A novel *Helicobacter pylori* phage to combat gastric infections: first characterizations

Ferreira, R.[1,3].; Sousa, C.[1]; Gonçalves, Raquel F.S.[1]; Pinheiro, Ana Cristina[1]; Oleastro, M[2].; Azeredo J.[1]; Figueiredo, C.[3,4,5]; Melo, L.D.R.[1]

[1]CEB –Centre of Biological Engineering, University of Minho, Braga, Portugal; [2]Department of Infectious Diseases, National Institute of Health Doctor Ricardo Jorge (INSA), Lisbon, Portugal; [3]i3S –Institute for Research & Innovation in Health, Porto, Portugal; [4]Ipatimup – Institute of Molecular Pathology and Immunology of the University of Porto; [5]Department of Pathology, Faculty of Medicine, University of Porto, Porto, Portugal

Helicobacter pylori, a Gram-negative microaerophilic bacterium in a spiral shape, is recognized as an etiological agent of active chronic gastritis and contributes to peptic ulceration[1]. Furthermore, some studies associate it to development of gastric carcinoma[2]. Currently, treatment of *H. pylori* is performed with conventional antibiotics that in addition to the several side effects, increase the appearance of antibiotic-resistant strains, which, consequently, increase their inefficiency[3]. Thus, novel strategies targeting *H. pylori* are a serious challenge. Bacterio(phages) have emerged in recent years as an effective alternative to fight bacterial infections[4], but very little is known about *H. pylori* phages[5]. In this study, we described a novel *H. pylori* phage named HPy1R, induced by UV radiation from a Portuguese clinical strain, and characterized in terms of morphology, host range, stability, and efficacy against a *H. pylori* cultures. Moreover, the phage HPy1R was characterized in an *in vitro* gastric model with simulated salivary fluid and simulated gastric fluid[6]. Overall, the developed research allowed the isolation of a new *H. pylori* podovirus, that forms small uniform plaques (1 mm diameter) in five of the 76 *H. pylori* strains tested. Additionally, in terms of genomic characterization, HPy1R has a genome length of 31 162 bp, and 37.1 % *G+C* content. HPy1R shares 80 % query coverage and 88.64 % identity with phage *Helicobacter phage* COL 23-PUJ. Moreover, using OrthoVenn2 software, we noticed that the COL 23-PUJ, Pt5771G, SwA626G and pHiHP33 phage genomes share between 66.67 % and 97.22 % orthologous proteins with HPyR1. Interestingly, HPy1R was stable from pH 3 to 11, and in *in vitro* gastric model small loss in the phage titer was observed in the gastric phase, suggesting that this phage could be adapted to the human stomach environment. Farther, this phage demonstrated to be capable of maintaining the *H. pylori* population at low levels for up to 24 h post-infection with MOIs of 0.01, 0.1 and 1. In conclusion, in this work we present the first complete characterization of an *H. pylori* phage, which has features that envisage a great therapeutic potential against *H. pylori* gastric infections, if safety measures are guaranteed.

Keywords: *Helicobacter pylori*, Phage, Phage therapy, Genomic analysis

References:

1. Azevedo, N. F., Huntington, J. & Goodman, K. J. The epidemiology of helicobacter pylori and public health implications. *Helicobacter* **14**, 1–7 (2009).
2. Cover, T. L. & Blaser, M. J. Helicobacter pylori in health and disease. *Gastroenterology* **136**, 1863–1873 (2009).
3. Chey, W. D., Leontiadis, G. I., Howden, C. W. & Moss, S. F. ACG Clinical Guideline: Treatment of Helicobacter pylori Infection. *Am. J. Gastroenterol.* **112**, 212–238 (2017).
4. Lin, D. M., Koskella, B. & Lin, H. C. Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. *World J. Gastrointest. Pharmacol. Ther.* **8**, 162 (2017).
5. Muñoz, A. B., Stepanian, J., Trespalacios, A. A. & Vale, F. F. Bacteriophages of Helicobacter pylori. *Front. Microbiol.* **11**, 12 (2020).
6. Minekus, M. *et al.* A standardised static in vitro digestion method suitable for food-an international consensus. *Food Funct.* **5**, 1113–1124 (2014).