Nature Precedings : doi:10.1038/npre.2007.1263.1 : Posted 27 Oct 2007

Streptomyces sp. as predators of bacteria

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Nature Precedings : doi:10.1038/npre.2007.1263.1 : Posted 27 Oct 2007

Abstract

Background: The ecological role of actinomycetes and their secondary metabolites is not yet completely understood.

Hypothesis: Actinomycetes and *Streptomyces* sp. in particular, are non-obligate predators of bacteria in soil.

Evidence: Ability to grow on live bacterial cells as a sole source of nutrients. Prey cell lysis accompanying growth. Circumstantial evidence for the involvement of antimicrobials along with enzymes.

Implications: This finding may open up a new source of novel secondary metabolites from the genus.

Are actinomycetes primarily *K* selected oligophilic bacteria?

For any soil sample the absolute number as well as proportion of actinomycete colonies on oligotrophic medium is substantially greater than any other conventional actinomycete media (1).

Growth yields of actinomycetes on oligotrophic media are substantially greater than conventional media (2)

Primary oligophilic isolates show antimicrobial activity significantly more frequently (3).

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A logical thread to predatory activity

Predation: potential benefits in oligotrophic environments

All known predatory bacteria are antibiotic producers (*Myxobacteria (4), Lysobacter (5), Burkholderia (6), Bacillus* sp.(7))

Suggestion of predation based on indirect bacteriophage analysis technique (8)

Why are secondary metabolites expressed more frequently in oligotrophic conditions?

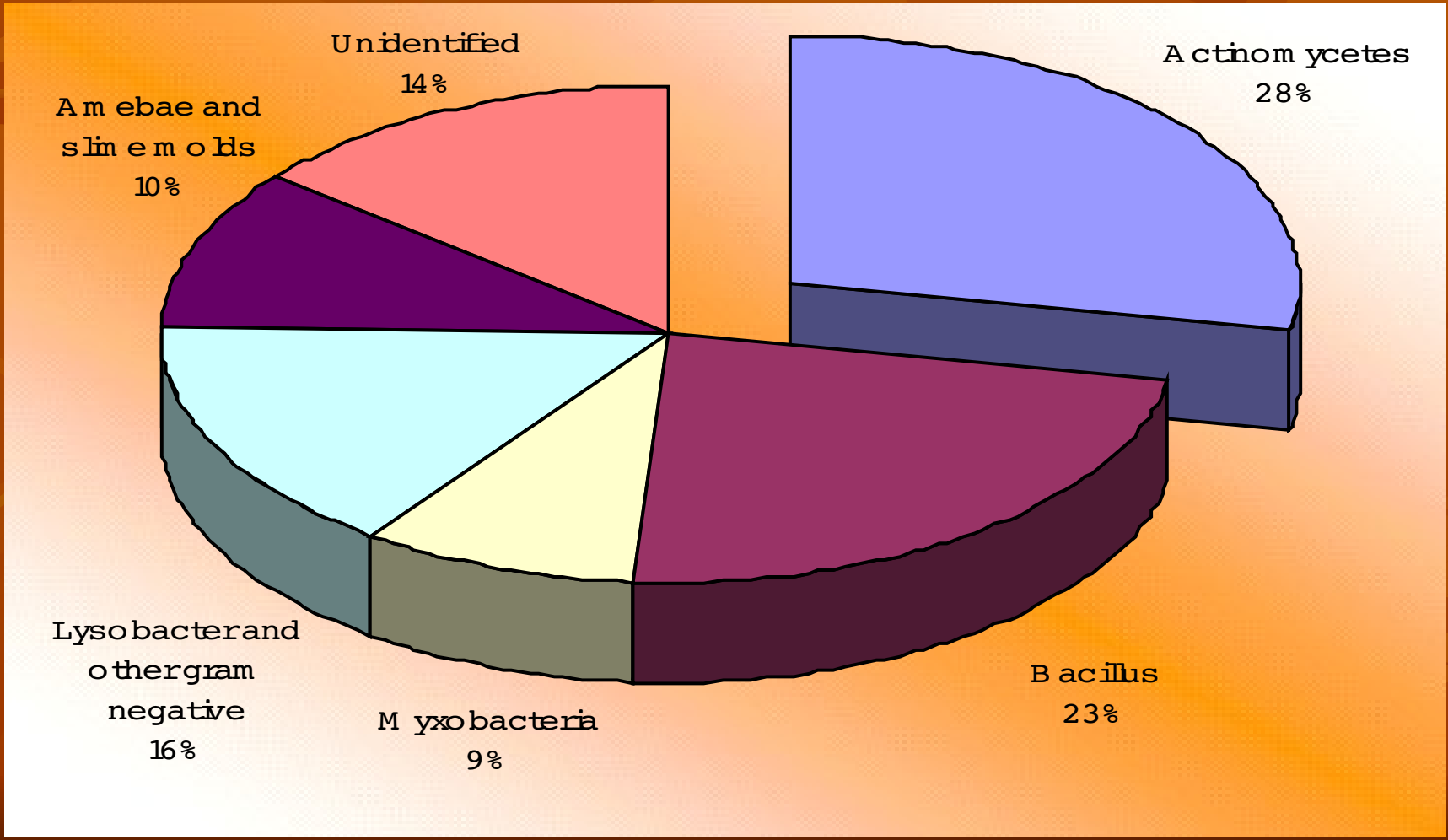
Competition or Predation?

