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A cheap, simple high throughput method for screening native *Helicobacter pylori* urease inhibitors using a recombinant *Escherichia coli*, its validation and demonstration of *Pistacia atlantica* methanolic extract effectivity and specificity

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ABSTRACT

Helicobacter pylori is the most frequent and persistent bacterial infection worldwide, and a risk factor for active gastritis, peptic ulcers, mucosa-associated lymphoid tissue lymphoma, and gastric cancer. Although combined antibiotics treatment is effective cases of antibiotic resistance are reported at an alarming rate. The *H. pylori* urease enzyme is essential for the bacteria establishment in the gastric mucosa, resulting urease inhibitors being sought after as effective and specific anti- *H. pylori* treatment. To-date, screening assays are based mostly on the analog plant urease enzyme but difference in properties of the plant and bacterial enzymes hamper these efforts. We have developed a screening assay based on recombinant *Escherichia coli* expressing native *H. pylori* urease, and validated this assay using thiourea and a methanolic extract of *Pistacia atlantica*. The assay demonstrated the thiourea and the extract to be potent urease inhibitors, with the extract having strong bacteriostatic activity against clinical isolates of *H. pylori*, including such with antibiotic resistance. The extract was also found to be neutral toward common probiotic bacteria, supporting its specificity and compatibility with digestive system desired microflora and suggesting it could be a good source for anti-*H. pylori* compounds.
The assay has proven to be cheap, simple and native alternative to the plant enzyme based assay and could allow for high throughput screening for new urease inhibitors and could expedite screening and development of novel, better *H. pylori* remedies helping us to combat this infection.

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1. Introduction

Helicobacter pylori is a gram-negative micro-aerobic bacterium described by Warren and Marshall (1983). To date it is considered the most frequent and persistent bacterial infection worldwide, with up to 84% of the population in some countries found positive for *H. pylori* (Mentis et al., 2015). Although many of those infected never report symptoms, *H. pylori* infection was identified as a risk factor for active gastritis, peptic ulcers, mucosa-associated lymphoid tissue lymphoma, and gastric cancer (Pişkipaşa and Pişkinpaşa, 2016).

The *H. pylori* specialized habitat, the human stomach mucosa, requires special adaptations. This ecological niche is characterized by very acidic pH (<2), a condition lethal for most microbes (but see Yang et al., 2013). *H. pylori* have been found to withstand these conditions by producing urease (EC 3.5.1.5), an enzyme that hydrolyzes urea, naturally found in the stomach, to ammonia, resulting in elevated pH and making conditions more favorable for the bacteria (Mobley, 1996). Indeed, patients with gastric ulcer showed elevated pH and ammonia concentration in their gastric fluids, which decreased when the *H. pylori* was eradicated (Furuta et al., 1998; Lee et al., 2004).

Currently, *H. pylori* infection is treated by a combination of proton pump inhibitor with multiple antibiotics (Ayala et al., 2014). Side effects of the treatment and increasing appearance of antibiotic resistance led to lower than 80% success rate in eradication of the bacteria and suggest there is an urgent need for alternative approaches (Megraud et al., 2013; Ayala et al., 2014; Camargo et al., 2014).

A promising approach to *H. pylori* eradication is the inhibition of its urease enzyme activity. This enzyme is produced in large quantities in the bacteria (up to 10% of total cell protein), and catalyzes the breakdown of one urea molecule to two ammonia and CO_2 molecules, the ammonia serving to raise the gastric fluid pH. Indeed, urease activity

