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Taxonomic characterization of pelagic and periphytic heterotrophic bacteria isolated from the tropical Ebrié lagoon, Côte d'Ivoire

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With 6 figures and 4 tables in the text

Abstract: Activity of heterotrophic bacteria is recognized as an important process governing the biochemical functioning of many aquatic ecosystems. The aim of this study is to compare (1) bacterial isolates of pelagic heterotrophic bacteria from two sites, each with a different trophic status (4 µg/l at Kpass and 23 µg/l at Layo for chlorophyll index) located in the Ebrié lagoon, and (2) the bacteria associated with periphyton growing on an artificial reef of bamboos stuck into the sediment of the two sites. Hierarchical classifications were performed from morphological and biochemical tests of 343 heterotrophic bacterial strains isolated from the two sites and for each habitat. Despite differences in trophic status, numerical taxonomic analysis of the pelagic bacterial strains (n = 165) showed a dominant cluster (72 % of strains) of mixed origin (close to 50 % of isolates from each site). The majority of these strains were non-fermentative rods producing neither amino-acids decarboxylases, nor acid from any carbohydrates. In the more eutrophic site (Layo), few differences of properties were observed between pelagic and periphyton-associated bacteria. On the contrary, a large part of bacteria isolated from bamboo at Kpass was characterized by specific particularities such as the degradation of arginine, tryptophan and citrate. Numerical taxonomic analysis allowed the distinguishing of specific characteristics of bacteria according to the habitat. Our results suggest that hydrological and biological conditions (especially photosynthetic exudates from periphyton) may induce an obvious specialization of the periphytic bacteria in the less eutrophic ecosystem (Kpass) compared to the bacteria isolated from the more eutrophic environment (Layo).

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Introduction

In various aquatic ecosystems, the composition of bacterioplankton assemblages is conventionally investigated by classical cultures (WARD et al. 1987) or numerical taxonomy (BOUVY & DELILLE 1987, SUGITA et al. 1993, DELILLE & RAZOULS 1994) applied to isolates grown on culture medium. Most members of the natural bacterial communities are non-culturable and their identity remains unknown (BROCK 1987). Molecular methods based on nucleic acids sequence analysis have reached a high level of acceptance in microbial ecology, due to new techniques for the identification of bacteria unbiased by the limitations of culturability (e.g. FUHRMAN & LEE 1989). For example, LAMBERT et al. (1993) made such an approach with the use of hybridization between purified DNA extracted from whole natural planktonic bacteria for comparison between different sites (rivers and ponds). These authors concluded that bacterioplankton from different sites can be separated into distinct groups and their conclusions agree with previous studies using traditional methods of isolation and identification. In terms of species diversity, the molecular technique is very interesting but in terms of trophic role, the knowledge of the physiological and biochemical properties of bacterial communities remains fundamental.

A complete trophic description of a bacterial species involves much information and it is not realistic to test all the potential properties of each natural species isolated. In most aquatic ecosystems, the dominant bacterial community is defined by the aerobic heterotrophic bacteria which perform the crucial role of decomposers. But the taxonomic composition of heterotrophic bacteria may change according to the environmental conditions without modifications in its trophic role in aquatic ecosystem.

Since 1991, a multidisciplinary program with an ecological aspect has begun with the study of artificial reefs made of bamboos stuck into the sediment in the shallow tropical lagoon (Ebrié lagoon, Côte d'Ivoire). These structures called "Acadja" established in an extensive aquaculture system allowed the fixation of a large biomass composed of periphyton (KONAN-BROU & GUIRAL 1994) and microorganisms such as bacteria, protozoa, bryozoa (unpublished data). This particular biotope attracts fishes (HEM & AVIT 1994) and may provide a substantial source of food for phytophage fishes as tilapias (LEGENDRE et al. 1989). Development of periphyton biomass (algae and bacteria) growing on bamboo seems to be influenced by hydrological conditions (ARFI et al. 1997). Nutrients were not a limiting factor but oligohaline situations associated to a high turbidity may explain the seasonally marked decrease of periphyton biomass.

In February 1994, new structures of bamboos were stuck into the sediment of two sites of the western part of Ebrié lagoon characterized by different trophic status. Colonization of periphyton growing on bamboos and bacterial

cells associated to the periphyton have been studied with six samplings between February and May. The purpose of this study was (1) to evaluate the taxonomic composition of pelagic heterotrophic bacteria isolated from the two sites and (2) to compare their characterization with those of bacteria isolated from periphyton growing on bamboos. Numerical taxonomic analysis from purified strains of aerobic heterotrophic bacteria are performed to solve this investigation.

Material and methods

Study area

The study was conducted between February and May 1994 (see schedule of samplings in Table 1) in two stations located in the western part of the Ebrié lagoon in Côte d'Ivoire (Fig. 1). Each station is characterized by their hydrological conditions. Layo station is located near the Agneby River mouth and is submitted to continental influence during the rainy season; throughout the year, the habitat is very turbid with relatively high nutrient concentrations (ARFI et al. 1997). Kpass station is located at the far end of a bay and no exogenous influence was detected in this environment which is

Table 1. Numbers of isolated strains for the heterotrophic bacteria for each site (Layo and Kpass) and for each origin (lagoon and bamboo) during the study conducted between February and May 1994.

