inner lagoonal reef flat in a formative stage offers a good environment for mucus samples because at low spring tides the inner reef flat is isolated from the lagoonal water by a detrital hoof-shaped shingle rampart. In this inner reef flat, two types of assemblages are developed:

1. Just behind the rampart, a built-up reef flat where reef corals are dominant (branched and massive forms);
2. Back of this coral reef flat, a zone where very large populations of soft corals *(Lobophytum)* are developed; this probably due not only to the sheltered position of this area but also due to the increase of organic matter flowing over or decanting into this zone (Fig. 1).

MATERIALS AND METHODS

A. SAMPLING METHODS

In contrast to other authors who artificially stress the reef coelenterates by spreading fine sediments over them (such as Mitchell 1974 and Mitchell and Chet 1975 with *Platygyra)* or by strong seawater flows on the polyps (such as Rublee et al. 1980), the mucus was sampled on the reef flat at the end of the low spring tide, directly at the surface of the reef coral *(Lobophyllia corymbosa* and a faviid) polyps, or at the water surface for the mucous aggregates of

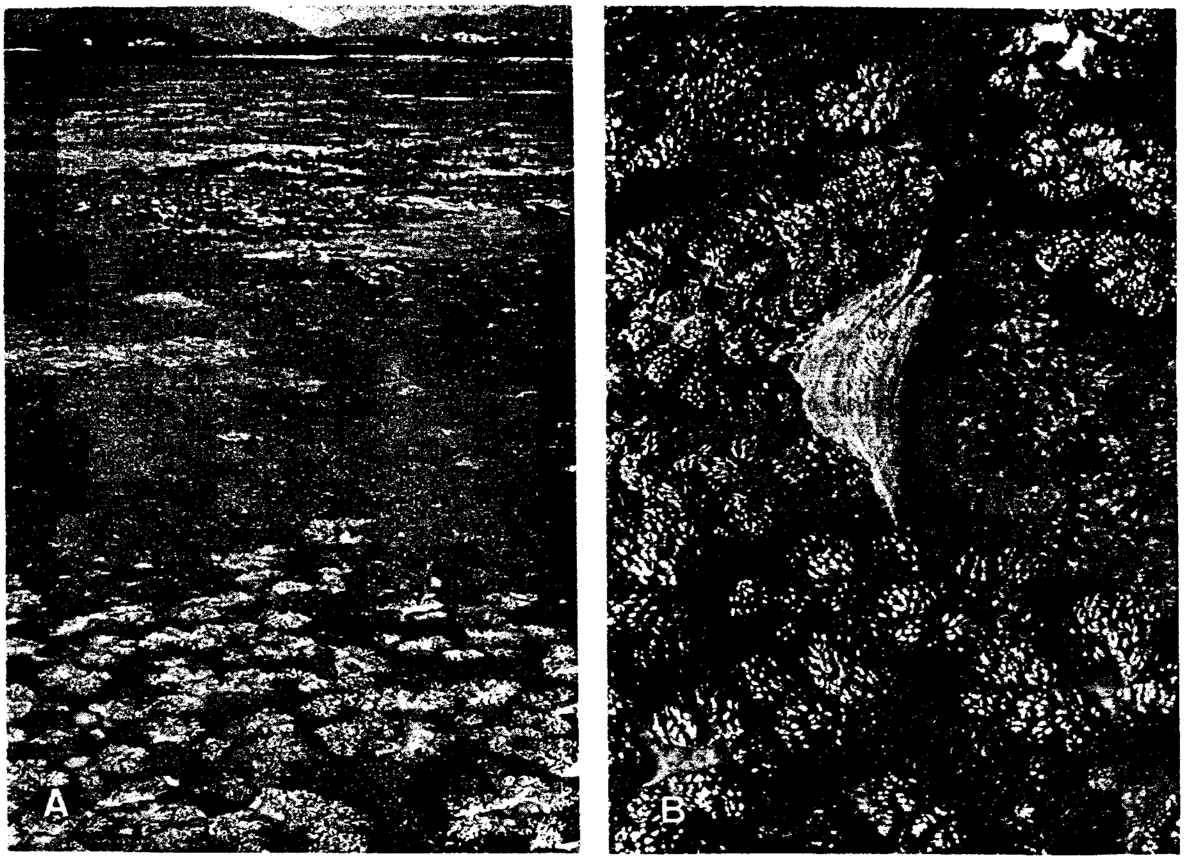

Figure 1. Croissant lagoonal reef flat (Ph. B.A. Thomassin). A. General view at low tide of the soft coral community on the backreef area. B. Mucoid aggregates at the water surface upon *Lobophytum* colonies at the end of the low tide.

