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Resistance of Marine Bacterioneuston to Solar Radiation

Hélène Agogué, Fabien Joux, Ingrid Obernosterer, and Philippe Lebaron*

Observatoire Océanologique, Université Pierre et Marie Curie, UMR 7621-INSU-CNRS, BP44, 66651 Banyuls-sur-Mer, France

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A total of 90 bacterial strains were isolated from the sea surface microlayer (i.e., bacterioneuston) and underlying waters (i.e., bacterioplankton) from two sites of the northwestern Mediterranean Sea. The strains were identified by sequence analysis, and growth recovery was investigated after exposure to simulated solar radiation. Bacterioneuston and bacterioplankton isolates were subjected to six different exposure times, ranging from 0.5 to 7 h of simulated noontime solar radiation. Following exposure, the growth of each isolate was monitored, and different classes of resistance were determined according to the growth pattern. Large interspecific differences among the 90 marine isolates were observed. Medium and highly resistant strains accounted for 41% and 22% of the isolates, respectively, and only 16% were sensitive strains. Resistance to solar radiation was equally distributed within the bacterioneuston and bacterioplankton. Relative contributions to the highly resistant class were 43% for γ-proteobacteria and 14% and 8% for α-proteobacteria and the *Cytophaga*/*Flavobacterium*/*Bacteroides* (CFB) group, respectively. Within the γ-proteobacteria, the *Pseudoalteromonas* and *Alteromonas* genera appeared to be highly resistant to solar radiation. The majority of the CFB group (76%) had medium resistance. Our study further provides evidence that pigmented bacteria are not more resistant to solar radiation than nonpigmented bacteria.

The marine air-water interface constitutes a unique microbial habitat (29, 34). Microorganisms in the surface microlayer are exposed to high intensities of solar radiation, in particular UV radiation, high concentrations of toxic organic substances and heavy metals, and unstable temperature and salinity conditions (16, 45). Despite these harmful conditions, the surface microlayer has been reported to have higher abundances of microorganisms than underlying waters (1, 16, 34). This suggests that the bacterioneuston (i.e., the bacterial community of the surface microlayer) has developed strategies to survive in this "extreme environment."

The effect of UV radiation on the ecology of microorganisms has been studied in detail with aquatic systems (14). Each type of UV radiation causes distinct but overlapping types of damage (24). UV-A radiation (320 to 400 nm) causes only indirect damage to cellular DNA, proteins, and lipids by catalyzing the intracellular formation of chemical intermediates such as reactive oxygen species (ROS). In contrast, UV-B (280 to 320 nm) radiation causes direct DNA damage by inducing the formation of DNA photoproducts, of which the cyclobutane pyrimidine dimers and the pyrimidine (6-4) pyrimidinone photoproducts are the most common.

Bacteria are particularly vulnerable to UV damage because their small size limits effective cellular shading or protective pigmentation (11) and their genetic material comprises a significant portion of their cellular volume (22). Moreover, UV-absorbing compounds, such as mycosporine-like amino acids and scytonemin, that confer some protection to eukaryotic organisms and cyanobacteria appear not to be widespread antioxidant molecules in bacterioplankton (12, 38). The potential ecological importance of pigmented bacteria was recently reinforced by the discovery of aerobic anoxygenic phototrophs (AAnPs) in surface waters (6, 27) and the presence of proteorhodopsin in some marine α- and β-proteobacteria (5, 9). For the surface microlayer, larger percentages of pigmented bacteria, primarily red and yellow, have been reported (19). It was suggested that pigments are important for the resistance of bacteria to solar radiation. However, a relationship between bacterial pigmentation and resistance to solar radiation has never been demonstrated thus far.

Results from field studies on marine bacteria indicate that exposure to natural solar UV radiation results in a decrease in total cell abundance, a reduction in amino acid uptake, a depression of the activity of degrading enzymes, and a significant inhibition of protein and DNA synthesis (21). Bacterial activity can also be indirectly affected by solar radiation due to the photochemical transformations of dissolved organic matter. The exposure of dissolved organic matter to solar radiation can result in an increase or decrease in its biological reactivity, subsequently stimulating or inhibiting bacterial activity (36). Most studies are based on measurements of metabolic activities of natural bacterioplankton communities, while studies of photobiological responses of marine bacterial species are scarce and have examined few isolates (3, 18, 23). To our knowledge, the resistance of bacterioneuston to solar radiation has not been investigated thus far.

For the present study, we investigated the resistance of 90 marine bacterial strains to simulated solar radiation (UV and photosynthetically active radiation [PAR]). These strains were isolated from both the sea surface microlayer and underlying waters collected from coastal waters in the northwestern Mediterranean Sea, and the isolates were classified according to their growth pattern following exposure to simulated solar radiation.

* Corresponding author. Mailing address: Observatoire Océanologique, Université Pierre et Marie Curie, UMR 7621-INSU-CNRS, BP44, 66651 Banyuls-sur-Mer, France. Phone: 33 4 68 88 73 53. Fax: 33 4 68 88 73 98. E-mail: lebaron@obs-banyuls.fr.

MATERIALS AND METHODS

Sampling and isolation of marine bacterial strains. Bacterial strains were isolated from coastal waters from the northwestern Mediterranean Sea during four field campaigns in March and September 2001 and March and June-July 2002. The surface microlayer was collected with different types of devices as described in detail by Agogué et al. (1). Most of the surface microlayer samples were collected with a metal screen and a glass plate, and to a lesser extent, with a nylon screen, a Harvey roller, and two types of membranes (Teflon and polycarbonate) (1). Samples from underlying waters were collected by submerging a polycarbonate bottle and opening it at a depth of 0.5 m. For isolation of the bacterial strains, 100-μl subsamples were spread on marine agar 2216 plates (MA 2216; Difco, Detroit, Mich.). After incubation in the dark at 20°C for 7 to 14 days, isolates were selected from the plates according to differences in color and shape. Isolates were then picked and purified. The strains were named S, U, and SU when originating from the sea surface microlayer, the underlying waters, or both environments, respectively.

