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Rupture of Protozoal Cells in Presence of *Chromobacterium violaceum*

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***Chromobacterium violaceum*, a violet pigmented Gram negative bacterium, constitute an important link among water and soil microbial system. Few of its properties has been utilized for commercial purpose such as antibiotic aztreonam, many still remain unexplored. We report here the series of events leading to the rupture of three protozoal species when incubated with *C. violaceum* as observed under microscope. Such type of cell rupture has never been reported in literature.**

Key words: Protozoal rupture, *Chromobacterium violaceium*, Anti-protozoal activity.

Chromobacterium violaceum is a Gram negative bacteria which is found commonly in water and soil of tropical and sub-tropical areas. As name suggests, violacein secreted by the bacterium gives characteristic dark violet metallic sheen colonies. This bacterium has been well known among the researchers for its anti bacterial, anti cancer, quorum sensing, and immune response enhancing properties and for industrial products such as textile and in cosmetics[1,2,3]. Anti protozoal activities of *C.violaceum* has been studied on typanosoma, leishmania and plasmodium[4,5]. In this study, we directly observed the events leading to rupture of 3 protozoal species *Tetrahymena*, *Chilomonas* and *Euglena* under microscope when incubated with *C.violaceum*.

C.violaceum was isolated from moistened garden soil and placed on filter paper inside a petriplate[6]. Soaked rice grain sprinkled with an antifungal agent (Mycoderm C {1:100}) was placed on the soil. After 48 hrs, some grains became violet, which were then inoculated into nutrient broth for 48 hrs. Loopful of the pellicle was streaked on Nutrient agar plates for purification of the isolate. The isolate was identified from other violet pigment producing bacteria like *Jhanthinobacterium* and *Iodobacter* by different biochemical tests[7].

Protozoal species were isolated from pond water filled in measuring cylinders and allowed to stand for 5-6 days. On carrying protozoal mounts from various layers of the column of cylinder, different protozoans were observed in different layers of the column, according to the density; the column thus being used as a winogradsky column. Markings were made on the column to roughly

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differentiate the location of different protozoans. Repeated dilution was done in separate cylinders to get pure isolates of *Tetrahymena*, *Euglena* and *chilomonas*.

Isolated Tetrahymena, *Euglena* and *chilomonas* were then inoculated into their respective media in conical flasks (wheat grain medium for *chilomonas*, split pea medium for *Euglena* and proteose peptone medium for Tetrahymena). *C.violaceum* at a concentration of about 10^9 cfu/ml. were added to the each media containing the respective protozoa and incubated for 3 hours. As control, *E.coli* at a concentration of 10^9 cfu/ml was added to 3 Medias containing the respective protozoans and the flasks were incubated for 3 hours.

After 3 hours, we prepared a wet mount from each protozoan flask and observed the mount for next 2 hours under microscope, we saw many bacteria which were near the protozoa were ingested, protozoal motility became sluggish. Slowly the membrane of the protozoa started to get distorted and ruptured. The protozoa became irregular shaped and stopped moving completely. No rupture of protozoal cells or slugging of motility was seen in the control flasks.

These findings clearly indicate there is direct toxic effect of *C.violaceum* on these protozoans. However, it is uncertain whether violacein is responsible for this rupture or there is some other unknown mechanism. There is lack of scientific evidence in literature regarding role of violacein in such type of cell rupture. These findings may hold a key role in understanding the bacteria and protozoal interactions and search of anti protozoal agent.

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