Date	Site	Origin	Strains
23/02	Kpass	Lagoon	34
23/02	Layo	Lagoon	33
25/02	Kpass	Bamboo	17
25/02	Layo	Bamboo	17
3/03	Kpass	Bamboo	33
3/03	Layo	Bamboo	11
30/03	Kpass	Bamboo	29
30/03	Layo	Bamboo	25
27/04	Kpass	Lagoon	29
27/04	Layo	Lagoon	28
25/05	Kpass	Bamboo	26
25/05	Kpass	Lagoon	19
25/05	Layo	Bamboo	23
25/05	Layo	Lagoon	22
	Site	Layo	159
		Kpass	187
	Origin	Lagoon	165
		Bamboo	181
	Total		346

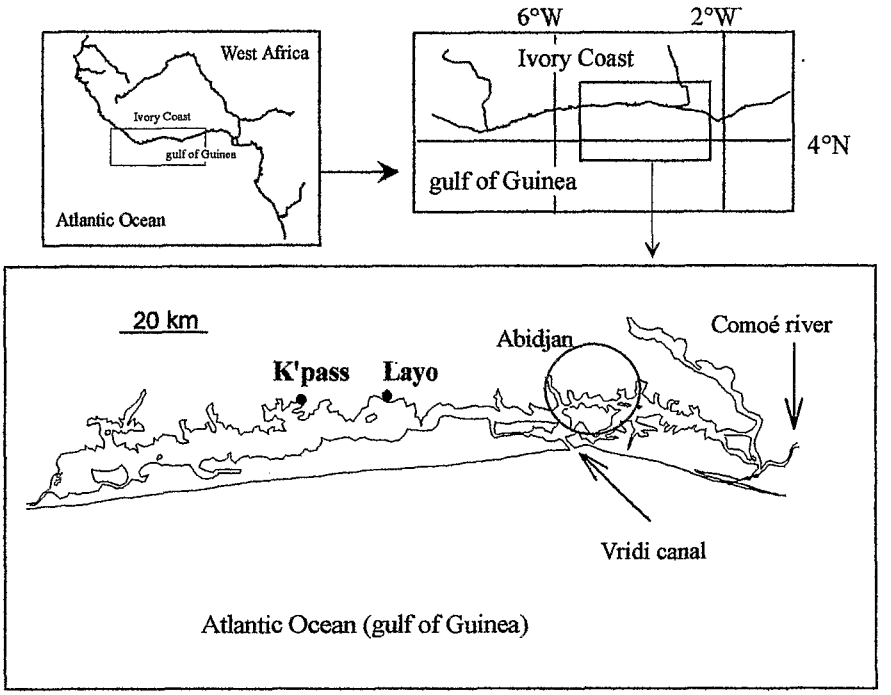

Fig. 1. Map of the Ebrié lagoon and location of the two sampling sites (Layo and Kpass).

considered as a mesotrophic zone (PAGANO & SAINT-JEAN 1988). Artificial structures called "Acadja" made of bamboos stuck vertically into the sediment were installed in shallow water (1 m depth) at each station. All sticks (average length and diameter of 120 cm and 6 cm, respectively) were placed in the sediment in February 1994 at regularly spaced intervals (4 to 5 sticks per m^2).

Hydrological and biological parameters

Lagoon water was sampled 10 cm below the surface. Conductivity and turbidity (expressed in NTU, Nephelometric Turbidity Unit) were measured, respectively, using a Tacussel conductimeter and a HE 9 turbidimeter. Dissolved nutrient concentrations (ammonia, nitrate and orthophosphate) were analysed using an autoanalyzer according to the procedure described by STRICKLAND & PARSONS (1972).

Concentrations of chlorophyll-a retained on GF/F filters were measured in a Turner Designs fluorometer after methanol extraction (YENTSCH & MENZEL 1963). Abundance of pelagic bacteria was estimated from direct counts using an epifluorescence microscopy procedure after staining cells by DAPI (PORTER & FEIG 1980).

Previous work has demonstrated that colonization of periphyton began at the upper level of bamboo (ARFI et al. 1997). Thus bamboos were sampled at the 10 cm below the surface level by scraping a known surface (close to 2 cm^2) with a sterile scalpel. The

periphyton collected was immediately subsampled after thorough mixing. Fixed algae pigment concentration was estimated as described above. For epiphytic bacteria, periphytic material was diluted in 0.22 µm filtered lagoon water, and samples were sonicated (30 W during 30 s) in order to disperse the attached bacterial cells. Direct counts were estimated as described above when accurate determinations were possible.