Molecular characterization of strains. The initial identification of each isolate was done by sequencing PCR-amplified regions of the 16S rRNA gene. For most of the isolates, the DNA suspension for PCR consisted of colonies picked from agar plates and resuspended in 500 μl of sterile water. For refractory isolates (i.e., highly pigmented isolates and isolates with polysaccharides), cells were lysed and the DNA was extracted. Colonies were picked, resuspended in 500 μl of lysis buffer (40 mM EDTA, 50 mM Tris, pH 8, 750 mM saccharose), and incubated with lysozyme (final concentration, 1 mg ml^{-1}) at 37°C for 45 min with gentle agitation. Sodium dodecyl sulfate (final concentration, 0.5% [wt/vol]) and proteinase K (final concentration, 0.1 mg ml^{-1}) were added, and the samples were incubated at 55°C for 1 h. DNA was extracted with equal volumes of phenol-chloroform-isoamyl alcohol (25:24:1 [vol/vol/vol]) and chloroform-isoamyl alcohol (24:1 [vol/vol]). The DNA was then precipitated with 2 volumes of isopropanol and recovered by centrifugation. Pellets were washed with 70% cool ethanol (−20°C), air dried, and resuspended in 50 μl of sterile water.

The 16S rRNA gene was amplified by PCR using two primers, SAdir (5′-AG AGTTTGATCATGGCTCAGA-3′; *Escherichia coli* 16S rRNA gene positions 8 to 27 [forward primer]) and S17 Rev (5′-GTTACCTTGTTACGACTT-3′; *E. coli* 16S rRNA gene positions 1491 to 1508 [reverse primer]). Reaction mixtures of 50 μl contained 5 μl of 10× PCR buffer (supplied with the enzyme), 200 μM deoxynucleoside triphosphate mix (Eurogentec, Seraing, Belgium), 100 pmol of each primer, 1 U of Super *Taq* (HT Biotechnology, Cambridge, England), 5 μl of washed cells (or 1 μl of DNA), and MilliQ water to a 50-μl volume. PCR was carried out in a Robocycler 96 (Stratagene, La Jolla, Calif.). The thermal PCR profile was as follows: initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 1 min, primer annealing at 48°C for 1.5 min, and elongation at 72°C for 1 min. The final elongation step was 5 min at 72°C. The 16S rRNA gene products were analyzed by electrophoresis in 1% agarose gels. Restriction fragment length polymorphism analysis was performed by digesting the 16S rRNA gene PCR products with the restriction endonuclease Hin6I (Eurogentec) at 37°C overnight, and the resulting electrophoretic patterns obtained in 2% agarose gels were used to group the isolates. The 16S rRNA gene products representing each distinct pattern were then sequenced with an automatic DNA analysis system (Genome Express, Meylan, France). Sequences were compared with sequences available in the GenBank database by using the BLAST (Basic Local Alignment Search Tool) service to determine their approximate phylogenetic affiliations (2).

Characterization of solar radiation sensitivity. Bacterial isolates were grown in marine broth 2216 medium (MB 2216; Difco) on a laboratory shaker at 25°C, and cells were harvested in the early stationary phase by centrifugation (6,000 × *g* for 10 min at 10°C). The pellets were washed twice with filtered and autoclaved seawater. The bacterial abundance was determined after staining with a nucleic acid dye (SYBR green I; final concentration, 0.01% [vol/vol]; Molecular Probes Inc., Eugene, Oreg.) on a FACS-Calibur flow cytometer (Becton Dickinson, Franklin Lakes, N. J.) using CellQuest software (28).

To avoid self-shading during irradiation, bacterial suspensions were diluted to a final concentration of 10^4 cells per ml with filtered and autoclaved seawater. One milliliter of each diluted bacterial suspension was then dispensed in duplicate into a 24-well microtiter plate (Multiwell; Becton Dickinson) and exposed to simulated solar radiation at a distance of 30 cm. During exposure, the microplates were shaken at 100 rpm and maintained at a constant temperature (~25°C) using a cooled plate. To determine possible contamination during the exposure period, 1 ml of filtered and autoclaved seawater was also dispensed into duplicate wells. Samples maintained in the dark were used as controls. Irradiation was conducted with a 1,000-W xenon lamp solar simulator (Oriel Corporation, Stratford, Conn.) equipped with AM0 and AM1 air mass filters. This set of filters allows the simulation of solar radiation at the earth surface. The intensities of UV-B, UV-A, and PAR measured with a broad-band Eldonet (European Light Dosimeter Network) radiometer (15) are presented in Table 1. The hourly dose received by the solar simulator is comparable to the noontime hourly dose at Banyuls-sur-Mer, France (Table 1).

TABLE 1. Intensities and doses of UV-B and UV-A radiation and PAR for simulated and natural solar radiation[a]

Parameter	Value for indicated type of radiation		
	UV-B	UV-A	PAR
Simulated solar radiation			
Intensity (W m^{-2})	1.2	40	352
Hourly dose (kJ m^{-2})	4.32	144	1,267
Natural solar radiation			
Maximum intensity (W m^{-2})	1.7	72	452
Daily dose (KJ m^{-2})	43.5	2,122	13,440

[a] The intensity of natural solar radiation was measured on 21 June 2003 at 14:00 h at Banyuls-sur-Mer.

Replicate microplates were removed after 0.5, 1, 2, 3, 5, and 7 h of exposure to simulated solar radiation, and 1 ml of concentrated (2×) MB 2216 was added to each well to investigate the growth pattern of each strain following exposure. Microplates were incubated in the dark at 25°C with agitation (100 rpm), and bacterial growth was followed for 6 days. Bacterial growth was determined by measuring the optical density at 450 nm with an automated microplate reader (FLUOstar Optima; BMG Labtechnology, Offenburg, Germany).

Nucleotide sequence accession numbers. The sequences determined for this study were deposited in the GenBank database under accession numbers AY576689 to AY576777 (see Table 3).

RESULTS

Determination of different classes of resistance. We determined four classes of resistance according to the growth of the bacterial isolates following different exposure times to simulated solar radiation. Isolates not growing after 30 min of exposure were considered sensitive (S) strains (Fig. 1). Isolates growing after 1 h of exposure, but not after 2 h, were considered weakly resistant (R) strains (Fig. 1). Bacterial strains that grew after 2 or 3 h of simulated solar radiation, but not after 5 h, were considered to have a medium resistance (R+) (Fig. 2). Finally, highly resistant (R++) strains were able to grow after 5 or 7 h of exposure (Fig. 2). The class of highly resistant strains (R++) displayed different responses with respect to the lag time of the growth curve, and therefore these strains were divided into three categories (C1, C2, and C3) (Table 2).

