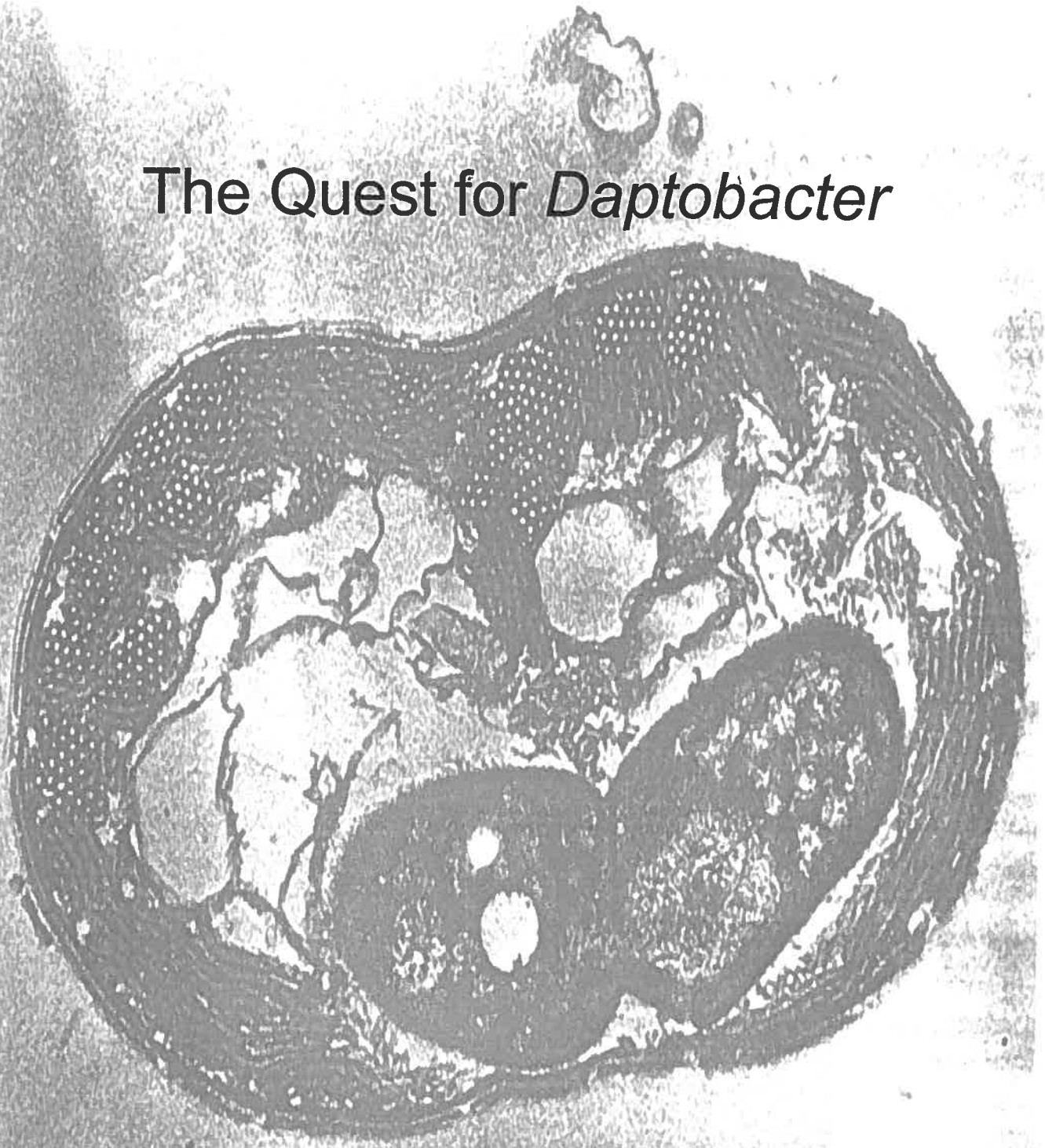
The Quest for *Daptobacter*



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Introduction

It is now clear that microbes are critical and quantitatively significant components of food webs and have numerous roles. While much attention has focused on the production of bacterial biomass, it is often more important to understand the fate of that biomass, particularly the bacterial mortality (Fuhrman and Noble, 1999)
Possible Causative Agents of Mortality:

- Protist Grazing
- Viral Infection
- Predatory Bacteria
- Starvation
- Antibiosis ("Microbe-wars")
- Aggregation Followed by Sinking and Grazing (Fuhrman and Noble, 1999)

What is predation?

In the microbial world, the distinction between predation and parasitism is not sharp. Predation typically occurs when one organism, the predator, engulfs and digests another organism, the prey. Normally, predator-prey interactions are of short duration and the predator is larger than the prey where as in parasitism the interaction time is usually much longer and the parasite is smaller than the host. (Atlas and Bartha, 1998)

Different kinds of predation:

1. Extracellular predation

In most cases bacterial cell lysis and digestion requires contact between the bacterium and its prey, while in a few cases it is caused by extracellular lytic enzymes. Although the ability of excreting enzymes outside the cell is a property widely spread in different bacterial groups, those enzymes capable of dissolving and digesting live bacterial cells are only produced by a small number of groups. The best known examples are those of the gliding bacteria, especially the myxobacteria (Guerrero *et al.*, 1986):

1.1 Lysis by Exoenzymes:

- *Myxobacteria*

1.2 Attaching to the prey under aerobic conditions:

- *Ensifer adhaerens*
- *Micavibrio*
- *Vampirovibrio chlorellavorus*

1.3 Attaching to the prey under anaerobic conditions:

- *Vampirococcus*

2. Intracellular predation

2.1 Periplasmic predators:

- *Bdellovibrio*

2.2 Cytoplasmatic predators:

- *Daptobacter*

Daptobacter

Daptobacter is a facultative anaerobic, gram negative, straight rod (0.5-0.6 by 1.5-2.0 □m), which penetrates inside the cytoplasm of its prey and divides there (Esteve *et al.*, 1992). *Daptobacter* attaches to its prey cells outer membrane of the cell wall and penetrates inside where it degrades the cytoplasm and then divides by binary fission; during this process the cytoplasm of the prey is dramatically altered. It is possible to see lytic zones appearing in the areas near the predator and the photosynthetic vesicles are destroyed. As *Daptobacter* bursts out of the prey cell, only the fragmented cell wall and the cytoplasmic inclusions remain. Several *Daptobacter* can be seen at a time inside a single prey cell. *Daptobacter* has complex metabolic capabilities: it can ferment or respire, it can live under aerobic or anaerobic conditions. The optimal growth conditions for *Daptobacter* are 33°C and it can tolerate up to 1.75 mM concentration of sulfide. *Daptobacter* has a narrow range of known prey, which are microorganisms that belong to the family Chromatiaceae. (Guerrero *et al.*, 1986).

Section 1: Who is *Daptobacter*?