Lobophytum, back of the colonies, using a 50 cc syringe.

A volume of around 200 cm^3 was collected for the mucus and mucoid aggregates of soft corals, and a volume of nearly 100 cm^3 was sampled from the hard corals. Samples were transported at low temperature to the laboratory, where after one hour, they were frozen. Then they were lyophilized.

B. ANALYTICAL METHODS

The lyophilized material (salts and organic material) was reduced to powder and homogenized.

The following analyses were conducted by Daumas and Galois.

1) Determination of organic carbon and nitrogen ratio after decarbonation (using a C.H.N. analyser);

2) Determination of the mineral and organic fractions of the materials by calcination at 450°C;

3) Identification of the chlorophyll pigments and xanthophylls by spectrometry of the acetonic extract;

4) Study of the glucidic fraction (total glucids) after hydrolysis (method of Dubois et al. 1956);

5) Measurement of total amino acids, after hydrolysis by hydrochloric acid (6N);

6) Study of the lipid fraction: total lipids and lipid classes, after extraction according to the method of Bligh and Dyer (1959), then separation of the lipid extract after weighing using the method of Freeman and West (1966). The lipids identified and estimated were phospholipids, sterols, acylglycerols, wax and sterol esters, free fatty acids and hydrocarbons.

Ducklow and Mitchell (1979a) processed by dialysis the nearly freshly excreted mucus before lyophilisation. This technique has the advantage of eliminating the mineral salts and the small organic molecules.

RESULTS AND DISCUSSION

A. MINERAL CONTENT AND ORGANIC MATTER (Tables 1, 2; Fig. 2)

The observed mineral content comprises between 75 to 79% of the total and is constituted by non-carbonate substances; a high proportion of salts coming from the seawater is also included in the samples. This could correspond to the presence of the siliceous fractions revealed by the scanning observations of Ducklow and Mitchell (1979b). Total organic components identified represent only a small percent of the samples. The organic fraction constituted by the small molecules, mainly the free amino acids, was never evaluated in the mucus. It could not be a part of the organic frame formed by the constitutive glycoproteins and lipoproteins, but it could come from the excretion processes of the coral symbiotic association or from the bacterial activity that is developed in the mucoid "floc". A study of this free organic fraction (Tables 1 and 2) could give more arguments for a dialysis process of the samples.

Table 1. Composition of reef coral and soft coral mucus, and of floating mucous aggregates from alcyonarian colonies (percent of sampled material).

	C organic %	total N %	protein N %	protein N / total N	C org./ N org.
REEF CORALS					
Lobophyllia corymbosa	0.29	0.042	0.027	64.3	10.7
Faviidae	0.74	0.036	0.017	47.2	43.5
SOFT CORALS					
Lobophytum pauciflorum? ech. 1	1.27	0.099	0.032	32.3	39.7
——— ——— ech. 2	0.77	0.088	0.026	29.5	29.6
MUCOUS AGGREGATES from					
Lobophytum spp. ech. 3	5.80	0.820	0.376	45.8	15.4
——— ——— ech. 4	1.86	0.036	0.017	47.2	43.5

Table 2. Composition of the organic fraction of reef coral and soft coral mucus, and of floating mucous aggregates from alcyonarian colonies.

	Lipids %	Glucids (glucose equivalent) %	Proteins (Σ amino-acids) %	Chlorophyll pigments
REEF CORALS				
Lobophyllia corymbosa	31.3	11.0	57.7	−
Faviidae	44.4	31.7	23.8	−
SOFT CORALS				
Lobophytum pauciflorum? ech. 1	35.7	32.8	31.5	+
——— ——— ech. 2	36.2	24.4	39.4	+
MUCOUS AGGREGATES from				
Lobophytum spp. ech. 3	24.3	21.8	53.8	+++
——— ——— ech. 4	21.8	26.8	51.4	++

The mucus excreted in the water was subjected to a polymerisation that gave it a fibrous structure. The floc is composed of particulate material, and at the same time creates a microcosm where bacterial activity begins to develop. This leads to an increase in the protein fractions during the time spent in the water (Fig. 2).

