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Commensal bacteria living with two multicellular marine algae : *Antithamnion plumula* (Ellis) Thuret and *Cladophora rupestris* (L.) Kützing (Linné), Kützing. Phenotypic characterization

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Abstract : From three strains of the red algae *Antithamnion plumula* and one strain of the green alga *Cladophora rupestris*, twenty seven strains of marine bacteria have been isolated and characterized. The various genera met were *Pseudomonas, Alteromonas, Acinetobacter, Moraxella, Deleya, Flavobacterium, Cytophoga* and *Vibrio*. Some species were found on both algae : *Pseudomas gelidicola, Pseudomonas elongata, Acinetobacter* sp., *Deleya marina* and *Alteromonas macleodii*.

In view of obtaining axenic algae, the reactions of these bacteria to different antibacterial products and to 19 antibiotics, have been studied. The most efficient antibiotics are rifampicin and carbenicilion.

Résumé : A partir de 3 isolats de l'algue rouge *Antithamnion plumula* et d'une souche de l'algue verte *Cladophora rupestris*, 27 souches de bactéries marines ont été isolées et caractérisées. Les bactéries appartiennent aux genres *Pseudomonas, Alteromonas, Acinetobacter, Moraxella, Deleaya, Flavobacterium, Cytophaga* et *Vibrio*. Quelques espèces sont communes aux deux types d'algues : *Pseudomonas gelidicola, Pseudomonas elongata, Acinetobacter* sp., *Deleya marina* et *Alteromonas macleodii*.

Dans le but d'avoir des algues axéniques, la réponse de ces bactéries à divers agents antibactériens dont 19 antibiotiques a été étudiée. Les antibiotiques les plus efficaces sont la rifampicine et la carbenicilline.

INTRODUCTION

Identification of marine bacteria using only phenotypic features is very difficult because of the paucity of the literature in this field. Thirty years ago, several *Pseudomonas*, *Achromobacter, Empedobacter, Flavobacterium* and *Acinetobacter* species from the marine environment have been described (27), but discriptions are incomplete. More recently Baumann *et al.* (1, 2) have studied different pseudomonads and, on the basis of their DNA G+C content, created the genus *Alternomonas* (1, 4). This was completed by an investigation of the biochemical differences between *Alteromonas* and *Pseudomonas* (14, 23). From the genus *Alteromonas*, a new genus has been created : *Marinomonas,* containing two species : *M. vaga* and *M. Communis* (22). Fresh water *Acinetobacter* have been studied by Pagel *et al.* (25), who identified two species : *A. calcoaceticus* and *A. lwoffi*. More recently, new pathogenic species of *Acinetobacter* have been described : *A. baumanii, A. haemolyticus, A. johnsonii* and *A. junii* (11). The marine *Achromobacter-Alcaligenes* group was recently reviewed by Baumann *et al.* (3). They created the genus Deleya to accomodate for the previously named *Alcaligenes aestus, A. pacificus, A. cupidus* and *A. venustus*. Surprisingly, *Pseudomonas marina* was included in this new genus although it differed from other species by the presence of polar flagella. The *Virbionacea* family has been

extensively studied by Kalina *et al.* (18), who created a new genus, *Allomonas*, by R.R. Colwell and coworkers (15, 16, 19, 31, 32) and by Baumann *et al.* (5).

The study of bacteria living within or on algal cell walls is interesting, especially if one wants to grow the algae axenically. In this paper we have studied the bacteria living with two different species of multicellular marine algae, *Antithamnion plumula* and *Cladophora rupestris* in an attempt to axenise those algae. We have phenotypically characterized the bacterial strains we found and we have investigated their behaviour in relation to various antibiotics and antiseptics.