Material & Methods

Strains. *Thiocapsa rosea* (DSM235), *Thiocystis gelatinosa* (DSM215) and *Chromatium vinosum* (DSM180) were obtained from the DSMZ as pure cultures. The green sulfur environmental isolate was obtained from Great Sippiwissett salt marsh sediments after enrichment and successive agar shakes.

Growth curve of an axenic *Daptobacter* culture. Cells were grown both in LB and MB media over 14h. O.D. and cell density were measured at frequent intervals. Cell density was determined by plating 20 µl drops (triplicate) from serial dilutions of the growing culture.

Growth curve of a co-culture of *Daptobacter* and potential preys. Purple sulfur and green sulfur bacteria were grown in liquid culture as described in the manual (MBL microbial diversity 2002). 10ml of high cell density cultures were transferred to 20 ml tubes and flushed with N_2 / CO_2. 10 µl of an overnight *Daptobacter* LB culture was diluted 100 fold in PSB/GSB media. 100 µl of this dilution was added to the green sulfur and purple sulfur tubes. *Daptobacter* cell density was determined at frequent intervals as described for the axenic culture. Prey cells did not grow on LB with *Daptobacter* from the co-culture when plated to determine cell density. Motility of *Daptobacter* cells was assayed at each time point.

FISH. Fluorescent in-situ hybridization was performed as describe in the manual (MBL microbial diversity 2002). α, β and γ-Proteobacterial specific probes were first hybridized to a filtered pure cultured of *Daptobacter*. Further hybridizations were performed using β or γ-Proteobacteria specific probes along with the corresponding competitor probe. Probes are labelled with cyber green 3 and have the following sequences: α-Proteobacteria (5'-GGTAAGGTTCTGCGCGTT-3'), β-Proteobacteria (5'-GCCTTCCCACTTCGTTT-3') and γ-Proteobacteria (5'-GCCTTCCCACATCGTTT-3').

PCR and sequencing. The SSU genes of *Daptobacter* were amplified by PCR using the Universal bacterial specific primers 8F and 1492R. The PCR product was purified and cloned in the TOPO-TA 4.0 vector. 850 bp were sequenced from the 5' end of the cloned SSU genes.

Phylogeny. Small ribosomal subunit (SSU) sequences were retrieved using ARB. They were subsequently aligned with the *Daptobacter* sequences using clustalX and edited by eye in BioEdit to remove positions of questionable homology. The final alignment included 800 bp. Trees were constructed using PAUP* (distance with K2P mutation model and 100 bootstrap replicates).

Results

Fluorescent in-situ hybridization (FISH)

Hybridization using the β-Proteobacteria specific probe along with an unlabelled competitor γ-Proteobacteria specific probe gave very little detectable signal under the fluorescence microscope. On the other hand, very strong signal was obtained for the Proteobacteria specific probe in presence of a competitive β-Proteobacteria specific probe (Fig. 1).

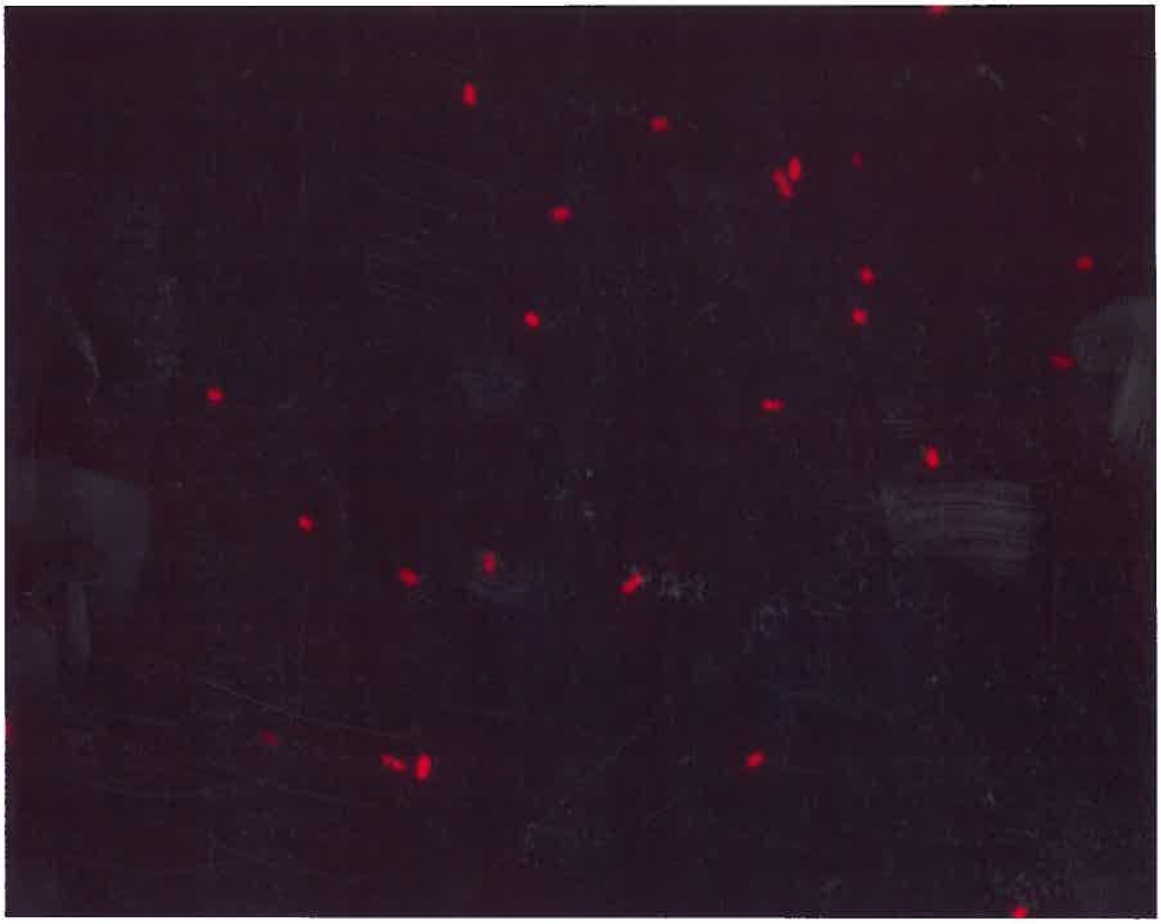


Figure 1. FISH using a γ-Proteobacteria specific probe targeting *Daptobacter* immobilized on a filter.

Phylogenetic analysis of *Daptobacter*.

The distance tree of γ-Proteobacteria rooted with β-Proteobacteria clearly shows that *Daptobacter* is part of the Enteobacteriaceae family (Fig. 2). Its placement within this family is less certain, the complete sequence of the SSU would be required to get a well-supported answer.

