

Phylogenetic connections and comparisons of the amino acid sequences of several peptide inducers of microbial origin-A foundation study for development of novel biopesticides

Suvala Shalini Devi, Chanduri Akshitha, Aparna Bandaram, Bomma Sharath Chandra and Vulishe Vaishnavi

ABSTRACT

Peptides of microbial origin stand out as one of the prominent tools to elicit plant immunity. These peptides comprise one of the key strategies of integrated pest management and are considered as candidates to develop novel biopesticides. Many research investigations have proved their potential in fending off plant pathogens and were described as sustainable plant protection strategies. The present study was attempted to discover phylogenetic relationships and compare amino acid sequence alignments of various peptide elicitors of microbial origin. Phylogenetic analysis of 33 microbial peptide elicitors resulted in two clusters, one cluster contained 19 flagellin sequences, which is further divided into one major (15 peptide sequences) and one (4 peptide sequences) minor subclusters. Further amino acid sequence alignments were carried out based on the evolutionary relationships among the peptides. The amino acid sequence alignment of flagellin sequences using Clustal W did not present conservative amino acid sequences except Serine (S), Alanine (A) and Aspartic acid (D). These conserved amino acids (SAxD) that are positioned in the protruding loop may play a vital role in recognition by plant surface receptors. Alignment of amino acid sequences of cold shock protein, xylanase, elongation factor and harpin from various sources did not present conservative amino acid sequences except glycine. These investigations lay a theoretical foundation for exploring many more microbial peptides for inducing plant resistance.

Keywords: plant immunity, peptide elicitors, microbial peptides, flagellin, Alignment of amino acid sequences.

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INTRODUCTION

Plant diseases are responsible for major share of crop losses worldwide (Agrios, 2005). Most of the plant disease management strategies being implemented were curative measures (Maloy, 2005). "Prevention is better than cure"-deployment of preventive measures for plant disease control not only protects the crop but also prevents financial losses (Maloy, 2005).

Introduction of elicitors that activate plant defense system is considered as one of the best strategies for disease prevention (Thakur and sohal, 2013; Tamm *et al.*, 2011). Utilization of these elicitors can reduce the scope of chemical control, thus paves the path for sustainable agriculture, the need of the day (Caicedo-López *et al.*, 2021). Boosting the natural plant immunity can combat pathogens causing minimal loss to the plant. Peptides

elicitors from microbial sources are the most promising tools to spike the plant immune system. Several peptides like elf18, flg22, flg15, pep13, HaX23, axYS22 etc. from beneficial and pathogenic bacteria were proved as potential triggers of plant resistance. Other novel protein elicitors like PeBL1 from *Brevibacillus laterosporus* (Wang *et al.*, 2015), PeBA1 from *Bacillus amyloliquefaciens* (Wang *et al.*, 2016), BAR11 from *Saccharothrix* (Zhang *et al.*, 2018), AMEP412 from *Bacillus* and Hpa Xpm, novel thermo stable harpin peptide from *Xanthomonas phaseoli* were described (Liu *et al.*, 2020). These peptides are sensed by LRR-RLKs or LRR-RLPs of plant cells (Albert, 2013). Small exogenous peptides of a plant could be sensed by plants belonging to other taxa resulting in resistance induction of the latter. Systemins and HypSys from Solanaceae were found to induce resistance in *Arabidopsis thaliana* to the necrotrophic fungus *Plectosphaerel lacucumerina* (Pastor *et al.*, 2020). Identification of key amino acid residues that are involved in perception by specific receptors can pave path for quick and accurate screening of more number of peptide elicitors produced by various microbes which can be employed for activating plant immunity. Future research on screening microbial biopesticides/biocontrol agents should not only focus on direct effect of biocontrol agent on pathogen, but should also study its capacity to strengthen plant defense system which can aid in the control of broad-spectrum pathogens. In the current study, we used bioinformatic techniques to evaluate the amino acid sequence alignments of different peptide elicitors and analyze the evolutionary relationships among them. Further, sequence comparison of well-known microbial peptide elicitors is done to understand the amino acid sequence homology. Also molecular modelling of flagellin peptide was performed to predict the spatial location of homologous amino acids.