A high proportion of the nitrogen contained in the mucus is in a non-protein form (Table 1). This fraction seems to decrease during the transformation of polyp mucus to floating mucous aggregates. It represents 60 to 70% of the total nitrogen of *Lobophytum pauciflorum* and could be constituted by purine or pyrimidine bases, or by fixed ammonium (NH_4). This second observation must be linked to the elevated ratio of ammonia found in the aggregates sampled at the seawater surface by Shanks and Trent (1979). This seems in agreement with the works of Muscatine and D'Elia (1978) who demonstrated the excretion and reutilization of the ammonia by symbiotic reef coral species.

The suspected presence of a high non-organic fraction in the nitrogen ratio has a direct effect on the value of the C/N ratio of the organic matter. This ratio is higher than the values usually given in the literature (Coles and Strathman 1973, Rublee et al. 1980).

B. AMINO-ACID COMPOSITION
(Tables 3, 4, Fig. 3)

Generally, mucus is classified among the glycoproteins. The presence of a higher proportion of lipids according to species considered must be mentioned, however. Mucus is a glycolipoprotein dif-

Table 3. Amino-acid composition of reef coral and soft coral mucus, and of floating mucous aggregates from alcyonarian colonies.

	Reef corals				Soft corals				Mucous aggregates from *Lobophytum* colonies			
	Lobophyllia corymbosa		Faviidae		*Lobophytum pauciflorum?* 1		2		3		4	
	weight µg / 100 mg dry matter	%	weight µg / 100 mg dry matter	%	weight µg / 100 mg dry matter	%	weight µg / 100 mg dry matter	%	weight µg / 100 mg dry matter	%	weight µg / 100 mg dry matter	%
LYS	113	5.3	72	6.1	105	4.3	77	3.7	984	3.6	234	3.5
HIS	tr.	–	63	5.3	tr.	—	tr.	—	1677	6.2	341	5.1
ARG	106	4.9	92	7.8	140	5.7	95	4.5	1901	7	482	7.2
ASP	258	12	144	12.2	297	12.1	270	12.9	2545	9.4	768	11.5
THR	153	7.1	69	5.8	156	6.4	113	5.4	1705	6.3	453	6.8
SER	174	8.1	84	7.1	169	6.9	133	6.3	1868	6.9	522	7.8
GLU	368	17.1	141	12	299	12.2	322	15.3	2968	11	802	12
PRO	138	6.4	92	7.8	169	6.9	116	5.5	1681	6.2	387	5.8
GLY	126	5.9	68	5.8	213	8.7	222	10.6	1457	5.4	380	5.7
ALA	107	5	63	5.3	160	6.5	145	6.9	1634	6.1	364	5.4
CYS	tr.	—	tr.	—	tr.	—	tr.	—	496	1.8	134	2
VAL	89	4.1	43	3.6	114	4.7	126	6	1710	6.3	233	3.5
MET	tr.	—	tr.	—	33	1.3	tr.	—	688	2.5	105	1.6
ILE	87	4	39	3.3	129	5.3	97	4.6	1335	4.9	346	5.2
LEU	182	8.5	90	7.6	192	7.8	148	7	1801	6.7	536	8
TYR	99	4.6	44	3.7	107	4.4	109	5.2	766	2.8	213	3.2
PHE	146	6.8	75	6.4	163	6.7	126	6	1745	6.5	381	5.7
Total % dry matter	2.146		1.179		2.446		2.099		26.961		6.681	
N ratio (coeff. 6.25)/ 100 mg dry matter	0.34		0.19		0.39		0.33		4.31		1.068	
amino-acids di-COOH		29.1		24.2		24.3		28.2		20.4		23.5
basic amino-acids		10.2		19.2		10		8.2		16.8		15.8
ashes	79%		78%		78%		75%		76%		79%	

ferent, by its constitution, from the mucoid secretions, such as the sublingual glycoproteins, but the presence of N- acetylneuraminic acid was never recorded.