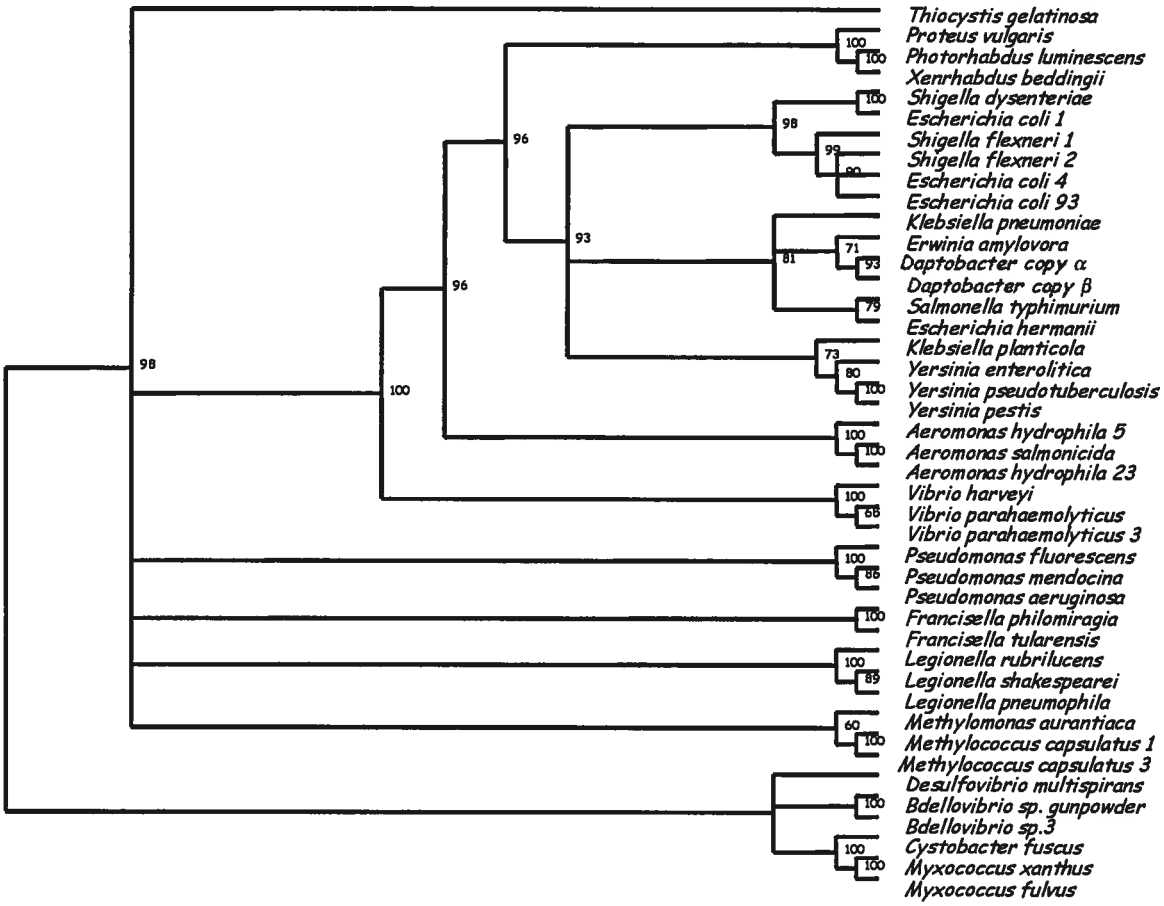


Figure 2. Bootstrap distance tree (using K2P mutation model) of SSU (800 5' bp) of γ-Proteobacteria rooted with Δ-Proteobacteria.

Sequence heterogeneity among the different SSU genes of *Daptobacter*.

In the clone library obtained from the SSU PCR amplification, heterogeneity was noticed among the different clones sequenced. Over 850 bp sequenced, 7 nucleotides were found to be different in independent clones (Fig. 3). Only two types of variant were found among the clones, meaning that *Daptobacter* has at least two divergent operons. The variability occurs in the stem of a loop of the SSU rRNA (around 450 b in *E. coli*). Most of these mutations are complementary on each side of the stem (Fig. 4). This region is an highly variable one in most bacteria but is the only one were variability is observed between the different SSU genes present in *Daptobacter*.

Daptobacter α	GGAAGGCGATAAGGTTAATAACCTTGTCGATTGACGT
Daptobacter β	GGAAGGTGTTGAGGTTAATAACCTCAGCAATTGACGT
E. coli	GGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGT

Figure 3. Sequence variability in the 450 bp stem (*E. coli* positions) of *Daptobacter* SSU genes.

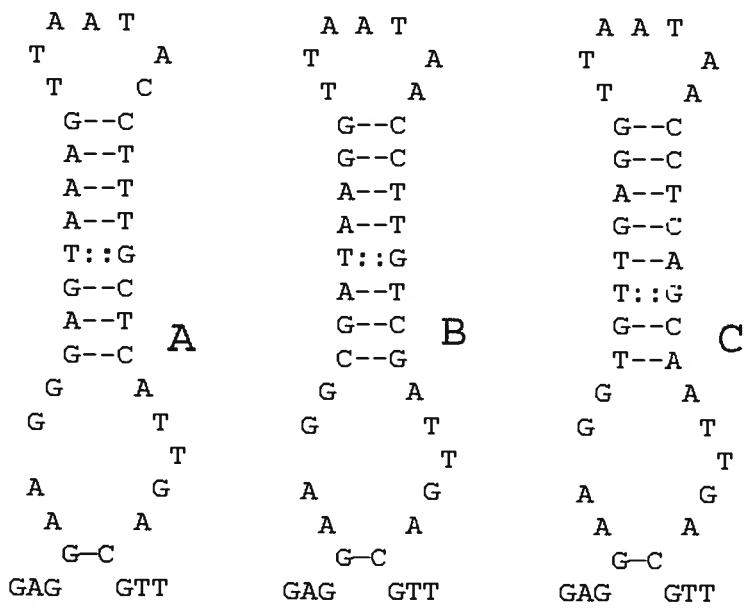


Figure 4. Secondary structure of two divergent *Daptobacter* SSU rRNA compared to *E. coli*. Positions diverging between the two *Daptobacter* genes are colored in red. A) *E. coli*, B) *Daptobacter* copy α, C) *Daptobacter* copy β.

Growth of *Daptobacter* on rich media and bacterial cells.

Daptobacter grow very well axenically on LB media and can reach high cell densities (Fig. 5). It grows more slowly on MB media and cell density is lower. Biofilms tend to form more readily in MB culture flasks than in LB. In co-culture in PSB/GSB media, *Daptobacter* only grew if the other bacterium present was a PSB (Fig. 6). Its growth rate was also different for the two different species of PSB tested.

