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Saprospira grandis: A Flexibacterium That Can Catch Bacterial Prey by "Ixotrophy"

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Abstract

A marine, multicellular, filamentous flexibacterium, *Saprospira grandis* Gross, can not only live heterotrophically on dissolved organic substrata, but can subsist on other microbes and can even catch motile bacteria by their flagella before killing and digesting them.

Introduction

Saprospira grandis is not a spirochaete, though it was originally so described [6]. Although motile, it does not swim, but glides, and has been assigned to the Flexibacterales [11, 26, 27] or Cytophagales [10, 19]. The filaments (trichomes) are about 1 μm wide and of indefinite length (5–500 μm), with a shallow helical pitch of 5–10 μm. They consist of cells 1–5 μm long, which tend to separate as they die. They glide on solid substrata at 2 to 5 μm sec^{-1} (at 23–33°C), the movement being presumably effected by streams of surface mucilage, as in other flexibacteria (such as *Cytophaga*) [9].

Saprospira grandis is common in marine littoral sand and other coastal habitats; clones have been isolated from seashores around California, Hawaii, Massachusetts, Texas, Costa Rica, Mexico, the Galàpagos, Japan, Australia, New Zealand, Jugoslavia, and so forth [16]. Filaments, at a density of about 15 cm^{-2}, were also found at a depth of 1.6 km (in darkness, at less than 5°C), growing on a glass slide submerged offshore for 4 weeks near La Jolla, California (SEM observations, unpublished). Corresponding molecular-biological "signatures" have been recorded from suspended particles of marine detritus [4]. The cells (observed en masse) are peach colored and contain a unique carotenoid, saproxanthin [1], which may be photoprotective against damage by solar ultraviolet light. The guanine + cytosine proportion in the DNA is approximately 47% [17]. In old cultures the filaments disarticulate into single cells which lyse spontaneously, liberating cylindrical rhapidosomes of unknown function [12, 14, 15, 20].

It was earlier assumed that *Saprospira* lives only heterotrophically, although supplies of organic solutes are limited in its natural environments. However, it was subsequently discovered that it can also exist as a predator, killing and lysing microbes, including bacteria, on the surfaces over which it glides and grazes [26]. Among 58 strains of non-marine bacteria, 4 were lysed by all 8 freshwater *Saprospira* strains, and 28 others were lysed by at least some (V. Sangkhobol Arunpairojana, personal communication). Presumably, like other gliding organisms such as *Sorangium* [5], it employs enzymes that digest bacterial cell walls and intra-

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cellular proteins. It has also been reported to kill and digest eukaryotes, forming clear plaques on agar sown with a diatom, *Chaetoceros,* or a chrysophyte, *Isochrysis* [23–25]. However, it has not been clear how *Saprospira* in nature could obtain sufficient prey bacteria to sustain growth. The main purpose of this note is to report that it can catch them by ixotrophy. I define ''ixotrophy'' as feeding on prey caught on a sticky substance such as bird-lime or flypaper.

Methods

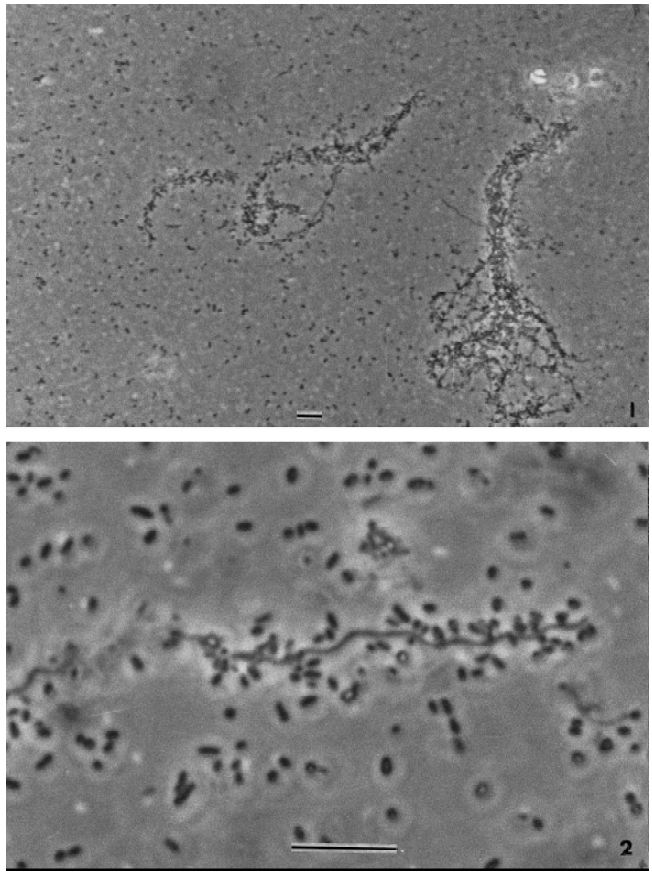
Three axenic cultures, taken from subtidal sand, polyethylene sheeting, and dead didemnid ascidian colonies in the Bahama Islands, were isolated by serial streaking on seawater agar supplemented with Tryptone and yeast extract (TY, 5.0 g l^{-1} each). They produce discrete, rounded colonies on this medium. On agar media containing low nutrient concentrations (e.g., TY 0.5 g l^{-1}), the trichomes migrate over the surface and the colonies are, consequently, more diffuse. In liquid cultures, prepared with synthetic seawater, the TY can be replaced by a mixture of the 10 amino acids essential for animals, together with asparagine (as an assimilable source of nitrogen) and an unidentified factor in nucleic acid hydrolysates [13]. Aspartic or glutamic acid can serve as a respiratory substrate; glucose cannot (Lewin and Leira, unpublished data). Disodium ethylenediaminetetraacetate (EDTA) was used to chelate calcium ions, and an organic buffer (HEPES) served to poise the pH for the experiment described below.