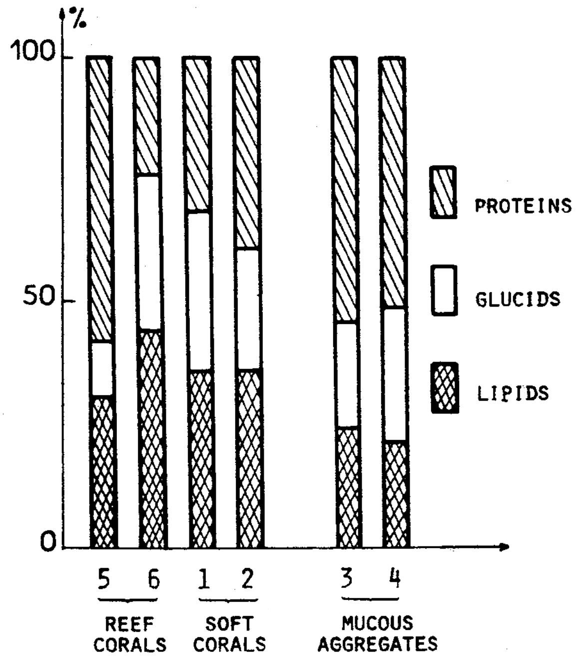

Figure 2. Histogram of the principal constituents in the mucus and the mucoid aggregates.

Table 4. Comparison between the amino acid composition of soft coral mucus and floating mucous aggregates from alcyonarian colonies.

	Soft coral mucus *Lobophytum pauciflorum* mean %	Mucuous aggregates from soft *Lobophytum* mean %	increase	decrease
LYS	4	3.55	—	
HIS	tr.	6.7	+++	
ARG	5.1	7.1	+	
ASP	12.5	10.55		+
THR	5.9	6.55	—	
SER	6.6	7.35	+	
GLU	13.75	11.50		+
PRO	6.2	6.0		
GLY	9.65	6.55		++
ALA	6.7	5.75		
CYS	tr.	1.9	+	
VAL	5.35	4.9		
MET	0.6	2.05	++	
IsoLEU	4.95	5.05	—	
LEU	7.4	7.35	—	
TYR	4.8	3.0		+
PHE	6.35	6.1		

The composition of the protein fraction confirms the relative abundance of dicarboxylic amino acids (glutamic acid and aspartic acid) that allow the classification of this protein as an acid-protein. However, we cannot state precisely if a part or all of these dicarboxylic acids comes from a deaminization of asparagine or of glutamine during the hydrolysis of the protein fraction. Mitterer (1978), studying the organic matrix of several coral skeletons, links the richness in aspartic acid to the fixation of calcareous ions. He mentions the absence of asparagine in his samples. Previous works (Daumas and Thomassin 1977, Mitchell 1974, Ducklow and Mitchell 1979a) agree with this composition in amino-acids of the protein fraction of the freshly excreted mucus, with the glutamic acid content higher than that of the aspartic acid.

C. LIPID COMPOSITION (Table 5, Fig. 4)

1. Total lipids

From a general point of view, the total lipid ratios of the mucus sampled on the hard corals and soft corals comprises between 35 and 45% of the total organic matter (mean = 37%).

The lipid levels of Alcyonaria and Scleractinia are very similar and comparable to those given by Ducklow and Mitchell (1979a), particularly for the soft coral *Sarcophyton glaucum*.

The lipid levels of the mucus do not seem to be too different from those generally known for the tissues of the colonies. In the sea-anemones, Bergmann et al. (1956) pointed out that the lipids represented nearly a third of tissue dry weight. In the scleractinian *Pocillopora capitata*, the analyses made by Patton et al. (1977) gave a mean average of 34.5% of the dry weight of the tissue.

Floating aggregates from *Lobophytum* are less rich in lipids than those collected at the surface of the colonies. A degradation process probably occurs during the time spent in the seawater.

2. Lipid classes

In the mucus sampled on the colonies, the lipids more often represented are, in decreasing order; phospholipids, monoacylglycerols (and analogue ethers) wax and sterol esters, and triacylglycerols.

