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Discovery of plant antimicrobial peptides and laboratory scale production

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Abstract: Plant antimicrobial peptides (PAMPs) are natural peptide molecules derived from plants with antimicrobial properties. Their targets range from viruses, bacteria to fungi with the activities of replication or growth inhibition, slow and abnormality growth induction, bactericides and fungicides. With the availability of genome, transcriptome and metabolome databases, bioinformatics has been successfully identified these beneficial molecules and *in silico* studied of their properties. Four peptides including P5, P9, P25 and P36 were selected from retrieved genomic and transcriptome data of monocots including maize, sorghum, rice and sugarcane. Their properties were analysed online tools such as the predicted protein server, Phyre2 and AMPA. The molecules had potential to be antimicrobial peptides. In order to determine their activities, *E. coli* expression system was used to produce these peptides in fusion form. The fusion proteins were selected, digested, purified and concentrated using affinity absorption and size selection methods. Spectrophotometry indicated that the purified peptides had high concentration and can be used for further study.

Keywords: defensin, bioinformatics, *E. coli* expression system, laboratory scale production, protein stability

Introduction

Antimicrobial peptides (AMPs) are found in invertebrates, vertebrates, plant and bacteria. Most AMPs are cationic or amphiphilic. Hence their interactions are to anionic charge molecules. Modes of AMP functions include 1) binding to negatively charged surfaces of gram negative bacterial outer membrane, 2) interact with bacterial cell wall and 3) interact with bacterial cell membrane. These actions cause the restriction cell division, causing cell wall remodeling and subsequently cell lysis. In some cases, AMPs interact with specific targets within the bacterial cells causing the disruption of cellular functions such as protein synthesis (Bechinger and Gorr, 2018). They are, therefore, valuable not only for plant pathogen protection but also medical and veterinary treatment. Some plant AMPs reportedly had antifungal and antibacterial activities against both plant and human pathogens (Sathoff et al., 2019). Although share modes of functions, plant microbial peptides have a distinct character of high cysteine residues which form multiple disulfide bonds. Most of them are low and moderate in size (molecular weight). These features provides advantages of chemical, thermal and proteolytic activities. There are seven major PAMP families; thionins, defensin, hevein, knottin, hairpinin, lipid transfer proteins (LTPs) and snakins (Tam et al., 2015).

Recently, many plant genome and transcriptome databases are available in public domain. Therefore, bioinformatics approach becomes a power tool in identifying and retrieving PAMPs data. Schmitt et al., (2016) reported the link between variations of AMP amino acid sequences and AMP binding capacity to bacterial membranes. Plant AMPs are generally accumulated from specific organs such as leaves, flower and seeds. Several purification steps are required for purified AMPs (Tang et al., 2018). Alternatively, *in silico* approach combining with *de novo* synthesis via chemical reaction has become an alternative approach to conventional method for production and an improvement of AMP (Mikut et al., 2016). The aim of this study is to 1) identify target AMPs and retrieve the genome/transcriptome data from monocots databases using *in silico* study 2) to amplify AMP genes from monocots and 3) produce purified PAMPs in laboratory scale.

Materials and Methods

Plant databases

There are many plant genome and transcriptome databases available on line. However, only those with monocots data (sugarcane, rice, maize and sorghum) were included (Table 1). Criteria such as keywords and sequence similarity were used in BLAST and FASTA search. The outcome from various sources were compared and analyzed. The PAMP genomic DNA and cDNA sequences were retrieved. They were aligned using MacVector 12.6.

***In silico* study on protein properties and antimicrobial activity**

Protein secondary and tertiary structures and other important characters including solubility, transmembrane probability and stability were investigated. The antimicrobial activities were determined using on line software (Table 1).

Primer design and PCR amplification

Primers were designed based on retrieved sequences using Primer3 (Untergasser et al., 2012). The target genes were amplified using Reverse transcription PCR. The DNA were cloned and automate sequenced. The authenticity were determined using BLASTX software (Altschul et al., 1997).

***E. coli* Expression system**

DNA fragments were cloned into *pMAL-c2* (New Englan Biolab, USA) and recombinant plasmids were transformed into *E. coli*. The optimal temperature were investigated in small scale conditions. The fusion protein was produced in laboratory scale under optimized conditions. It was purified and digested with factor Xa. PAMPs was further purified and concentrated.

Result & Discussion

Four plant microbial peptides (P5, P9, P25 and P36) were successfully retrieved from plant databases. They were identified as defensin and snakin. They displayed antimicrobial activities and relatively stable in cytoplasm. The coding regions were cloned into *pMALc2 E. coli* expression vector. The work processes were summarized in Figure. 1 *E. coli* expression system is widely used for heterologous protein expression because of the cost effective and routine/standard protocol for *E. coli* production (Chen, 2012).The temperature, aeration and duration for induction were tested to fine optimized condition for protein production (Papaneophytou and Kontopidis, 2014; Singha et al., 2017). The fusion proteins were produced in laboratory scale and maintained within bacterial cells. They were released using sonication and purified from other bacterial proteins via specific binding. PAMPs were digested out of fusion proteins, purified and concentrated using size exclusion chromatography. The concentration and protein content were summarized in Table 2. The results indicated variation of protein recovery and yield. Interestingly, the deviation results from the last steps of factor Xa digestion and protein recovery. This could be specific protein tertiary structure that reduce availability of factor Xa site or the current process caused misfolding of specific protein (Overton, 2013).