Antibiotics should be produced in early and mid growth phase so that they help in competition.

Antibiotics should be produced when other nutrients are scanty.

Most antibiotics are known to be produced during end of growth phase, stationary phase or starvation. Supports predation hypothesis.

Proportion of different classes of isolates (potential predators) when soil is sprinkled on a bacterial lawn on water agar.



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Adaptation and selection of oligophilic isolates for predation

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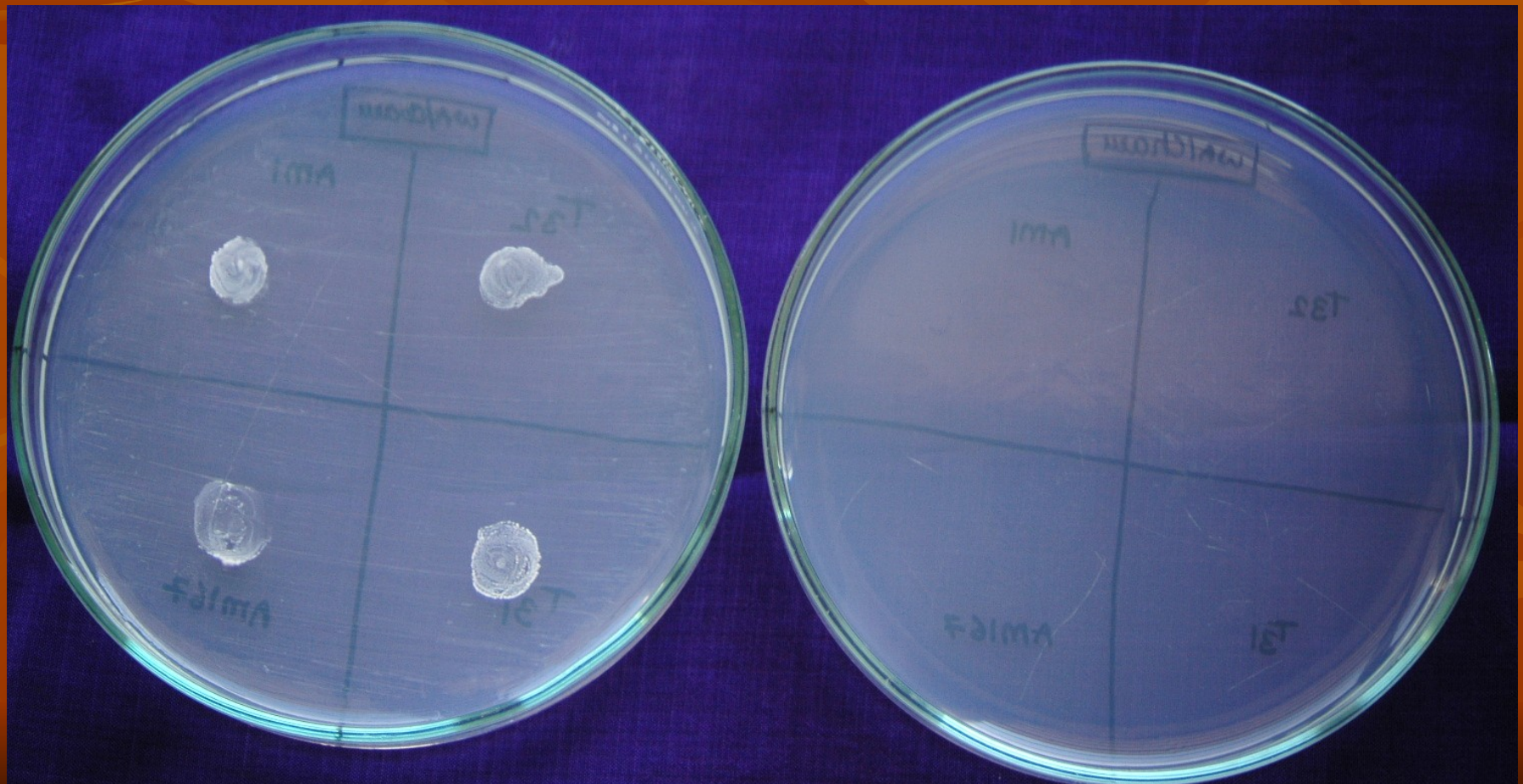
The isolates were sub-cultured on water agar with a lawn of bacterial cells as sole source of nutrient. Repeated transfers on the same.