For the floating mucous aggregates of soft corals, a few differences are noted, particularly an increase in phospholipids and sterols and a decrease in monoacylglycerols and wax esters.

The polar lipids (phospholipids) are well represented in the mucus studied. Their levels are comparable with those of animal tissues, and of Cnidaria in particular, such as the sea anemone *Metridium senile* (Hill-Manning and Blanquet 1979).

The ratios of neutral lipids/polar lipids (LN/LP) are around 0.70-0.72 for the polyps collected on soft and hard corals but decrease to 0.47 for the floating mucous aggregates. This could be induced by a loss

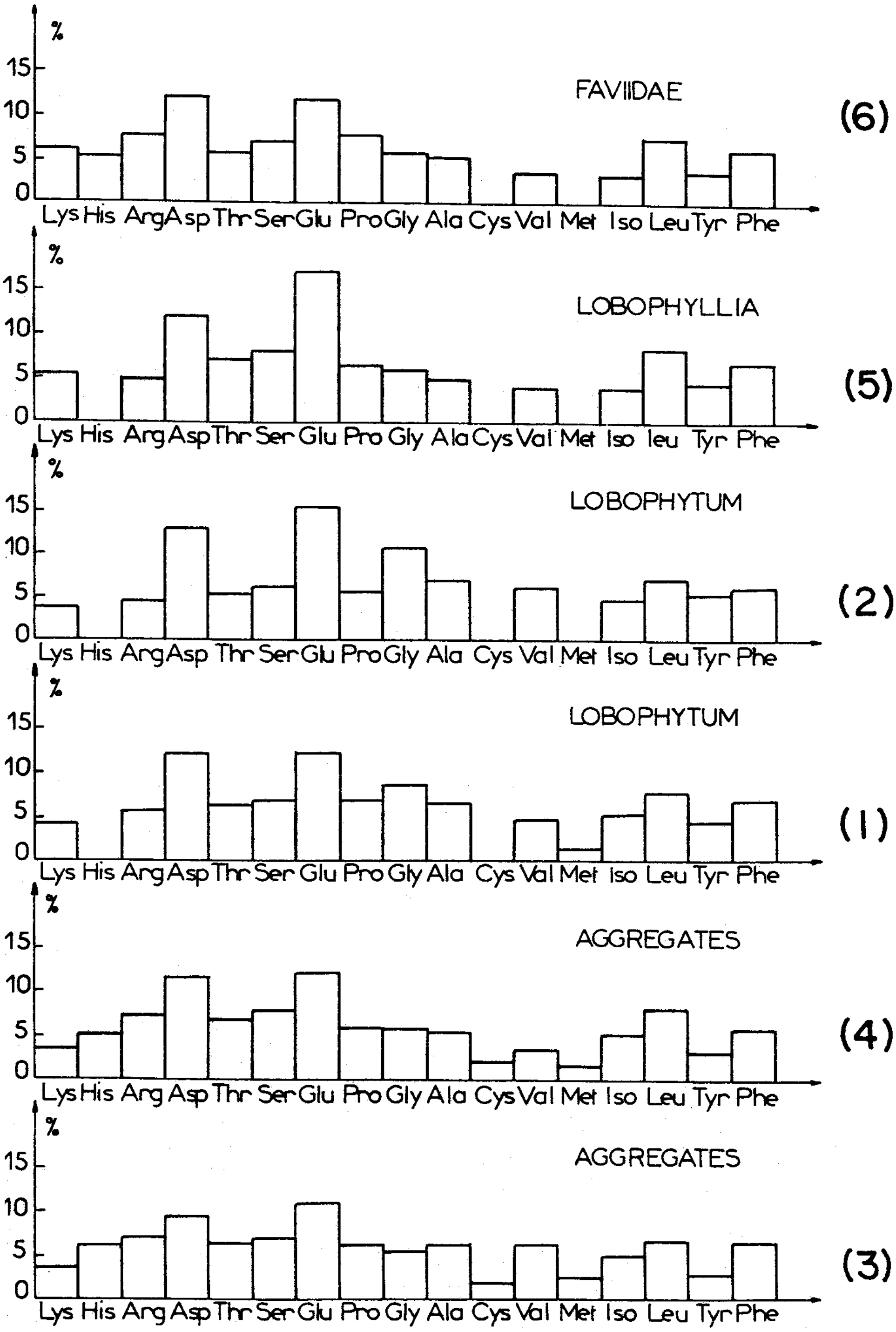

Figure 3. Histograms of the amino acid composition in the mucus and the mucoid aggregates.

of one or several neutral lipids, or by an increase in phospholipids probably in relation with the colonization of this material by microorganisms.