Isolation and characterization of the strains

In order to isolate bacterial strains, a spread plate technique was applied with the selective medium Nutrient Agar, Biomérieux. From subsamples of lagoon water and periphytic material, appropriate dilutions were made in order to obtain 40–50 colonies per plate after an incubation at 30 °C for 2 days. All colonies of each plate were picked for purification with the same medium. Before the test, all isolates were subcultured (48 hours at 30 °C) on marine agar plates (same medium as above) to ensure purity and viability of each isolate. A total of 343 isolates were purified and maintained throughout the study. The distribution of isolated strains from each bacterial community (two sites and two origins) is reported in Table 1. In order to assess the qualitative changes in heterotrophic bacterial communities during the study, the biochemical characters of the API System were used, knowing that this system has been successfully used in natural environments by many authors (e.g. Le Chevallier et al. 1983, Di Siervi & Mariazzi 1982, Bouvy & Delille 1987, Delille & Razouls 1994). Convenient for routine studies, this method represents a substantial improvement over more tedious procedures available some years ago (Troussellier & Legendre 1981).

The following morphological and biochemical tests were performed for each strain: presence of beta-galactosidase (ONP) and presence of arginine (ADH), lysine (LDC) and ornithine decarboxylases, citrate utilization (CIT), H_2S production (H_2S), urease (URE), presence of tryptophandesaminase (TDA), indol (IND) and acetoin production (VP), hydrolysis of gelatin (GEL), presence of carboxylases for utilization of glucose (GLU), mannitol (MAN), inositol (INI), sorbitol (SOR), rhamnose (RHA), sucrose (SAC), melibiose (MEL), amygdalin (AMY) and arabinose (ARA), presence of cytochrome-oxidase (OX), nitrate reduction (NO_2), presence of catalase, cells mobility (MOB), oxidative and fermentative tests (OFO and OFF), reaction to gram test (GRAM), cells as cocci (COC) and presence of spores (SPO). Numerical taxonomic analysis based on the similarity among strains was performed using the simple matching coefficient of Sokal & Michener (1958) in association with the UPGMA algorithm (Sneath & Sokal 1974). The functional evenness indexes were calculated as an estimation of the taxonomic structure of the bacterial isolates (Troussellier & Legendre 1981).

Results

Hydrological and biological conditions

Pelagic environmental conditions encountered at the two sites are given in Table 2. For all parameters studied, the means noted at Layo site are always higher than those reported at Kpass site. Rain and inflow of local rivers are the

Table 2. Environmental conditions in the two sites during the investigation. Abbreviations: Cond, conductivity; Turb: turbidity; Chloro: amount of chlorophyll-a; concentrations of nutrients (ammonia, nitrate and phosphate).

		Kpass			Layo		
	Units	min	max	mean	min	max	mean
Cond	mS/cm	10.5	13.1	12.1	3.5	17.6	12.9
Turb	NTU	0.9	1.5	1.2	6.9	28.1	13.9
Chloro	µg/l	1.2	7.6	4.2	9.7	42.2	22.8
NH_4	µM	1.8	5.1	3.2	3.2	16.1	7.6
NO_3	µM	3.2	6.9	4.6	6.8	43.1	19.3
PO_4	µM	0.4	0.7	0.6	0.4	0.8	0.7

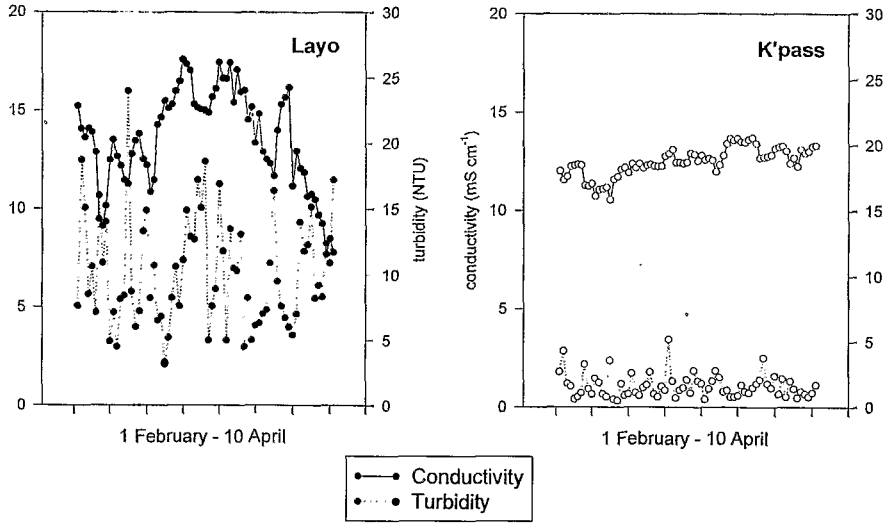

Fig. 2. Hydrological conditions at Layo and Kpass during the study. Conductivity values (right scale) expressed in mS/cm and turbidity values (left scale) expressed in NTU unit.

