

PAPER • OPEN ACCESS

Peptidomic and Bioinformatic Analysis to Evaluate the Application of Peptides from Latex of *Hevea brasiliensis* Clone BPM24

To cite this article: P Havanapan and N Phungthanom 2019 *IOP Conf. Ser.: Mater. Sci. Eng.* **526** 012037

View the [article online](#) for updates and enhancements.

Peptidomic and Bioinformatic Analysis to Evaluate the Application of Peptides from Latex of *Hevea brasiliensis* Clone BPM24

P Havanapan^{1,*}, N Phungthanom¹

¹Institute of Molecular Biosciences, Mahidol University, Salaya, Nakhonpathom, Thailand.

*Corresponding author: phattaraorn.hav@mahidol.ac.th

Abstract. Peptidomic analysis coupled with mass spectrometry identification was employed to investigate the constitutively expressed peptides in latex serum *Phytophthora*-resistant rubber tree (*Hevea brasiliensis*), BPM24. The utilization of proteins and peptides as a strategy to study new methods can lead to a therapeutic application. However, a high-efficiency method to extract peptides from latex serum is not well established. After optimization of latex serum isolation from natural rubber latex, using of lysis solution for peptide extraction exhibited superior serum peptide recovery than using other chemical reagents. Ultrafiltration followed by solid-phase extraction was selected to achieve high extraction yield of low molecular weight proteins and peptides. Mass spectrometric and bioinformatics analysis characterized and predicted the identified protein and peptide contents. All latex serum peptide fractions from C₁₈ reversed-phase chromatography possessed effectiveness of antifungal activity against three tested plant fungal including *A. brassicicola*, *C. lunata* and *M. grisea* with different MIC in the range 250-2,000 µg/ml from different peptide fraction. Moreover, Etotol (E1-E5 pooled together) from lysis solution extraction was found to be able to inhibit three tested plant fungal at MIC 500 µg/ml. Moreover, *B. cereus* and *E. faecium* were the sensitive gram-positive bacteria with MIC in the range 250-4,000 µg/ml. However, all gram-negative bacteria were not affected by 4,000 µg/ml. Among these tested pathogens, plant fungal was quite sensitive to our peptide product. Etotol from lysis solution extraction will be the best candidate for using further in antifungal testing of other plant fungal both *in vitro* and *in vivo*. The further investigation and application will lead to possible use of latex serum as a great biotechnological resource to develop peptide –based product for the treatment of plant infection.

1. Introduction

The widely cultivated Para rubber tree, *Hevea brasiliensis*, clone BPM24 produces a high yield of latex, is highly tolerant to *Phytophthora* disease. Therefore, it might be possible clone BPM24 may be produced antimicrobial proteins or peptides to protect itself from any pathogens. The term “peptidomics” was defined as the quantitative and qualitative analysis of endogenous or native low molecular weight proteins in biological samples [1]. However, peptide extraction efficacy of plant serum, especially *H. brasiliensis*, for industry application remains a technological challenge for increased coverage of the serum peptidome. The major problem for small peptide preparation is that their concentration is comparatively lower than the protein concentration. Peptide may diffuse from their tissue of origin and be diluted in rubber latex. Therefore, the specificity and sensitivity of processing for enhance the abundant peptides is necessary to further study. The aim of this research was to conduct an evaluation of peptide extraction method from *H. brasiliensis* latex serum to maximize the total number of peptides detected by mass spectrometric analysis



together with the screening of extracted proteins for antimicrobial activity. These obtained and further data would be promising valuable peptides for new antifungal agent development.

2. Materials and methods

2.1 Latex serum isolation

Fresh latex samples were collected from the Para rubber trees, *H. brasiliensis* clone BPM24 by tapping method with Jeh-Bong knife. Thirty rubber trees, planted in the same crop at Khao Cha Mao district, Rayong province were selected for sample collection. To avoid contaminants, the fresh latex was collected directly into plastic container with or without addition of the preservative (0.6% ammonia). The latex serum was obtained by centrifugation of latex sample at 4 °C using the optimized condition. Then, the latex serum (supernatant) was collected and used for peptide extraction process.

2.2 Serum peptide extraction and subfractionation

To extract peptides from latex serum, serum sample was mixed with three different reagents at the final concentration of 80% acetone, 80% methanol, and lysis solution (8 M Urea, 2 M Thiourea, 2% CHAPS, 0.4% Triton X-100, 50 mM DTT). The filtrate was passed through consecutively on three molecular weight cut-off membranes (30, 10, and 3 kDa) using an Amicon ultra-15 centrifugal filter (Millipore, Germany). The filtrate (after passed 3kDa MWCO) was collected, pooled together, and stored in aliquots at -30 °C until used for peptide subfractionation by C₁₈ reversed-phase chromatography. The filtrate mixed with 0.1% trifluoroacetic acid was subsequently loaded onto Solid-phase extraction (SPE) cartridges, SEP-PAK C18 reversed-phase chromatography [Waters]. The 50 % acetonitrile/0.1 % TFA was used as elution buffer. The eluate from the 50 % acetonitrile/0.1 % TFA elution were divided into five fractions, designated as E1 to E5, collected depending on volume to volume or pooled each fraction together (Etotal). Each fraction was concentrated and dried until becoming the powder by Freeze dryer [Thermo Fisher Scientific].