The paucity of sterols in the mucus at the polyp surface in comparison with the floating aggregates must be pointed out. Neutral lipids of the mucus

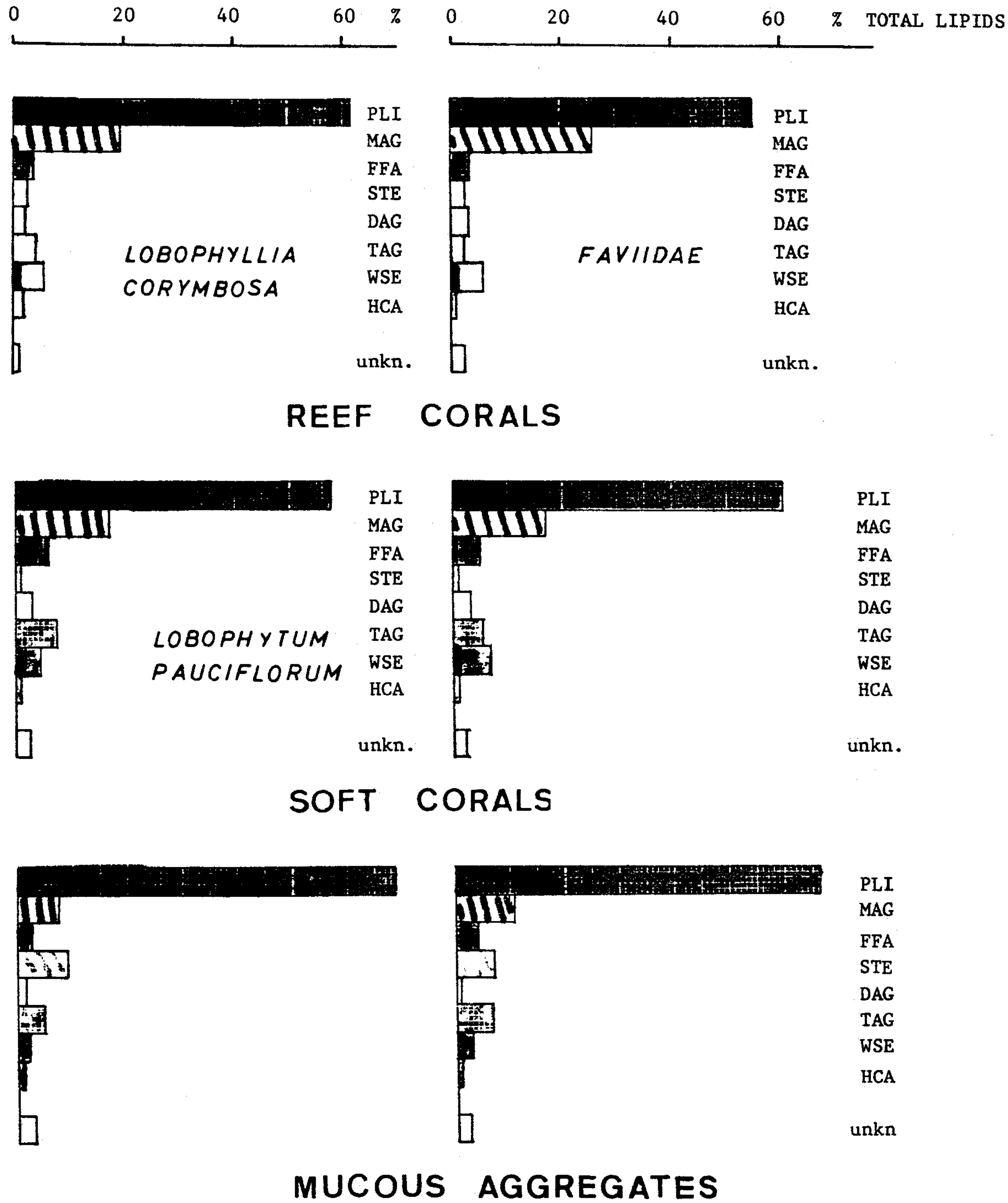

Figure 4. Histograms of the lipid classes in the mucus and the mucoid aggregates.