MATERIALS AND METHODS

Phylogenetic analysis

We have selected 33 peptide sequences that were reported to elicit various defence responses in plants. These sequences were aligned using Clustal W and phylogenetic analysis was carried out using Molecular Evolutionary Genetic Analysis (MEGA X) software (Kumar *et al.*, 2018). The best model was selected according to the Bayesian Information Criterion (BIC). The best model for construction of phylogenetic tree for peptide sequences was found to be WAG as analyzed by Mega Caption expert: best fit substitution model. The phylogenetic tree was constructed by maximum likelihood joining method. The Bootstrap value was set to 1000.

Amino acid sequence alignment and structure prediction

Multiple alignments of amino acid sequences were carried out by following the results of phylogenetic analysis of the sequences. Flagellin sequences (19) and other peptides (15) were aligned separately by utilizing Clustal W. Further, the three-dimensional structure of flagellin peptide was predicted by Swiss model software.

RESULT AND DISCUSSION

Phylogenetic analysis of microbial peptides

From the phylogenetic analysis, two main clusters were identified. One cluster contained 19 flagellin sequences, which is further divided into one major (15 peptide sequences) and one (4 peptide sequences) minor subclusters. The second main cluster is also divided into one major and one minor subcluster. The major and minor subclusters contained eleven clades and four clades respectively (Fig.1). The evolutionary divergence of elicitors of plant origin was studied by Martina *et al.* (2015). Similarly, (Liu *et al.*, 2020) analyzed the diversity of amino acid sequence s of *Xanthomonas* Hpa1 proteins using the MEGA 6.0 program. A maximum likelihood bootstrap tree derived in this study divided 8