Richer growth and larger zones of clearance with subsequent subcultures.

Evidence for predation

Growth with washed live bacterial cells as a sole source of organic nutrients.

No growth on control washed agarose gel.



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Evidence for predation

The edge of an unidentified actinomycete colony invading microcolonies of bacteria on a oligotrophic medium

Low resolution (40 X) time lapse pictures with 20 minute interval. Note that the edge grows faster where there are microcolonies **(black arrow**). Also note the fragmentation of a large colony surrounded by the predator (**white arrow**).

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Evidence for predation

Zones of lawn-clearing around colonies. Lysis of cells towards the predator colony visible at high resolution.

Click here for a high resolution pan of this area.

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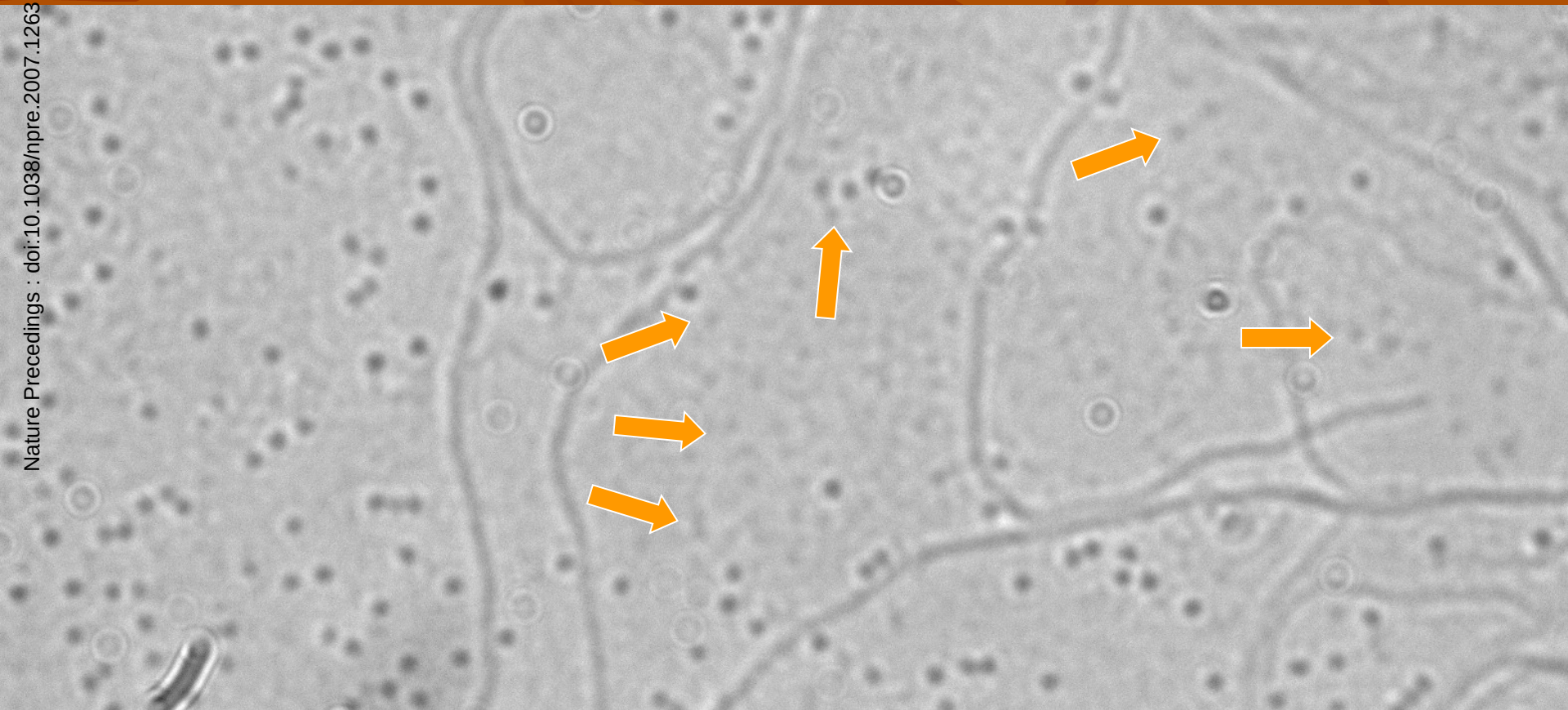
Evidence for predation

