Predator prey and the third beneficiary — **Source link** ⬈

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3   **Title: Predator prey and the third beneficiary**

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16   **Abstract:**

17   All bacterial epibiotic predators are rich in secondary metabolites and most genera rich in

18   secondary metabolites have demonstrable predatory abilities. Therefore it is likely that an

19   antibiotic resistant and thereby predation resistant species may benefit not only by escaping

20   predation but also by utilizing nutrients released by lysis of prey cells by predatory bacteria.

21   The resistant organisms may enjoy greater fitness benefits than the predator since they get

22   the benefit without investing in the predation machinery. In our experiment, a marine

23   isolate of *Streptomyces atrovirens* showed good predatory activity on a range of species

24   including *Staphylococcus aureus* and *Proteus vulgaris*. *Escherichia coli* was resistant to

25   predation by this species. On slide culture with water agar when the predator, *S. aureus* and

26   *E. coli* were grown together *S. aureus* population declined whereas the predation resistant

27   *E. coli* increased their population as compared to controls. However the growth of *E. coli* did

28   not affect growth of the predator unfavorably. This strengthens the possibility that

29   evolution of antibiotic resistance not only gave a selective advantage of escaping predation,

30   it also would have increased the fitness of the resistant organism by promoting growth on

31   nutrients released from the prey cells lysed by the predator. When the predator was grown

32   with *S. aureus* and *P. vulgaris* as prey, *S. aureus* declined rapidly whereas *P. vulgaris* was

33   spared. This suggests that the predator appears to show preference towards prey and in

34   that case even a partial or relative resistance may give substantial advantage to a

35   population.

36   Keywords: Predation, *Streptomyces atrovirens,* antibiotic-resistance*.*

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Introduction:

Predation is defined as the act of consumption of a living organism by another living organism. Predation is a major ecological force, shaping the structure of communities, driving diversity and evolution of life histories (Stanley 1973; Day, Abrams and Chase 2002). Since bacteria do not have phagocytic abilities predatory bacteria lyse and degrade the prey cells using extracellular weapons including enzymes and secondary metabolites (Senges *et al.* 2018). Involvement of antibiotics and other secondary metabolites in predation is inferred from the strong association between predatory activity and genomic richness of secondary metabolites (Kumbhar and Watve 2013); differential expression of secondary metabolite genes when co-cultured with different prey species (Kumbhar *et al.* 2014) and loss of predatory abilities after mutating the antibiotic genes (Xiao *et al.* 2011). Kumbhar and Watve (2013) argued that antibiotics primarily evolved for predation, later diverging into mutualistic, signalling (Davies and Davies 2010) and other functions. Based on the association between antibiotics and predation Leisner et al (2016) argued that antibiotic resistance primarily evolved to resist predation but further this ability allows the resistant organisms to cross feed on the nutrients released from the lysis of susceptible prey cells by the predator. Resistant organisms can thus hitch hike on the predator and they might enjoy a fitness advantage over the predator since the predator needs to invest in the predation machinery which the hitch hiker doesn't have to. Although this is a logical possibility, this has not been experimentally demonstrated so far.

Streptomyces sp. is known for producing an array of secondary metabolites (Watve *et al.* 2001). *Streptomyces spp.* are non-obligatory predators which are suspected to utilize a diversity of small molecules for predation (Kumbhar and Watve 2013; Kumbhar *et al.* 2014). The lysis of prey cells adjoining *Streptomyces* mycelium has been demonstrated microscopically using Differential Interference Contrast (DIC) microscopy (Kumbhar *et al.* 2014). In the present study we monitored the populations of susceptible and resistant prey species in presence of a predatory *Streptomyces*. We screened *S. atrovirens,* a predatory marine isolate against 14 lab strains and environmental isolates out of which 6 were susceptible to predation (*S.aureus, P.vulgaris, Mycobacterium smegmatis, Serratia*

69   *marcencesus, Micrococcus leuteus and Bacillus subtilis*). After studying predatory activity

70   against individual cultures we studied the responses of mixed populations of prey cells. The

71   population response of a predation susceptible and resistant species, as well as two

72   susceptible species co-cultured in presence of predator is reported here.

