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# Metabolite identification of Helicobacter Pylori supernatant using near-IR Raman Spectroscopy

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**ABSTRACT**

Helicobacter pylori, a non-invasive gram-negative bacteria is well known for its direct cause and effect relationship with peptic ulcer, gastric or duodenal ulceration. Its severe effect on human begin with its colonization and production of various enzyme like Vacuolating cytotoxin, Urease and Heat shock proteins. Urease is complex spherical protein which causes hydrolysis of gastric urea to ammonia and $CO_2$. The enzymatic reaction results in increase of gastric pH and hence results in gastric cancer. We present a photonic based technique to identify these proteins in its natural supernatant condition. The Raman spectrum of Helicobacter pylori is reported and the vibrational peaks are identified with corresponding chemical bonds in the biochemical enzyme. This can lay the foundation for future diagnostic tool for clinical detection of peptic ulcer, gastric or duodenal ulceration.

**Keywords**: Helicobacter pylori, Urease, Raman Spectroscopy, VacA, CagA.

## 1. INTRODUCTION

Helicobacter pylori (H. pylori), a non-invasive gram-negative bacteria is well known for its direct cause and effect relationship with peptic ulcer [1]-[4]. Numerous studies have reported that infection due to H. pylori results into gastric or duodenal ulceration [5]-[7]. It colonizes in the underlying layer of the gastric mucosa, where the H. pylori promotes the production of various proteinaceous enzymes into the extracellular space, namely; (i) vacuolating cytotoxin (VacA), (ii) cytotoxin-associated gene A (CagA), (iii) urease, and (iv) heat shock proteins (HspA and HspB).

VacA is a toxic protein which alters the morphology and function of the gastric epithelial cells. It has also been reported that strains of H. pylori are differentiated into two broad families: type I and type II. Only Type I strains suggest infection due to CagA cytotoxin, resulting into duodenitis, duodenal ulcers and gastric tumours. By comparison, the Type II strains are not known to have causing inflammation or ulceration [8]. Urease acts as a catalyst for enzymatic reaction, during which the gastric urea is hydrolysed into ammonia and carbonic acid. The release of ammonia increases the gastric pH via: $NH_3+H_2O \rightarrow NH_4^+ + OH^-$. It is this high pH environment, which supports the sub-mucosal ecology of H. pylori for colonisation. A schematic diagram of enzyme reaction supporting the eco-system of H. pylori is shown in Figure 1. Since the H. pylori colonies are buried into the mucosa, they remain insular from the strong acidic pH in the stomach. Figure 1 shows this colonization and changing morphology of the epithelial layer (steps [1] to [4]). The inset (layers of stomach) shows the colonization of Helicobacter pylori into the sub-mucosa where it sustains to grow into peptic ulcer. Finally, HspA and HspB are localized in the bacterial cytoplasm.

In this study, we have investigated the presence of these extracellular proteins in the supernatant of Helicobacter pylori using the near-IR Raman spectroscopic technique.

