UNIVERSITI TEKNOLOGI MARA

GLYCOPROTEOMICS ANALYSIS OF SHIGELLA FLEXNERI AND ITS CONTRIBUTION TOWARDS PATHOGENICITY

MUHAMMAD IQBAL BIN MUSTAFA

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

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Name of Student	:	Muhammad Iqbal Bin Mustafa
Student I.D. No.	:	2010926529
Programme	:	Doctor of Philosophy (Proteomics) – AS990
Faculty	:	Applied Sciences
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Signature of Student	:	 ……..
Date	:	September 2021

ABSTRACT

Shigellosis or bacillary dysentery is caused by *Shigella flexneri* and affects over 165 million people annually resulting in more than 1.1 million deaths especially in children under the age of five. To date, the focus has been directed mainly on the role of lipopolysaccharides (LPS) in *Shigella's* pathogenicity apart from recent concerns addressing the increased resistance against antibiotics as well as the continuous efforts for vaccine development. The lack of data in Shigella pathogenicity especially in protein glycosylation and limited number of targets in vaccines and therapies development have hindered the development of an alternative drugs and vaccines. This study was designed to assess the glycoproteins of *S. flexneri* and its potential role in pathogenicity by utilizing two strains; the virulent *S. flexneri* 2457T and its avirulent counterpart, *S. flexneri* 2457O. Treatment using glycosylation inhibitors tunicamycin and tri-O-benzyl showed changes in morphology and reduced antibiotic resistance as well as virulence based on Congo Red binding assay and Caco-2 cells infection assay. It was found that 25 μg/mL of glycosylation inhibitors increased the susceptibility of *S. flexneri* towards 30 μg novobiocin. Next, introduction of glycosylation inhibitors which decrease the internalization of *S. flexneri* into Caco-2 cells. In addition, tunicamycin treatment also reduces Congo red binding ability of *S. flexneri* 2457T in a dose-dependent manner. It can assume that there is interaction between eukaryotic cell surface receptor and bacterial ligands which play an important role in the internalization of the bacteria which potentially being glycosylated. In silico glycosylation site prediction using GlycoPP, found twenty-one proteins of *S. flexneri* 2457T and five proteins of *S. flexneri* 2457O carrying potential *N* and *O*-glycosylation sites. Following this, the glycosylation of *S. flexneri* OM proteins was characterized by lectin binding assay and 1D PAGE. Further study using agarose-bound lectin revealed the existence of outer membrane glycoproteins with several types of terminal sugars which suggested to play roles in *S. flexneri* virulence Differential lectin binding was observed with Mannose/Glucose being the predominant terminal monosaccharides present. Next, the outer membrane proteins from both strains (wild-type and treated with glycosylation inhibitors) were extracted and profiled by 2D PAGE before being subjected to LC-MS/MS for protein identification. Twenty-six potential glycosylated proteins were successfully separated and identified. Two out of twenty-six glycoproteins were exclusively detected in the treated samples namely CDP-diacylglycerol pyrophosphatase (Tri-o-benzyl treated sample-possibly *N*-glycosylated) and protein YciI (Tunicamycin treated sample-possibly *O*-glycosylated). As a conclusion, *S. flexneri* OM proteins is glycosylated and these glycans may contribute towards pathogenicity and antibiotic resistance which warrants further investigation.

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May this work benefit others

InsyaAllah

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