IDENTIFICATION OF LIPID RAFTS-LIKE IN *TRITRICHOMONAS FOETUS*

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Tritrichomonas foetus is an extracellular parasite that causes bovine trichomoniasis, which is a sexually transmitted disease that results in reproductive failure and considerable economic loss in areas of the world where natural breeding are practiced [1]. *T. foetus* is an extracellular parasite that adheres to epithelial cells, but however, the cellular mechanism by which colonizes mucosal surfaces is not well defined. The involvement of lipid rafts have been described in a variety of cellular functions, including cell polarization, signal transduction, endocytosis, secretion, and cell-cell and cell-pathogen adhesion [2]. The presence of the lipid raft has been demonstrated in parasitic protozoa such as *Trypanosoma brucei*, *Entamoeba* and *Leishmania.* However, these membrane domains are not reported in *Trichomonas* yet. Because trichomonads membranes contain cholesterol and sphingolipids [3], it is conceivable that raft-like domains exist in the plasma membrane of this organism. Therefore, the purpose of the study reported here were to identify raft-like domains in *T. foetus*. For this study *T. foetus* were cultured for 2 h in serum-free TYM at 37°C. In some trials, rafts were disrupted by depleting cholesterol with methyl-β-cyclodextrin (MBCD) (15mM) or by sequestering cholesterol with filipin (5μM) for 30min at 37°C. To determine whether *T. foetus* cells possess raft-like microdomains and to distinguish them from more-fluid-phase membrane regions, cells were stained with either the order-preferring lipid analog DiIC16 (1.1 μM) or the non-order-preferring lipid analog FAST-DiI (1 μM) with or without chemical disruption of rafts for 2 min. The cells were then fixed with 4% paraformaldehyde and viewed on fluorescence microscope. In addition, cells were stained with cholera toxin B subunit FITC conjugated (FITC-CTX-B), another marker of membrane rafts, diluted 1/10 for 2 hours. Both lipid analogs and FITC-CTX-B were localized to the plasma membrane and some intracellular structures in *T. foetus*. Treatment with MBCD or filipin abolished staining in the plasma membrane by DiIC16. In addition, disrupt treatment resulted in alteration in the DiIC16 staining pattern. Similar results were observed with FITC-CTX-B staining. As expected, the raft-disrupting agents did not affect FAST-DiI staining. Together, these data authenticate the colocalization of DiIC16 and FITC-CTX-B with cholesterol-rich membrane regions and suggest the existence of raft-like domains in *T. foetus*.

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