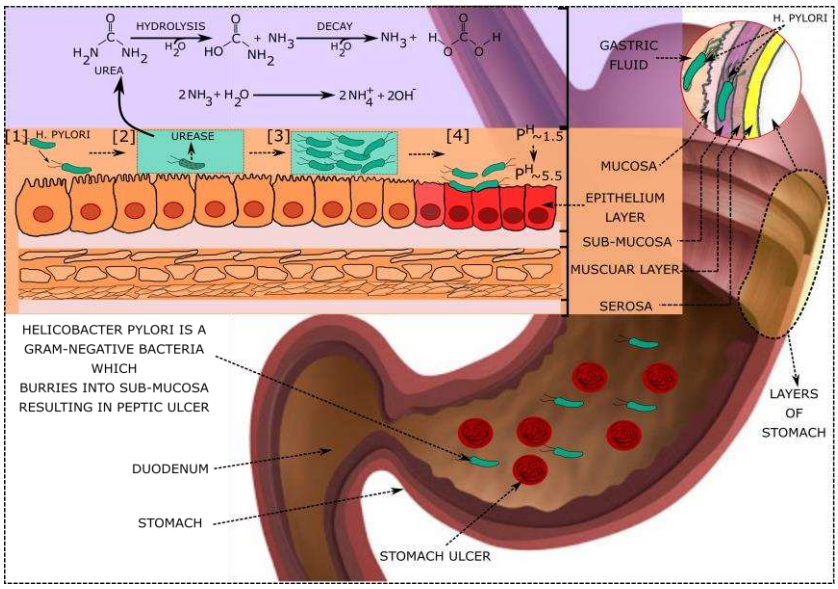


**Figure 1**: Schematic of enzymatic reaction in the stomach

Since the Raman spectroscopy offers a unique tool for the characterisation of molecular vibrations, which then represent the signatures for the presence of molecules in the tissue structure. In addition, any change due to enzymatic reaction can therefore be monitored using the Raman spectroscopy. The article therefore aims to set out a protocol for demonstrating the characterisation of metabolic by-products in vitro enzymatic reaction, which can be then adopted for mapping H. pylori colonies in the gastric mucosa.

## 2. MATERIALS AND METHODS

**Bacterial culture and Helicobacter pylori strain:** The H. pylori strain (NCTC 11916 [9]) was grown on blood agar plates at 37°C. The culture was injected into 0.9% saline solution by scrapping the bacteria from the plates. This saline solution was then added to a liquid media and agitated in rotary shaker. The shaken solution was incubated at 37°C (at 8% $CO_2$) for 3-5 days. The culture broth, thus obtained, was centrifuged to precipitate the proteins in form of pellets, which was then removed. The balance liquid was filtered through filter paper (pore size 0.22microns) and added with glycerol to avoid cytosolic crystallization. Seven (1ml) aliquots were prepared for analyses in the Raman spectrometer.

**Raman Spectroscopy of the supernatant:** A portable Raman spectrometer (Ocean Insight [10]) equipped with a diode laser source (785nm), spectrometer, Raman probe, sample holder was utilized for further studies. A blank vial was placed in the sample holder to obtain the background vibrational spectrum. Thereafter, the prepared supernatant aliquots (inside glass vial) were placed in the sample holder in sequence (from 1 to 7). The 785 nm laser source (power 350mW) was focussed on sample to obtain the vibrational spectrum (obtained via suitable photonic filter and mirrors) on customized software (spectrum analyser). Figure 2 shows the experimental setup including the supernatant sample.

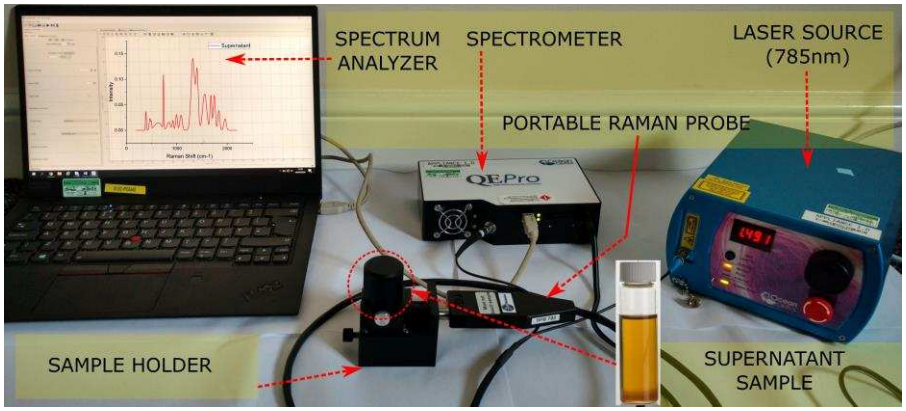


**Figure 2**: Portable Raman Probe for Urease supernatant analysis

## 3. EXPERIMENTAL RESULTS

Figure 3 shows the normalized Raman vibrational peak obtained for Helicobacter pylori supernatant. Each identified peak refers to a particular enzymatic bond which is explained as follows.

