Stalking Streptomyces on Hunt

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The Andes Flight Disaster is a unique incident of what human beings can do when faced with ultimate threat to survival. In 1972, when a plane crashed on a high altitude glacier in the Andes and no help was likely for several days, the crash survivors ate others in order to live. This may not be common in humans but our lab discovered that this happens routinely in bacteria. When they perceive conditions of starvation, they start eating each other. Many adaptations and characteristics of several genera of bacteria have specifically evolved the abilities to do so and this adaptation has shaped bacterial evolution to a large extent. This work is a long and difficult series of experiments of which I was fortunate to be a part, but only a part, because it was necessarily a team work being pursued for over a decade.

Predatory bacteria are not new. Some forms of bacteria such as *Bdellovibrio* and bacteria are known to be predatory in nature. But what we found was much beyond that. Many bacteria that we normally know as saprophytic and happily grow on many of the commonly used media can suddenly start eating each other if they are exposed to habitats where other nutrients are almost absent. Environments such as the nutrient media that we prepare in the lab are exceedingly rare in nature. Over 70% of earth surface is water which is a very dilute environment. Most of the rest is soil where again soluble nutrients are at a very low concentration. When we grow bacteria on media that have 1 or 2% of protein digests or sugars or other extracts they are face varying unnatural conditions. So studying pure cultures of bacteria on such artificial conditions tells us very little

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about their life in nature. Our lab is more interested in looking at how bacteria live out there rather than what they do in petri-plates and test-tubes.

While I was exploring how the aging response in bacteria works in dilute environments, in 2011 a former PhD student of our lab and my contemporary, Charu Kumbhar demonstrated that *Streptomyces* are predators of bacteria (https://www.youtube.com/watch?v=5OwWfkgr9-8) in soil and that they seem to use antibiotics as their canines to kill prey. Here, we define predation as the ability to kill other bacteria and grow by consuming them when no other source of nutrition is available. In oligotrophic, i.e. nutritionally extremely dilute conditions or during starvation predator attacks the prey using secondary metabolites and enzymes to kill them, break the cell open and enjoys the contents as a meal. We also had several reasons to think that antibiotics might have evolved primarily for predation. All predatory bacteria that kill their prey from outside are rich in secondary metabolite genes. We tested the reverse hypothesis that all antibiotic-producing bacteria should be able to survive by predatory existence and that is turning out to be largely true for most genera that we tested from soil and the marine environment. The classical belief that antibiotics evolved for competition does not sustain because antibiotics are not produced during growth phase when competition should be the highest.

Predators and prey are known to have a co-evolutionary arms race. If predators evolved chemicals and enzymes to kill prey, prey should evolve resistance. It is simple to conceive that resistance to antibiotics would evolve in nature and be extremely common. But, the advantage of resistance is not limited to escaping predation. Resistance may also be a strategy for obtaining nutrients without energetic cost associated with production of antibiotics and extracellular enzymes. If you have a predator and prey in the neighbourhood, let the predator kill the prey, you only absorb nutrients released from the kill. This way you can do better than the predator because the predator has to invest in making and secreting the chemicals and enzymes. You don't invest in this effort but only skim the benefits.

To understand the mechanism of predation better, we studied marine isolates which show good predatory activity against different prey. By this time, I was a post-doc in the same lab shouldering the responsibility of coordinating a project to understand what actinomycetes and other bacteria associated with marine sponges do in nature. Now our team was bigger and two project assistants Ketaki Holkar and Anagha Pund had joined me in exploring predation by marine actinomycetes. The team studying marine microbiota consisted of more researchers including Uttara Lele, Srinu Meesala, Neha Shintre, Tejal Gujrathi, Avantika Jakati, Ruby Joshi and Harshada Vidwans who contributed to our understanding of the microbial community in shallow sea and intertidal pools. They kept on supplying us many interesting strains. During screening, we found that *Streptomyces atrovirens* isolated from a sponge showed excellent predatory activity against many species of bacteria. One unique property of this strain was that it had the ability to eat the spores of *Bacillus species* which no other predator has shown so far.

