

### **Stain free implementation to differentiate *Helicobacter pylori* strains, and its correlation with protein pattern by RAPD**

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The bacterium *Helicobacter pylori* survives in the human stomach for decades. Because of its capacity to capture and assimilate foreign DNA, it is considered a natural competent Gram (-) bacterium. This characteristic allows it to change its DNA while colonizing the host. For most microorganisms, the Rapid amplification of Polymorphic DNA (RAPD) has been the method of choice to distinguish between strains from the same species confirming or refuting similarity between isolates. However, because of *H. pylori*'s genetic variability, the Multilocus Sequencing Typing (MLST) is required to confirm the identity in isolates in this species. This method, requires the amplification and sequencing of 7 genes, raising the cost for diagnostic and research laboratories around the world. Tryptophan is an essential aminoacid and its indole group makes it an aromatic aminoacid and confers the proteins where it is present a fluorescence at 280 nm wavelength. It is known that by adding chlor to the indole ring, the fluorescence was present after excitation with a 305 nm light source and detectable at ~ 500 nm. This feature is used to stain proteins in polyacrylamide gels using 2,2,2 trichlorethanol (TCE). Here we describe a new and low cost method to determine the uniqueness of *H. pylori* strains with the analysis of the protein profile and their tryptophan content produced. Chromosomal DNA was extracted from *Helicobacter pylori*'s culture and RAPD and Stain free was carried out. The software ImageLab™ of the ChemiDoc MP imaged the bands automatically. We analyzed the RAPD signal pattern of a number of different wild type bacteria and some strains obtained from patients, and we observed a different band pattern, which allow us to differentiate them from each other. We saw distinct protein patterns in gels after staining with Stain-Free method, and these depend on the source of the strain. We compared both methodologies, especially in patients who present different band pattern by RAPD, and we could see that protein expression was different as well, based on the distinct patterns of tryptophan-rich proteins by Stain-free. The bands' intensities between the strains were different as well, which leads us to think that production of proteins containing tryptophan varies between different strains. These observations gave us a base to consider that stain-free methodology might be used also as a differentiation technique. It is a complement for RAPD technique, or even a new technique, to differentiate between strains from *H.pylori* based in the production of tryptophan-rich proteins.