Distribution of Lipolytic, Proteolytic, and Amylolytic Marine Bacteria between the Lipid Film and the Subsurface Water

S. Kjelleberg and N. Håkansson

1 Department of Marine Microbiology, University of Göteborg; Göteborg, Sweden
2 Department of Microbial Ecology, University of Lund; Lund, Sweden

Abstract

The biochemical activity of marine bacteria adhering to the lipid film at the air-water interface was compared to that of bacteria inhabiting the subsurface water. The bacteria were isolated from the two levels at 7 stations, all located along Brofjorden on the Swedish West Coast. The isolates were tested for their ability to attack lipids, proteins, and carbohydrates. A higher percentage of active isolates was found in samples from the subsurface water than from the surface film. The accumulation of marine bacteria at the air-water interface is not due to their biochemical activity.

Introduction

During recent years, the physics and chemistry of the air-sea interface has been intensively studied and the microbial interaction with the surface film has received some attention (e.g. Garret, 1970; Jeffrey, 1970; Bezdek and Carlucci, 1972).

Triglycerides, free fatty acids and wax esters are the predominant constituents of the layered surface film (Garret, 1966). Proteinaceous surface molecules are attached from beneath in a more random way than the oily and fatty surface molecules forming the layer (MacIntyre, 1974). The substances at the air-water interface differ chemically from those dissolved in the subsurface water. When competitive adsorptive processes occur, short-chain fatty acids and alcohols are forced out from the film by longer-chain entities. The latter are less water-soluble and more surface-active (Garret, 1966).

Measurements have shown that there is an accumulation of microorganisms in the surface microlayer (Blanchard and Syzdek, 1970; Bezdek and Carlucci, 1972). In model systems, a positive correlation also exists between the total count of microorganisms and the lipids collected (Norkrans and Sörensson, in preparation). Furthermore, Sieburth (1971) has shown the dominant film isolate, Pseudomonas sp., to be 95% lipolytic, 94% proteolytic and 28% amylolytic. On the other hand, he reports a much lower percentage of subsurface bacteria able to attack lipids and proteins, with the relative percentage of amylolytic ones being about twice as high here. The bulk (subsurface) water contains aggregates of bacteria, combined with adsorbed surface-active material (Baylor and Sutcliffe, 1963; Riley, 1963) or attached to micelles (Larsson et al., 1974).

Do the bacteria interact at the surface by decomposing the surface material? If this is the case, this could explain the bacterial enrichment at the surface. The present investigation was designed to study the biochemical activity of the bacteria, and their enrichment when interacting with the surface film. Use of the surface-balance technique in model systems (Kjelleberg et al., 1976) clearly revealed an interaction between bacteria and lipid monolayers at the air-water interface.

Materials and Methods

Samplings and Isolation of Bacteria

Samplings were made at 7 stations in Brofjorden on the Swedish West Coast. The area (around 58°20'N; 11°20'E) is free of contaminating products and with a salinity in the range of 25 to 30%.
Fig. 1. Map of Brofjorden on the Swedish West Coast, showing location of sampling stations. Wave-height and water temperature for each station are also shown.

Fig. 1 gives the locations of the hydrographic stations, as well as the water temperatures and approximate waveheights. The investigation was made during the first week of July, 1975. All samplings were performed in the daytime. At each station, 6 surface and 6 bulk-water samples were taken, the latter at a depth of 1 m. A method for collecting surface films at the air-water interface has been developed by Larsson et al. (1974). The sampler is a Teflon plate measuring 15 x 15 cm, densely perforated with conical holes. As Teflon is strongly hydrophobic, dipping through the water surface results in deposition of lipids on the plate. In model systems with solid condensed phases of monomolecular films, 70 to 90% of the surface film was recovered after one dipping (Larsson et al., 1974). The Teflon plate was also efficient in the collection of microorganisms associated with the lipid monolayer (Odham et al., in press; Norkrans and Sörensson, in preparation). The amount of water remaining on the plate has been determined to be 2 g ± 0.5 g. At each sampling the plate was dipped once through the water surface. The organic material and organisms withdrawn was washed off with 100 ml solution of 2% Tween 80 in 2.5% NaCl. All samples were treated within 2 to 4 h. The bulk-water samples were taken with a Valäs-apparatus for water collection (Swedish patent, no. 177629). All samples were filtered through a 0.45-µm Millipore filter in a Millipore-field-monitor attached to disposable tubing. The filters were detached and placed on plates with a ZoBell medium for marine bacteria. This medium (2216 E) has the following composition: peptone 5.0 g; yeast extract 1.0 g; FePO₄ 0.01 g; aged seawater 1000 ml. In order to obtain an even distribution of bacteria on the filters, 0.5 ml of each surface "Tween suspension" and 1.0 ml of each bulk-water sample were mixed with 10 ml sterile seawater before filtrating. After incubation at ambient temperature (23°C) for 1 week, the colonies were counted using a stereo magnifier. The enrichment factors for the bacteria accumulated at the surface microlayer were determined.

According to a fixed pattern of evenly distributed points, 12 colonies from each filter were picked and streaked out on Medium 2216 E. These plates were incubated for a further week at room temperature, after which individual colonies from each plate were again streaked as above.

Assays for Biochemical Activity of the Isolates: Media and Multipoint Technique

From each station, between 117 and 144 isolates (see Table 2) were tested by the multipoint technique for their ability to split lipids, proteins and carbohydrates. For each of these classes of substances, two different media were used from the following: (1) LI (lipid I), LII (lipid II), PI (protein I), PII (protein II), CI (carbohydrate I) and CII (carbohydrate II).

The media used for assaying the biochemical activity were:

1. LI: Peptone 10.0 g, CaCl₂ 2H₂O 0.1 g, agar 13.0 g, aged seawater 1000 ml, and 10 ml of Tween 80 (Sierra, 1957). Tween 80 was sterilized separately and added when the basal solution was about 50°C.

2. LII: Peptone 5.0 g, yeast extract 3.0 g, glycercyltributyrate 10.0 g, agar 15.0 g, and aged seawater 1000 ml (Holding and Collee, 1971).

3. PI: Gelatine 125 g and aged seawater 1000 ml (Falser, personal communication).

4. PII: Gelatine 4.0 g, nutrient agar 15.0 g, and aged seawater 1000 ml (Holding and Collee, 1971).