Overall, 46 and 23 strains were isolated from the sea surface microlayer and underlying waters, respectively, and 21 strains originated from both layers (Table 3). Most of the isolates (41%) had a medium resistance (R+), whereas highly and weakly resistant strains represented 22% and 21% of the isolates, respectively (Table 3). Only 16% of the strains were sensitive. Highly resistant strains (R++) were isolated from the surface microlayer ($n = 10$) or from both layers ($n = 10$) (Tables 3 and 4). No isolate collected only from underlying waters was highly resistant. A slightly larger fraction of the isolates from underlying waters (22%) were sensitive than that of isolates collected from the surface microlayer or both biotopes (17% and 5%, respectively) (Table 3).

Taxonomic affiliation of isolates. Exposure to simulated solar radiation revealed that 41% of γ-proteobacteria were highly resistant, while only 14% and 8% of α-proteobacteria

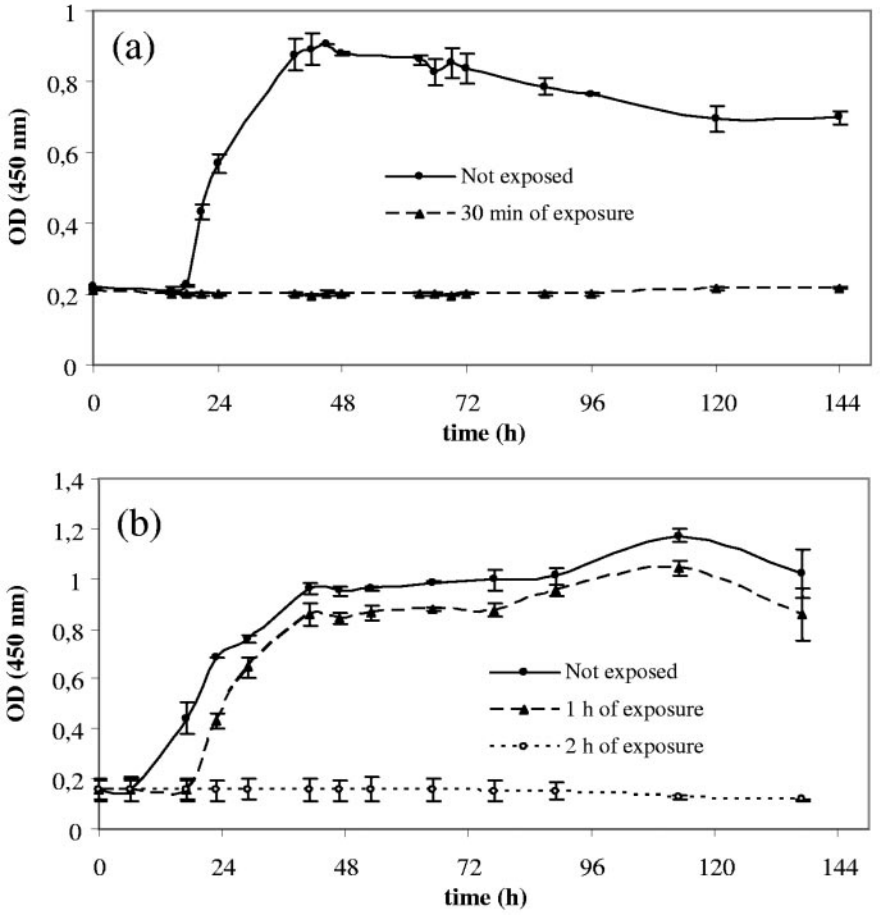

FIG. 1. Representative growth curves of a sensitive strain (S-140; Table 4) (a) and a weakly resistant strain (S-068; Table 4) (b).

and members of the *Cytophaga*/*Flavobacterium*/*Bacteroides* (CFB) group, respectively, belonged to this class of resistance. Similarly, only 9% and 15% of the *Actinobacteria* and the low-G+C gram-positive (LGC) strains, respectively, were highly resistant to simulated solar radiation. A high percentage of the isolates (31% to 46%) of α-proteobacteria, *Actinobacteria*, and LGC strains belonged to the class of medium resistance (Table 3). The relative contribution of isolates with medium resistance was particularly high for the CFB group (76%). Within the R++ γ-proteobacterial strains, the dominant genera were *Pseudoalteromonas* (61%) and *Alteromonas* (23%) (Table 4). For the class of weakly resistant strains (R), the contribution of each taxonomic group varied between 8% and 29%. The LGC group showed the largest relative contribution to sensitive strains (S) (31%) (Table 3). In contrast, strains belonging to the γ-proteobacteria and the CFB group attributed only 9% and 8% of the sensitive strains (Table 3).

No relationship was observed between the resistance to simulated solar radiation and the G+C content of the species (Table 3). The R++ class was characterized by isolates with a G+C content ranging from 35.5 to 62.4%. This range of values was similar to that of sensitive strains, which shared a G+C content of 30 to 67%.

Pigmentation of isolates. The numbers of pigmented and nonpigmented strains were fairly similar (41 and 49 isolates, respectively). Overall, similar percentages of pigmented strains were isolated from the surface microlayer (43% of strains), underlying waters (48% of strains), and both layers (48% of strains). The majority of pigmented strains (53%) had a medium resistance (R+), but pigmented strains had a smaller relative contribution (10%) to the highly resistant class (R++) than nonpigmented strains (33%) (Table 3). Among the sensitive and weakly resistant strains, pigmented and nonpigmented strains were equally distributed.

Within the γ-proteobacteria, 84% of the strains were nonpigmented. In contrast, all of the strains belonging to the CFB group ($n = 13$) were pigmented (Table 4). Of the R++ γ-proteobacterial strains, all of the strains belonging to the *Pseudoalteromonas* and *Alteromonas* genera were nonpigmented (eight and three isolates, respectively) (Table 4). Among the pigmented isolates, we determined that four isolates belonged to the AAnPs (37), including *Erythrobacter litoralis*, which was sensitive; *Roseobacter gallaeciensis* and *Erythrobacter flavus*, which had a medium resistance; and *Erythrobacter citreus*, which was highly resistant (Table 4).