MATERIALS AND METHODS

- *Algal strains and growth conditions. Antithamnion plumula* (Ceramiales, Ceramiaceae) n° 6 and A are the male gametophyte and the tetrasporophyte, respectively of the laboratory strains R3556 of the collection of Maine University (Maine, France). The wild types of *Antithamnion plumula* and *Cladophora rupestris* (Cladophorales, Cladophoraceae) were collected at Roscoff (Brittany, France). These algae were kept or cultivated at 14°C in Von Stosh's medium (30) with a 12h-12h photoperiod and a light intentsity of 20 uE m^{-2} sec^{-1}. Solutions of antibiotics, antiseptics and vitamins were sterilized by filtration through a 0.22 µm Millipore membrane. Manipulations were done aseptically within a laminar flow hood.

- *Bacterial strains and growth conditions.* Bacterial strains were isolated from the above algae which had undergone or not a treatment by antibiotics or antiseptics (Tables I, II). Bacteria were grown on Zobell's medium or on a basal salt medium (BSM) (28) at the temperature of 25°C.

- *Investigations of phenotypic features.* Morphological observations were performed with cells grown on Zobell's medium with a photonic microscope at the magnification 1100 X. The motility and its type as well as cell morphology was noted. Flagella were stained according to Rhodes' method (29) and the flagellation types were recorded. To determinate the respiratory types, bacteria were inoculated in Veillon's tubes containing Zobell's medium solidified with 6‰ (w/v) agar. The metabolic type was determined with the modified medium of Hugh and Leifson containing 0,5 % glucose (17). Strains were characterized as inert, alkaline, oxidative or fermentative. The ability to use various carbon sources was tested in BSM containing the investigated sugar at the concentration of 0,5 % (w/v). Oxidase activity was assayed with disks impregnated with dimethylparaphenylene diamine oxalate (Diagnostic Pasteur) (20). Catalase activity was assayed by mixing one colony from a Zobell's agar plate with a drop of hydrogen peroxide at 10 volumes. Strains were investigated for amylase and DNase activity using starch at the concentration of 1 % (w/v) in Zobell's agar plates and DNase Agar (Diagnostics Pasteur) supplemented with 25 g/l NaCl as respective substrates. Activities were revealed with a lugol solution on starch agar and HCl 1 N on DNase agar. The nitrate reduction test was run with Griess reagent. Other tests

Table I : Time course of the appearance of different bacterial strains upon treatment of *Antithamnion plumula* with various antibiotics

Antibiotic treatment	Algal strain		
	Wild type	strain 6	Strain A
0	W1 ; W2	6-1 ; 6-2 ; 6-3	Ap ; Ag
1	WRC 1		
1 + 2	WG-1 ; WG-2		
1 + 2 + 3	WGR_21 ; WGR_22 ; WGR_23		
	WGR_24		
1 + 2 + 3 + 4	WRG1 ; WRG2		
5		6RC1 ; 6RC2	
5 + 4		6RG1 ; 6RG2	

Antibiotic treatments were chosen from antibiograms results. Treatments were performed subsequently on the same algal fragment.

Treatment 1 : Rifampicin 20 µg/ml and Chloramphenicol 7 µg/ml for 45 hours simultaneously

Treatment 2 : Gentamycin 2 µg/ml for 48 hours.

Treatment 3 : Gentamycin 10 µg/ml and rifampicin 5 µg/ml for 24 h followed by rifampicin 5 µg /ml for 24 hours.

Treatment 4 : Rifampicin 30 µg/ml for 7 days followed b y gentamycin 10 µg/ml for 5 days.

Treatment 5 : Rifampicin 5 µg/ml and chloramphenicol 5 µg/ml for 24 hours simultaneously.

Table II : Time course of appearance of different bacterial strains upon treatment of *Cladophora rupestris* with various antiseptics.

Antiseptic treatment	Algae strain
0	ClJ ; ClT ; ClB ; ClP
1	Cl70
2	ClT60 a ; ClT60 b

Treatments 1 and 2 were performed with different ex-plants because the fragment having undergone the treatment n° 1 did not survive.

0 : untreated

Treatment 1 : Ethanol 70 % for 5 mn.