major factors controlling the hydrology of the western part of the Ebrié lagoon. Average conductivities were similar for the two sites, but the range was greater at Layo. At Kpass, conductivity values did not change with time (Fig. 2) whereas at Layo this parameter showed a seasonal pattern with a maximum on 30 March (17.6 mS/cm) and a minimum on 25 May (3.5 mS/cm). Water turbidity was low at Kpass (Table 2) whereas values may increase at Layo until 28 NTU in May (Fig. 2). At this site, the regular rainfall observed in May induced a progressive shift from a mesohaline to an oligohaline situation, with high turbidity due to the Agneby River. Ammonia, nitrate and orthophosphates were characterized by the same pattern with high concentrations during the

oligohaline periods and low values during the mesohaline sequence. Chlorophyll-a values also showed higher amounts during the dry season in April (7.6 µg/l at Kpass and 42.2 µg/l at Layo, Table 2 and Fig. 2). Direct counts of pelagic bacteria ranged between 2.6×10^6 cells/ml to 4.4×10^6 cells/ml at Kpass and between 2.7×10^6 cells/ml and 6.0×10^6 cells/ml at Layo (Table 3). Ratios between direct and viable counts (DC/VC) for the pelagic bacteria were higher in the more eutrophic site. Direct count of periphytic bacteria has been accurately possible on 30/03 and the ratios obtained between DC and VC are higher than those reported for pelagic bacteria (Table 3).

Taxonomic analysis

A total of 343 strains of heterotrophic bacteria were isolated and purified from lagoon water and bamboo at both sites. Hierarchical classifications of the strains isolated from lagoon water (165 isolates) determined 7 clusters at the 75 % similarity level; only 13 isolates failed to cluster at this level (Table 4).

Table 3. Comparison between viable plate counts (V.C.) and direkt counts (D.C.) of pelagic and periphytic bacteria in the two sites studied during the investigation conducted in 1994.

		Kpass			Layo		
Lagoon	Units	23/02	27/04	25/05	23/02	27/04	25/05
V.C.	CFU/ml	$3.3*10^3$	$9.0*10^3$	$5.3*10^3$	$1.8*10^3$	$9.8*10^3$	$3.9*10^3$
D.C.	cells/ml	$2.6*10^6$	$4.4*10^6$	$3.2*10^6$	$2.7*10^6$	$6.0*10^6$	$4.0*10^6$
D.C./V.C.		788	489	606	1500	612	1039
Bamboo		25/02	30/03	25/05	25/02	30/03	25/05
V.C.	CFU/ml	$2.3*10^3$	$5.5*10^3$	$6*10^3$	$7.2*10^3$	$2.1*10^4$	$5*10^4$
D.C.	cells/ml	nd	$2.6*10^7$	nd	nd	$2.8*10^7$	nd
D.C./V.C.		nd	4727	nd	nd	1333	nd

Table 4. Number of strains for each cluster determined by hierarchical classification for each site (Kpass and Kayo) and for each origin (lagoon and bamboo). Number of isolates non clustered is mentioned for each numerical analysis.

Kpass = 187 strains		Lagoon = 165 strains		Layo = 159 strains		Bamboo = 181 strains	
KP1	70	LG1	109	LY1	63	BB1	49
KP2	49	LG2	15	LY2	61	BB2	48
KP3	25	LG3	11	LY3	11	BB3	32
KP4	11	LG4	10	LY4	7	BB4	27
KP5	11	LG5	7	LY5	6	BB5	14
KP6	6	Isolates	13	Isolates	11	Isolates	11
KP7	5						
Isolates	10						

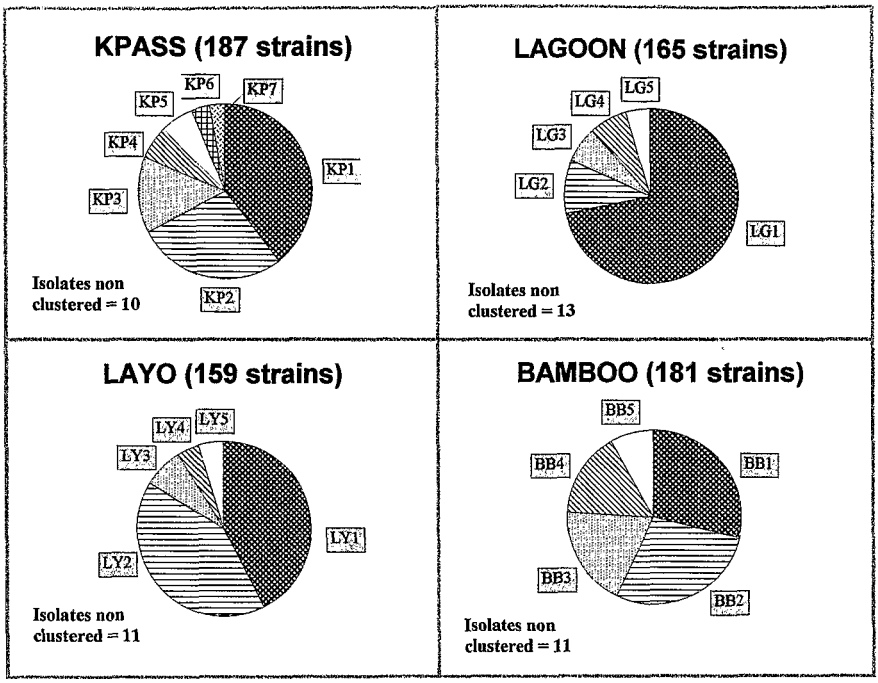

Fig. 3. Schematic representation of the different clusters isolated from the two sites (Kpass and Layo) and from the two origins (Lagoon and Bamboo). See the strain numbers of each cluster in Table 4. Numbers of isolates non clustered are noted for each numerical analysis.