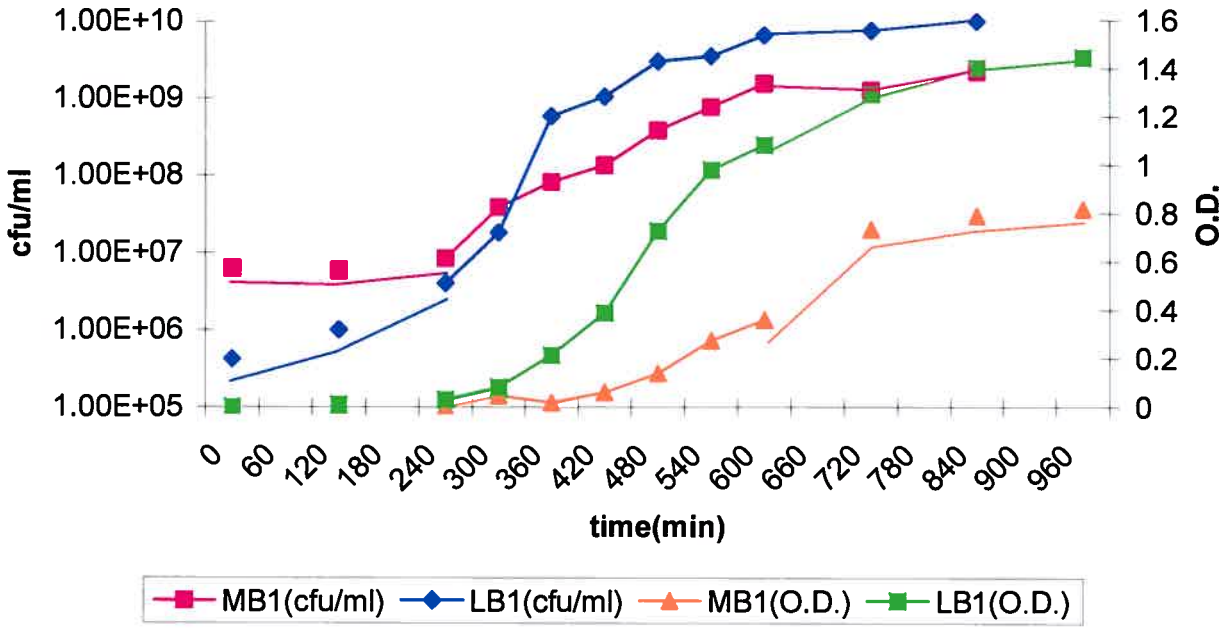


Figure 5. Axenic growth of *Daptobacter* in liquid LB and MB under aerobic conditions.

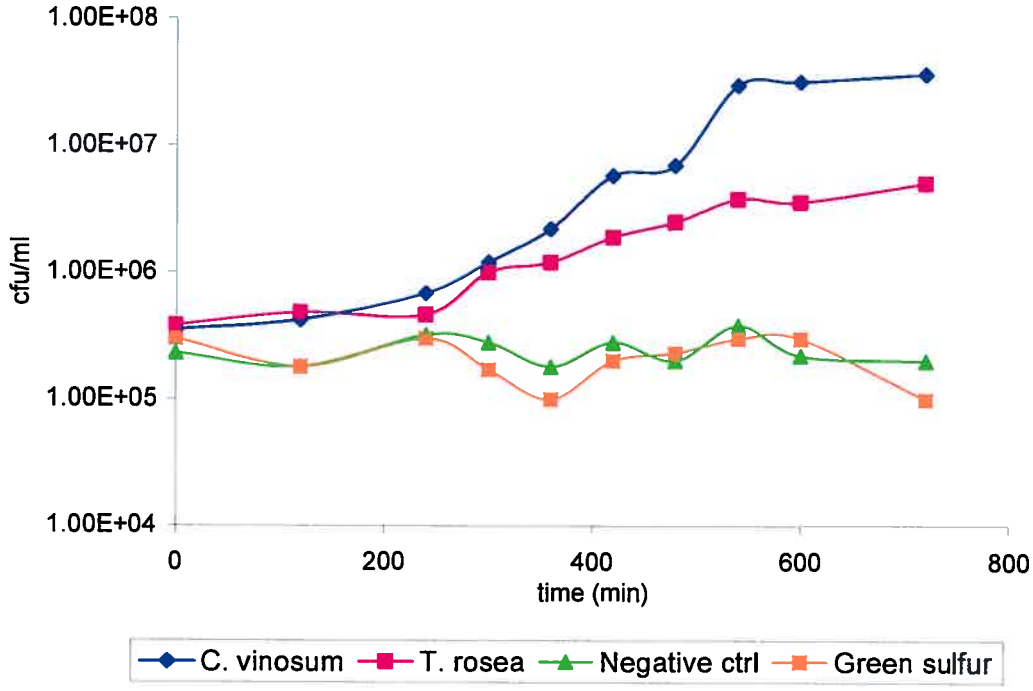


Figure 6. Growth of *Daptobacter* in presence of different types of prey.

Conclusion

The FISH results and the sequence of the SSU gene are showed that *Daptobacter* is a γ-Proteobacteria, more specifically a member of the Enterobactericeae family. It is separate from other known bacterial predators, like myxobacteria or *Bdellovibrio*, which are part of the Δ-Proteobacteria (Guerrero *et al.,* 1987). This is the first γ-Proteobacteria to be identified as a bacteriovorous predator. This highlights that predatory bacteria are not monophyletic and that this mode of life arose multiple times independently in evolutionary history of the bacteria.

Daptobacter has a least two SSU genes, which display some heterogeneity. It is unclear if this heterogeneity is positively selected for, the result of neutral drift given a low homologous recombination rate with other copies of the gene or a recent recombination event with another bacterium. This phenomenon could be fairly common within the bacterial domain, as suggested by a vast study on *Streptomyces* (Ueda *et al.*, 1999).

Prey selectivity was clear from the growth curves, were *Daptobacter* grew in co-culture with purple sulfur bacteria but not with purple non-sulfur or green sulfur. Mechanisms for this selectivity are not known, but some form of cell-to-cell recognition might be involved (Esteve *et al.*, 1983).

Part 2: Who is afraid of *Daptobacter.*

1. Introduction

(a) Goal

In this part of the project, we aimed at studying what the influence of a predator like *Daptobacter* would be on the microbial mats in Sippiwissett Marsh. We targeted to determine the host-range of *Daptobacter* to investigate the possible impact on the different layers in the mats. Originally, the idea was to use isolates derived from all layers (cyanobacteria, purple sulfur bacteria (PSB), green sulfur bacteria (GSB), colorless sulfur bacteria, sulfate reducers), but after realizing the technical challenges the predation assays created, we chose to limit ourselves to the PSB and GSB.
In a second part we tried to determine whether or not predation occurred in Sippiwissett Marsh microbial mats. Again, we limited ourselves to the study of the GSB and PSB layers.

