J Ecosyst Ecography 2017, 7:2 (Suppl)
DOI: 10.4172/2157-7625-C1-030

The effect of double-stranded RNA on the expression of the homologous gene in *Toxoplasma gondii*

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The double-stranded RNA has been used in many organisms to interrupt gene expression at the post-transcriptional level. We have explored the use of in vitro synthesized double-stranded RNA for gene expression study in *Toxoplasma gondii*. We produced double-stranded RNAs homologous to the three well documented selectable markers the green fluorescent protein, the uracil phosphoribosyl transferase and the hypoxanthine-xanthine-guanine phosphoribosyltransferase. Each dsRNA was efficiently electroporated into the parasites and monitored for its effect on the expression of the homologous gene. The parasites electroporated with the double-stranded RNA homologous to the green fluorescent protein exhibited reduced fluorescence for the green fluorescent protein. The parasites electroporated with the double-stranded RNA homologous to the uracil phosphoribosyl transferase had low enzymatic activity for the uracil phosphoribosyl transferase, while the parasites electroporated with the dsRNA homologous to the hypoxanthine-xanthine-guanine phosphoribosyl transferase had low enzymatic activity for the hypoxanthine-xanthine-guanine phosphoribosyl transferase. To investigate the in vivo longevity of the effects of the electroporated double-stranded RNA, we utilized the uracil phosphoribosyl transferase. An operative uracil phosphoribosyl transferase assimilates 5-fluoro-2'-deoxyuridine ultimately leading to parasite clearance. Parasites electroporated with the dsRNA homologous to the uracil phosphoribosyl transferase became resistant to 5-fluoro-2′-deoxyuridine as a result of inhibited uracil phosphoribosyl transferase expression. Moreover, the effects of the double-stranded RNA homologous to uracil phosphoribosyl transferase persisted for three successive propagations of the parasites. Our study suggests that the double-stranded RNA could be a useful tool for gene silencing in *T. gondii*.

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