Among these clusters, five clusters have at least 5 strains (Fig. 3). The major cluster (called LG1) with 109 strains represented 72 % of the total population originating from both sites almost equally (Fig. 4). The majority of these strains were non fermentative motile rods with presence of catalase and oxidase, and producing neither amino-acids (arginine, lysine and ornithine) decarboxylases, nor acid from any carbohydrates tested (Fig. 5). Half of the strains have been identified as gram-positive rods with presence of beta-galactosidase (ONP) and gelatin hydrolase (GEL). A minority of strains (30 %) were able to produce acetoin from sodium pyruvate (VP test). The other clusters were of minor importance without any information of characterization.

Analysis of the strains isolated from the bamboo (181 isolates) revealed 5 clusters at the 74 % similarity level and only 11 non clustered isolates (Fig. 3; Table 4). Predominance of one cluster was not observed and the sole characteristic of this analysis was a cluster (called BB2) represented by 48 strains with 83 % of strains isolated from Kpass site (Fig. 4). Physiological and biochemical properties of this cluster showed a great majority of non-fermentative gram-negative rods producing catalase and oxidase (Fig. 5). Therefore,

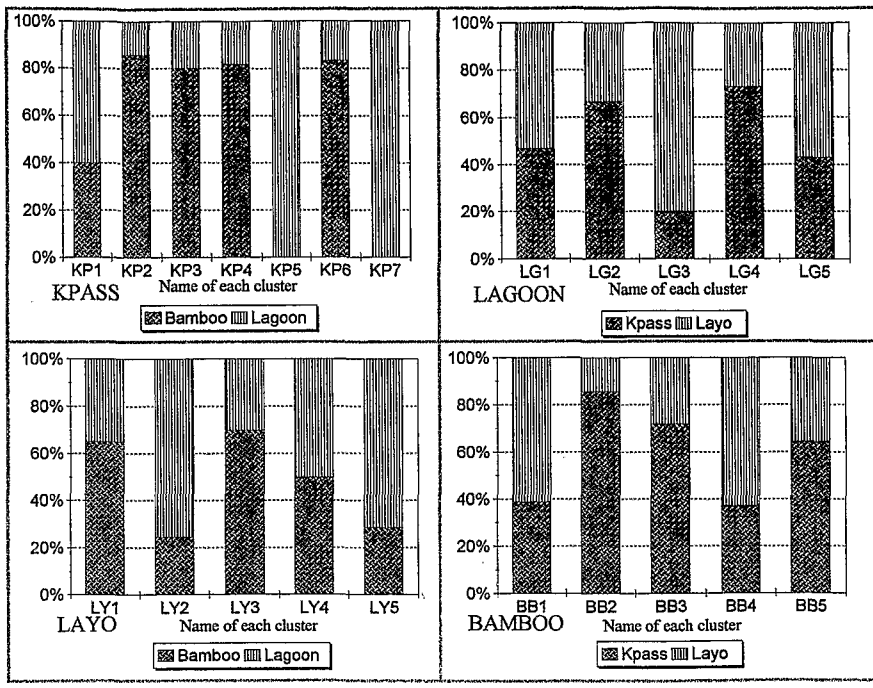

Fig. 4. Diagrams representating the percentage of strains for each cluster; – according to the site (Kpass and Layo) for each origin (Lagoon and Bamboo); – according to the origin (Lagoon and Bamboo) for each site (Kpass and Layo). See details in Table 4 for strain number of each cluster.

all the strains were characterized by the presence of arginine dihydrolase (ADH test), were positive for oxidase (OX) and were able to hydrolyze citrate (CIT).

Bacteria isolated from each site were also analyzed by hierarchical classifications. At Kpass station, a total of 187 strains were isolated and 7 clusters were distinguished at 78 % of similarity level with only 10 isolates failing to cluster at this level (Fig. 3; Table 4). The predominant cluster with 70 strains (called KP1) was formed by a mixture of the two origins (bamboo and pelagos) without specific properties from tests (Fig. 4). Another cluster (called KP2) was represented by 49 strains (28 % of strains isolated) with a majority of the strains (86 %) originating from bamboo. The physiological and biochemical characteristics of these strains were similar to those isolated from the cluster which characterized the bamboo analysis (see above; cluster BB2 defined by 48 strains from bamboo with 83 % of strains isolated from Kpass). Two more clusters (KP5 and KP7) represented 6 and 3 % of the total bacteria isolated from Kpass site (11 and 5 strains, respectively) but all these strains were isolated from lagoon water. Biochemical tests revealed that part of the