(b) Literature background

Only one study has been carried out to determine the range of hosts, susceptible to predation by *Daptobacter*. Guerrero et al. determined this by performing filter plaque assays (see material and methods). The following results were obtained:

- Susceptible:
  PSB (*Cromatium minus, C. vinosum, C. minutissimum, Thiocapsa roseopersicina, Lamprocystis* sp.)
- Non-susceptible:
  PNS (*Rhodobacter capsulatus, Rhodopseudomonas palustris, Rhodocyclus gelatinosus*)
  Heterotrophic bacteria (*E. coli, Ps. Aeruginosa, B. subtilis, Staphylococcus aureus*)

2. Material & Methods

(a) Bacterial strains and media

Three strains of PSB were acquired from DSMZ, Germany. *Allochromatium vinosum* (DSM 180), *Thiocystis gelatinosa* (DSM 215), *Thiocapsa rosea* (DSM 235). 2 uncharacterized isolates (isolate 3 and 10) (from Sipiwissett marsh samples) of PSB were used as well as one isolate of GSB (isolate 5). Enrichments for PSB and GSB were obtained from microbial mat samples from Sippiwissett marsh. The liquid media were prepared as described in the course manual.

(b) Growing sulfur bacteria on solid media as colonies or lawns

To grow sulfur bacteria on solid medium 1.5% agar plates were prepared containing 1x SB medium final. SB medium is very similar to the liquid

medium described in the course handouts, except for the pH, S^{2-} concentration and addition of thiosulfate.

2x SB medium (500 ml):

(+) 50x Basal SW medium (w/o NaCl)	: 20 ml
(+) NaCl	: 19 g
(*) $NaHCO_3$	: 2.5 g (in 10 ml separately)
7 Vitamin solution	: 1 ml
Se-Wo solution	: 1 ml
SL 10	: 1 ml
(*) Na_2S	: 0.48 g (in 20 ml separately)
(+) $Na_2S_2O_3$	: 0.62 g

(+) autoclaved together in 450 ml ddH_2O
(*) autoclaved separately
Cool down under N_2 flushing. Then add unmarked solutions, bicarbonate buffer and Na_2S. Adjust pH to 7.2. Add equal amount of 3% agar when ready to pour plates.

(c) Filter plaque assays

The filter assays were performed as previously described by Guerrero et al. We only used this approach for determining whether or not predation activity could be found in the S. marsh. A 0.20 μm filter is used to collect the cells grown in a PSB or GSB enrichment. The filter was placed face up on top of a 3% agar and covered with a second 3% agar layer to create microaerophilic conditions. In the original protocol, plates were incubated at 30°C for 3-10 days after which > 0.5 cm pale pink circles appeared on the background of the deep purple lawn of *Chromatium*. In our case the filter was incubated at room temperature in the dark for 1.5 weeks after which it was transferred to a dark 30°C incubator for another 1.5 weeks.

(d) Soft agar overlay plaque assays

Another approach was derived from the classical plaque assay to determine the activity of phages. As determined in 2b, PSB could be grown in overlays on solid media. An 0.5% agar overlay containing a high-density inoculum of a sulfur bacteria enrichment, pure culture or isolate, was poured on top of solid SB medium.
Daptobacter was added to the overlays using three strategies: by adding a defined number of cells to the overlays before pouring on top of the SB medium (mix); by spreading a defined number of cells on top of the stiffened overlay (spread); by adding drops of LB medium or sterile ddH_2O containing a defined number of cells on top of the stiffened overlay (drop).
After the drop or overlay was absorbed into the overlay, the plates were incubated upside down for several days. Incubation was carried out in anaerobic jars with a Gaspak (H_2 + CO_2) at 25-30°C in the light.

3. Results and discussion

(a) Growing PSB/GSB on plates as colonies or lawns

Considering Jochen's words that "it will be a successful mini-project if you can show me a single PSB colony growing on a Petri plate", we included this section in the results.

It was found that colonies could easily be grown when using the overlay method on top of SB medium plates. For colonies 0.7% was found to be ideal, while for a lawn 0.5 % proved to be the most practical. Lower percentages make it impossible to invert the plates, which creates problems with condensation because of the high humidity in Gaspak anaerobic jars.

1 ml of a dense PSB enrichment in 3 ml total of 0.7% overlay on a SB medium (1.5% agar) resulted in a dense growth of PSB colonies. Liquid PSB medium was inoculated from colonies from this plate to confirm the identity of the colonies. An identical experiment was setup with an overlay on top of MB solid medium. Plates were incubated anaerobically (Gaspak) for 4 days in the light at 25 - 30°C, liquid cultures anaerobically (N_2/CO_2) for 7 days in the light at 25°C.

Purple-colored colonies were observed on both, but only in the former case growth was observed in the liquid medium. Most likely the purple colonies observed on MB medium were purple non sulfur bacteria.

Trials with GSB were less successful. When an overlay containing a dense inoculation of 1x transferred GSB enrichment was made, a purple lawn was the result. More optimization of the growth medium is required.

(b) Filter plaque assays

We used 10 ml of a dense enrichment of PSB from a Sippiwissett Marsh microbial mat sample. The filter was incubated at room temperature for 1.5 weeks + at 30°C for 1.5 weeks. After this period we observed opaque yellow spots of about .5 cm in diameter, which were very similar to the plaques described by Guerrero et al. No further examination was done since the plaques only appeared by the end of the course.

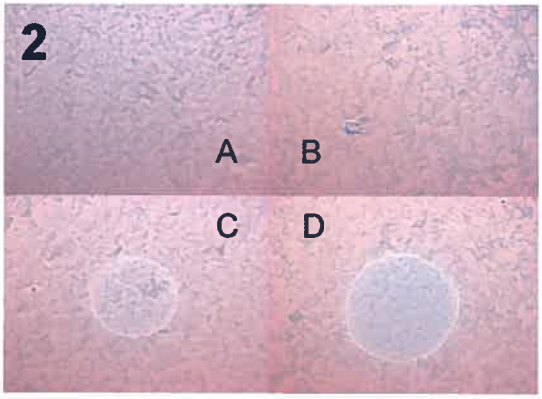
(c) Overlay plaque assays

As a positive control, *Daptobacter* was grown on LB medium, while for negative control, it was grown on SB medium as drops or spread. Growth was observed on LB and noth on SB, which means that if growth is observed in the presence of PSB/GSB, *Daptobacter* is growing on a metabolite of these cells or on the cells themselves. Only in case clearing zones would be observed, we could conclude that *Daptobacter* is indeed predating on the tested cells.

Spreading or mixing *Dpatobacter* with pure cultures did not result in clearing zones after 5 days of incubation. A mix of 10^9 cells of *Daptobacter* with a PSB enrichment (figure 1a) in an 0.7% agar overlay on 1.5% SB agar did result in a clear aggregation effect, not observed when growing the same overlay without *Daptobacter* (figure 1b).