DISCUSSION

Resistance of neustonic versus nonneustonic strains. The interspecific variability of the sunlight-induced inhibition of

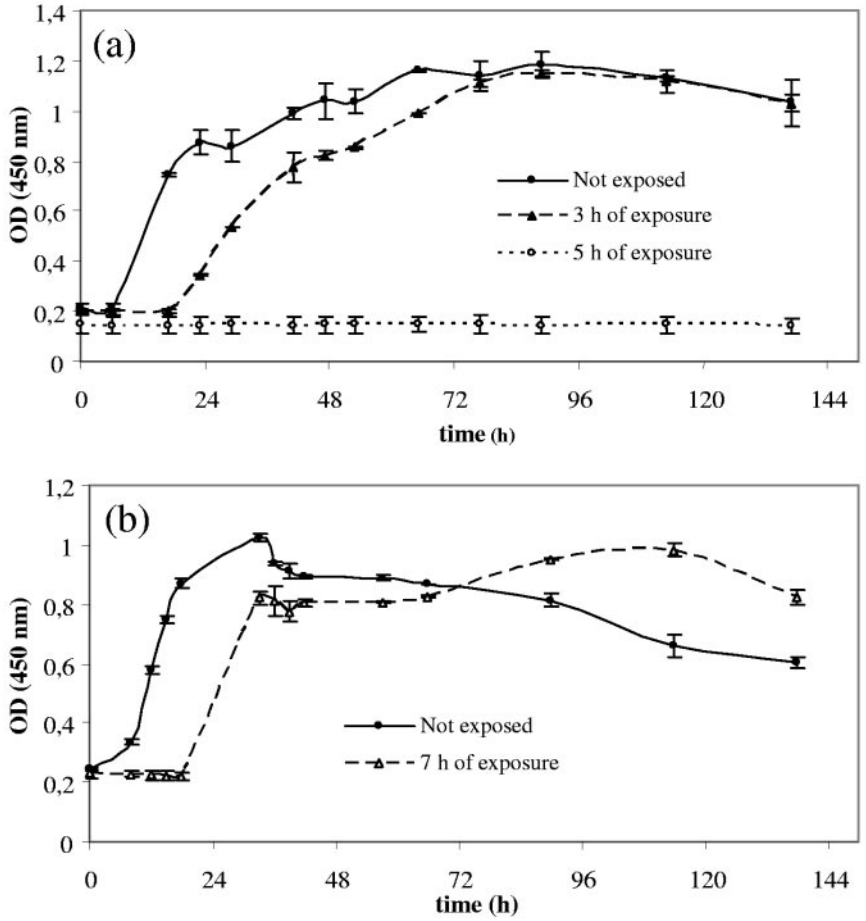

FIG. 2. Representative growth curves of a strain with medium resistance (U-220; Table 4) (a) and a highly resistant strain (SU-003; Table 4) (b).

growth of selected marine bacterial isolates was determined under laboratory conditions using a solar simulator. The conditions of exposure were very close to those found in the natural environment from which the bacterial species were isolated. Seven hours of radiation corresponded to two-thirds of the daily dose received by the bacterial community at the air-water interface in the Bay of Banyuls-sur-Mer during a sunny summer day (Table 1).

The underlying hypothesis of the present study is that the bacterioneuston is more resistant to solar radiation due to adaptive strategies developed in the surface microlayer. However, in the present study, no relationship was found between the sensitivity of the isolates to solar radiation and the biotope from which they were isolated (i.e., the surface microlayer or underlying waters). This suggests that resistance to radiation is well distributed among bacterial species present in the surface microlayer and subsurface waters. Similarly, no significant differences in the inhibition of bacterioneuston and bacterioplankton activity (determined as [^{3}H]leucine incorporation) were observed when natural bacterial communities from the respective environments were exposed to solar radiation (G. J. Herndl, unpublished data). In a 1-year study in the Chesapeake Bay, Bailey et al. (4) found no correlation between the depth of sampling (6 mm and 8.5 m) and the survival of bacteria exposed to surface solar radiation. For the northern Adriatic Sea, Herndl et al. (20) reported that bacterioplankton from near-surface (0.5-m depth) waters of a highly stratified water column were as sensitive to surface UV-B radiation as subpycnocline bacteria (20-m depth). They concluded that adaptive mechanisms against surface solar radiation are not present in near-surface bacterioplankton consortia. Similarly, when investigating the sensitivity of bacteria isolated from various marine environments (i.e., marine snow, sediment, and ambient water) which received different intensities of UV radiation, Arrieta et al. (3) found no relationship between the

TABLE 2. Categories of highly resistant strains (R++) according to the lag time of the growth curve after different time periods of exposure to simulated solar radiation

Category of R++ strains	Abbreviation	Lag time (h)		
		After 1 h of exposure	After 3 h of exposure	After 5 h of exposure
1	C1	0	0	<6
2	C2	<6	<6	$6 < t < 12$
3	C3	>6	>12	>24

TABLE 3. Relative contribution of strains in each class of resistance according to the depth layer where they were collected, their taxonomic affiliation, their pigmentation, and their G+C content

Class of resistance	No. of isolates (n = 90)	% of isolates from indicated origin[a]			% of isolates with indicated taxonomic affiliation					% of isolates with pigmentation[b]		G+C content (%)
					Gram-negative organisms			Gram-positive organisms				
		S (n = 46)	SU (n = 21)	U (n = 23)	γ-*Proteobacteria* (n = 32)	α-*Proteobacteria* (n = 21)	CFB group (n = 13)	*Actinobacteria* (n = 11)	LGC group (n = 13)	P^+ (n = 41)	P^- (n = 49)	
S	16	17	5	22	9	19	8	18	31	17	14	30–67 (n = 7)
R	21	24	9	26	19	29	8	27	23	20	22	31.7–73 (n = 12)
R+	41	37	38	52	31	38	76	46	31	53	31	32–73 (n = 22)
R++	22	22	48	0	41	14	8	9	15	10	33	35.5–62.4 (n = 18)

[a] S, strains isolated from the surface microlayer; U, strains isolated from underlying waters; SU, strains isolated from the both layers.
[b] P^+, visible pigmentation; P^-, no visible pigmentation.

UV sensitivity of the isolates and the environments from which they originated.