Treatment 2 : Temerol 12 µl/ml for 4 hours.

were performed by utilization of API 20 NE and API 20 B galeries (API System SA). Antibiograms were determined with antibiotic disks (Diagnostic Pasteur). Finally, to discriminate between *Pseudomonas* and *Alteromonas* we followed the identification protocol of Lee *et al.*, (23) and Gilson *et al.* (14) (see Table VI).

RESULTS

- *General*

Twenty seven bacterial strains were isolated from the untreated algae and from the ex plants treated with various antibiotics (Table I and II). Their nutritional requirements as well as their morphological and physiological features are summarized in table III. Antibiograms are given in table IV.

All strains grew between 24 h and 48 h on Zobell's agar plate. Colonies were generally smooth, entire, round. All strains were catalase positive, strict aerobes (excepted the strain CIT 60b). They all had a respiratory metabolism, with the exception of the strain CIT60b which had a fermentative metabolism. No cocci shaped cells were observed. No strain could grow on Zobell's solidified medium without NaCl excepted the strain WGR_2-2. They all were gram negative and did not fix nitrogen ; none could use H_2 and CO_2 as energy and carbon sources, respectively. No growth occurred at 43°C. With the exception of the strain WGR_2-2, no strain was capable of denitrification. With the exception of the strain Clt 60b, no strain produced indole from tryptophan. No strain synthesized a fluorescent pigment in king A and B media.

- *Description of individual strains*

Strain 6-1 : Small, round, smooth colonies with a slow growth rate (48 hours at 25°C). Cells were small (2 µm x 0,5 µm), motile rods with polar flagella. The strain was oxidase positive, oxidative (produced acid from glucose), did not reduce nitrate to nitrite, did not produce amylase, desoxyribonuclease and gelatinase and did not cut esculine.

Strain 6-2 : Flat, not pigmented colonies which spread on the plate with a slow growth rate (48 hours at 25°C). Cells were motile rods with polar flagella. The strain was oxidase positive, alkalin, did not reduced nitrate no nitrite and did not cut esculine. This strain exhibited a very few positive phenotypic characters (Table IV). The main difference with the strain 6-3 was the aspect of the colonies.

Strain 6-3 : Small, round, smooth colonies, pigmented in red in primary isolates but loosing their pigmentation upon sub-culture. Growth was slow (48 hours at 25°C). Cells were small (2 µm x 0,5 µm), motile rods with polar flagella. The strain was oxidase positive, alkaline, did not reduce nitrate to nitrite, did not produce amylase, gelatinase and desoxyribonuclease and did not cut esculine. As for the previous strain, it exhibited a very few positive phenotypic characters (Table IV).

Strain Ap and ClJ : Big, round, smooth, slimy yellow-pigmented colonies which grew within 24 hours. Cells were polymorph, motile cocobacilli with polar flagella. They were oxidase positive, oxidative, did not reduce nitrate to nitrite, dit not produce amylase and desoxyribonuclease and cut esculine. These strains did not survive more than six days in Zobell's bath at 25°C.

Strains Ag, W2, WRG1, ClP : White, round, smooth colonies which grew within 24 hours. Cells were motile coccobacilli with polar flagella. The strains were oxidase positive, oxidative, did not reduce nitrate to nitrite, did not produce amylase but produced gelatinase and desoxyribonuclease and cut esculine.

Strain W1 : Round, smooth, white colonies growing within 24 h. Cells were motile coccobacilli with peritrichous flagella. The strain was oxidase positive, oxidative, reduced nitrate to nitrite but did not denitrify. It did not produce amylase, gelatinase for desoxyribonuclease and did not cut esculine.

Strains WRC1, WG2, WRG2 and ClT60a : Colonies were round, smooth and white and grew within 24 h. Cells were non motile coccobacilli. The strains were oxydase negative, oxidative. They did not reduce nitrate to nitrite, did not produce amylase, gelatinase, or desoxyribonuclease and did not cut esculine.