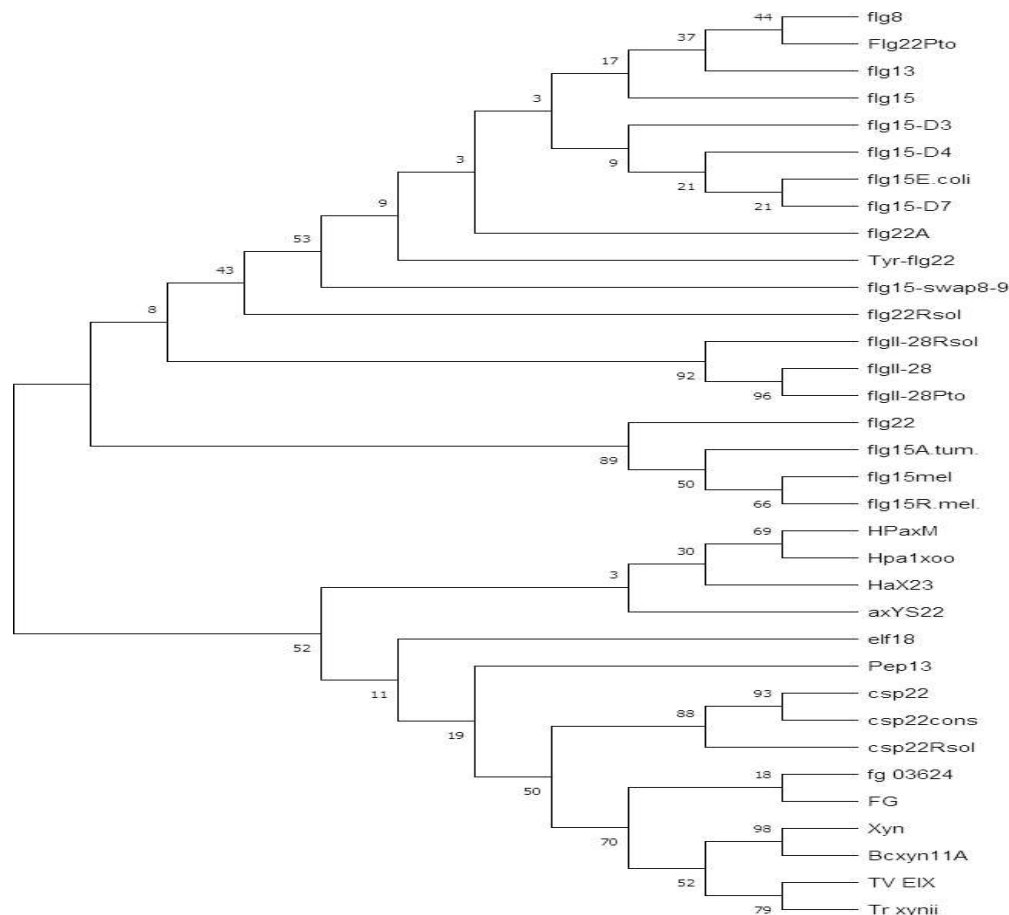


Figure 1. Phylogenetic analysis of 33 peptide sequences (*Botrytis cinerea* xylanase BcXyn11A (Acc.No.Q2LMP0), FGSG_10999: *Fusarium graminearum* xylanase FGSG_10999 (Acc.No.XP_011325371), FG_03624: *Fusarium graminearum* xylanase FG_03624 (Acc.No.XP_011322077), Tv_EIX: *Trichoderma viride* xylanase EIX (Acc.No. CAB60757), Tr_XynII: *Trichoderma reesei* xylanase II (Acc.No. AAB50278), csp22Rsol, csp22cons (Wei *et al.*, 2018), csp22 (Felix and Boller 2003), axYS22 (Lee *et al.*, 2009), Pep 13 (Brunner *et al.*, 2002), elf18 (Kunze *et al.*, 2004), flgII 28 (Cai, Lewis, & Yan, 2011) flg22Rsol (Bauer, Gómez-Gómez, Boller, & Felix, 2001), flg22, flg15A.tum., flg15mel (Felix, & Boller, 2003), flg15Rmel, flg15D3, flg15E.coli, flg13, flg8, flg15-swap8-9, flg22A, Tyr- flg22 (Meindl, Boller, & Felix, 2000) flg15 (Mueller *et al.*, 2012), *hpa1-Xm* (DQ643828.1), *hpa1Xoo* (AP008229.1), flgII- 28Rsol, flg22Rsol, Flg22Pto (Wei *et al.*, 2018) AMEP412 (Zhang *et al.*, 2018)) that were reported to elicit various defence responses in plants. The phylogenetic tree was constructed by maximum likelihood joining method.

harpin proteins into two clades, one major and one minor.

Amino acid sequence alignment

The amino acid sequence alignment of flagellin sequences using Clustal W did not present conservative amino acid sequences except Serine (S), Alanine (A) and Aspartic acid (D) (Fig.2). These conserved amino acids (SAXD) that are

positioned in the protruding loop may play a vital role in recognition by plant surface receptors (Fig.3). These results were in agreement with a recent report by Yuan *et al.* (2022), in which the significance aspartic acid (D) (42Asp, 175Asp) in signaling plant defense was evident by its presence in the cavity on the surface of H1AD43, a microbial peptide elicitor from *Bacillus*

licheniformis BL06. Previous research reports also suggested the prominence of hydrophobic and charged amino acids in the activity of certain peptides (Sagaram *et al.*, 2011). Alignment of amino acid sequences of cold shock protein, xylanase, elongation factor and harpin from various sources did not present conservative amino

acid sequences except glycine (Fig.4). A comparison of various harpin amino acid sequences and prediction of secondary structure of a novel harpin protein was done by Liu *et al.* (2020). Three- dimensional structure and amino acid sequence alignment of various plant defensins