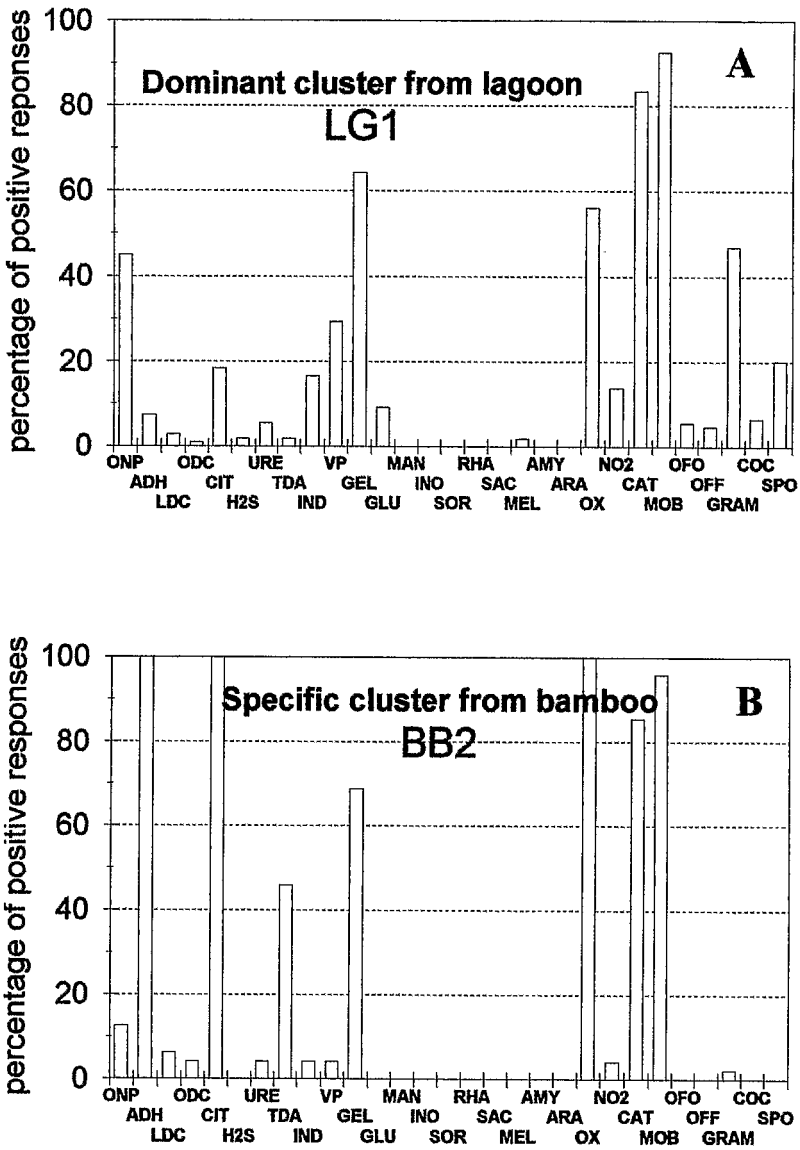

Fig. 5. Percentage of positive responses for each physiological and biochemicals tests (see abbreviations in Material and Methods section). **A:** dominant cluster from lagoon (called LG1); **B:** specific cluster from bamboo (called BB2: 48 strains with 82% from Kpass site).

pelagic strains were able to hydrolyze citrate (30 % of strains) and were positive for arginine dihydrolase (80 % of strains).

At the Layo site, the hierarchical analysis clustered a total of 159 strains into 5 clusters at 75 % of similarity coefficient with 11 non clustered isolates (Fig. 3; Table 4). Two major clusters were predominant (called LY1 and LY2) with 83 % of the total bacteria and each cluster represented one origin (Fig. 4). One cluster (LY1 with 63 strains) was representative of the bamboo habitat (63 % of strains) with a majority of non-fermentative motile rods with the presence of catalase and oxidase (Fig. 6). The other dominant cluster (LY2 with 61 strains) was representative of lagoon water (75 % of strains); the great majority of the strains were non-fermentative, Gram-positive, motile and spore-forming rods with presence of catalase, beta-galactosidase, gelatine hydrolase and producing acetoin (Fig. 6).

Functional evenness indexes were similar for the bacteria isolated from Layo site with a value of 0.55. On the contrary, the index was high for the pelagic bacteria isolated from Kpass whereas a low index was calculated for bacteria originating from bamboo (0.48).