Although bacterial isolates from the sea surface microlayer do not seem to be more resistant to solar radiation than bacterioplankton isolates, several environmental factors could explain the survival of bacterioneuston exposed to a high level of solar radiation. Exopolysaccharides secreted by bacteria, algae, and other marine organisms accumulate in the surface microlayer (30, 39). Exopolysaccharides have been reported to provide protection from environmental stresses, such as pH shifts, osmotic shock, desiccation, and UV radiation (10). Furthermore, the surface microlayer is characterized by higher concentrations of chromophoric dissolved organic matter and particulate organic matter than those in underlying waters (8, 17, 35, 43). The accumulation of organic matter of different origins in the surface microlayer could provide in situ protection from solar radiation to bacterioneuston. Efficient DNA repair mechanisms likely also account for the high abundance and activity of bacterioneuston.

Interspecific variability of resistance to solar radiation. A large variability in the resistance to solar radiation was found among species, but γ-proteobacteria and CFB bacteria have high contributions to the R+ and R++ classes. Similar results have recently been reported by others (3, 23). Sunlight could therefore potentially influence the species composition of marine bacterioplankton in surface waters. Within the γ-*Proteobacteria*, some genera were dominated by highly resistant isolates. The genera *Pseudoalteromonas* and *Alteromonas* contained seven and two highly resistant species, respectively. The fraction of sensitive bacteria was the lowest for the CFB group and γ-*Proteobacteria*. This may partly explain the occurrence of these groups in marine surface waters (26).

The harmful effects of UV-B radiation on DNA are mostly explained in terms of the formation of dimeric photoproducts involving two adjacent pyrimidine bases. Moreover, it was recently suggested that AT (adenine and thymine)-rich DNA contributes to UV damage by enhancing the generation of ROS, which cause oxidative damage (42). Therefore, as proposed by Singer and Ames (40), bacteria adapted to sunlight exposure may have evolved a higher guanine-plus-cytosine content (G+C content) in the DNA to avoid dimeric pyrimidine photoproducts and oxidative damage. Some evidence supporting this hypothesis has been obtained (30, 34). Kellogg and Paul (25) reported a high correlation between the G+C contents of marine phage DNAs and the degree of DNA damage. However, we found no correlation between the resistance of bacterial species and their G+C content. These results are consistent with other observations reported in the literature (13, 23). Consequently, the most resistant strains may have developed other resistance mechanisms that allow them to survive high doses of UV radiation.

Role of pigmentation in resistance to solar radiation. In contrast to the case in previous studies, UV sensitivity was not related to pigmentation in the present study. Maki (31) and others (19, 34) have suggested that pigments are effective at protecting bacterioneuston against solar radiation. Similarly, Wu et al. (44) reported that a colorless mutant of the extreme halophilic archaebacterium *Halobacterium cutirubrum* was more sensitive to UV light than the wild-type strains, which possessed bacteriorhodopsin and bacterioruberin, two major carotenoid pigments. For these authors and Mathews and Sistrom (33), carotenoid pigments appeared to contribute to the resistance to UV irradiation. Carotenoids were found to protect microorganisms from UV and visible light damage by quenching triplet-state photosensitizers and ROS (7, 32). In the surface microlayer of the Black Sea, the number of pigmented cells, primarily yellow, often exceeded that in underlying waters (41). A significantly higher percentage of pigmented cells, primarily red (i.e., pink, red, or brown), were found in the surface microlayer (52% ± 22%) than in underlying waters (12% ± 7%) for four stations near the Swedish west coast (19). The larger proportion of pigmented cells may be indirect evidence of resistance to intense solar radiation at the interface. However, this protective effect of pigments was never demonstrated, and the results reported in the present study do not support this hypothesis. The heterogeneity of resistance observed within the AAnPs (aerobic anoxygenic phototrophs) indicates that resistance to solar radiation is not attributable to bacteriochlorophyll *a*. We observed that most of the highly resistant isolates were nonpigmented strains. R++ γ-proteobacterial strains belonging to the *Pseudoalteromonas* and *Alteromonas* genera were nonpigmented. Also, Gascon et al. (13) reported that strains of *Rhodobacter sphaeroides* with high levels of pigment (associated with phototrophic growth) were more sensitive to UV-C irradiation than strains with less pigment (associated with heterotrophic growth). From the present study, there is clear evidence that there is no direct correlation between pigmentation, high solar radiation levels,

TABLE 4. Resistance to simulated solar radiation of bacterial strains isolated from the sea surface microlayer (S), underlying waters (U), and both layers (SU)

