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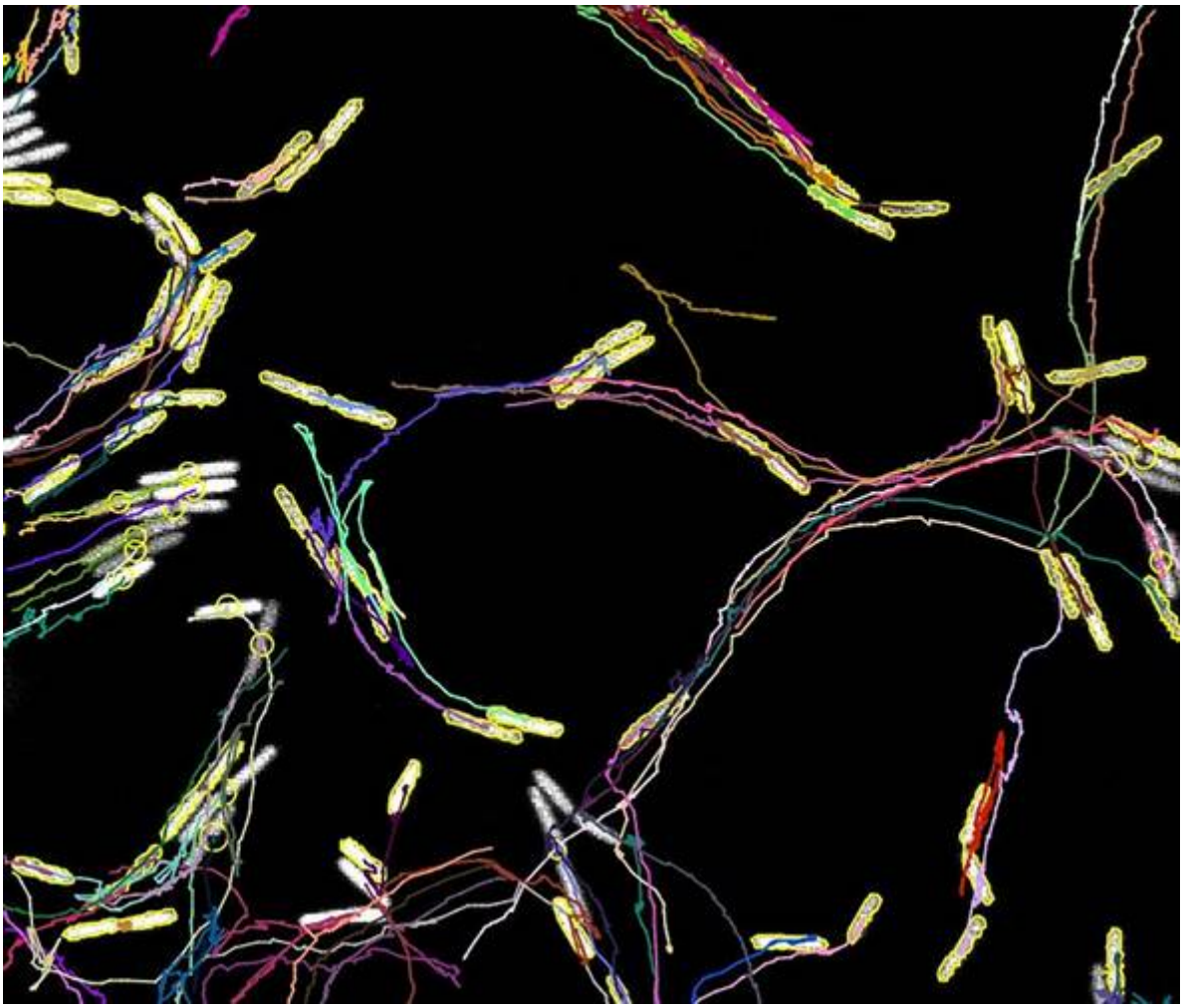

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

SELECTION AND CHARACTERIZATION OF A TRIMETHYLAMINE MONOOXYGENASE WITH POTENTIAL BIOTECHNOLOGICAL USE FOR THE TREATMENT OF TRIMETHYLAMINURIA

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Trimethylamine (TMA) is a nitrogenous compound that has a strong odor of decomposing fish. In humans, TMA is produced in the intestine by microbial action on certain compounds present in food. Under normal conditions, the TMA generated passes into the circulatory system and, upon reaching the liver, is oxidized by the enzyme flavin monooxygenase 3 (hFMO3) to trimethylamine oxide (TMAO), an odorless compound that is then eliminated in the urine. Trimethylaminuria (TMAU), also known as “fishy odor syndrome”, is a rare disease associated with a decrease in TMA oxidation, caused by hFMO3 failure or inactivity. Thus, TMA accumulates in the body and is excreted in urine, sweat, saliva and other bodily fluids, causing the person to emanate an odor similar to decomposing fish. As a result, patients can suffer severe psychosocial sequelae. Currently, there is no cure for TMAU, and available treatments are both limited and unsustainable in the long term. This work is part of a project that aims to develop a genetically modified bacterium, safe for human consumption (GRAS), with potential use in the treatment of TMAU. For this purpose, it is proposed to express a trimethylamine monooxygenase (Tmm) with the capacity to oxidize TMA. The developed bacterium could be consumed by patients with trimethylaminuria, and it would eliminate the trimethylamine generated in the intestine, thus reducing the body odor of those suffering from this disease. In order to have an effective Tmm for the elimination of TMA, bacterial Tmm from *Roseovarius* sp. 217 (RsTmm) and *Methylocella silvestris* (MeTmm) were cloned and expressed in *Escherichia coli*, as well as two variants of human hFMO3: the complete protein (hFMO3) and a truncated version without the transmembrane region (hFMO3 27-351). The expression of the different Tmm was assessed by SDS-PAGE, and their activity to oxygenate substrates was initially confirmed by the production of indigo blue from tryptophan. In addition, the ability of these Tmm to oxidize TMA was evaluated by incubating bacteria expressing the different Tmm in the presence of TMA and monitoring over time the concentration of TMA. It was determined that three of the proteins studied significantly reduced the amount of TMA after 24 hours. From the results obtained, we selected RsTmm as it was the most efficient, achieving a reduction of approximately 80% of TMA in only 3 hours of incubation. RsTmm was purified, kinetically characterized and its crystal structure was determined at a resolution

of 1.4 Å. Our results indicate that RsTmm is a promising candidate for the development of a genetically modified bacterium with potential use in the treatment of TMAU.

Palabras clave: Trimethylaminuria – Trimethylamine monooxygenase – Trimethylamine – Trimethylamine oxide – Crystal structure