1a


1b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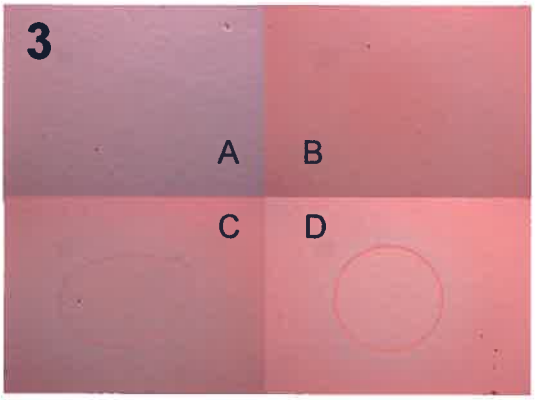
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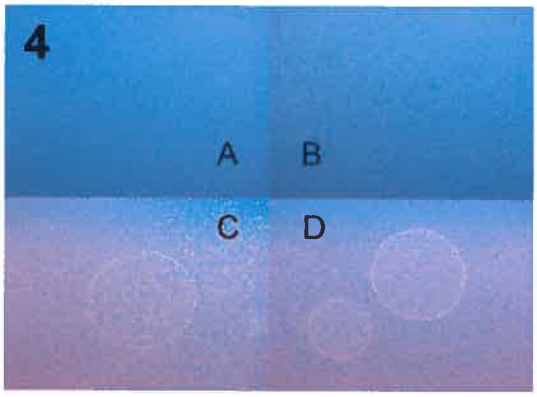
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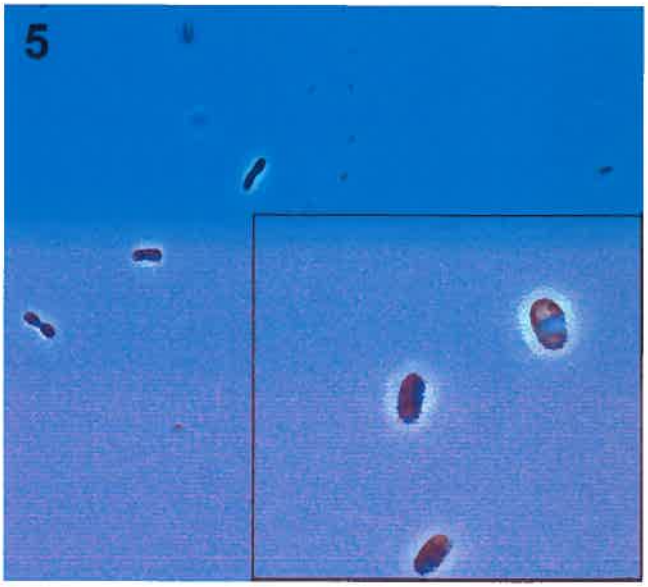
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The drop method did work for the pure cultures of PSB. On the GSB isolate 5, growth of *Daptobacter* was observed while there was no clear lawn of GSB being formed. This result can only be explained if there was enough growth substrate (cells or metabolites) in the inoculum for *Daptobacter* to grow on.
Figure 2-4 show the results of the plaque assays performed on the DSMZ strains.
Figure 2: Growth and clearing zones are observed on lawns of DSM 180 (*Allochromatium vinosum*) when growing a 0.5% agar overlay on 1.5% SB agar. (a) No drop, (b) 10 µl of LB, (c) 10 µl of LB with ~100 cells, (d) 10 µl of LB with ~10000 cells of *Daptobacter*. This is a confirmation of the work of Guerrero et al.
Figure 3: Growth and a paler halo are observed on lawns of DSM 215 (*Thiocystis gelatinosa*) when growing a 0.5% agar overlay on 1.5% SB agar. (a) No drop, (b) 10 µl of LB, (c) 10 µl of LB with ~100 cells, (d) 10 µl of LB with ~10000 cells of *Daptobacter*.
Figure 4: Growth is observed on lawns of DSM 235 (*Thiocapsa rosea*) when growing a 0.5% agar overlay on 1.5% SB agar. (a) No drop, (b) 10 µl of LB, (c) 10 µl of LB with ~100 cells, (d) 10 µl of LB with ~10000 cells of *Daptobacter*.
Figure 5: A 100x magnification of a wet mount of cells taken from the edge of the "plaque" of DSM 180 (10000 cell drop). Large cells are the PSB while the smaller cells observed are *Daptobacter* cells. A detail is shown of an invagination observed in several cells. No obvious proof of predation was observed though.

So *Daptobacter* can grow on lawns of all three strains, but the effect of the growth is different depending on which one. The halo on the DSM 215 and the clearing on the DSM 180 lawns suggest that the growth is actually resulting in elimination of the PSB cells in that zone, although no microscopical evidence was found that backs up the predation hypothesis.

(d) Alternative PSB isolation technique
We examined whether we could develop a faster and easier method for the isolation of purple sulfur bacteria than using shake tubes. We prepared PSB inoculated 0.5-0.7 % agar overlays on top of 1.5% SB agar. From dense enrichments and a DSM 235 culture serial dilutions were made up. A 1/3000 dilution of 1 ml DSM 235 and 1/30000 dilution of 1 ml of 1x transferred PSB enrichment gave distinct single colonies after a 3 day incubation.

4. Conclusions

- Filter assays work… slowly
- Faster for isolates: Overlay on SB medium
- *Daptobacter* grows on tested PSB strains, effect ~ which one.
- GSB less succesful to grow on thiosulfate plates, growth on isolate ?
- Overlay instead of shakes to get isolated colonies

Section3: Does *Daptobacter* shake its booty?

Materials and Methods

The complex media Difco Marine Broth 2216 (MB) was used for all motility tests, since the nutrient concentration is easily adjusted. Bacto-Agar (Difco) was used for all plates.

Swimming assays

To isolate motile *Daptobacter* cells, *Daptobacter* was first inoculated in MB and Luria-Bertani (LB10) broth. Samples were microscopically examined at two-hour intervals throughout the growth curve (described in Section 1). As motility was not identified samples were taken from co-cultures with the purple sulphur bacteria (DSM215 and DSM235) and the unknown green sulphur bacterial isolate from Sippiwissett Marsh at two hour intervals throughout the experiment (described in Section 1).

Swim plates were then used to induce motility in *Daptobacter*. Swim plates were set up in duplicate with the following variables:

(1) 0.2 and 0.3% agar concentration
(2) 0, 10, 40, 60 and 100% MB

Swim plates were stab inoculated at the centre of the plates and observed under the dissecting and light microscopes over three days.

Twitching 'taste'assay

A twitching 'taste' assay was designed to determine if *Daptobacter* was able to chemotactically respond to potential prey by twitching rather than swimming. 0.7% agar plates were amended with various MB and *Daptobacter* concentrations:

(1) MB concentrations (0, 10 and 100%) as *Daptobacter* twitched at 10 and 100% MB (with 0% MB acting as a control).

(2) *Daptobacter* cells were washed in PBS and resuspended to a known concentration. A variety of *Daptobacter* concentrations (10^6, 10^7, 10^8 and 10^9) were plated, as the predator-prey relationship may be density dependent.