Discussion

The development of living communities on a substratum (bacteria, algae, fungi, protozoa, micro- and macrometazoa), usually referred to as periphyton, is recognized as a major trophic component in shallow aquatic ecosystems. The substratum "bamboo" was utilized in an extensive aquaculture system and was installed in shallow zones located in the Ebrié lagoon. This was carried out to develop a large biomass composed of periphyton considered as a trophic resource, attracting or supporting the fish requirements (Hem & Avit 1994). Previous studies conducted at Layo have described the specificity and the productivity of phytoplanktonic and periphytic microflora growing on bamboo (Guiral et al. 1993, Konan-Brou & Guiral 1994). Colonization studies conducted in the same area have demonstrated that bacteria are the pioneer colonizers of bamboo, followed by adnate diatoms (Arfi & Bouvy 1997). Most of these algal species (except filamentous species) were euryhaline organisms frequently observed in pelagic environments in the Ebrié lagoon (Arfi & Bouvy 1995). Studies based on interrelations between periphyton and phytoplankton communities demonstrated that periphyton and phytoplankton may exchange organisms and may compete for nutrients. In a eutrophic lake (British Columbia), Brown & Austin (1973) reported that at least 12 taxa were found to be shared by both communities.

The west zone of Ebrié lagoon is considered eutrophic with high levels of inorganic nutrients (see Table 2), oligohaline and non polluted (Pagano &

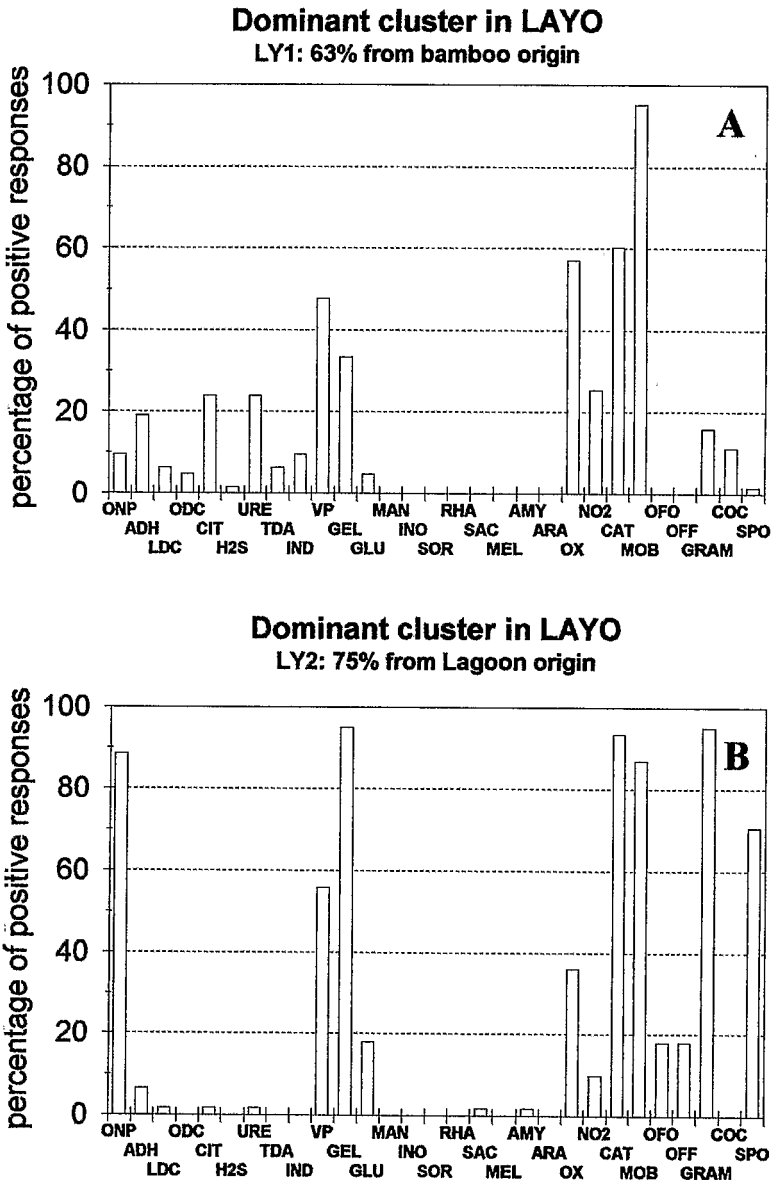

Fig. 6. Percentage of positive responses for each physiological and biochemicals tests (see abbreviations in Material and methods section). **A:** dominant cluster in Layo (called LY1: 63 strains with 63 % from bamboo origin). **B:** dominant cluster in Layo (called LY2: 61 strains with 75 % from lagoon origin).

SAINT-JEAN 1988). Average values of chlorophyll-a concentrations were 4.2 and 22.8 µg/l in Kpass and Layo, respectively. The values observed at Layo are characteristics of eutrophic pelagic environments and are linked to the location of the station. Indeed weak and regular winds, blowing from the southwest and characterized by a diel pattern of velocity, can induce resuspension in favorable conditions of fetch, depth, wind speed, wave height and bed roughness (ARFI et al. 1993, BOUVY et al. 1994). All these conditions are present in the Layo station and not at Kpass station and, therefore, explained the high level of turbidity, amount of chlorophyll and nutrient concentrations noted at Layo. Due to the high light attenuation (10 % of the photosynthetic active radiation reach the sediment), the energy decrease can explain the vertical distribution of the various species colonizing the bamboo (GUIRAL et al. 1993).