Urease peaks are identifiable at 427, 667, 832, 932, 1004, 1082, 1098, 1164, 1244, 1325, 1536, 1608, 1668, 1736, 1827, 1898, 1956, 2037cm$^{-1}$. Urease comprises of 4 molecules, i.e. $\alpha_A$, $\beta_B$, $Ni^{2+}$, $H_2O$ [11]-[12]. The $\alpha_A$ molecule consists of 238 residues of C, N, O and S atoms. Similar composition exists in $\beta_B$ with 569 residues. There are two atoms of $Ni^{2+}$, which forms the active site in water based urease.

Vac A [13] is again a complex protein of 12 long chains of 750 residues each, which consists of C, N, O and S atoms.

Cag A [14] also consists of a molecule comprising of two long chain of 103 residues each (with C, N, O and S atoms), a second molecule of isopropyl Alcohol, a third molecule of Di(hydroxyethyl) ether and water.

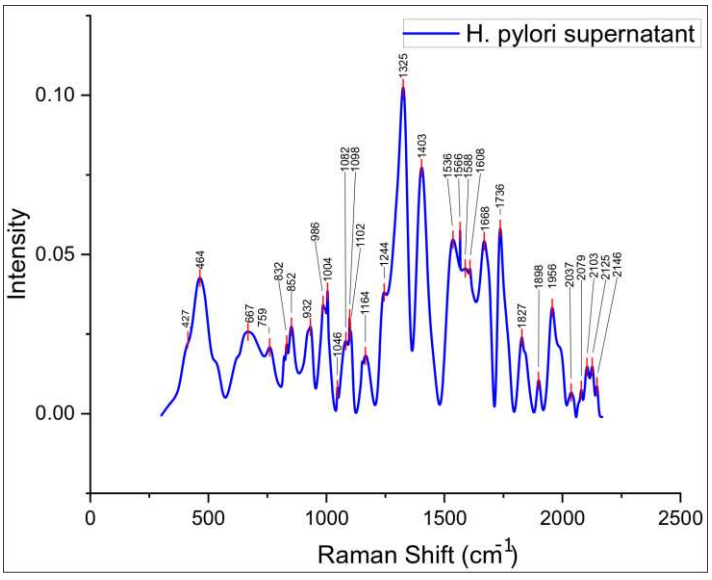


**Figure 3**: Raman Vibrational Spectrum of H. pylori Supernatant

Table 1 shows the Raman peaks assigned to specific chemical bond in urease, Vac A and Cag A.

**Table 1**. Raman frequencies of supernatant and their assignments.

| | **Assignment** | **Raman ($cm^{-1}$)** | **Assignment** | **Raman ($cm^{-1}$)** |
|---|---|---|---|---|
| **Urease** | S-S | 427 | N-C-N | 932, 1164 |
| | Ni-N | 1004 | C-S | 832 |
| | C-C | 1082, 1098, 1244, 1608, 1736, 1827, 1898 | $CO_2$ | 667, 1325 |
| | $NH_2$ | 1668 | C-O | 1536, 1956 |
| **Vac A** | $C_8$, $C_9$ | 2037, 2079, 2103, 2125, 2146 | | |
| **Cag A** | Isopropyl Alcohol ($C_3H_8O$) | 464, 759, 852, 986, 1102 | | |
| | Di(Hydroxyethyl) Ether ($C_4H_{10}O_3$) | 1403, 1566, 1588 | | |

## DISCUSSION AND CONCLUSIONS

Detection of different proteins of the Helicobacter pylori supernatant is demonstrated using Raman spectroscopy. The vibrational bonds of urease, VacA and CagA are understood to overlap for C, N, O and S bonds as they all have similar residue structure of different length. The long carbon chain ($C_8$, $C_9$) of Vac A and Cag A's components like Isopropyl Alcohol and Di(Hydroxyethyl) Ether differentiate the vibrational spectrum. One of potential benefit of identification of proteinaceous components of supernatant is understanding the mechanism and progression of gastric cancer in human patients. This can lay the foundation for future diagnostic tool for early clinical detection of peptic ulcer, gastric or duodenal ulceration.

## ACKNOWLEDGEMENTS

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