# **Study of the Effect of Bacterially Produced Secondary Metabolites on SARS-CoV-2 (COVID-19) *in Vitro***

Alexis C. R. Hoste1,2, Aurélien Cugnet1, Willy Smeralda3, Magali Deleu3, Mutien Garigliany2 and Philippe Jacques1

1*MiPI, TERRA Teaching and Research Centre, Joint Research Unit BioEcoAgro, UMRt 1158, Gembloux Agro-Bio Tech, University of Liège, Avenue de la Faculté, 2B, B-5030 Gembloux, Belgium*

2*Animal Pathology, Faculty of Veterinary Medicine, University of Liège, Avenue de Cureghem 6 B43, 4000 Liège, Belgium*

3*LBMI, TERRA Teaching and Research Centre, Joint Research Unit BioEcoAgro, UMRt 1158, Gembloux Agro-Bio Tech, University of Liège, Avenue de la Faculté, 2B, B-5030 Gembloux, Belgium*

In the past 20 years, three coronaviruses have emerged with the potential to cause pandemics, as showcased by the newly emerged SARS-CoV-2, the causative agent of COVID-19. Vaccines have rapidly been developed, however, to further reduce viral transmission, and mortality, effective antiviral treatments are still needed.

Lipopeptides are secondary metabolites produced by *Bacillus* spp., which are promising microbial biosurfactants and were shown to have antiviral properties against a wide range of enveloped DNA and RNA viruses, including coronaviruses. In the present work, lipopeptides from the surfactin family (surfactin, lichenysin and pumilacidin) were produced to test their antiviral activity against SARS-CoV-2. Different surfactin-like isoforms, varying in their fatty-acid chain length and their peptide chain composition, were purified. The *in vitro* cytotoxicity of each isoform was determined, and each was tested for its antiviral activity against SARS-CoV-2 on Vero E6 cells.

Surfactin isoforms exhibited the lowest cytotoxicity of the surfactin family, followed by lichenysin and pumilacidin, respectively. Based on these cytotoxicity results, variants were selected and tested for their antiviral activity. Some variants significantly reduced the viral RNA concentration in infected cells by 3-log to 6-log compared to control infected cells. Further experiments on SARS-CoV-2 were carried out to determine the mechanisms by which these lipopeptides inhibit SARS-CoV-2. Some isoforms significantly reduced the binding of SARS-CoV-2 to the cells, with some of them inhibiting the binding of the virus to levels comparable to neutralizing antibodies. Finally, experiments to study the impact of these lipopeptides on the fusion and on the budding steps are being carried out to fully understand the inhibitory mechanism of these lipopeptides.

The present work provides insights to better understand the link between structure and antiviral activity of surfactin-like lipopeptides and could lead to the design of new lipopeptides exhibiting a low cytotoxicity and a high antiviral activity, and, potentially, an effective treatment.