After *Daptobacter* was spread over the plate, twitching plates were dried at 37°C for one hour. Six prey were selected to be spotted on the taste assay. The purple sulfur bacteria DSM180, DSM215 and DSM 235, (described in Section 1) and unknown environmental isolates from Sippiwissett Marsh belonging to the purple non-sulfur (PNS), green sulfur (GSB) and colourless sulfur (CSB) bacteria. Cultures of these six strains were washed and resuspended in PBS. 10 μl of each strain was then spotted onto the twitching plates and 10 μl of PBS was spotted in the centre of the plate as a control. Plates were observed under the dissecting microscope after 24 hours. All twitching assays were performed during a period of two days and in duplicate.

Results

An isolation of motile *Daptobacter* cells was attempted from a variety of growth conditions including MB and LB media, co-cultures with PSB and GSB, and swim plates with varying concentrations of MB. Motility was not observed in any of the liquid cultures. *Daptobacter* did appear motile in the 40 and 60% MB swim plates (see Fig. One). However, no motile cells from these swim plates could be identified under the light microscope.

Daptobacter did appear to twitch along the bottom of the swim plate in the 10 and 100% MB swim plates (see Fig. One and Fig. Two). Consequently the twitching 'taste' assay was developed to determine if *Daptobacter* was able to use twitching to pursue prey. *Daptobacter* did not appear to respond in the 'taste' assay (see Fig. Three) although no positive control for twitching was available.

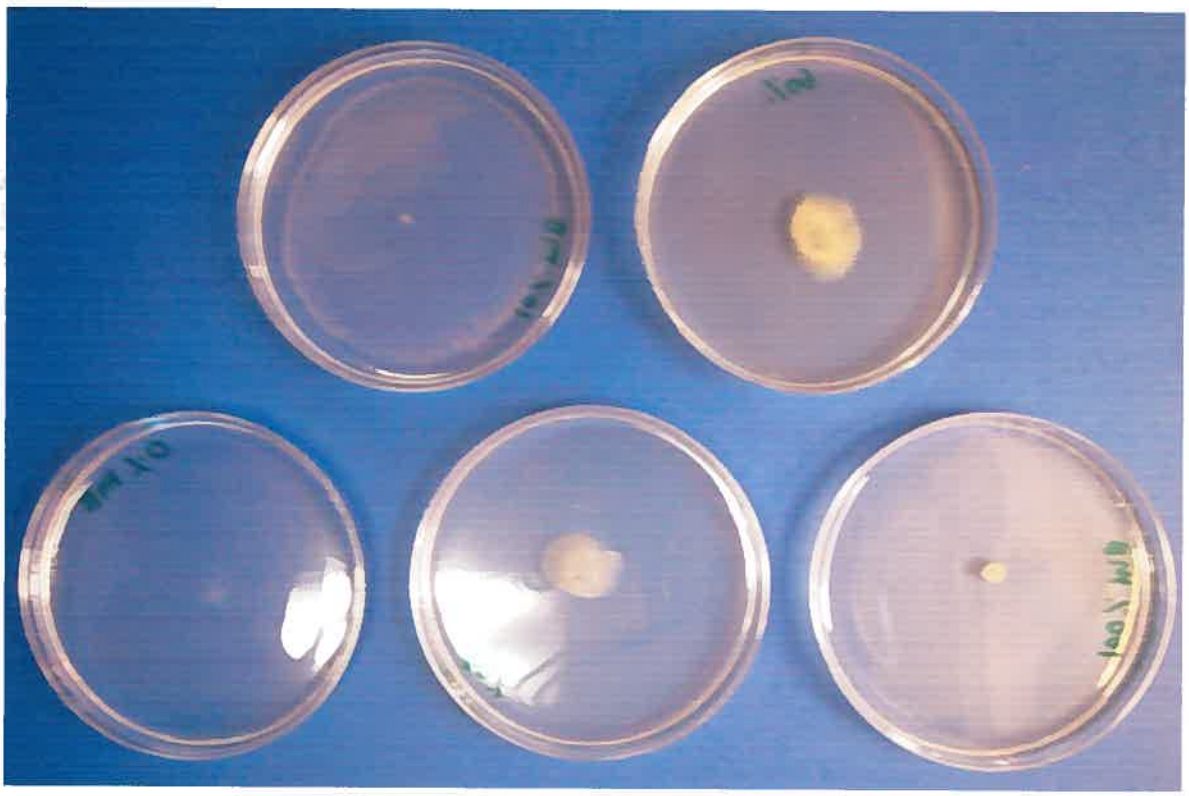
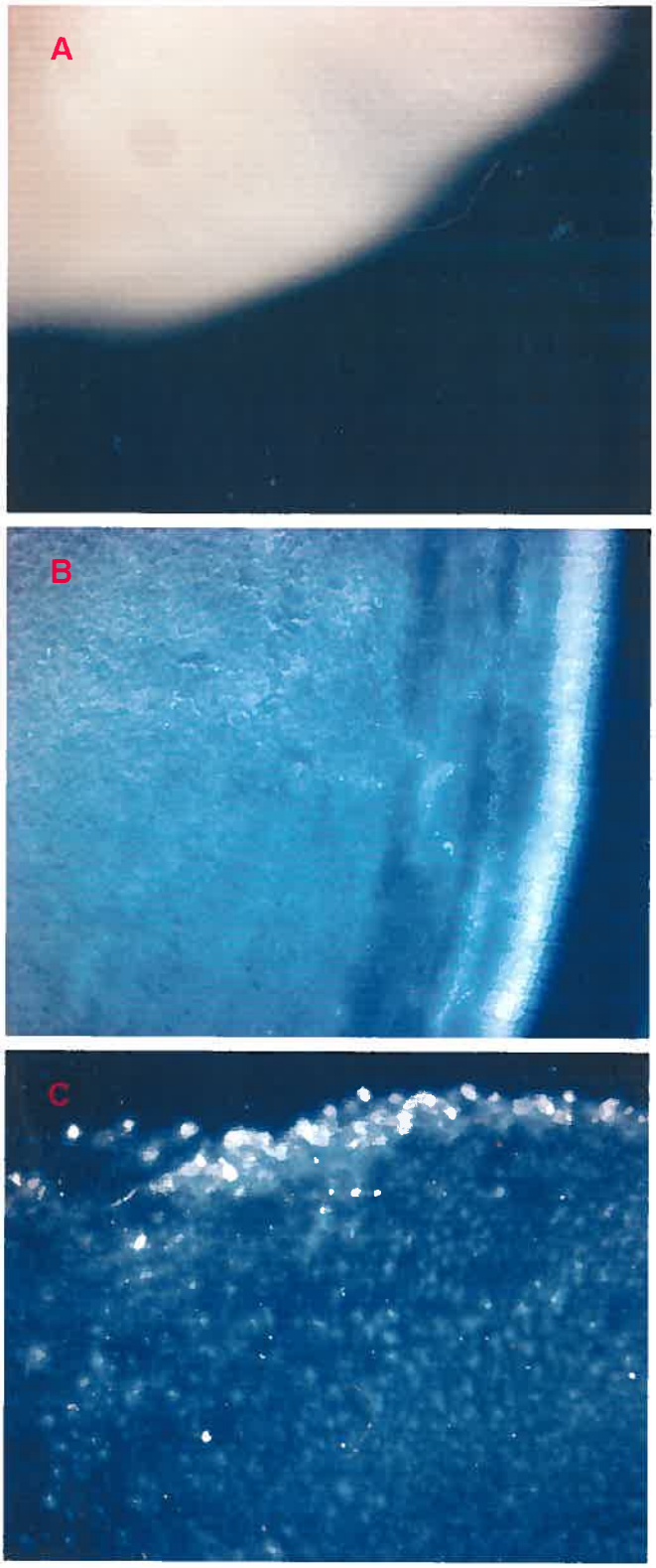


