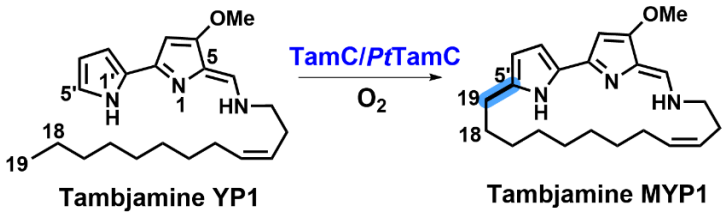
PhD Departmental Seminar – Manasa Ramachandra

November 3rd, 2023

The Study of Rieske Oxygenase Enzymes Catalysing Oxidative Cyclisation and the Identification of Serine Proteases with Anti-nociceptive Activity

ABSTRACT

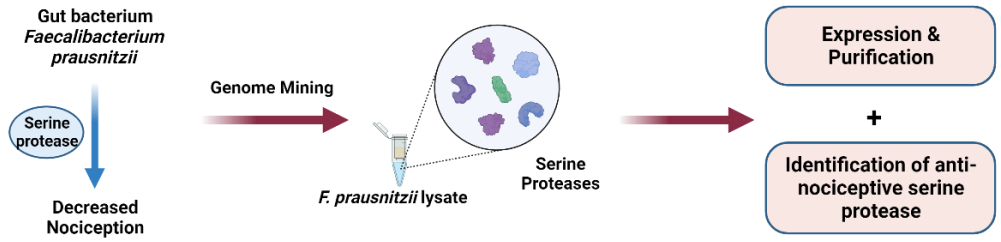
A.



Tambjamine YP1 Tambjamine MYP1

Tambjamines are alkaloids containing methoxy-bipyrrole cores with variable imine moieties isolated from marine invertebrates and bacteria. Tambjamines possess several biologically useful properties. The first cyclic analogue, tambjamine MYP1, was isolated from a marine bacterium, *Pseudoalteromonas citrea* (also proposed to exist in *Pseudoalteromonas tunicata*). The *tam* cluster in *P. citrea* contains TamC (*Pt*TamC in *P. tunicata*), a Rieske oxygenase, proposed to catalyse the cyclisation of tambjamine YP1, a linear analogue, to MYP1. Interestingly, the macrocyclisation in MYP1 would involve a C-C bond formation at a primary carbon atom (terminal methyl) of the alkyl chain in YP1. I will be presenting our experiments involving heterologous co-expression of TamC and its identified redox partner enzymes in *E. coli*, and substrate-feeding assays that have identified TamC and *Pt*TamC to be the Rieske oxygenases that catalyse the oxidative carbocyclisation of YP1 to MYP1. This is the first reported C-C bond formation being initiated at the terminal methyl group of an alkyl chain in oxidative cyclisations and therefore, the first successful activation of an unreactive terminal C(sp^3)-H bond in an oxidative carbocyclisation reaction catalysed by the Rieske oxygenase enzymes, TamC and *Pt*TamC.

B.



There exists a symbiotic relationship between humans and the gut microbiota. These gut microbes modulate physiological processes via a bidirectional communication network called the 'gut-microbiota-brain axis'. Visceral pain associated with inflammatory bowel disease (IBD) is mediated through peripheral pathways and the central nervous system, thereby allowing for IBD to be recognized as a disorder of the gut-brain axis. The neurons that perceive pain (nociceptors) have cell bodies in the dorsal root ganglia (DRG) and connect the gut to the brain via the spinal cord. IBD induces hyperexcitability in DRG nociceptors, the major cause of visceral pain. Proteases act on pain perception by activating cell-surface receptors that are present on sensory neurons and are responsible for the transmission of nociceptive signals. Recent findings indicate that heat-sensitive serine proteases from

Faecalibacterium prausnitzii, a bacterial strain found in the colonic microbiota of healthy individuals can reduce visceral pain. So far, there have been no known reports of the identification and expression of anti-nociceptive proteases from *F. prausnitzii*. Herein presented is our successful identification, expression, and purification of the anti-nociceptive serine protease from *F. prausnitzii*, the first step in the microbial modulation of IBD-related visceral pain.