Strain	Bacterial group	Closest relative species in the 16S rRNA gene sequence database	% Sequence similarity	Accession no.	Pigmentation	Class of resistance
Strains from surface microlayer						
S-156	γ-*Proteobacteria*	*Alteromonas infernus*	98	AY576745	None	R++ C1
S-242a	γ-*Proteobacteria*	*Alcanivorax venustensis*	100	AY576775	None	R
S-151	γ-*Proteobacteria*	*Enterobacter sakazakii*	99	AY576743	Orange	R++ C2
S-218	γ-*Proteobacteria*	*Glaciecola mesophila*	95	AY576759	None	R+
S-219	γ-*Proteobacteria*	*Marinobacter litoralis*	99	AY576760	None	R++ C2
S-131	γ-*Proteobacteria*	*Marinobacterium stanierii*	90	AY576729	None	R
S-240	γ-*Proteobacteria*	*Microbulbifer maritimus*	96	AY576773	None	R+
S-031	γ-*Proteobacteria*	*Oleispira antarctica*	91	AY576709	None	S
S-058	γ-*Proteobacteria*	*Pseudoalteromonas agarivorans*	99	AY576713	None	R++ C1
S-016	γ-*Proteobacteria*	*Pseudoalteromonas agarivorans*	92	AY576698	None	R+
S-067	γ-*Proteobacteria*	*Pseudoalteromonas anguilliseptica*	96	AY576718	None	R++ C2
S-137	γ-*Proteobacteria*	*Pseudoalteromonas mariniglutinosa*	99	AY576733	None	R++ C1
S-235	γ-*Proteobacteria*	*Pseudomonas pseudoalcaligenes*	90	AY576769	Brown	R+
S-066	γ-*Proteobacteria*	*Pseudomonas putida*	98	AY576717	None	R+
S-025	γ-*Proteobacteria*	*Pseudoxanthomonas broegbernensis*	97	AY576708	None	R
S-023	α-*Proteobacteria*	*Agrobacterium tumefasciens*	99	AY576704	None	R+
S-136	α-*Proteobacteria*	*Brevundimonas intermedia*	98	AY576732	None	S
S-140	α-*Proteobacteria*	*Erythrobacter litoralis*	98	AY576736	Red	S
S-143	α-*Proteobacteria*	*Jannaschia helgolandensis*	95	AY576739	None	R
S-069	α-*Proteobacteria*	*Paracoccus aminophilus*	98	AY576720	None	S
S-070	α-*Proteobacteria*	*Paracoccus marcusii*	99	AY576721	Orange	R+
S-132	α-*Proteobacteria*	*Salipiger mucescens*	97	AY615725	None	R+
S-236	α-*Proteobacteria*	*Ruegiera atlantica*	95	AY576770	Pink	R
S-232	α-*Proteobacteria*	*Stappia aggregata*	93	AY576766	None	R
S-032	α-*Proteobacteria*	*Sulfitobacter deliciae*	94	AY576710	None	R+
S-139	α-*Proteobacteria*	*Sulfitobacter pontiacus*	99	AY576735	None	R++ C3
S-061	CFB group	*Algibacter lectus*	95	AY576741	Yellow	R+
S-010	CFB group	*Maribacter sedimenticola*	97	AY576693	Yellow	R+
S-169	CFB group	*Mesonia algae*	92	AY576752	Orange	R+
S-155	CFB group	*Muricauda ruestringensis*	97	AY576744	Orange	R++ C2
S-068	CFB group	*Salegentibacter salegens*	95	AY576719	Yellow	R
S-030	*Actinobacteria*	*Arthrobacter agilis*	99	AY576708	Pink	R+
S-028	*Actinobacteria*	*Arthrobacter nitroguajacolicus*	100	AY576707	Yellow	S
S-017	*Actinobacteria*	*Brachybacterium tyrofermentans*	98	AY576699	Yellow	R+
S-014	*Actinobacteria*	*Corynebacterium ammoniagenes*	97	AY576697	None	R++ C2
S-021	*Actinobacteria*	*Dietzia maris*	100	AY576702	Orange	R
S-148	*Actinobacteria*	*Microbacterium esteraromaticum*	99	AY576742	Yellow	R
S-011	*Actinobacteria*	*Microbacterium kitamiense*	99	AY576694	Orange	R+
S-022	*Actinobacteria*	*Micrococcus luteus*	99	AY576703	Yellow	R
S-020	*Actinobacteria*	*Nocardioides jensenii*	96	AY576701	Yellow	S
S-135	LGC group	*Bacillus cereus*	100	AY576731	None	R
S-007	LGC group	*Bacillus firmus*	99	AY576692	None	R++ C2
S-142b	LGC group	*Paenibacillus glucanolyticus*	99	AY576738	None	S
S-167	LGC group	*Planococcus rifietoensis*	99	AY576750	Orange	R+
S-027	LGC group	*Staphylococcus aureus*	99	AY576706	None	R+
S-063	LGC group	*Staphylococcus warnerii*	99	AY576715	Yellow	S
Strains from underlying waters						
U-071	γ-*Proteobacteria*	*Aeromonas media*	100	AY576722	None	R
U-072	γ-*Proteobacteria*	*Acinetobacter johnsonii*	99	AY576723	None	R+
U-222	γ-*Proteobacteria*	*Glaciecola mesophila*	91	AY576763	None	S
U-168	γ-*Proteobacteria*	*Marinobacter sedimentalis*	90	AY576751	None	R
U-082	γ-*Proteobacteria*	*Photobacterium leiognathi*	99	AY576728	None	R+
U-220	γ-*Proteobacteria*	*Photobacterium leiognathi*	95	AY576761	None	R+
U-237	γ-*Proteobacteria*	*Pseudomonas jessenii*	91	AY576771	Black	S
U-241	γ-*Proteobacteria*	*Shewanella baltica*	99	AY576774	Brown	R+
U-080	γ-*Proteobacteria*	*Shewanella putrefacienos*	99	AY576727	Brown	R
U-233	α-*Proteobacteria*	*Brevunodimonas alba*	97	AY576767	None	R
U-215	α-*Proteobacteria*	*Hyphomonas johnsonii*	92	AY576758	Orange	S
U-160	α-*Proteobacteria*	*Paracoccus aminovorans*	98	AY576746	Orange	R
U-210	α-*Proteobacteria*	*Porphyrobacter sanguineus*	99	AY576755	None	R+
U-234	α-*Proteobacteria*	*Roseobacter gallaeciensis*	98	AY576768	Red	R+

Continued on following page

TABLE 4—*Continued*

Strain	Bacterial group	Closest relative species in the 16S rRNA gene sequence database	% Sequence similarity	Accession no.	Pigmentation	Class of resistance
U-075	α-*Proteobacteria*	*Stappia aggregata*	99	AY576725	None	R+
U-244	CFB group	*Cytophaga latercula*	98	AY576777	Brown	R+
U-211	CFB group	*Hongiella mannitolivorans*	99	AY576756	Pink	R+
U-243	CFB group	*Muricauda ruestringensis*	97	AY576776	Brown	R+
U-012	CFB group	*Tenacibaculum mesophilum*	93	AY576695	Yellow	S
U-077	*Actinobacteria*	*Salinibacterium amurskyense*	96	AY576726	Yellow	R+
U-145	LGC group	*Bacillus megaterium*	99	AY576740	None	R+
U-073	LGC group	*Enterococcus faecium*	100	AY576724	None	R
U-033	LGC group	*Staphylococcus epidermidis*	99	AY576711	None	S
Strains from surface microlayer and underlying waters						
SU-003	γ-*Proteobacteria*	*Alteromonas macleodii*	98	AY576689	None	R++ C2
SU-229	γ-*Proteobacteria*	*Alteromonas marina*	98	AY576765	None	R++ C2
SU-053	γ-*Proteobacteria*	*Pseudoalteromonas atlantica*	99	AY576712	None	R++ C1
SU-209	γ-*Proteobacteria*	*Pseudoalteromonas citrea*	99	AY576754	None	R++ C2
SU-208	γ-*Proteobacteria*	*Pseudoalteromonas elyakovii*	98	AY576753	None	R++ C1
SU-213	γ-*Proteobacteria*	*Pseudomonas stutzeri*	100	AY576757	None	R++ C2
SU-221	γ-*Proteobacteria*	*Pseudoalteromonas tetraodonis*	100	AY576762	None	R++ C1
SU-018	γ-*Proteobacteria*	*Vibrio splendidus*	99	AY576700	None	R+
SU-162	α-*Proteobacteria*	*Brevundimonas aurantiaca*	99	AY576748	Orange	R
SU-065	α-*Proteobacteria*	*Erythrobacter citreus*	99	AY576716	Brown	R++ C3
SU-228	α-*Proteobacteria*	*Erythrobacter flavus*	99	AY576764	Yellow	R+
SU-004	α-*Proteobacteria*	*Roseobacter gallaeciensis*	96	AY576690	None	R++ C1
SU-134	CFB group	*Algibacter lectus*	95	AY576730	Yellow	R+
SU-164	CFB group	*Cellulophaga lytica*	88	AY576749	Orange	R+
SU-006	CFB group	*Flexibacter tractuosus*	94	AY576691	Yellow	R+
SU-239	CFB group	*Hongiella ornithinivorans*	99	AY576772	Pink	R+
SU-013	*Actinobacteria*	*Blastococcus aggregatus*	99	AY576696	Pink	R+
SU-161	LGC group	*Bacillus horikoshii*	99	AY576747	Orange	R+
SU-146	LGC group	*Bacillus thuringiensis*	99	AY576741	None	R
SU-141	LGC group	*Exiguobacterium aurantiacum*	99	AY576737	Orange	R++ C2
SU-138	LGC group	*Staphylococcus pasteuri*	99	AY576734	None	S