Edge of a *Streptomyces* colony growing by predation on *Staphylococcus aureus*. Arrows point to ghost cells of recently lysed bacteria. Some cells show persistence.



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How common is predatory ability in *Streptomyces?*

From 40 oligophilic isolates 16 showed distinct predatory activity on first challenge.

Half of the remaining showed predation after three to six subcultures under selective conditions.

Approximately one third of conventional *Streptomyces* isolates and standard strains showed evidence of predation after three to six subcultures of selection.

This suggests that predatory abilities are widespread within the genus.

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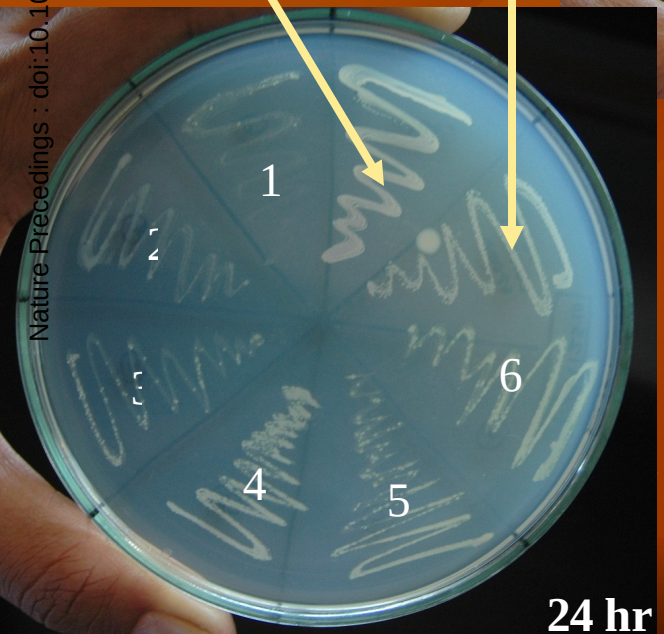
Are antibiotics involved in predation?

Before visible lysis appeared prey cells were killed up to a considerable distance from predator colony.

This suggest involvement of diffusible antimicrobial compounds.

S. aureus culture contol

S. aureus on water agar



S. aureus on water agar

S. aureus culture contol

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Unexplored potential of *Streptomyces* species

Trend analysis (9) and genome analysis (10) indicate that only a minority of secondary metabolites from the genus have been discovered so far. A much greater number remains unexpressed.

Why are most of the secondary metabolites unexpressed?

The most likely answer is that we do not understand the ecological conditions under which they are expressed.

Predation may be the key!

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Materials and Methods

Screening of soil for predators.

Lawns of *Staphylococcus aureus*, *E.coli* and *Bacillus subtilis* were spread on water agar plates and fine soil sprinkled on it.

After incubation for 72-96 hrs colonies/swarms showing visible growth and clearance of the lawn were selected as potential predators.

Demonstration of Predation.

Washed cells of *S.aureus* were spread on washed agarose plates. Test cultures were spot inoculated on this lawn and on washed agarose control. Growth, lysis of cells on the lawn and absence of growth on control indicated predation.

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Materials and Methods

Selection for predatory ability

Cells of *S. aureus* were spread on water agar plates and test organisms were spot inoculated. The test organisms that showed growth after 72 – 96 hr were subcultured on fresh lawns. Subculturing was repeated up to 20 times.

Microscopic observations

Slide culture technique was used with a thin layer of water agar covered with a lawn of *S. aureus*. The test culture was spot inoculated. The slide was incubated for 48-96 hrs and observed under 100x oel. Time lapse pictures with a 20 min interval were taken using digital imaging by Leica DFC 340 FX.

Acknowledgements

Anagha kale, Kalyani Damle, Anuja Damle, Tejashree Modak, Uttara Lele, Ashwini Keskar, Geeta Khaladkar, all from Anujeeva Biosciences and NCIM, Pune for isolates.

LabIndia Instruments Pvt. Ltd. and Leica Microsystems for Microscopy and photomicrography.

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