Conclusion

Plant antimicrobial peptides (PAMPs) were effectively produced in laboratory scale using bioinformatics approaches combining with *E. coli* expression system. This method can be applied to other AMPs production.

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References

Bechinger, B., and Gorr, S. U. 2017. Antimicrobial peptides: mechanisms of action and resistance. Journal of Dental research 96(3): 254–260.

Chen, R. 2012. Bacterial expression system for recombinant protein production: *E. coli* and beyond. Biotechnology Advances 30: 1102–1107.

Mikut, R., Ruden, S., Reischl, M., Breitling, F., Volkmer, R., and Hilper, K. 2016. Improving short antimicrobial peptides despite elusive rules for activity. Biochimica et Biophysica Acta (BBA)-Biomembranes 1858:1024–1033.

Overton, T. W. 2014. Recombinant protein production in bacterial hosts. Drug Discovery Today 19(5): 590–601.

Papaneophytou, C.P. and Kontopidis, G. 2014. Statistical approaches to maximize recombinant protein expression in *Escherichia coli*: A general view. Protein Expression and Purification 94: 22–32.

Sathoff, A. E., Velivelli, S., Shah, D. M. and Samac, D. A. 2019. Plant defensing peptides have antifungal and antibacterial activity against human and plant pathogens. Phytopathology 109: 402–408.

Schmitt, P., Rosa, R. D. and Destoumieux-Garzón, D. 2016. An intimate link between antimicrobial peptide sequence diversity and binding to essential components of bacterial membranes. Biochimica et Biophysica Acta (BBA)-Biomembranes 1858: 958–970.

Singha, T. K., Gulati, P., Mohanty, A., Khasa, Y., Kapoor, R. K. and Kumar, S. 2017. Efficient genetic approaches for improvement of plasmid based expression of recombinant protein in *Escherichia coli*: A review. Process Biochemistry 55: 17–31.

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman D. J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research. 25(17) : 3389–3402.

Tam, J. P., Wang, S., Wong, K. H. and Tan, W. L. 2015. Antimicrobial peptides from plants. Pharmaceuticals 8(4): 711–757.

Tang, S. S., Prodhan, Z H., Biswas, S. K., Le, C. F. and Sekaran, S. D. 2018. Antimicrobial peptides from different plant sources: isolation, characterization and purification. Phytochemistry 154: 94−105.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M. and Rozen, S.G. 2012. Primer3-new capabilities and interfaces. Nucleic Acids Research 40(15): e115.

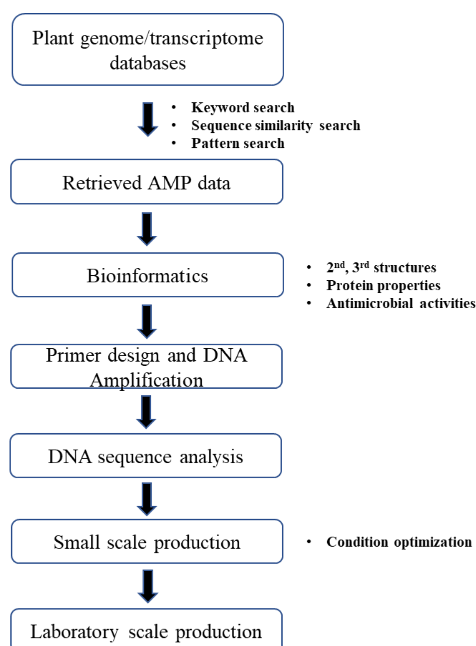

Figure 1. The overview of plant AMP protein production at laboratory scale.

Table 1. Websites used in this study for A) Plant genome and transcriptome databases and B) Protein analysis and antimicrobial activity.

Name	Location	Tools
Ensembl Plants Home	http://plants.ensembl.org/species.html	BLAST/BLAT
NCBI	https://www.ncbi.nlm.nih.gov/	BLAST, keyword search
Phytozome12	https://phytozome.jgi.doe.gov/pz/portal.html	BLAST, keyword search
PlantGDB	http://www.plantgdb.org/prj/GenomeBrowser/	BLAST, pattern search
Sugarcane genome hub	http://sugarcane-genome.cirad.fr/	BLAST, keyword search, primer design
PredyFlexy	http://www.dsimb.inserm.fr/dsimb_tools/predyflexy/index.html	Flexibility and Local Structure prediction from sequence
Pro Pi	https://www.protpi.ch	Protein tools, peptide tools
AntiBP2	http://crdd.osdd.net/raghava/antibp2/index.html	Server for antibacterial peptide prediction
Antimicrobial Sequence Scanning System (AMPA)	http://tcoffee.crg.cat/apps/ampa/do	A theoretical approach to spot active regions in antimicrobial proteins.

Table 2. Protein concentration and total protein content of four plant antimicrobial peptides. Bradford assay was used for determination of protein concentration with bovine serum albumin (BSA) as a protein standard.

Step	Protein concentration (mg/mL)	Total protein (milligram)
Cell suspension	0.455 - 1.083	182-433.2
Cell disruption	7.65 -8.27	153.0-169.5
Fusion protein	4.15-7.19	4.15-7.19
Purified protein	0.014-0.765	0.0032-137.7