and the occurrence of bacteria in the surface microlayer. Therefore, pigmentation may have only an indirect effect on the resistance of bacterial cells to solar radiation.

Conclusion. Our results demonstrate (i) similar distributions of resistant bacterial isolates in the surface microlayer and subsurface waters, (ii) a large interspecific variability of resistance to solar radiation, and (iii) the lack of a direct relationship between pigmentation and the resistance of marine isolates to solar radiation.

Physiological traits such as carotenoids, sunscreen molecules, and polysaccharides could be additional factors determining the resistance of bacteria to solar radiation. The rapid recovery from UV stress of several species, as determined in the present study, should encourage further investigations in order to characterize the mechanisms involved in the resistance of marine bacteria to solar radiation.

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REFERENCES

1. **Agogué, H., E. O. Casamayor, F. Joux, I. Obernosterer, C. Dupuy, F. Lantoine, P. Catala, M. G. Weinbauer, T. Rheinthaler, G. J. Herndl, and P. Lebaron.** 2004. Comparison of samplers for the biological characterization of the sea surface microlayer. Limnol. Oceanogr. Methods **2:**213–225.
2. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. Lipman.** 1990. Basic Local Alignment Search Tool. J. Mol. Biol. **215:**403–410.
3. **Arrieta, J. M., M. G. Weinbauer, and G. J. Herndl.** 2000. Interspecific variability in sensitivity to UV radiation and subsequent recovery of selected isolates of marine bacteria. Appl. Environ. Microbiol. **66:**1468–1473.
4. **Bailey, C. A., R. A. Neihof, and P. S. Tabor.** 1983. Inhibitory effect of solar radiation on amino acid uptake in Chesapeake Bay bacteria. Appl. Environ. Microbiol. **46:**44–49.
5. **Beja, O., L. Aravind, E. V. Koonin, M. T. Suzuki, A. Hadd, L. P. Nguyen, S. B. Jovanovich, C. M. Gates, R. A. Feldman, J. L. Spudich, and E. F. DeLong.** 2000. Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. Science **289:**1902–1906.
6. **Beja, O., M. T. Suzuki, J. F. Heidelberg, W. C. Nelson, C. M. Preston, T. Hamada, J. A. Eisen, C. M. Fraser, and E. F. DeLong.** 2002. Unsuspected diversity among marine aerobic anoxygenic phototrophs. Nature **415:**630–633.
7. **Carbonneau, M. A., A. Melin, A. Perromat, and M. Clerc.** 1989. The action of free radicals on *Deinococcus radiodurans* carotenoids. Arch. Biochem. Biophys. **275:**244–251.
8. **Carlson, D. J.** 1982. Surface microlayer phenolic enrichments indicate sea surface slicks. Nature **296:**426–429.
9. **de la Torre, J. R., L. M. Christianson, O. Beja, M. T. Suzuki, D. M. Karl, J. Heidelberg, and E. F. DeLong.** 2003. Proteorhodopsin genes are distributed among divergent marine bacterial taxa. Proc. Natl. Acad. Sci. USA **100:**12830–12835.