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74   **Material and method:**

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76   *Escherichia coli* (NCIM 2184), *Staphylococcus aureus* (NCIM 2121) and *Proteus vulgaris*

77   (NCIM 2172) were inoculated in nutrient broth and incubated for 24 h at 37^0C. Prey cells

78   were centrifuged (Eppendorf centrifuge 5810R) at 6000 rpm for 10 min to concentrate cells

79   followed by washing with sterile distilled water to remove traces of nutrients. After washing

80   cells were suspended in sterile distilled water making thick slurry of cells. Prey cell number

81   was standardized based upon a relationship between optical density of the washed

82   suspension and the cell density obtained using Neubauer chamber (Rohem India BS 748). To

83   study three species interaction we used two different co-culture combinations namely *E.*

84   *coli* and *S. aureus* in approximately 1:1 ratio and *P. vulgaris* and *S. aureus* as prey in the

85   same ratio. In these combinations it was possible to identify the cells based on morphology

86   alone. It was not possible to study the *E. coli - P. vulgaris* combination owing to the difficulty

87   of differential identification microscopically. The prey population was spread over the

88   surface of water agarose bed on a slide culture over which *S. atrovirens* the predator, was

89   spot inoculated. The agarose bed was covered with sterile coverslips so that same slide

90   could be observed every day. The slides were incubated at 30°C for up to five days in moist

91   chambers. Three controls were prepared similarly with (i) individual prey species in pure

92   culture (ii) individual prey species in presence of predator and (iii) co-culture of two prey

93   species without the predator.

94   Observations were made daily using DIC microscopy on Zeiss Axioimager M-1 upright

95   microscope (100X/1.40 Oil DIC M27), alpha plan apochromat objective, with a digital

96   camera, HAL 100 illuminator and quartz collector controlled by Axio software 4.8. DIC

97   images were taken and resulting images were used for the quantification of cells. Ten

98   randomly selected fields were imaged from every slide every day. The number of cells of

4

99   each of the prey species per field was counted from the images. *S. atrovirens* being a

100 mycelial species, we recorded in every image the number of times a diagonal crossed

101 mycelial threads. The change in the population densities thus measured during the five day

102 incubation period was studied.

103 **Reproducibility runs:**

104 Experiment was repeated three times at different time points (data not shown here) and

105 similar trends were observed in all the repetitions. Since the starting populations were

106 substantially different in every run we did not pool the results. In all the three experiments

107 the results were qualitatively the same in that in every run, *S. aureus* population declined

108 while *E. coli* population and the predator population increased in three species experiment.

109 In case of three species experiment with two sensitive organisms *S.aureus* and *P. vulgaris*

110 populations of both the organisms declined; *S. aureus* being first to show decline followed

111 by decrease in *P. vulgaris* population. Predator, *S. atrovirens* increased in both the

112 experiments.

113 **Results:**

114 Since the medium contained no nutrients, in pure culture controls the prey cells did not

115 show a significant trend in population during the incubation period. In prey co-culture there

116 was no significant trend in the populations of *S. aureus and E. coli* however *S. aureus and P.*

117 *vulgaris* populations showed a growing trend when co-culturing indicating some synergistic

118 interaction. We did not investigate the nature of this interaction. In one to one predator

119 prey interactions *S. aureus and P. vulgaris* populations showed a monotonic declining trend

120 while the predator population increased. In the *E. coli* predator interaction, neither the

121 population of the predator nor that of *E. coli* showed a time trend.

122 In the three species interaction experiment with *S. aureus* and *E. coli* along with the

123 predator, the *S. aureus* population declined by 97 % whereas the E. coli population

124 increased by 60 % (Fig. 1C). The predator population increased by 72 % (Fig. 1D).