Generally, environmental change is the principle driving force that alters bacterial structure and composition (KLUG & TIEDJE 1993). It is clear that a direct method would be the best for the analysis of indigenous bacteria, but the selective methods can be informative if representative bacteria are isolated from the community. Understanding of the ecological role of bacteria includes morphology, development, metabolism and ecology (TRÜPER 1993). This author also stated that molecular ecology is a fascinating development but isolation, cultivation and characterization of strains remain indispensable in order to judge their ecological role in an ecosystem. The approach used in this study is questionable because the traditional culture based on identification of bacteria gives a rough focus of the members of indigenous populations. It is known that a relatively small proportion of the total bacterial population is capable of colony formation. However, our goal is not to determine the specific diversity of bacteria, but to evaluate the possible differentiation in the biochemical and physiological properties of heterotrophic bacteria according to various environmental trophic status. Heterotrophic bacteria are the predominant members of many bacterial communities and their activity is known as an important process governing the biochemical functioning of aquatic ecosystems. Aerobic heterotrophic bacteria were the sole bacteria studied which represented a small part of the total bacteria (see Table 3). Indeed, the ratio between direct and viable counts for pelagic and periphytic bacterial communities was close to 0.1 % and 0.4 %, respectively. But one of the assumptions in our investigation is based on the representativity of the 343 colonies purified.

Pelagic bacteria isolated from the two sites located in Ebrié lagoon were characterized by a great homogeneity of physiological and biochemical properties with a predominance of non fermentative rods with presence of catalase and oxidase. This observation is in agreement with previous work performed in this area (CAUMETTE et al. 1994) which also reported a majority of rods, isolated from surface water at different stations in the Ebrié lagoon, with

a strict oxidative metabolism (70 to 90 % of strains). GUIRAL (1984) demonstrated the importance of the mineralizing bacterial activities in the surface water of a very polluted bay (Bietri bay) located in the Ebrié lagoon, where cultured bacteria were also dominated by rods with presence of catalase and oxidase. In our study, the population of pelagic bacteria showed a homogeneous picture, whatever the site studied. Indeed, bacteria isolated from pelagic environment must be combined to form one large group (cluster called LG1 with 72 % of all pelagic strains). This was confirmed by the measure of functional eveness indexes, with higher values for the pelagic bacteria. On the contrary, a strong differentiation of the strains isolated from bamboo is clearly observed between the two sites. Strains isolated at Kpass were very different with a great specificy compared to those isolated from Layo. A large part of bacteria isolated from bamboo at Kpass is non-fermentative gram-negative rods characterized by specific particularities as the metabolism of arginine, tryptophan and citrate. The low functional evenness index confirms this more marked specificity compared to the pelagic area. Between the two sites, bacteria isolated from Kpass appeared more diverse with 7 clusters (with two clusters represented by strains originating from lagoon) as opposed to the 5 clusters at Layo (see Table 4). One of the two dominant clusters in Layo (called LY2) was represented by strains isolated at 75 % from lagoon origin (non-fermentative and positive-gram rods with presence of spores). Generally, carbohydrates except glucose were not utilized by strains and especially those isolated from bamboo.

During the colonization phase, the first microorganims colonizing the bamboo were bacteria which came from the pelagic environment (ARFI & BOUVY 1997). In a review, BRADING et al. (1995) clearly explained the dynamics of bacterial biofilm formation and detailed the maturing of the biofilm with time. Cells in the upper regions have easier access to nutrients and oxygen. Consequently these cells are similar, in terms of physiology, to those grown planktonically. But the development of multiple layers of cells in thick biofilms induces, for the cells enmeshed in the glycocalyx, a lack of nutrients and oxygen. Thus these cells must utilize other compounds to maintain metabolism, which may explain the differentiation of strains isolated from bamboo. Non-fermentative gram-negative rods were isolated from periphytic and pelagic heterotrophic bacteria at Kpass site but the physiological properties of strains were different according to the biotope.

In this paper, we showed that numerical taxonomic analysis from aerobic heterotrophic bacteria isolated from different sites allows to distinguish specific characteristics of bacteria according to the habitat. The strains growing on bamboo isolated at Kpass are distinct in their properties with a great specificy compared to those isolated from Layo. DAVIS et al. (1983) demonstrated with a similar analysis that bacterial assemblages in adjacent kelp-dominated

habitats segregate into discrete populations in response to physical, chemical and biological factors. In most aquatic ecosystems, it is recognized that the photosynthetically produced dissolved organic carbon (PDOC) released from natural autotrophic communities (SELL & OVERBECK 1992 for phytoplankton; COUCH & MEYER 1992 for wood) is used by heterotrophic bacteria. The presence of periphytic algae growing on bamboo may provide by their exudates new sources of dissolved organic materials for the periphytic bacteria. This would explain the differentiation of strains isolated from bamboo, and especially at Kpass which is considered as the less eutrophic site studied.

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