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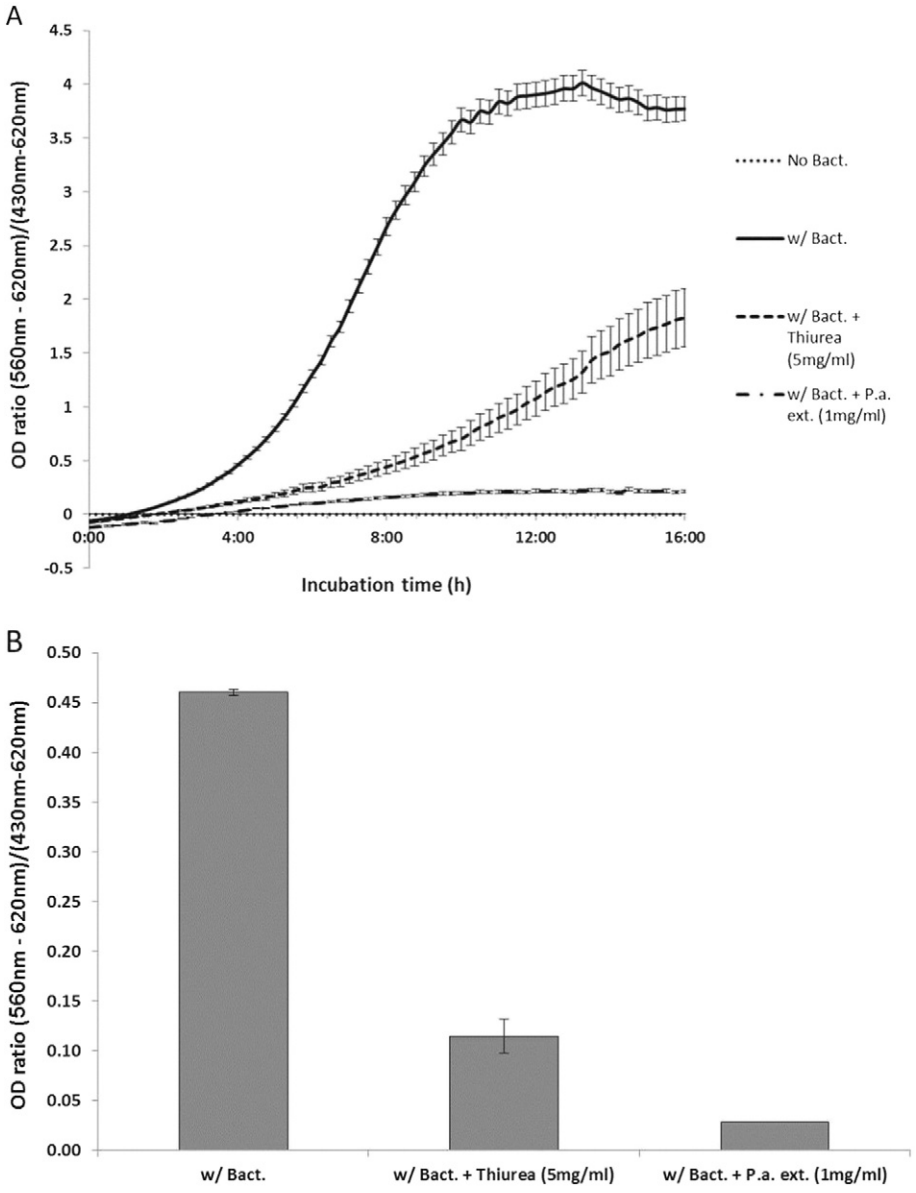

Fig. 1. (A). Urease activity under different treatments (Avg + S.E). Urease activity of *E. coli* pHP808/pHP902 strain with addition of 5 mg/ml Thiourea - a standard urease inhibitor; 1 mg/ml *P. atlantica* methanolic extract; or DDW as "no inhibition" control (+Bac). No bacteria control with extract added (−Bac) was used as blank. (B). OD ratio at 4 h time point.

was found to be essential to the bacterium's survival (Mobley, 1996; Bauerfeind et al., 1997) and for successful life-lasting colonization of the human stomach by *H. pylori* (Weeks et al., 2000).

An emerging approach to inhibition of the urease enzyme is the use of natural products; especially plant extracts (Ayala et al., 2014; Bonifácio et al., 2014). Many such have been demonstrated to be effective against *H. pylori* including many flavonoids from different plants, such as glabridin in licorice, luteolin in celery, quercetin in caper (Moon et al., 2013), curcumin (De et al., 2009), and honey (Ayala et al., 2014). Nevertheless, the search for inhibitory extracts and/or compounds is complicated by the need to work with live *H. pylori*, a known pathogen, or by the use of model enzymes that might have different properties.

This study describes the development of a screening system based on non-pathogenic *Escherichia coli* expressing the *H. pylori* urease in an active form, its validation using standard inhibitor (thiourea), a using it to study the effectivity of the methanolic extract of *Pistacia atlantica*.

Fig. 2. Demonstration of the extract effect on *H. pylori* clinical isolate #2. Whatman 3 paper disk contained 12.5 μg of *P. atlantica* extract. **Bold Circle** - Diameter of inhibition zone.

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