Strain 6RG2 : Colonies were round and rough in primary cultures then became and not pigmented smooth upon sub-culture. Grow was slow (48 h at 25°C). Cells were very small (1 µm x 0,5 mm) non motile coccobacilli. The strain was oxidase positive and inert on glucose, did not reduce nitrate to nitrite, did not produce amylase, gelatinase and desoxyribonuclease, did not cut esculine.

Strains 6RG1 and ClT : Colonies were small and smooth. Growth occurred within 24 h. Cells were motile rods with polar flagella. The strains were oxidase negative, inert on glucose, did not reduce nitrate to nitrite, did not produce gelatinase or desoxyribonuclease but had amylase activity. They did not cut esculine.

Strain WGR_2-3 : This strain grew into round, smooth, cream-pigmented colonies. Cells were motile coccobacilli with peritrichous flagella. The strain was oxidase negative, oxidative, did not reduce nitrate to nitrite, did not produce amylase, gelatinase or desoxyribonuclease and did not cut esculine.

Strains WGl and ClB : Small (1 mm), round, smooth. Colonies growing within 24 h. Cells were very motile rods with polar flagella. Those strains were oxidase positive, inert on glucose, did not produce nitrate to nitrite and did not produce amylase. They did produce gelatinase and desoxyribonuclease and cut esculine.

Strain WGR_2-2 : Round, flat, adherent, brown, rough colonies with a wrinkled appearance. Cells were motile rods with polar flagella. The strain was oxidase positive and oxidative, capable of vigorous denitrification. It did not produce gelatinase or desoxyribonuclease and did not cut esculine.

Strain WGR_2-1 and 6RC1 : Colonies at 24 h were not pigmented very small (<1 mm), round and smooth. Cells were rod shaped and were very motile by polar flagella. The strains were oxidase positive and inert on glucose. They reduced nitrate to nitrite but did not denitrify.

Table III : Morphological, physiological and nutritional of the bacterial strains

	6-1	6-2	6-3	Ap	ClJ	Af	W-2	WRG-1	ClP	W-1	WRC-1	WG-2	WRG-2	ClT60n	6RG-2	6RG-1	ClT	WGR_2-3	WG-1	C1B	WGR_2-2	CG	JC	ClT60b	Cl70
Morphology																									
Pigmentation	-	-	-	yellow	yellow	-	-	-	brown	-	-	-	-	-	-	-	-	-	-	-	-	-	yellow	-	orange
Cell	b	b	b	cb	cb	cb	cb	cb	b	cb	cb	cb	cb	cb	cb	b	b	cb	b	b	b	b	b	b	b
Motility	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	-	+	-
Flagellation	p	p	p	p	p	p	p	p	p	pe	-	-	-	-	-	p	p	pe	p	p	p	p	-	p	-
Gram	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Physiology																									
Zobell broth	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0% NaCl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
6,5% NaCl	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	ND	-	ND
+3° C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	-	ND
Respiratory type	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	Ana	A
Hugh & Leifson	OX	AI	AI	OX	OX	OX	OX	OX	OX	OX	OX	OX	OX	OX	I	I	I	OX	I	I	OX	I	I	F	I
Enzymology																									
Oxydase	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arginine dihydrolase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Indole from tryptophan	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
H_2S production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate reductase	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	+	-	+	+
Denitrification	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Esculine hydrolysis	+	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+
Galactosidase	+	-	-	+	+	+	+	+	+	-	+	+	+	+	-	-	-	+	+	+	-	-	-	+	+
Gelatinase	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	+
Amylase	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	+	+	-	-	-	+	-	ND	+	ND
Desoxyribonuclease	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-	ND	-	ND
Utilisation of																									
Xylose	+	-	-	+	+	+	+	+	+	-	-	-	-	-	ND	ND	ND	ND	-	ND	-	-	ND	+	ND
Arabinose	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glucose	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	+
Galactose	+	-	+	+	+	+	+	+	+	+	+	+	+	+	ND	ND	ND	+	+	ND	-	-	ND	+	ND