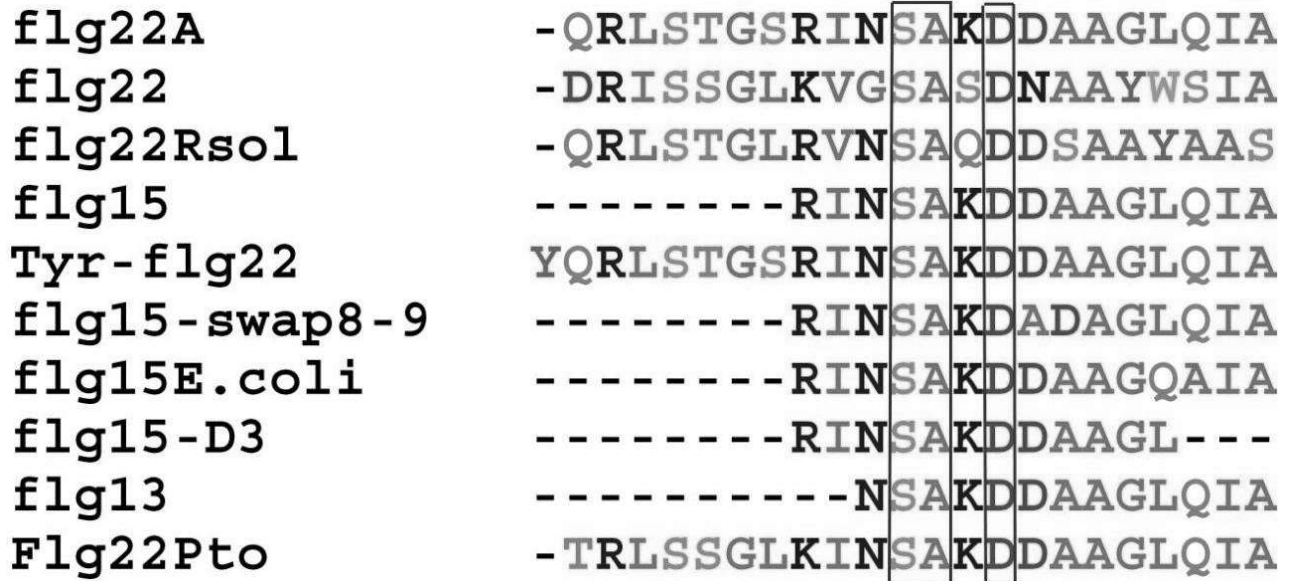


Figure 2. The amino acid sequence alignment of flagellin sequences using Clustal W (conservative amino acids Serine (S), Alanine (A) and Aspartic acid (D) were highlighted)

