

2004 TAIWAN INTERNATIONAL SCIENCE FAIR

CATEGORY : Biochemistry

**PROJECT : Isolation and Expression of an
Eoinephrine-Synfhesizing Enzyme
(PNMT) from Entamoeba Parasites**

AWARDS : Biochemistry First Award

SCHOOL : Roslyn High School

FINALISTS : David Alexander Gottesman

COUNTRY : United States

Isolation and Expression of an Epinephrine- Synthesizing Enzyme(PNMT) from Entamoeba Parasites

Biochemistry

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Entamoeba histolytica is a protozoan parasite known to cause infectious colitis and amoebic dysentery in humans. Its life cycle consist of two parts: the infectious cyst stage and the multiplying trophozoite stage.

Epinephrine, a neurotransmitter in vertebrates, is released by the trophozoites during the process of cyst formation. The addition of epinephrine to in vitro cultures of amoebas causes them to encyst, and addition of compounds that prevent epinephrine's activity inhibits encystations. Therefore, epinephrine plays a critical role in encystation in vitro. An understanding of the molecular intricacies of epinephrine-induced encystations may allow for pharmacological manipulation of epinephrine metabolism to control cyst formation in vitro. Drugs that either prevent cyst formation or induce it before a large amoebic population is present would result in the release of fewer cyst forms of the parasite, reducing parasite transmission from person to person.

Although trophozoites release epinephrine, it is no known if *E.histolytica* synthesizes epinephrine or extracts it from the growth medium. Phenylethanolamine N-methyltransferase(PNMT) is the enzyme that catalyzes production of epinephrine norepinephrine. This study aims to determine the source of epinephrine by determining if *E.histolytica* contains a PNMT-type enzyme.

PNMT amino acid sequences from several higher organisms were compared to identify conserved regions of the enzyme. These conserved amino acid sequences were then used to search for similar sequences in a database containing the recently sequenced amoeba genome. A PNMT-like gene was found in the *E.histolytica* database and cloned in bacteria. Yeast cells containing the cloned *E.histolytica* PNMT gene expressed PMT enzyme activity. This suggests that *E.histolytica* produces its own epinephrine, and is the most evolutionarily ancient eukaryote shown to do so. The use of inhibitors against PNMT activity is under investigation.