Mannose	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	ND	ND	ND	+	+	ND	+	+	ND	+	ND
Sucrose	+	-	-	+	+	+	+	+	+	+	+	+	+	+	ND	ND	ND	-	+	ND	-	-	ND	-	ND
Maltose	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-	+	-	-	+	+
Lactose	+	-	-	+	+	+	+	+	+	-	+	+	+	+	ND	ND	ND	ND	+	ND	+	-	ND	+	ND
Fucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	ND	ND	ND	-	ND	+	-	ND	-	ND
Rhamnose	+	-	-	+	+	-	-	-	-	-	+	+	+	+	ND	ND	ND	-	-	ND	-	-	ND	-	ND
Starch	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	+	+	-	-	-	+	-	ND	+	ND
Glycerol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	ND	ND	ND	+	+	ND	+	-	ND	-	ND
Inositol	+	+	+	-	-	-	-	-	-	+	-	-	-	-	ND	ND	ND	ND	-	ND	+	+	ND	-	ND
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	-	-	+	+
N.Acetylglucosamine	+	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Acetate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	ND	ND	ND	ND	+	ND	+	-	ND	-	ND
Phenylacetate	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Tartrate	-	-	-	+	+	-	-	-	-	-	-	-	-	-	ND	ND	ND	ND	-	ND	-	-	ND	-	ND
Lactate	+	+	-	+	+	-	-	-	-	+	+	+	+	+	ND	ND	ND	ND	-	ND	+	+	ND	+	ND
Citrate	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	+	-	+	-	-	+	-	-	+	+
Pyruvate	-	+	-	+	+	+	+	+	+	+	+	+	+	+	ND	ND	ND	ND	-	ND	+	-	ND	-	ND
Caprate	-	-	-	-	-	+	+	-	-	+	+	+	+	+	-	-	-	+	-	-	+	-	-	-	-
Adipate	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gluconate	-	-	-	+	+	-	-	+	-	+	+	+	+	+	-	+	+	+	+	-	+	-	-	+	-
Malate	-	+	+	-	-	-	-	-	+	+	+	+	+	+	-	+	+	+	-	-	+	-	-	+	-
Ethanol	-	+	-	+	+	-	+	ND	ND	+	+	+	+	+	ND	ND	ND	ND	-	ND	+	-	ND	-	ND
Butanol 1	-	+	-	+	+	-	-	-	-	+	-	-	-	-	ND	ND	ND	ND	-	ND	+	-	ND	-	ND
Butanol 2	-	-	-	-	-	-	-	-	-	+	-	-	-	-	ND	ND	ND	ND	-	ND	-	-	ND	-	ND
Isobutanol	-	-	-	+	+	-	+	ND	ND	+	-	-	-	-	ND	ND	ND	ND	-	ND	+	-	ND	-	ND
Methanol	-	+	+	-	-	-	-	-	-	-	-	-	-	-	ND	ND	ND	ND	-	ND	-	-	ND	-	ND
Acid from																									
Arabinose	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glucose	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	+	-	-	+	-
Galactose	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	-	-	+	-
Mannose	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Fructose	+	-	-	+	+	+	+	-	-	+	+	+	+	+	-	-	-	+	-	-	-	-	-	+	-
Sucrose	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Maltose	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	-	-	+	-
Rhamnose	+	-	-	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Starch	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
Mannitol	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-	-	-	+	-
Sorbitol	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glycerol	+	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	+	-	-	+	-	-	-	-

cb : cocobacilli ; b : rod ; p : polar ; pe : peritrichous ; A : Aerobic ; Ana : Anaerobic facultative ; OX : oxydative ; Al : Alkalin ; I : Inert ; ND : ND : Not determinate.