Cultures were maintained in liquid medium with weekly transfers. After about 2 weeks at room temperature old cultures tended to die out. However, suspended in seawater containing 10% glycerol and conserved at −80°C, they remained viable for at least several months. Cultures have been deposited with the American Type Culture Collection.

Results and Discussion

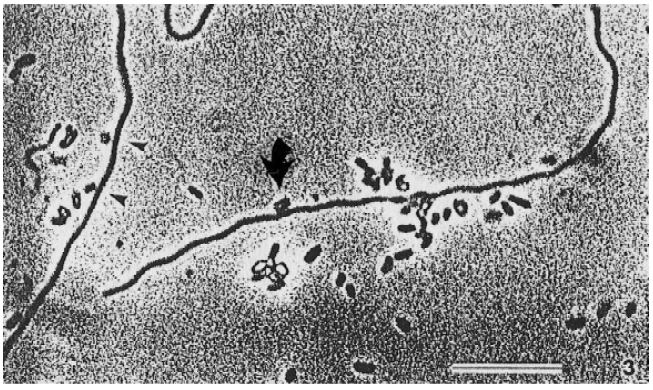
Cells of the prey bacteria were observed by phase-contrast microscopy (×400 diam.) to become attached by the tips of their flagella to the surfaces of the filaments. They apparently adhered to the thin, mucilaginous streams responsible for the gliding of *Saprospira,* or to goblet filaments extending from the outer cell membrane like those described for *Flexibacter polymorphus* [21]. However, no evidence for chemotactic attraction of the bacteria was observed. This ixotrophic phenomenon is very similar to particle adhesion and movement along the flagella of *Chlamydomonas* [2] and the haptonema of *Chrysochromulina* [8].

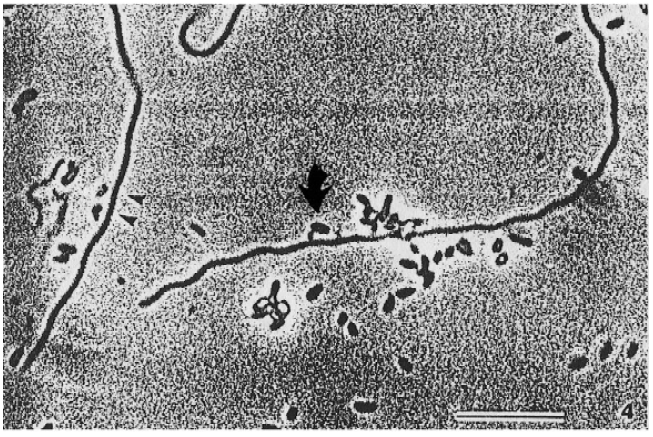
Within a few seconds after mixing, flagellated cells of an unidentified bacterial strain from the same Bahaman habitat adhered to filaments of *Saprospira* grown for 2–3 days in a seawater medium supplemented with TY 0.5 g l^{-1} (Figs. 1, 2). Filaments grown in richer media (containing TY 2.0 g l^{-1}) exhibited the phenomenon less clearly. From a dense suspension of similar susceptible prey bacteria (e.g., *Photobacterium leiognathi*), dozens of cells soon became attached to a single filament (Figs. 1, 2). The agglutination was unaffected by pretreatment of the filaments for 5 min with concanavalin A (Sigma, 1 g l^{-1}), an agent that specifically interferes with many cell-surface recognition phenomena involving mannose-containing glycoproteins. However, heat-killed cells were not caught.