Figure One: Swimming plates inoculates with *Daptobacter*. Clockwise from top left: 10% MB, 60% MB, 100% MB, 40% MB and 0% MB. All plates were incubated for three days.

Figure Two: Images of the edge of *Daptobacter* motility on swim plates. In 2A a 60% MB swim plate inoculated with *Daptobacter* produces a diffuse edge to the spreading colony that is characteristic of swimming cells. The edge of the spreading *Daptobacter* colony shown in 2B &2C significantly differs with cells localised between the media and the bottom surface of the plate.



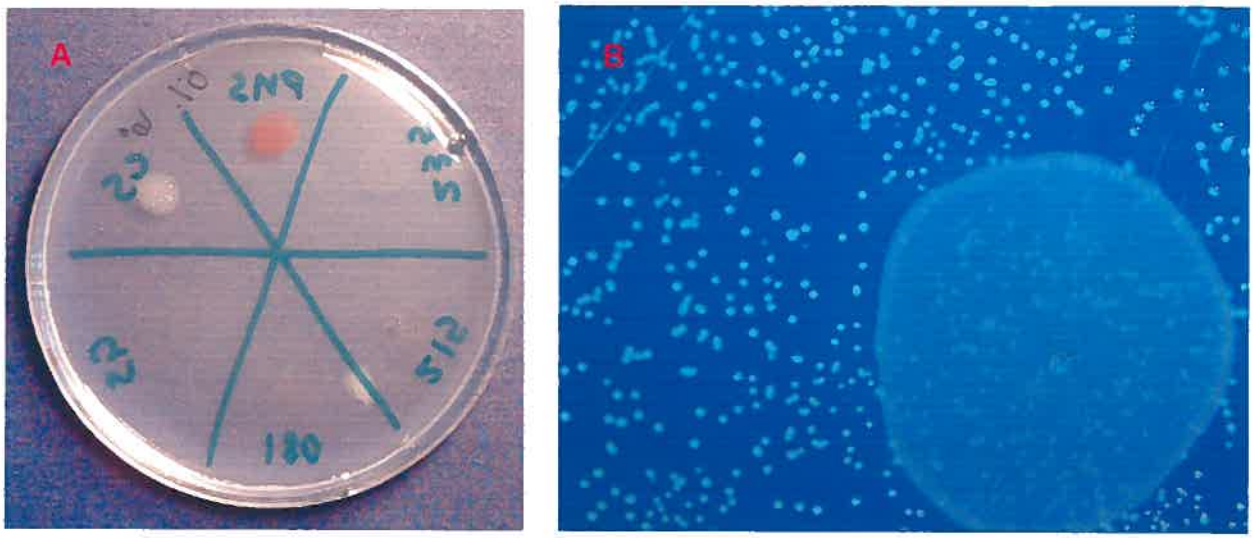


Figure Three: The twitching 'taste' assay. 3A illustrates the assay with prey spotted onto a sparse lawn of *Daptobacter*. 3B shows strain DSM215 spotted onto a lawn of *Daptobacter* on 10% MB. *Daptobacter* is forming colonies on the plate and in the DSM215 spot, but the even density of colonies indicates *Daptobacter* is not twitching toward this potential prey.

Discussion

Swimming is an important phenotype for bacteria as it provides the capacity to move toward and away from heterogeneity (e.g. nutrient pulses) in the environment. For *Daptobacter* swimming could play an important role in prey selectivity as it provides the mechanics for *Daptobacter* to pursue prey.

Daptobacter appears to be selective for purple sulfur bacteria as prey. The mechanism for prey selectivity for bacterial predators is not known but could possibly arise from a number of mechanisms:

1. Chemotaxis: a chemotactic response to leaky metabolites or signal molecules
2. Cell-cell recognition: a stochastic meeting with some cell surface recognition before killing

Daptobacter is reported to have 1-3 polar flagella so chemotaxis seemed a likely mechanistic candidate. Induction of the swimming phenotype was attempted by looking at different phases in the growth curve, swim plates at various nutrient concentrations and co-culturing with prey in the absence of any other carbon source. The swimming phenotype appears to be lost in this *Daptobacter* strain that could arise from the selectivity of the culturing process through loss of genotype (*fla* genes) or phenotype (non-functional *fla* genes).

Daptobacter did appear to twitch, which is another form of motility. Twitching is a surface associated motility that comprises a co-coordinated effort by a species to move across a surface. Mechanistically, tufts of type IV pili allow the cells to longitudinally orientate themselves to one another and through extension and retraction of the pili, pull themselves along a surface. Such surface associated motilities allow a species to rapidly colonise a surface environment to access resources, extent range or escape an exhausted habitat. In *Neisseria gonorrhoeae* twitching provides the intimate interaction necessary for infection. *Daptobacter* could potentially use twitching for both or either of these functions. *Daptobacter* was never identified infecting bacterial cells so no conclusions can be made for the role of twitching in infection.

Purple sulfur bacterial enrichments had been seeded with *Daptobacter* and plated previously (see Section Two) and aggregation of cells similar to *Daptobacter* had occurred around clumps of purple sulfur bacteria. This suggested that *Daptobacter* might twitch toward purple sulfur bacteria.

To explore to possible role of *Daptobacter*'s twitching in prey selectivity a twitching 'taste' assay was designed. A sparse lawn of *Daptobacter* was spotted with alternative prey to see if *Daptobacter* could preferentially twitch toward prey. However no twitching was identified in the assay as *Daptobacter* colonies formed uniformly over the plates.

Conclusion

Daptobacter appears to have lost its ability to swim since its initial culturing, however this does not appear to affect its ability to prey on purple sulfur bacteria (see Section One). The twitching phenotype identified for *Daptobacter* potentially has a role in the pursuit and predation of purple sulfur bacteria; this has yet to be demonstrated.

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