They did not produce amylase, gelatinase, or desoxyribonuclease and did not cut esculine. Only the strain WGR_2-1 was investigated further for its biochemical characters (Table III).

Strain WGR_2-4 and $6RC_2$: Round, smooth lemon yellow- coloured colonies. Cells were rod shaped and not motile. Those strains were oxidase positive and inert on glucose. They did not reduce nitrate to nitrite, produced gelatinase but did not cut esculine. Only the strain WGR_2-4 was investigated further fot its biochemical characters (Table III).

Strain Cl70 : Round, rough, orange. Cells were very long rods (20 to 50 µm), not motile. This strain was slightly oxidase positive, inert. It reduced nitrate to nitrite but did not denitrify. It produced gelatinase and cut esculine.

Strain CIT60b : Colonies were round, smooth, and grew well within 24 h. Cells were very motile polymorph rods with polar flagella. The strain was oxidase positive and fermentative (it produced acid from glucose in closed tube). It reduced nitrate to nitrite but did not denitrify. It had gelatinase activity, produced indole from tryptophan and did not cut esculine.

Given the above results, all the strains we found could be assigned a species or a genus name (Table V). Phenotypic features used to distinguish between *Alteromonas* and *Pseudomonas*, are summarized in table VI.

DISCUSSION

In order to identify with certitude bacteria, especially marine bacteria, one generally compares their DNA to that of reference strains by hybridization or assaying for the G+C content. Such methods are heavy, expensive and can only be done in well-equiped laboratories. In this paper we show that it is possible to have a good presumptive determination of different heterotrophic bacteria using easy and unexpensive methods such as the utilization of two types of API galeries (API system) adjusted to the marine environment, as well as different staining methods and the investigation of the mode assimilation of glucose and other carbon sources. In spite of a few discrepancies with the taxonomic investigations published previously, these methods enabled us to give most of the bacteria we found species names already assigned in the literature (Table V), the remaining strains could be assigned to groups already described (1). Some of these strains might be new species, whose identification would require a detailed genetic analysis. Globally (Table VII) results indicated phenotypic percentages similar with those found by Berland *et al.* (7). In this study, however, more strains were able to acidify the medium from various sugars. Notably, the percentage of strains which were capable of utilization of glucose was much higher (80 %). This result is similar to those of Banchi A (94 %) (9). The sugars more utilized were glucose (80 % of strains), maltose (72 % of strains) and galactose (94 % of 17 strains tested). The less used sugar was arabinose (1.2 %). These results are again very close to these obtainly Berland *et al.* (8). It is interesting to note that galactose is a main wall component of red algae and that glucose is constituent of cellulose, the main wall polysaccharide in *Cladophora rupestris* (26).

Table IV : Antibiograms of the various bacterial strains and minimal inhibitory concentration (ug-ml-^{1}).