Table 5. Lipid composition of reef coral and soft coral mucus, and of floating mucous aggregates from alcyonarian colonies. (Values expressed as percent of total lipid extract).

	Reef corals		Soft corals		Floating mucous aggregates from *Lobophytum* colonies		
			Lobophytum pauciflorum?				
	Lobophyllia corymbosa	Faviidae	1	2	3	4	
PLI	61.45	54.92	57.80	60.16	69.14	66.59	
MAG	19.43	25.73	16.94	17.09	7.39	10.22	decrease
FFA	3.12	3.23	6.00	4.43	2.64	3.39	decrease
STE	2.35	2.73	0.99	0.91	8.87	6.84	increase
DAG	1.80	2.97	3.03	3.04	1.31	1.02	decrease
TAG	3.99	2.26	7.27	5.30	4.69	6.44	
WSE	5.64	5.35	4.62	6.38	1.85	2.24	increase
HCA	1.38	0.48	0.72	0.47	1.15	1.07	decrease
unkn.	0.84	2.13	2.63	2.22	2.96	2.19	

PLI : phospholipids; MAG : mono acyl-, alkyl- and alkenyl glycerols; FFA : free fatty acids; STE : sterols; DAG : diacylglycerols; TAG : triacylglycerols; WSE : wax and sterol esters; HCA : hydrocarbons; unkn : unknown.

studied are principally constituted by monoacylglycerols, wax esters and triacylglycerols.

The separation method of the lipid classes used by us (thin layer chromatography according to Freeman and West 1966) does not allow the resolution of the wax and the sterol esters. Nevertheless, it is proved that the sterol esters are not well represented, or are absent, in the Cnidaria (Hill-Manning and Blanquet 1979) so that the ester ratio obtained refers to the wax esters. The abundance of the wax esters in the tissues and in the coral mucus was largely pointed out by Benson and Muscatine (1974), Patton et al. (1977) and Benson et al. (1978). However, the values found here are less than those given by these authors.

These authors also reported the richness in triacylglycerols of the coral tissues and mucus. The study of the ratio of triacylglycerols/wax esters is very interesting here because it shows distinct differences in the mucus sampled:

— Mucus from the polyps of alcyonarians is richer in triacylglycerols (R = 1.14) than that from scleractinians which is richer in wax esters (R = 0.56).

— Floating mucous aggregates of soft corals are very poor in wax esters, probably because this type of lipids is more rapidly degraded or consumed.

One of the principal characteristics of the mucus studied is its richness in monoacylglycerols. Following some authors (Benson et al. 1978, Hill-Manning and Blanquet 1979), and based on the migrating Rf on the chromatography plates, it seems that the main constituents of this fraction are not monoacylglycerols *sensu stricto*, but their analogous derivatives: glycerylmonoethers, monoalkyl- and monoalkenyl-glycerols.

CONCLUSIONS

In conclusion, we will point out that the processes that induce the excretion of mucus for analysis could have consequences on the composition of the obtained material. However, processes that cause stress (such as decrease of the salinity, sand projection, pressing of the soft-corals) must not be used. Exposure to air for short periods could be used but only on organisms in situ and during low spring tides in the tropics in order to avoid abnormal stress on the animals. We are conducting a study to examine the consequences of the sampling procedures on the excreted products. Analyses of the obtained results could lead to an agreement on the sampling technique and on the treatment of the mucus samples, allowing comparisons of the results obtained by different research teams.

ACKNOWLEDGEMENTS

Materials were sampled during a 1979-2 survey in Noumea. in the scope of the joint research programme ORSTOM/CNRS "Studies of reef and lagoonal ecosystems of new Caledonia" (CNRS/ATP - PVD n° 4067). We are grateful to the staff of the biochemical division of the ORSTOM center.

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