Abstract of Master's Dissertation

No.1

Course	Master of Tropical Medicine	Name	Augustin Tshibaka KABONGO
Thesis Title	"**Purification and biochemical characterization of malate: quinone oxidoreductase from *Campylobacter jejuni*, a potential drug target**"		

Abstract of Master's Dissertation

Background: *Campylobacter jejuni* is the most common cause of bacterial foodborne illness worldwide. Infection is asymptomatic in most patients, and occasionally this disease leads to severe complications such as Guillain-Barré syndrome. Recently, resistance of *Campylobacter* spp. to antibiotics commonly used for clinical treatment has been reported. Therefore, the development of new drugs is an important strategy, along with other measures. There is a growing interest in electron transport chains (ETC) of microorganisms as drug target. Many bacterial species express malate:quinone oxidoreductase (MQO); this enzyme catalyses the oxidation of malate to oxaloacetate, a crucial step in the tricarboxylic acid cycle, but also transfers electrons to ETC, contributing to cellular bioenergetics. MQO is an interesting drug target because it is not conserved in mammalian cells and has been found to be essential for survival in several pathogens. In *C. jejuni*, the gene encoding MQO can be identified in the genome, but no study has been conducted to characterize this enzyme or evaluate its potential as a drug target.

Objective : This thesis aims to investigate and evaluate MQO from *C. jejuni* (CjMQO) as a novel drug target.

Methods: Putative CjMQO sequence was codon optimized for expression in *Escherichia coli.* TOPO cloning vector (pET101/pET151) was used to prepare the expression plasmids. The positive colonies were selected using carbenicillin as the marker with plasmid purified, sequenced and confirmed. The plasmid harbouring the correct sequence was used to transform the conventional *E. coli* expression system [NiCo21(DE3)]. Next, the CjMQO was overexpressed on the membrane fractions of NiCo21(DE3). Furthermore, I have optimized the protocol for CjMQO purification and conducted a biochemical characterization. Finally, the effect of two classic inhibitors of MQOs (ferulenol and embelin) were assessed on the activity of CjMQO and growth of *C. jejuni* strain JCM2013 *in vitro*.

＊ The abstract, containing background, objectives, methods, results and conclusion should not exceed 300-500words and printed double sided on A4 paper)

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Results : In solution, the purified CjMQO exhibited dimeric and tetrameric states. It had an optimal temperature of 40°C and a pH of 7. CjMQO showed higher affinity for ubiquinones (UQ) with long side chains and a maximum velocity of 48.8, 106.7, 111.4, 9.9, 76.3 µmol/min/mg for UQ0, UQ1, UQ2, UQ4, and decyl-UQ (dUQ), respectively. Substrate inhibition of CjMQO was observed above 10 µM for UQ2 and dUQ and 2 µM for UQ4. CjMQO showed a bisubstrate sequential Bi Bi reaction mechanism. Ferulenol and embelin inhibited CjMQO at IC_{50} of 0.018 and 0.087 µM, and the growth of *C. jejuni* JCM2013 at IC_{50} of 0.322 and 20.130 µM, respectively.

Conclusion : This is the first study reporting the purification of an active MQO from *C. jejuni* and its biochemical characterization. Interestingly, CjMQO displayed specific activity higher than the one reported for *Plasmodium falciparum* and *Toxoplasma gondii* mitochondrial MQOs, and is likely essential for *C. jejuni* survival and an important drug target, as growth of this pathogen was severely impaired in the presence of both MQO inhibitors. Nevertheless, future studies are needed to further elucidate the physiological role of this enzyme in *C. jejuni*, and validate it as a drug target.
Key words: malate:quinone oxidoreductase; purification; biochemical characterization; drug target.

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