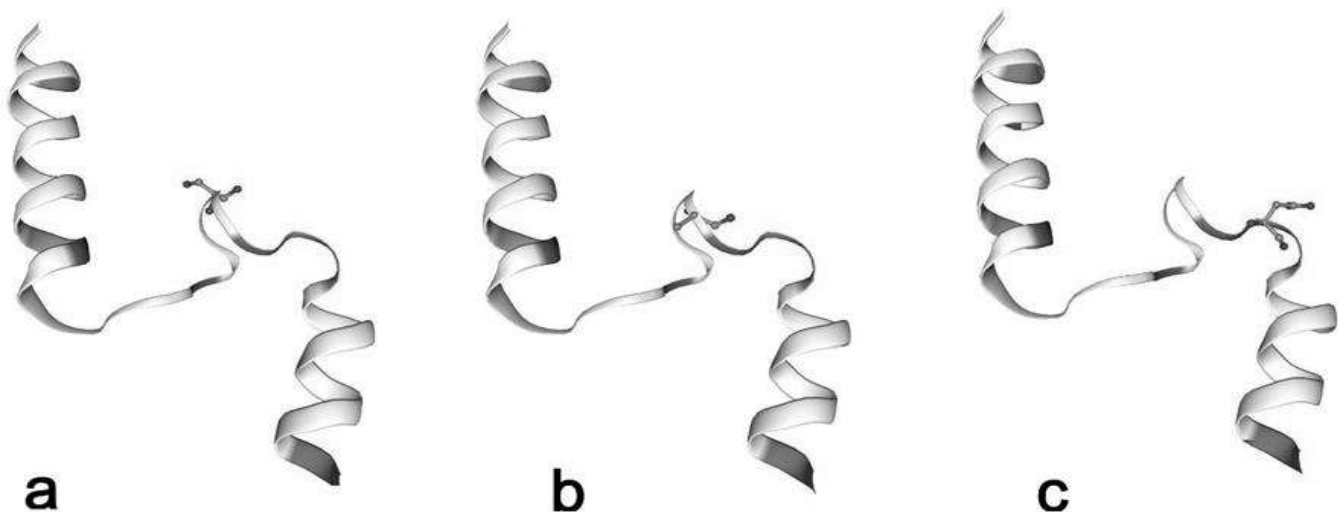


Figure 3. Flagellin peptide's three-dimensional structure as predicted using Swiss model software, revealing the locations of conserved amino acids (shown as ball and stick model) Locations of the amino acids Serine (S), Alanine (A), and Aspartic Acid (D).

were studied by Lacerda *et al.*, (2014). This investigation revealed the conservation of eight cysteine and one glycine residue among all the selected plant defensin peptides.

Microbial Biopesticides were defined as live microbes or their metabolites that can kill pests or plant pathogens. Much of the literature has focused on pesticidal activity of antimicrobial metabolites and live microbes. However, recent studies have highlighted the role of plant defense elicitor peptides of microbial origin as biopesticides (Montesinos *et al.*, 2022; Badosa *et al.*, 2022). This was evidenced by the remarkable increase in number of research publications in both web of science and SciFinder databases on peptides for plant protection in the past decade (Zhang *et al.*, 2023).

Many microbial peptides were known to stimulate plant immune system by acting as pathogen-associated molecular patterns (PAMPs) or plant elicitor peptides (Peps) conferring protection to plants against phytopathogens. The present study can lay a foundation for rapid screening of such functional peptides that can serve as novel candidates to develop biopesticides. Also, Synthetic peptides may be designed based on the amino acid sequence of natural peptides (Badosa *et al.*, 2022). Such peptides will have added advantage of environmental compatibility and low toxicity. Miyashita *et al.* (2011), have screened random hexapeptide library and discovered a novel small peptide (YGIHTh-amide) that elicited defense responses in tobacco cells. Similarly, Badosa *et al.* (2022), have designed and developed linear and cyclic peptides based on the common features of natural antimicrobial peptides or based on the AMPs. The activity of BPC16 (c(KLKLKFKLKK)), an antimicrobial peptide was improved by replacing Trp in the place of Phe6. This modified peptide displayed enhanced antifungal activity against notorious phytopathogens like *Erwinia amylovora*, *Xanthomonas axonopodis* and *Pseudomonas syringae*. These peptide sequences are regarded as promising candidates for the development of new plant protection agents (Camó *et al.*, 2017). In the

present approach, identification of conservative amino acids will be advantageous for preparing peptide sequences with improved biological properties.

In the present study, we describe the phylogenetic relationships and amino acid sequence alignments of various microbial peptide elicitors deployed in plant resistance induction. Comparison of amino acid sequences revealed the presence of conserved amino acid residues among the peptides. Further, the predicted 3D structures of revealed that conserved amino acids (SAxD) are charged and hydrophobic residues were positioned in the protruding loop may play a vital role in recognition by plant surface receptors. There were no conservative amino acid sequences except for glycine when the amino acid sequences of cold shock protein, xylanase, elongation factor, and harpin from different sources were aligned. These studies lay the theoretical groundwork for investigating numerous additional microbial peptides for producing plant resistance. This research can serve as a starting point for peptide production or screening to demonstrate plant immunity. The present study also offers benefit of quick/computational screening of peptide biopesticide formulations that have high probability to stimulate plant immunity.

CONTRIBUTION OF AUTHORS

SSD -Conceived and designed the analysis, Performed the analysis; Wrote the paper; CA- Collected the data and data analysis, AB- Performed the analysis, BSC- Data collection, VV- co-wrote the paper

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