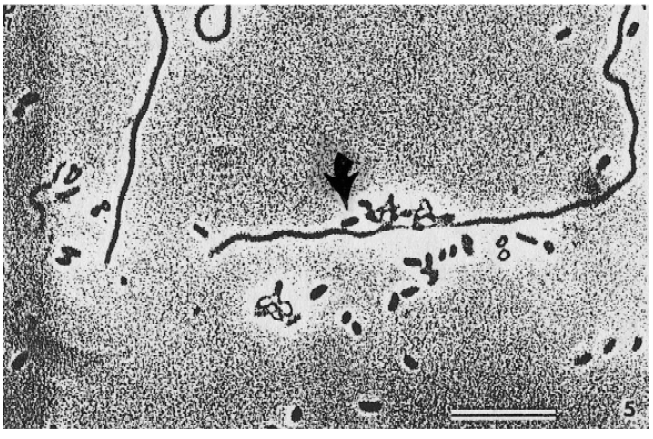


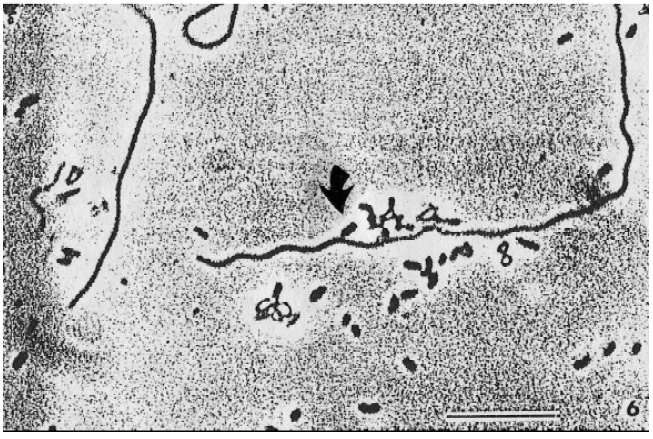
Figs. 1, 2. *Saprospira grandis* filaments 1 min after immersion in a suspension of flagellated cells of an oceanic *Vibrio* strain DS40.M5 [7]. Phase-contrast; *scale bar,* 10 μm.

When only a few cells were attached, they were observed to be moved, at rates comparable to the gliding speeds of the filaments, either toward the leading end or away from it (Figs. 3–6). Eventually, captured cells completely covered the surfaces of *Saprospira* filaments. Captured bacterial cells continued to spin on their axes, indicating that they were still alive when caught. From older (4- to 7-day) cultures of *P. leiognathi,* in which few or no cells were motile, few or









none were captured. In contrast, from 1-day cultures, in which almost all the cells had flagella, many cells were caught within a few seconds. This indicates that for potential prey species under some circumstances it might be selectively advantageous for them to shed their flagella, since "bald" cells are apparently not caught.

Attached bacteria may be moved simultaneously in both directions and pass one another, indicating that there are multiple mucilage strands streaming in opposite directions. When they reached an end, adherent bacteria were moved round the tip and back down the other side in the opposite direction, providing evidence for bidirectional movements among the motility strands, as has been reported for other gliding microbes [2, 3, 18]. Particles of India ink tended to adhere to the filaments, though at an efficiency considerably less than that exhibited by susceptible flagellated bacteria. The particles exhibited movements on helical translocational pathways, just like those described for *F. polymorphus* [22], although they were slower. As the helical *Saprospira* trichome glides and screws itself along, it rolls over and makes cell-to-cell contact with prey cells passing between it and its solid substratum.

In stationary cultures of the *Saprospira,* within a few days, sufficient extracellular polymer accumulated to hold the filaments at the bottom of the tube in a loose, readily dispersible clot. The mucilage may be an acidic polysaccharide, since it stains metachromatically (lilac) with Toluidine Blue O or Nile Blue A. When air-dried on a slide at room temperature and then de-salted by rinsing, the material retained some of its potential to catch bacteria by their flagella, as was observed when a droplet of a suspension of prey cells was applied to a patch of dried mucilage.

The following experiment indicated that few or no divalent ions (of calcium) were involved in the catching process. One milliliter of a suspension of *S. grandis* grown in enriched seawater was added to 9 ml of a calcium-free solution (700 mM NaCl, 10 mM EDTA, 10 mM HEPES, pH 7.5). Although the filaments were thereby rendered immotile, as reported also for *Cytophaga* [9], added bacterial prey cells, which retained their flagella and remained motile, were caught as usual. However, they were not transported along

Figs. 3–6. Squirming filaments of *Saprospira grandis* on which *Vibrio* cells have been caught by their flagella. Some prey bacteria (*arrows*) were translocated along the filaments by slime tracks. Phase-contrast photo-micrographs taken at 2-s intervals. *Scale bar,* 10 μm.