10. **Elasri, M., and R. V. Miller.** 1999. Study of the response of a biofilm bacterial community to UV radiation. Appl. Environ. Microbiol. **65:**2025–2031.
11. **Garcia-Pichel, F.** 1994. A model for internal self-shading in planktonic organisms and its implications for the usefulness of ultraviolet sunscreens. Limnol. Oceanogr. **39:**1704–1717.
12. **Garcia-Pichel, F., and R. W. Castenholz.** 1993. Occurrence of UV-absorbing, mycosporine-like compounds among cyanobacterial isolates and an estimate of their screening capacity. Appl. Environ. Microbiol. **59:**163–169.
13. **Gascon, J., A. Oubina, A. Pérez-Lezaum, and J. Urmeneta.** 1995. Sensitivity of selected bacterial species to UV radiation. Curr. Microbiol. **30:**177–182.
14. **Hader, D. P.** 2000. Effects of solar UV-B radiation on aquatic ecosystems. Adv. Space Res. **12:**2029–2040.
15. **Hader, D. P., M. Lebert, R. Marangoni, and G. Colombetti.** 1999. ELDONET—European Light Dosimeter Network hardware and software. J. Photochem. Photobiol. B **52:**51–58.
16. **Hardy, J. T.** 1982. The sea surface microlayer: biology, chemistry and anthropogenic enrichment. Prog. Oceanogr. **11:**307–328.
17. **Harvey, R. W., and L. Y. Young.** 1980. Enrichment and association of bacteria and particulates in salt marsh surface waters. Appl. Environ. Microbiol. **39:**894–899.
18. **Helbling, E. W., E. R. Marguet, V. E. Villafane, and O. Holm-Hansen.** 1995. Bacterioplankton viability in Antarctic waters as affected by solar radiation. Mar. Ecol. Prog. Ser. **126:**293–298.
19. **Hermansson, M., G. W. Jones, and S. Kjelleberg.** 1987. Frequency of antibiotic and heavy metal resistance, pigmentation, and plasmids in bacteria of the marine air-water interface. Appl. Environ. Microbiol. **53:**2338–2342.
20. **Herndl, G. J., G. Müller-Niklas, and J. Frick.** 1993. Major role of ultraviolet-B in controlling bacterioplankton growth in the surface layer of the ocean. Nature **361:**717–719.
21. **Jeffrey, W. H., J. Kase, and S. W. Wilhelm.** 2000. Ultraviolet radiation effects on heterotrophic bacterioplankton and viruses in marine ecosystems, p. 206–236. *In* S. De Mora, S. Demers, and M. Vernet (ed.), The effects of UV radiation in the marine environment. Cambridge University Press, Cambridge, United Kingdom.
22. **Jeffrey, W. H., R. Pledger, P. Aas, S. Hager, R. B. Coffin, R. von Haven, and D. L. Mitchell.** 1996. Diel and depth profiles of DNA photodamage in bacterioplankton exposed to ambient solar radiation. Mar. Ecol. Prog. Ser. **137:**283–291.
23. **Joux, F., W. H. Jeffrey, P. Lebaron, and D. L. Mitchell.** 1999. Marine bacterial isolates display diverse responses to UV-B radiation. Appl. Environ. Microbiol. **65:**3820–3827.
24. **Karentz, D., M. L. Bothwell, R. B. Coffin, A. Hanson, G. J. Herndl, S. S. Kilham, M. P. Lesser, M. Lindell, R. E. Moeller, D. P. Morris, P. J. Neale, R. W. Sanders, C. S. Weiler, and R. G. Wetzel.** 1994. Impact of UV-B radiation on pelagic freshwater ecosystems: report of working group on bacteria and phytoplankton. Arch. Hydrobiol. Beih. **43:**31–69.
25. **Kellogg, C. A., and J. H. Paul.** 2002. Degree of ultraviolet radiation damage and repair capabilities are related to G+C content in marine vibriophages. Aquat. Microb. Ecol. **27:**13–20.
26. **Kirchman, D. L.** 2002. The ecology of *Cytophaga-Flavobacteria* in aquatic environments. FEMS Microbiol. Ecol. **39:**91–100.
27. **Kolber, Z. S., C. L. Van Dover, R. A. Niederman, and P. G. Falkowski.** 2000. Bacterial photosynthesis in surface waters of the open ocean. Nature **407:**177–179.
28. **Lebaron, P., N. Parthuisot, and P. Catala.** 1998. Comparison of blue nucleic acid dyes for flow cytometric enumeration of bacteria in aquatic systems. Appl. Environ. Microbiol. **64:**1725–1730.
29. **Liss, P. S., and R. A. Duce.** 1997. The sea surface and global change. Cambridge University Press, Cambridge, United Kingdom.
30. **Maki, J. S.** 1993. The air-water interface as an extreme environment, p. 409–440. *In* T. E. Ford (ed.), Aquatic microbiology: an ecological approach. Blackwell Scientific Publications, Boston, Mass.
31. **Maki, J. S.** 2002. Neuston microbiology: life at the air-water interface, p. 2133–2144. *In* G. Bitton (ed.), The encyclopedia of environmental microbiology. John Wiley & Sons, New York, N.Y.
32. **Margalith, P. Z. (ed.).** 1992. Pigment microbiology. Chapman and Hall, London, United Kingdom.
33. **Mathews, M. M., and W. R. Sistrom.** 1959. Function of carotenoid pigments in non-photosynthetic bacteria. Nature **184:**1892–1893.
34. **Norkrans, B.** 1980. Surface microlayers in aquatic environments. Adv. Microb. Ecol. **4:**51–83.
35. **Obernosterer, I., P. Catala, T. Rheinthaler, G. J. Herndl, and P. Lebaron.** 2005. Enhanced heterotrophic activity in the surface microlayer of the Mediterranean Sea. Aquat. Microb. Ecol. **39:**293–302.
36. **Obernosterer, I., B. Reitner, and G. J. Herndl.** 1999. Contrasting effects of solar radiation on dissolved organic matter and its bioavailability to marine bacterioplankton. Limnol. Oceanogr. **44:**1645–1654.
37. **Oz, A., G. Sabehi, M. Koblizek, R. Massana, and O. Beja.** 2005. *Roseobacter*-like bacteria in red and Mediterranean sea aerobic anoxygenic photosynthetic populations. Appl. Environ. Microbiol. **71:**344–353.
38. **Shick, J. M., and W. C. Dunlap.** 2002. Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. Annu. Rev. Physiol. **64:**223–262.
39. **Sieburth, J. M. N., P. J. Willis, K. M. Johnson, C. M. Burney, D. M. Lavoie, K. R. Hinga, D. A. Caron, F. W. French III, P. W. Johnson, and P. G. Davis.** 1976. Dissolved organic matter and heterotrophic microneuston in the surface microlayers of the North Atlantic. Science **194:**1415–1418.
40. **Singer, C. E., and B. N. Ames.** 1970. Sunlight ultraviolet and bacterial DNA base ratios. Science **170:**822–826.
41. **Tsyban, A. V.** 1971. Marine bacterioneuston. J. Oceanogr. Soc. Jpn. **27:**56–66.
42. **Wei, H., Q. Ca, R. Rahn, X. Zhang, Y. Wang, and M. Lebwohl.** 1998. DNA structural integrity and base composition affect ultraviolet light-induced oxidative DNA damage. Biochemistry **37:**6485–6490.
43. **Whitehead, K., and M. Vernet.** 2000. Influence of mycosporine-like amino acids (MAAs) on UV absorption by particulate and dissolved organic matter in La Jolla Bay. Limnol. Oceanogr. **45:**1788–1796.
44. **Wu, L.-C., K.-C. Chow, and K.-K. Mark.** 1983. The role of pigments in *Halobacterium cutirubrum* against UV radiation. Microbios Lett. **24:**85–90.
45. **Zuev, B. K., V. V. Chudinova, V. V. Kovalenko, and V. V. Yagov.** 2001. The conditions of formation of the chemical composition of the sea surface microlayer and techniques for studying organic matter in it. Geochem. Int. **39:**773–784.