Sociedad Argentina de Microbiología General

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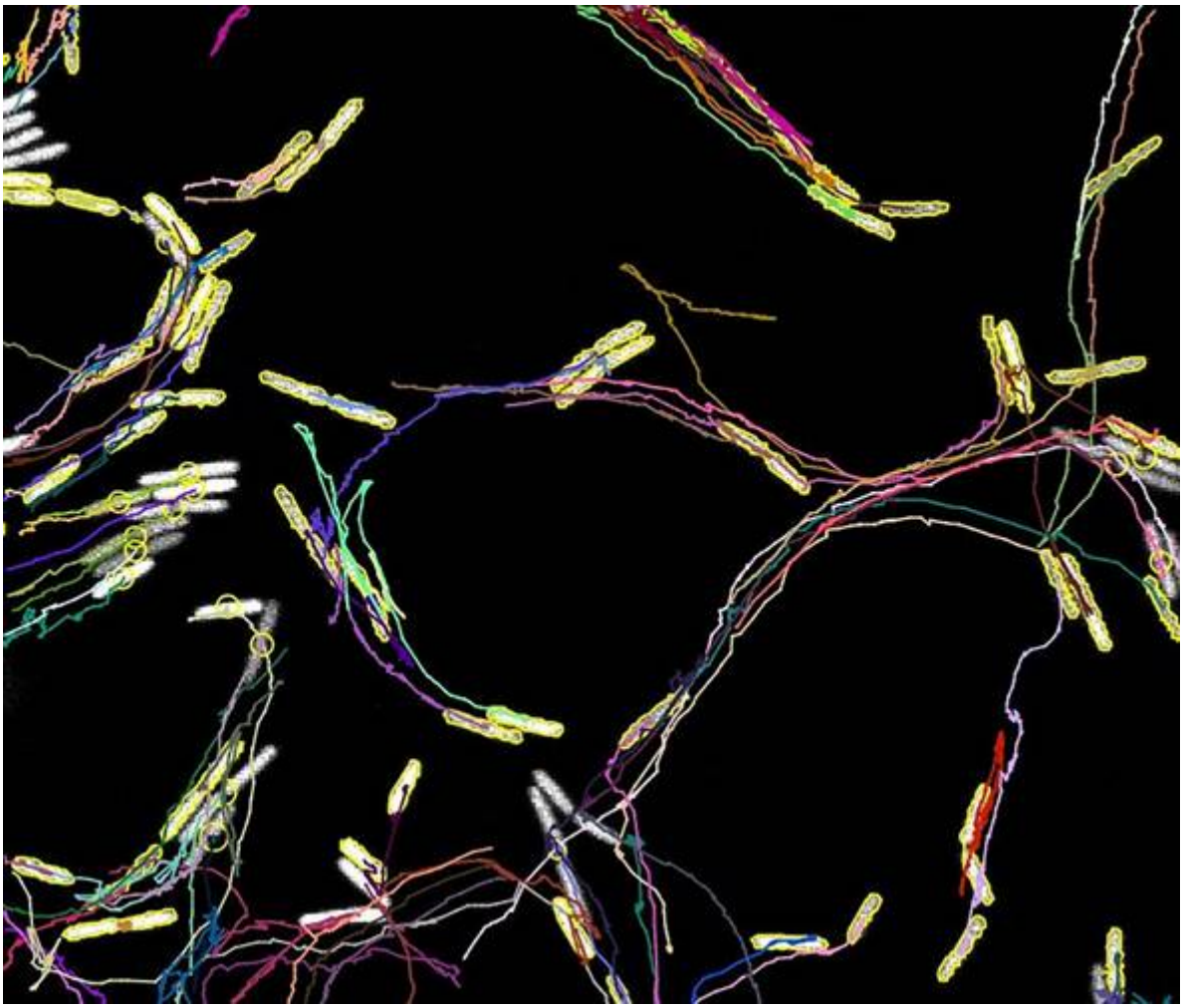

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

FUNCTIONAL GENOMIC TO DISCOVER ENZYMES INVOLVED IN REDOX METABOLISM OF TRYPTOPHAN

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Tryptophan (Trp) is an essential amino acid for humans, as we cannot synthesize it. It is acquired through the diet and incorporated directly into proteins during anabolic processes or taken directly from bacteria that are to do so. Trp is a precursor to a variety of metabolites, including neurotransmitters such as serotonin and immune response regulators such as indole-3-acetic acid which is derived from kynurenine (Kyn). There are two oxidative degradation pathways for Trp: the Kyn pathway, which depends on the action of the enzymes tryptophan-2,3-dioxygenase and indole-2,3-oxygenase, and the serotonin synthesis pathway, catalyzed by the enzyme 5-hydroxytryptophanase, which is mediated by the stable metabolite 5-hydroxytryptophan (5HT). The Kyn pathway is conserved in many eukaryotes and is also present in some bacterial species that are part of the human gut microbiome. The serotonin pathway has only been found in eukaryotes, although it is speculated that some bacteria may also be capable of this biotransformation. Our work is based on the assumption that the gut microbiota encodes a variety of enzymes as an yet unknown function, among them we expect to find enzymes that act on Kyn or 5HT catalyzing yet unknown reactions. In this first stage, we will focus on trying to discover enzymes capable of reducing Kyn and 5HT to Trp. For this purpose, we designed a method based on functional genomics in vivo using strains of Escherichia coli auxotrophic for aromatics and Trp (?aroA, ?trpB), produced in our laboratory, transformed with plasmid libraries and forced to grow on selective media containing Kyn or 5HT as the sole “precursor” of Trp. This system allows the selection of colonies that recover the growth phenotype, under the assumption that these colonies carry a gene coding for one or more enzymes capable of conferring auxotroph cells the ability to reduce or recycle 5HT or Kyn and thus reverse the auxotrophic phenotype. Positive hits will be tested in vivo and in vitro. This strategy makes it possible to recognize which gene is responsible for recovering the phenotype. Considering that Trp metabolism is of high relevance for gut microbiome homeostasis and bacterial-host dialogue, we focused our exploration on libraries constructed from metagenomes of the canine and human gut microbiome, as well as selected bacteria. In conclusion, this work aims to uncover novel enzymes encoded in gut microbiota that are capable of reducing 5HT or Kyn using a functional genomic approach. Hence, we present the methodology, explaining construction of host strains, a new plasmid library construction

protocol and positive hits that can revert the phenotype.

Palabras clave: Functional genomic - tryptophan - kynurenine - 5-hydroxytryptophan - reductases