Essential Oils do not Inhibit *E. coli* Peptidyl-tRNA Hydrolase

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Abstract

The purpose of the study was to purify peptidyl-tRNA hydrolase (Pth) and peptidyl-tRNA from bacterial cells in order to determine the inhibitory activity of essential oils from aromatic medicinal plants located in Nigeria, South Africa, Tajikistan and Huntsville.

A gel shift assay was used to determine the inhibitory factor of the essential oils against Pth.

The plant extracts that showed inhibitory activity were not essential oils.

Introduction

E. coli peptidyl-tRNA hydrolase (Pth) is an essential esterase that allows for the proliferation of the cell by cleaving tRNA from peptidyl-tRNA after the release from a stalled ribosome. Pth allows for the proliferation of the cell because of the need to recycle tRNA and recover the ribosome for further mRNA translation. If peptidyl-tRNA accumulates in the cell, protein synthesis will halt and cell death will occur.

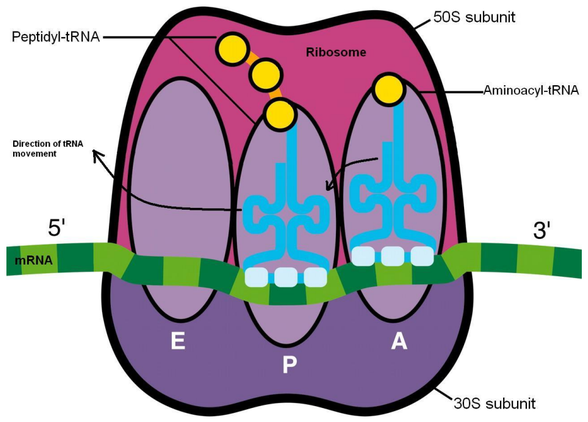

Figure 1: Protein Biosynthesis. Ribosome movement is from 5' to 3'.

The highly conserved, single form of Pth in bacteria enables researchers to study Pth inhibition and therefore study possible antibacterial agents.

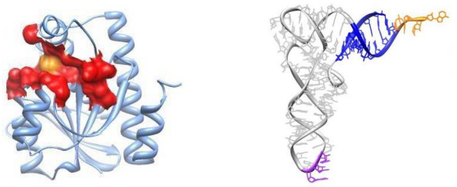

Figure 2: A representation of 21 kDa, globular α/β domain, peptidyl-tRNA hydrolase (Left) and peptidyl-tRNA (Right).

Plant extracts have been used for medicinal purposes for centuries. Plants have substantial secondary metabolites that have been found to have antimicrobial effects. Due to the development of resistance, the life span of antibiotics is limited. Plant sources have been recently studied to determine the effectiveness of antibacterials.

E. coli Pth Purification

E. coli Pth was purified using metal chelation chromatography. Pth was eluted with imidazole which competes with the hexa-histidine tag that coordinates with the Ni^{2+} sites. After the column, the resulting Pth-imidazole solution was pooled and dialyzed against activity buffer (10 mM Tris. Acetate pH 8.0, 10mM Magnesium Acetate, 20mM Ammonium Acetate in DEPC water).

Figure : Nickel Column

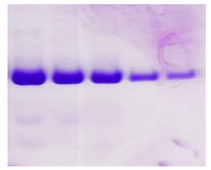

Figure : Purified Pth

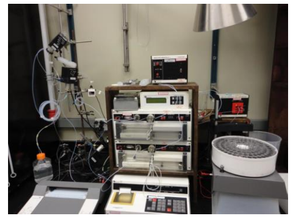

Figure : Nickel column (FPLC) apparatus

Optimization of Activity Assay

The optimal extract concentration and Pth concentration was determined using extracts with known inhibitory activity against Pth. Once the method was established, dimethyl sulfoxide (DMSO) and an inactive mutant of Pth, known as H20R, acted as the controls.

Acid urea polyacrylamide gels were used to perform the activity assays. Because tRNA is single-stranded and can form compact and stable secondary structures, urea, a denaturant is used to disrupt the hydrogen bonds. This prohibits reformation and allows for the fractionation of the tRNA, which is necessary to be viewable on a gel. A sodium acetate-methylene blue stain was used after electrophoresis to display the products of the reaction.