Table 1. List of bacteria (in Vibrionaceae) susceptible to ixotrophy and lysis by *Saprospira grandis*[a]

Potential prey species	ATTC #[b]	Caught	Lysed
Photobacterium leiognathi PL.721[c]		+	+
P. phosphoreum NZ.11D[c]		−	+
Vibrio aestuarius	35048	−	+
V. alginolyticus	17749	+	+
V. angustus B.70[c]		+	+
V. campbellii	25920	+	+
V. carcharinae	35084	−	+
V. cholerae	14033	−	+
V. damsela	33539	−	+
V. diazotrophicus	33466	−	+
V. furnissii	35016	+	+
V. fischeri MJ.1[c]		−	+
V. gazogenes	29988	−	−
V. harveyi B.392[c]		−	−
V. harveyi BB.7[c]		−	+
V. marinus	15381	−	+
V. meridianus nom prov.[c]		+	+
V. metschnikovii	7708	−	+
V. nereis	25917	−	+
V. opisthoproctus nom. prov.[c]		+	+
V. orientalis	33933	−	−
V. proteolyticus	15338	−	−
V. splendidus	33869	−	−
V. vulnificus	27236	−	+
V. vulnificus 46[c]		−	+

[a] Catching was recorded in liquid suspensions, lysis on agar medium in plates
[b] ATCC #, American Type Culture Collection accession number
[c] Other cultures from the Haygood culture collection

the *Saprospira* filaments, which was to be expected since flexibacterial motility is attributable to linear transport of superficial slime. In solutions containing higher concentrations of EDTA, and consequently even fewer free calcium ions, the *Saprospira* filaments disintegrated. In the same medium to which 1 mM $CaCl_2$ had been added, both adhesion and translocation were evidently unimpaired.

Not all kinds of flagellated bacteria can be caught by *Saprospira* filaments in this way. Among 25 tested cultures of marine Vibrionaceae from the culture collection of Margo Haygood (Table 1), only 7 could be caught, suggesting that in this family of bacteria the flagella (at least at the tips) are biochemically different. Tested under similar axenic conditions in seawater medium, cells of three strains of a nonmarine bacterium, *Escherichia coli* (J.100, TSM.39, XLI-blue), though flagellated, were not caught, nor were eukaryotic cells of *Isochrysis galbana* (CCMP 1323) (a flagellate chrysophyte), *Chaetoceros neogracile* (CCMP 1318) (a diatom), *Micromonas pusillus* (a flagellate chlorophyte), or a motile unicellular cyanophyte, *Synechococcus* sp. (strain WH 8102).

On the surface of agar medium seeded with prey bacteria and a few filaments of *S. grandis,* and incubated at 30°C, the *Saprospira* filaments propagated and formed clear plaques reaching 4 mm in diameter in 3 days. Within such plaques, the prey bacteria had been killed, lysed, and digested; the only intact organisms that remained were the ixotrophic filaments. Colonies of *Saprospira* and potential prey bacteria were placed at various distances (0–10 mm) from one another on nonnutrient seawater agar, but no evidence for chemotaxis of the filaments was observed. Cells of 13 strains of different marine bacteria, which were apparently not caught by *Saprospira* filaments, were nevertheless killed and lysed, forming clear plaques on agar. Evidence that the *Saprospira* utilizes components of the prey-cell lysates for growth was obtained by adding *Saprospira* filaments to suspensions in sterile seawater of *P. leiognathi* (0–10^9 cells ml^{-1}). In stationary cultures incubated for 3 days at 30°C, the *Saprospira* filaments reached densities approximately proportional to the initial concentrations of prey cells, while in the absence of prey the *Saprospira* did not multiply.

Neither toxicity nor growth inhibition of *V. leiognathi* cells could be demonstrated in cultures to which cell-free filtrates (0.2-μm pore-diameter filter) of *S. grandis* cultures were added. However, in view of the ability of *Saprospira* to lyse prey bacteria, it is, perhaps, worth mentioning that their rhapidosomes have structural features similar to those of the "tails" of certain lytic coliphages. Whether these enigmatic particles play any part in prey-cell lysis remains to be determined.

In similar tests, nonaxenic filaments of the freshwater species *S. albida* did not catch flagellated cells of *Photobacterium leiognathi,* as did filaments of *S. grandis,* although they were observed to erode bacterial colonies on agar media.

It may be concluded that, as a versatile bacteriovore, *Saprospira grandis* need not subsist solely on bacteria that it encounters on surface microbial films. It can feed on motile bacteria, too. Typically living in nitrogen-poor environments, *Saprospira,* like many spiders and insectivorous plants, has developed a kind of "fly-paper" system for catching the proteinaceous prey that it needs for its nutrition. The term coined for this process is ixotrophy.

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