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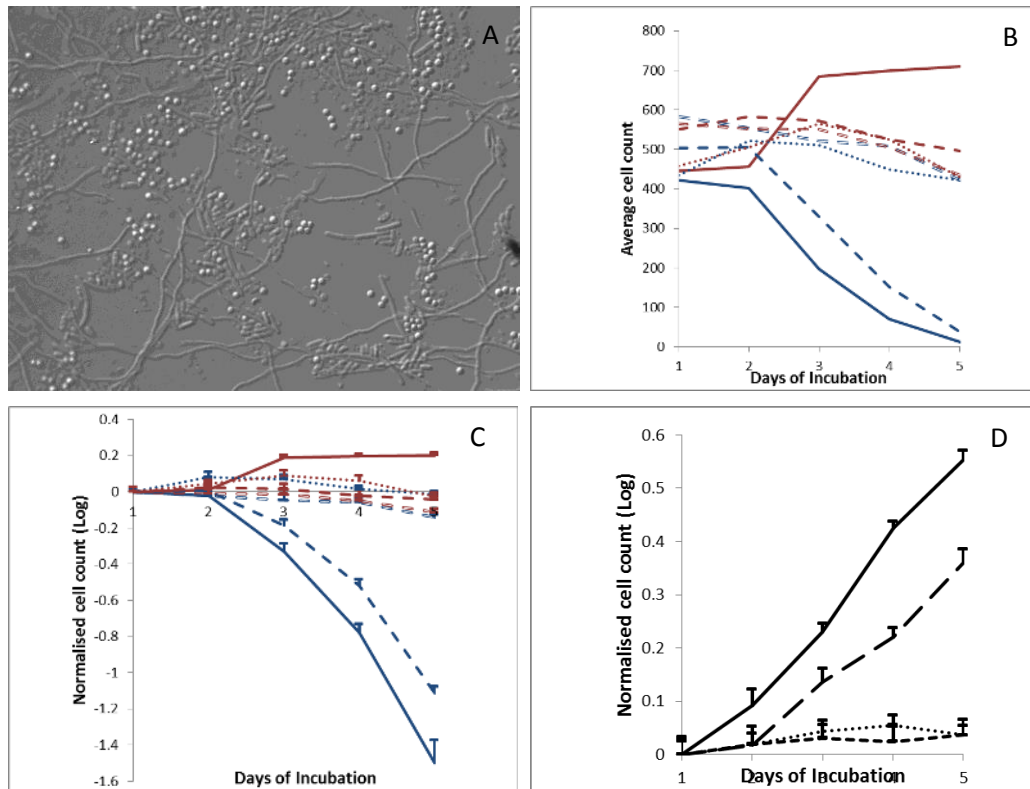
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Figure 1: Change in cell density of prey predator and resistant cells in three species interaction.

140 **A.** Microscopic image of slide culture for three species experiment with prey and resistant cells. **B.** Change in absolute
average cell count of *S.aureus* (Solid blue line) and *E.coli* (Solid brown line) along with controls (*S.aureus* predation in solid
141 dashed blue line, *S.aureus* in coculture with *E.coli* in hollow dashed blue line, pure *S.aureus* in dotted blue line and *E.coli*
142 predation in solid dashed brown line, *E.coli* in coculture with *S.aureus* in hollow dashed brown line, pure *E.coli* in dotted
brown line) over incubation period. **C.** Change in cell count of *S.aureus* and *E.coli* (normalized and on log scale) **D.** Change
143 in cell count of predator, *S.atrovirens* (Solid black line) along with controls (*S.atrovirens* count in *S.aureus* predation,
dashed black line, *S.atrovirens* count in *E.coli* predation, small dashed black line, pure *S.atrovirens* dotted black line.)

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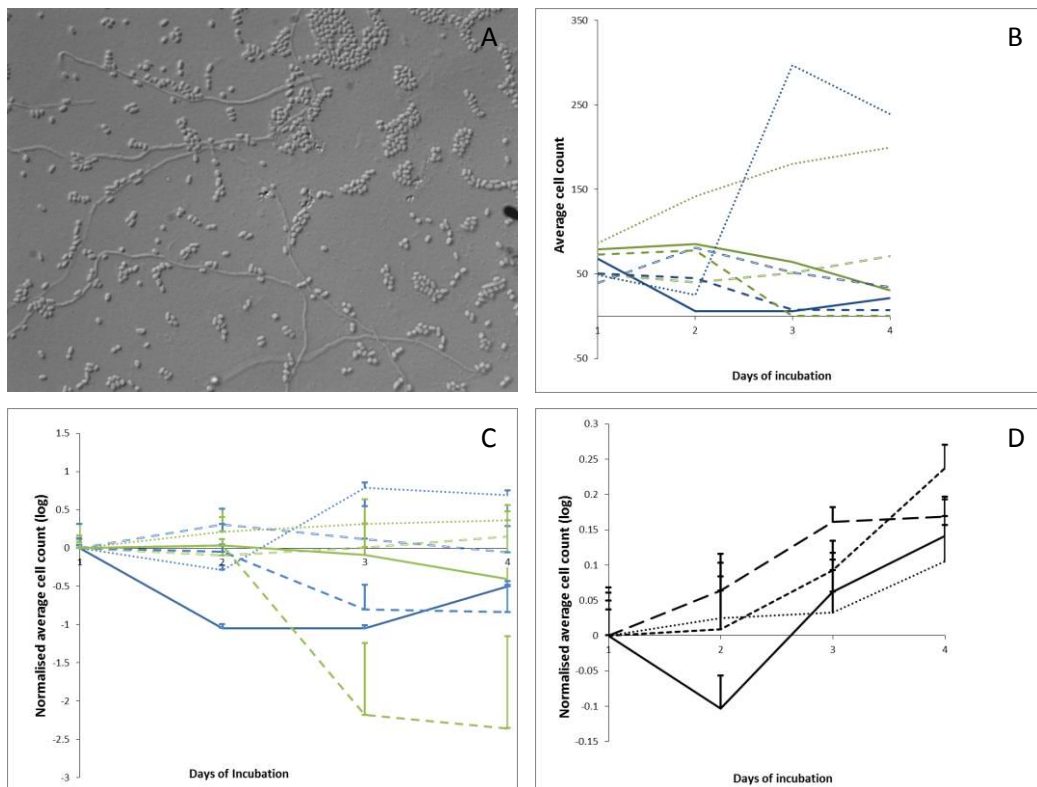
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Figure 2: Change in cell density of predator and two sensitive prey cells in three species interaction

