Determination of functional properties of spates dissertation conclusion samples

Serine protease autotransporters of enterobacteriaceae (SPATEs) are particularly secreted by gram-negative enteric bacteria via autotransporter pathway (Rawlings, 3093). SPATEs are a wide family of proteases that are secreted particularly by Escherichia coli and Shigella flexneri. Shigella has several SPATEs including SigA, SepA, and Pic (Leonard, 1176). These proteases show two different proteolytic activities. Firstly, an intramolecular cleavage is initiated by a C-terminal catalytic site. This triggers the release of the N-terminal domain of the proteins into the medium surrounding the cell. Secondly, the N-terminal portions themselves are proteases with each containing a conical serine- protease catalytic portion. These secreted portions have various effects on mammalian cells.

Choosing the Oligopeptide Substrate

Protease action is specific for each protein (Sagi, 164). Choosing the oligopeptide substrate was done to determine the PicU-specific oligopeptide with the appropriate amino acid chain to be used in validating the proposed thesis. On each sequence of amino acid on each SPATE, split decomposition was done on the N-terminal portion. Each of the SPATEs was reacted with different oligopeptides conjugated primarily at the C-terminal domain with pNA. Significant substrate specificity is to a large extent determined by protease domains. The experiment revealed that Suc-Ala-Ala-Pro-abu-pNA is the best PicU-specific oligopeptide to be used.

Analysis of Proteolytic Activity of CFT073, Wtecn, and Δ Picu

The pNA conjugated PicU oligopeptide, Suc-Ala-Ala-Pro-Abu-pNA, was used as the substrate in this assay. Data from the experiment showed that the

comparison of the proteolytic activity of wtEcN and UPEC strain to the PicU mutant indicates an increase in oligopeptide cleavage as the absorbance increases in the former cases. The data revealed that PicU contributes to the proteolytic activity of EcN. Therefore, it is justifiable to conclude that any factors contributing to the mutation of the PicU gene in EcN also leads to the lowering of the cleavage of PicU-specific oligopeptide. The absorbance data of EcNΔ PicU also reveals background cleavage. This indicates that other proteins in the EcN genome also contributes to the cleavage of the same substrate.

Proteolytic Activity and Probiotic Effect of Ecn

Probiotics can ease gastrointestinal diseases such as irritable bowel syndrome, diverticular disease of the colon, and inflammatory bowel disease. Proteolytic activity of EcN has been applied over the years in treatment and prevention of chronic constipation. EcN also plays an important role in biological protein degradation in the body through proteolysis. EcN helps break down proteins into short chain proteins and peptides. This helps in their movement along the alimentary canal, thus reduces the possibilities of constipation. EcN also contributes to the formation of short chain fatty acids that are responsible for modulating colonic motility.

Probiotics such as EcN are also able to fight and destroy pathogens through the action of protease enzymes they produce. Pathogens can produce lethal toxins that bind to the specific receptors on the epithelial cells of the intestines. This causes inflammation and mucosal damage. Eukaryotic Probiotics are able to destroy bacterial toxins by releasing various enzymes such as proteases (Liong, 43). Proteases can break down the peptide bonds,

and thus alter the structure of the toxins. This renders the toxins harmless. These proteases are also able to break down the protein coating of most pathogens through lysis. This stops the physiological activities of the pathogens and eventually kills them.

Works Cited

Johnson, Leonard R. Physiology of the Gastrointestinal Tract. Amsterdam: Elsevier Academic Press, 2006. Internet resource. pg. 1176

Liong, Min-Tze. Probiotics: Biology, Genetics, and Health Aspects. Heidelberg: Springer, 2011. Print. pg. 43

Rawlings, Neil D, and Guy Salvesen. Handbook of Proteolytic Enzymes. San Diego: Elsevier Science, 2012. Internet resource. pg. 3093

Sagi, Irit, and Jean Gaffney. Matrix Metalloproteinase Biology. , 2015. Print. pg. 164