Essential Oil Optimization

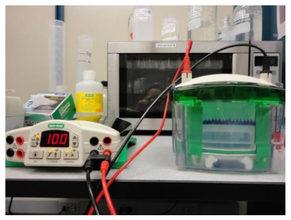

Figure : Electrophoresis Apparatus

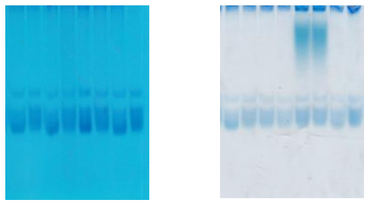

Figure : (Left) 3 µL aliquots of known inhibitory extracts; (Right) 5 µL aliquots

Optimization of Pth Concentration

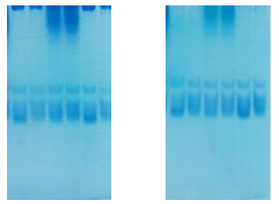

Figure : (Left) 3x diluted Pth Concentration (33.3 µM); (Right) 5x diluted Pth (20 µM)

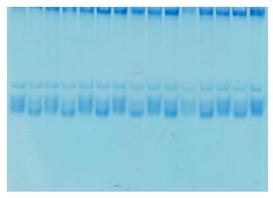

Figure : Activity Screen of Essential Oils numbered 1-6.

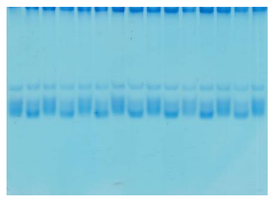

Figure : Activity Screen of Essential Oils numbered 7-12.

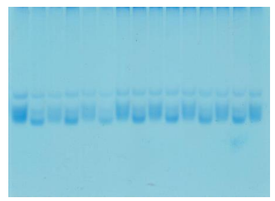

Figure : Activity Screen of Essential Oils numbered 19, 20, 21, 22, 24, 26

The Essential Oils

Mentha longifolia (aerial parts of the plant)
Aralia spinosa (leaf) - 4 different samples
Bursera graveolens (leaf)
Chenopodium ambrosioides (aerial parts)
Conradina canescens (aerial parts) - 2 samples
Cupressus lusitanica (leaf)
Humulus lupulus "Cascade" (hops)
Humulus lupulus "Norther Brewer" (hops)
Humulus lupulus "Vanguard" (hops)
Hyssopus vulgaris (aerial parts)
Magnolia grandiflora (flowers) – 4 samples
Pinus taeda – (bark), (leaf) and (wood)
Ptelea trifoliata (leaf)
Pulicaria jaubartii (aerial parts)
Sassafras albidum (bark)
Tagetes minuta (aerial parts)
Verbesina turbacensis (bark)
Verbesina turbacensis (leaf) – 2 samples

Results

Essential oils show no sign of inhibitory activity based on *in vitro* gel shift activity assays. Enzymatic activity was observed from Pth when incubated with each essential oil. Activity was based on whether or not peptides from peptidyl-tRNA accumulated and were visible by a distinct band on the gel. Therefore, cleavage by Pth occurred throughout all essential oils.

Matayba oppositifolia, is the source of an acetone-based plant extract from the bark of the tree, displayed strong inhibitory activity when tested against Pth. The extract, when diluted to 4% extract in DMSO, clearly inhibited Pth function (see figure).

Include table of extracts and essential oils here/somewhere?

Future Work

Matayba oppositifolia (Mo) will be used for future studies on Pth. The focus will be on why essential oils do not inhibit Pth function and why plant extracts, specifically Mo, inhibit Pth.

To analyze why plant extracts but not essential oils inhibit Pth activity, GC/MS will be performed on other essential oils in order to determine what compound allows plant extracts to inhibit Pth. In addition, analysis of Mo will determine what compound enables the extract to inhibit Pth function.

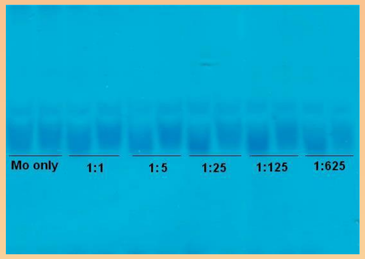

Figure : Serial Dilution of Mo (ratios reflect extract : DMSO)

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