Strains / Antibiotics	6-1	6-2	6-3	Ap	Ag	W-2	WRG-1	CIP	W-1	WRC-1	WG-2	CIT60a	6RG-2	6RG-1	WGR_2-3	WG-1	WGR_2-2	CG	JC	CIT60b
β-lactamines																				
Penicilin	I	I 8	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Ampicilin	I 4ug/ml	I 8	R	R	R	R	R	R	R	R	R		S I	R	R	R	R	R	R	R
Carbenicilin							S	S				S	S	S						R
Cefalotine							32 R	16 R				60	<8 S	40 I						I
													<0,5							>8
Aminosides																				
Streptomycin	R	I 8	R	R	R	R	I	R	R	S 5	S 5		R	R	I 8	I >8	R	R	R	R
Neomycin	R	I 8	R	R	R	I >8	S 4	R	R	S 5	S 5		I	I	I 8	I 8	S I	R	R	R
Framycetin							S 2	I					I 8	I 8						R
Kanamycin	R	I 8	R	R	R	R	S 4	R	R	S 7	S 5		I	R	I 8	I 8	S I	R	R	R
Amykacin							S I	I				I 8	I	R						
Gentamycin	R	S I	R	R	R	I >4	S I	I	R	S <1	S I	I 4	R	R	S 3	S 4	S 0.25	R	R	R
Tobramycin							S 2	R					R	R						R
Phenicol																				
Chloramphenicol	S 1ug/ml	S I.5	S I	I 8	I 8	S 7	S 2	R	R	R	R	I	S <2	I	R	R	R	R	I 8	I
Tetracyclin																				
Tetracyclin	S 1ug/ml	R	I 4	R	R	R	R	R	R	R	R		R	R	R	R	R	R	R	R
Oxytetracyclin							R	R					I	R						R
Macrolides																				
Pristinamycin							S I	R				R	S 0.5	R						R
Polypeptides																				
Bacitracin	S	I	R	R	R	R	R	R	R	R	R		R	R	R	R	R	R	R	R
Sulfamides																				
TSU	R	R	S	S	I	R	S	R	R	R	R		R	R	R	R	R	R	R	R
Quinolones																				
Acid Nalidixic	R	R	I 16	I	I	I >8	S 4	I	R	I 16	R		I	R	R	I	R	R	R	R
Divers																				
Rifampicin	S 0.1	I <4	S 1	S 2.5	S 3	S 3	S 0.25	S 0.25	I 16	R	R	R	S 0.25	S 1	R	S 2	S 4	I 16	S <0.25	I 4

S : Sensitive ; I : Intermediate ; R : Resistant ; TSU : Trimethoprime + Sulfamethoxazole.

Many of our strains did appear in the algal culture medium upon treatment of the algae with antibiotics or antiseptics. For example, the C170 strain only appeared after treatment of *Cladophora rupestris* with 70 % ethanol. We suggest two possible explanations to this finding. First, it is known that algae often excrete antibacterial substances (6, 10, 24). Such a secretion of antibiotics either by the alga or by some of the bacterial strains would inhibit the development of other bacteria. Weakening the plant or elimination of the former bacteria by antibiotic or antiseptic treatment might result in the development of the later strains. Another possibility is that treatments could liberate some bacterial strains previously stuck to the alga cell walls. In this respect, as has been observed by optical microscopy (not shown) the C1 70 strain was stuck perpendicularly into the cell walls of *C. rupestris*. This strain might have been physically released from cell walls upon treatment of the alga with 70 % ethanol. Bacteria were also noted more or less embedded within the cell walls of *A. plumula*.

Pseudomonas gelidicola, P. elongata, Acinetobacter sp., *Deleya marina* and *Alteromonas macleodii* were found with both species of algae. They displayed the same phenotypic features wherever they came from, with the exception of one strain of *Pseudomonas elongata* (WRG_1 strain) which had a different antibiogram pattern than the others. Other bacteria have been found with one algal strain only. This is the case of *Cytophaga* sp. (Cl70 strain). This genus, often cellulolytic was found, on *Cladophora rupestris*, the cell wall of which contains up to 70 % crystalline cellulose (26). We do not know, however, whether any of these is specific for one alga.

Very few antibiotics were effective in eliminating the contaminant bacteria (Table IV). The most effective was rifampicine (12 strains out of 20). Carbenicilline also was rather effective as 5 out of the 6 strains tested were sensitive. The strains which were resistant to rifampicine also were the only ones sensitive to the aminosides. This is the case of strains of *Acinetobacter* sp. (WRC1 ; WG2 ; WRG2 ; ClT60a strains). Two strains resisted to all the investigated antibiotics, *Vibrio parahaemolyticus* (ClT60b) and *Deleya aesta* (W1). Both of these species are characterized by an important metabolic activity. Perhaps these strains synthesize ensymes which destroy antibiotics by plasmid or episome attainment. The strain 6RG2, which we have assigned to the species *Moraxella* sp., was not sensitive to penicillin G, contrary to all other *Moraxella*, but was the only strain sensitive to ampicilline (Penicilline A). All the other distinctive characters of the genus were correct.