2.3 Peptidomic and Bioinformatic Analysis

The dried peptides were resuspended and vigorously mixed with MALDI matrix solution. The target samples were subjected to analysis in a Ultraflex III TOF/TOF mass spectrometer (Bruker Daltonics, Germany). The machine was run in the linear positive mode and mass spectra in the range of 500-5,000 m/z were collected. MALDI spectra of each sample from the mass spectrometer were subjected to spectral processing including peak detection, smoothing, baseline subtraction, and recalibration using flexAnalysis 3.0 software. For MS/MS analysis, the dried peptides were resuspended in 10 µL of 0.1 % formic acid. NanoLC-ESI MS/MS analysis was performed using an Ultimate 3000 LC system (Dionex, USA) coupled to an ESI-Ion Trap MS (Bruker Daltonics, Germany). These peptide fragment masses were detected and further processed by DataAnalysis software (Bruker Daltonics, Germany) to generate mass lists in the form of Mascot generic files. The MS/MS data from LC-MS were submitted to database search using the Mascot software *via* Matrix Science to identify peptide sequences. Moreover, the identified peptides were then blasted against a database of antimicrobial peptides through the bioinformatics program (AMPer) resource and find similarities to any known antimicrobial and anticancer peptides.

2.4 Antimicrobial testing

The pathogen that used in this experiment were 4 fungi [*Candida albicans* (ATCC 90028), *Alternaria brassicicola* (BCC 42724), *Curvularia lunata* (BCC 15558), *Magnaporthe grisea* (BCC 10261)] and 6 bacteria [*Acinetobacter baumannii* (ATCC 19606), *Pseudomonas aeruginosa* (ATCC 15692), *Klebsiella pneumoniae* (ATCC 700603), *Escherichia coli* (ATCC 25922), *Enterococcus faecium* (ATCC 51559),

Bacillus cereus (ATCC 11778)]. Antimicrobial activity was tested by broth microdilution assay. Briefly, each cell suspension was adjusted and further diluted to appropriated concentration. The assay was performed in 384-well plate in triplicate. Each well was added with extracted peptides (or positive or negative control agents) and cell suspension. Plates were then incubated at suitable temperature and hour depending on each microbial strain. Bacterial and fungal growth was observed by OD measurement at different excitation and emission wavelengths. Percent of bacterial/fungal inhibition was calculated. The concentration of crude peptide fraction required for 80% growth inhibition (MIC_{80}) was reported.

3. Results and discussion

3.1 Peptidomics analysis of *H. brasiliensis* clone BPM24 serum

Lysis solution extracted a higher number (93 ± 3) of low molecular weight peptides achieved more than that of 80% acetone and 80% methanol. Peptide extraction from fresh rubber latex by lysis solution followed by ultrafiltration (MWCO 30, 10, and 3 kDa, respectively) and then peptide purification and concentration (SEP-PAK C18) was the most efficient method to obtain superior serum peptide recovery than by other chemical reagents. There were some 31 peptides from 8 identified proteins found. The function of each identified proteins were also discussed. Most of identified proteins played roles in ATP synthesis and hydrolysis. The expression of ATP-binding cassette family was found to be high abundance in all extraction method of latex serum from pathogenic fungi tolerant *H. brasiliensis* BPM24. The bioinformatics program (AMPer) were then used to predict putative antimicrobial and anticancer peptides from the mass spectrometric-detected peptides. The prediction of peptide analysis revealed 15 putative antimicrobial or anticancer peptides. Most of these putative peptides showed broader spectrum activities or more potent activity against both Gram-negative and positive bacteria and fungi.

3.2 Antimicrobial activity of latex serum crude peptides

The fractionated peptides eluted from SEP-PAK column were collected. Each elution were subjected to perform antimicrobial testing. The results showed that all latex serum peptide fractions from C₁₈ reversed-phase chromatography possessed effectiveness of antifungal activity against three tested plant fungal including *A. brassicicola*, *C. lunata* and *M. grisea* with different MIC 250-2,000 µg/ml. Moreover, *B. cereus* and *E. faecium* were the sensitive gram-positive bacteria with MIC 250-4,000 µg/ml. However, all gram-negative bacteria were not affected by 4,000 µg/ml. Among these tested pathogens, plant fungal was quite sensitive with MIC₇₀ value as little as 125 µg/ml. The further investigation and application will lead to possible use of latex serum as a great biotechnological resource to develop bioactive agents for the treatment of human and plant infection.

4. Conclusion

Peptidomic analysis of latex serum *H. brasiliensis* BPM24 was investigated. Peptide extraction from fresh rubber latex without any preservatives by lysis solution extraction and then ultrafiltration passed MWCO 3 kDa prior to SPE using SEP-PAK C18 is the efficient method to obtain superior serum peptide recovery than using other chemical reagents. Moreover, the antimicrobial activity of latex serum proteins could be observed. Most of peptide fractions were highly active against all plant-pathogenic fungi and less active against gram-positive bacteria. Our perspective investigation and application will lead to possible use of latex serum as a great biotechnological resource to develop bioactive agents or peptide-based product for the treatment of *Phytophthora*-infected *Hevea brasiliensis* because various *Phytophthora* species cause leaf fall and black stripe diseases leading to a yield loss in rubber plantations.

5. Reference

1. Schulte I, Tammen H, Selle H, Schulz KP. Peptides in body fluids and tissues as markers of disease. *Expert Rev Mol Diagn.* 2005;5:145-157.

Acknowledgements

This work was financially supported by the Thailand Research Fund (TRF) and National Research Council of Thailand (NRCT) to PH.