158 **A.** Microscopic image of slide culture for three species experiment with two sensitive cells. **B.** Change in absolute average cell count of *S.aureus* (Solid blue line) and *P. vulgaris* (Solid green line) along with controls (*S.aureus* predation in solid dashed blue line, *S.aureus* in coculture with *P. vulgaris* in dotted blue line, pure *S.aureus* in hollow dashed blue line and 159 *P. vulgaris* predation in solid dashed green line, *P. vulgaris* in coculture with *S.aureus* in dotted green line, pure *P. vulgaris* in hollow dashed green line) over incubation period. **C.** Change in cell count of *S.aureus* and *P. vulgaris* 160 (normalized and on log scale) **D.** Change in cell count of predator, *S.atrovirens* (Solid black line) along with controls (*S.atrovirens* count in *S.aureus* predation, long dashed black line, *S.atrovirens* count in *P. vulgaris* predation, small dashed 161 black line, pure *S.atrovirens* dotted black line.)

162   In the three species interaction experiment with *S. aureus* and *P. vulgaris* along with the

163   predator by the second day, the *S. aureus* population declined by about 68% and remained

164   low on the third day but *P. vulgaris* population did not show a significant change. However,

165   during the last phase of growth the *P. vulgaris* population showed a decline by about 61%

166   whereas *S. aureus* showed a small but significant increase (Fig. 2C). *S. atrovirens* population

167   increased monotonically (Fig. 2D). This suggests that the predator might have selectively

168   preferred *S. aureus* in the first phase and when its population declined below a threshold

169   shifted to *P. vulgaris* allowing some comeback growth of *S. aureus*.

Discussion:

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171 The results highlight the complexity of multi-species interactions in bacteria. Earlier studies

172 have shown that in the absence of other soluble nutrients, some bacterial genera turn

173 predatory and grow at the expense of surrounding cells. We show here that different

174 bacteria show differential sensitivity to predation which is important in shaping the patterns

175 of interaction. Since bacterial predation is an extracellular phenomenon, a resistant

176 organism gets an added nutritional benefit when in proximity of a predator and a

177 susceptible organism. This was predicted earlier (Leisner, Jørgensen and Middelboe 2016)

178 but our experiments have demonstrated this advantage of resistance empirically for the first

179 time. The mechanism of predation resistance is not yet known. Secondary metabolites of

180 the predator are suspected to have a role in predation (Kumbhar *et al.* 2014), therefore it is

181 likely that resistance to the secondary metabolites might be the primary mechanism of

182 predation resistance. If this is true it gives an added dimension to the evolution of antibiotic

183 resistance.

184 More complex and more interesting appear to be the interaction between of two predation

185 susceptible species. The predator that showed successful predation on both when tested

186 separately appeared to exhibit preferential consumption of one species when challenged

187 with both together. This appears to have spared the other species at least for some time.

188 This demonstrates that even partial or relative resistance may give substantial selective

189 advantages in natural multispecies settings.

190 Microbiology has largely progressed by pure culture studies. Natural ecosystems, on the

191 other hand have a variety of multispecies interactions about which our understanding is still

192 quite primitive. The experiments suggest that studying multispecies interactions will reveal a

193 variety of novel dimensions of bacterial life in nature.

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