Antithamnion plumula resisted well to the treatments with carbenicillin, gentamycin, rifampicin and chloramphenicol (Table I) bud did not resist to a short immersion (1 h) in Temerol. By contrast *Cladophora rupestris* was resistant to treatments with Temerol shorter than 8 hours (Table II). The treatments that were investigated did reduce the number of bacteria but did not succeed in making the algae axenic. However, the knowledge we have gained on the various bacterial strains and the behaviour of algae towards antibiotics and antiseptics should prove useful in further axenisation attempts.

Table V : Assignement of strain

6-1 :	*Pseudomonas* sp. Baumann's group D_1 (Baumann 1972)
6-2 :	*Pseudomonas cruciviae* (Gray & Thorton 1928)
6-3 :	*Pseudomonas* sp. Baumann's group 1_2 (Baumann 1972)
Ap : ClJ :	*Pseudomonas gelidicola* (Kadota 1951)
W_2 WRG 1 Ag ClP	*Pseudomonas elongata* (Humm 1946)
W 1	*Deleya aesta* (Bauman, 1983)
WRC1 WG2 WRG2 ClT 60 a	*Acinetobacter* sp. (Brisou & Prevot 1954)
6RG2	*Moraxella* sp. (Zwoff 1939)
DRG1 ClT	*Deleya marina* (Baumann 1983)
WGR_2-3	*Deleya cupida* (Baumann 1983)
WG1 ClB	*Alteromona macleodii* (Baumann 1972)
WGR_2-2	*Pseudomonas stutzeri* (Zohmann & Neumann 1896) Sijderius 1946
WGR_2-1 6RC1	*Pseudomonas* sp. Baumann's group H_2 (Baumann, 1972)
WGR_2-4 6RC2	*Flavobacterium halmephilum* (Elazari & Volcani 1940)
Cl70	*Cytophaga* sp. (Winogradsky 1929)
ClT60 b	*Vibrio parahaemolyticus* (Fujino, Jibazaki, Iwanami & Eukumu 1963)

Table VI : Phenotypic characters of *Pseudomonas* and *Alteromonas* modified from Lee *et al.* (ref. 23) and Gilson *et al.* (ref. 14).

Tests	*Pseudomonas*	*Alteromonas*
Requirement for Na^+	d	+
Arginine dihydrolase	d	-
Growth in 7,5 % NaCl	d	+
Glucose Metabolism (Hugh & Leifson)	OX or I/Al	I/Al
DNase	d -	+

OX : Oxydative ; I : Inert ; Al : Alkalin ; d : differential.

Table VII : Global results of phenotypic features exprimed in percentage of tested strains

	a (%)	b (%)
Morphology		
White colonies	80	61
Yellow colonies	12	29
Orange colonies	4	5
Translucent colonies	20	18
Strains $Mobile^+$	72	68
Strains $Mobile^-$	28	26
Strains $Gram^-$	100	95
Physiology		
Growth in 0% NaCl	4	30
No growth at 43°C	100	63(40°C)
Fermentative strains	4	10
Oxydative strains	56	22
Inert strains	40	68
Aerobic strains	96	88
Anaerobic facultative strains	4	13
Enzymology		
Oxydase	72	90
Catalase	100	85
Gelatinase	40	43
Nitrate reductase	16	43
H_2S	0	35
Indole	4	1
Medium acidification with :		
Arabinose	12	11
Glucose	60	16
Galactose	56	9
Mannose	12	15
Fructose	48	15
Maltose	56	18
Saccharose	48	21
Manitol	56	10
Sorbitol	4	4
Antibiogram : Sensitive to :		
Penicillin G	0	35-55
Streptomycin	10,5	65
Chloramphenicol	30	75

a : our results ; b : Berland *et al.* 1975, *Hydrobiologia* 47, 481-497.

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