A typical experiment goes this way. You make a plate with water and agarose, devoid of any nutrient and spread a lawn of washed live cells of the potential prey on it. After placing *S. atrovirens* at the center of prey on water agar plates, the plates are incubated for several days at 30°C along

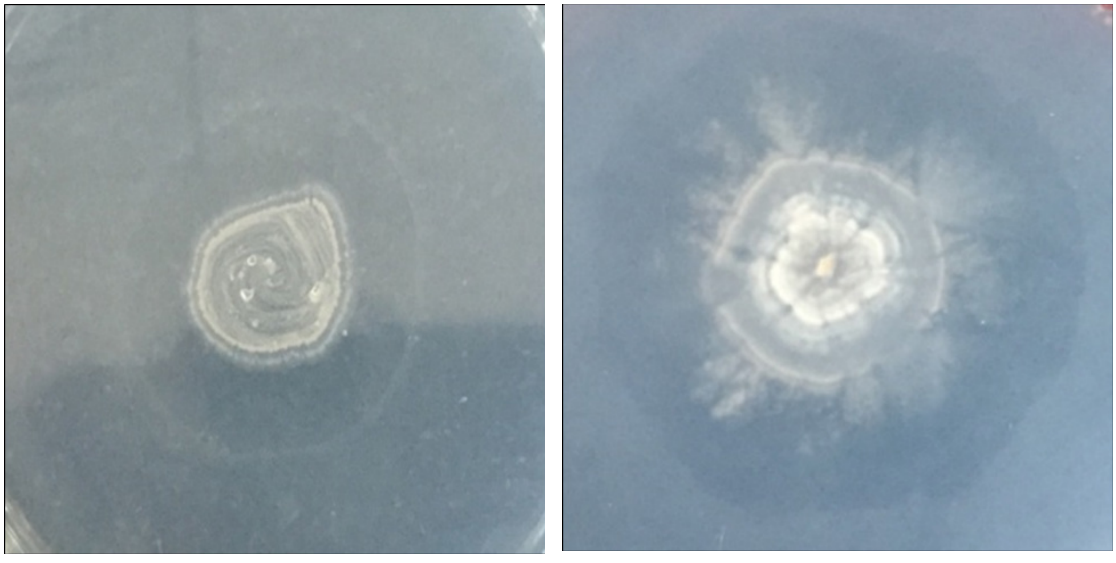

Figure 1A Zone of clearance for Bacillus (left) and Staphylococcus (right) and growth of Streptomyces atrovirens at the center.

with controls. The plates are monitored for the growth of predator and zone of clearance around the predator. In a few days, you see the predator growing and the making a clear zone around it whose diameter keeps in increasing for several days. Since there are no other nutrients, only predation can enable growth. This is simple but does not let us know what is actually happening at the level of the cell.

To watch things happen under a microscope, a water-agarose bed was prepared on a slide to view live predation using a 100X objective with differential interference contrast microscopy. Here, if you observe patiently, you can observe individual prey cells being lysed and the tips of predator mycelium growing. After a while we see that the density of prey cells decreases near to the predator and remains high away from it.

More fun and high drama started when we thought of observing interaction between more than two species. In two species, interaction different combinations for co-culture of *Staphylococcus aureus* and *Proteus vulgaris* showed susceptibility to predation, while *Escherichia coli* is resistant. In all combinations whenever S. *aureus* was present, it was lysed first. When *E. coli* was present with *S. aureus* and *S. atrovirens, E. coli* grew most luxuriantly although it could not kill any one when in pairs. This appears to be because when *S. atrovirens* kills *S. aureus*, *E. coli* benefits the most. In paired interaction, *P. vulgaris* can be preyed upon by *S. atrovirens*. But, in the presence of *S. aureus*, *P. vulgaris* was spared by the predator presumably because a more favourite prey was there.

On a different line, we tried to isolate compounds involved in predation. This job was too tough since they are produced in extremely minute quantities and are not produced in liquid media at all. In fact, predation is a strictly surface phenomenon and does not happen in submerged cultures. For *S. atrovirens,* after extracting from 3,000 experimental plates, we got 4mg of purified

compounds whose expression was specific to predation. Interestingly, this compound did not kill any of the prey bacteria in conventional MIC assays, but proved to have biofilm inhibitory activity against gram positive as well as gram negative bacteria. We hope we will be able to explore the nano-chemistry of predation and expect a number of novel compounds to surface, but it is going to be a tough job indeed.

This work is never-ending. We have just begun probing into how different species of bacteria interact in nature. We take commonly known bacteria to see how differently they behave when grown under conditions more closely simulating natural environments. Today, research in microbiology has compulsorily gone genomic. You can't publish papers in high-impact journals unless you generate large volumes of genomic, epigenomic, proteomic or any other omic data. But this does not necessarily give us an understanding of the life of bacteria. An attempt to understand the life of bacteria is both sentimentally and intellectually more rewarding than publishing papers in high-impact journals.