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Alanine rich peptide from *Populus trichocarpa* inhibit growth of *Staphylococcus aureus* via targetting its extracellular domain of Sensor Histidine Kinase YycGex protein

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ABSTRACT

Background: Due to growing concern towards microbial resistance, ongoing search for developing novel bioactive compounds such as peptides is on rise. The aim of this study was to evaluate antimicrobial effect of *Populus trichocarpa* extract, chemically identify the active peptide fraction and finds its target in *Staphylococcus aureus.*

Methods: In this study the active fraction of *P. trichocarpa* crude extract was purified and characterized using MS/MS. This peptide PT13 antimicrobial activity was confirmed by in-vitro agar based disk diffusion and in-vivo infection model of *G. mellonella*. The proteomic expression analysis of *S. aureus* under influence of PT13 was studied using LTQ-Orbitrap-MS in-solution digestion and identity of target protein was acquired with their quantified expression using label-free approach of Progenesis QI software. Docking study was performed with peptide PT13 and its target YycG protein using CABS-dock.

Results: The active fraction PT13 sequence was identified as KVPVAAAAAAAAAVVASSMVVAAAK, with 25 amino acid including 13 alanine having M/Z 2194.2469. PT13 was uniformly inhibited growth *S. aureus* SA91 and MIC was determined 16 μg/mL for SA91 *S. aureus* strain. Sensor histidine kinase (YycG) was most significant target found differentially expressed under influence of PT13. *G. mellonella* larvae were killed rapidly due to *S aureus* infection, whereas death in protected group was insignificant in compare to control. The docking models showed ten docking models with RMSD value 1.89 for cluster 1 and RMSD value 3.95 for cluster 2 which is predicted to be high quality model.

Conclusion: Alanine rich peptide could be useful in constructing as antimicrobial peptide for targeting extracellular Domain of Sensor Histidine Kinase YycG from *S. aureus* used in the study.

1. Introduction

There is growing concern towards rise of microbial resistance to many antibiotics and the demand for alternative medicine led to an ongoing search for novel molecules and drugs. Antimicrobial components mainly proteins/peptides are the new possibilities for combating the microbial resistance and as many infectious diseases [1]. Recent studies have identified several peptides to act against different targets against pathogenic microorganisms, with the purpose of developing novel bioactive compounds [2–6]. Palindromic or multifunctional peptides which use to have higher probability to formα-helical structures, open the prospect to design such conformation that show the interaction with bio membranes [7]. Migliolo et al. first explored the characterization of a multiple active peptide analogue, which was rationally designed based on an antifreeze peptide motif [7]. In the prior study reported, alanine rich antimicrobial peptide (Pa-MAP) derived from the polar fish *Pleuronectes americanus*, exhibited an ability to prevent *E. coli* infections in mice [8,9].

The rapid development of antimicrobial peptides (AMPs) is presently very essential to combat uncontrolled proliferation in Multi Drug Resistant pathogen such as *Staphylococcus aureus* [10]. *S. aureus* is associated as nasopharyngeal colonizer and readily causes skin infection. It is evident from the history of chemotherapy that *Staphylococcus aureus* develop resistance to any antibiotic; antibiotic resistance is the natural consequence of the production of new target antimicrobial compounds. *S. aureus* known to have an extracellular domain histidine

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kinase YycG with two important components, which has an important role in regulation of cell wall metabolism, cell viability, biofilm formation, virulence, and antibiotic resistance [11,12]. In recent study has examined the structure in crystal form, at 2.0 resolution two subunits with an extracellular topology- PAS (Per-Arnt-Sim) packed into a dimer with interloop interactions and the cross-linking studies using integral YycG confirmed that has capacity of creating high molecular weight oligomers on the cell membrane [12]. In any cellular processes interactions between protein-peptide are common often involve peptide-mediated interactions [13,14].

In present study we have identified and characterized the antimicrobial activity of an alanine rich peptide derived from *Populus trichocarpa* during previous study. We used a proteomic methodology to identify differentially expressed proteins in *S. aureus* after exposure to alanine rich peptide molecule named PT13. A high mass resolution instrument (LTQ-Orbitrap-MS) were used to see evaluate this peptide effect on protein expression in *S. aureus* and target protein and its sequence was obtained through MASCOT, Swiss-Prot, and TrEMBL search engines. Differential expression profile for targets protein in *S. aureus* were acquired through label-free quantification (Progenesis QI). This PT13 alanine rich peptide target YycGex protein of *S. aureus* and the results of dry lab were complimentary to that of in-vitro and in vivo experiment.

2. Methods and materials

2.1. Study molecule and bacterial strains

In earlier (unpublished) work we used various plant extract to evaluate their bioactive properties. From that previous study we identified the crude extract obtained from *Populus trichocarpa* showed its growth inhibitory bioactivity against few clinical strain of *Staphylococcus aureus* randomly selected from stock (Fig. 1). In this work, we purified the active fraction from P. *trichocarpa* (PT13), an alanine rich peptide and completely chemically characterized and confirmed its structure.

2.2. Extraction, purification of PT13 and its characterization from plant Populus trichocarpa

2.2.1. Extraction of crude protein sample

The seeds and leaves of *Populus trichocarpa* were dried for couple of days and kept at 60 °C to reduce moisture. Dried materials were ground in phosphate buffer at pH 7.4. After getting homogenous suspension, the supernatant was salt precipitated and used for the estimation of total protein. Further, the suspension was centrifuged at 6000 rpm for 20 min and proteins were precipitated using ammonium sulfate precipitation (75%). Precipitated samples containing proteins were further purified by buffer exchanged with 0.1M phosphate buffer by stirrer.

2.2.2. Purification of peptides

The dialyzed protein samples were passed through 5 kDa cutoff spin column. The flow through obtained after centrifugation at 5000 rpm for 10 min was filtered through a 0.45 mm or 0.2 mm filter. The crude peptide was dissolved in 50% H20/50% CH3CN and a run was started in preparative HPLC (SHIMADZU (Shimadzu Corporation) using buffers (buffer a 2% CH3CN with 0.02% TFA; buffer b 80% CH3CN and 0.02% TFA). The column was re-equilibrate to conditions for the run initially and specific separation method were standardized. For peptides, 214 nm (the absorption frequency of peptide bonds) and 280 nm (the absorption of tyrosine and tryptophan) were recommended. During purification different output in peptide were carefully analyzed and we have collected all the relevant peaks. All the peaks were recorded with their retention time and M/z by mass spectrometry. Each of these ppeptide sequences were generated by MS/MS and searched against nucleotide or protein databases (MASCOT & EMBL). All fractions obtained in purification step were evaluated for their antimicrobial activity as per protocol below described to find out the active fraction.

2.3. In vitro functional analysis

2.3.1. In-vitro antimicrobial activity of peptide PT13 against S. aureus

Few clinical strain of *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) were used for agar based antimicrobial assays. These bacterial species were tested against the different purified peptide (2 μL) fraction in accordance with guidelines from the Clinical & Laboratory Standards Institutes (CLSI), 2016. Inhibitory zone were absorbed after 16–18 h of incubation period. All antibacterial experiments were carried out in triplicate.

In addition, PT 13 showed max inhibitory zone against to *S. aureus* SA91 that lead us to determine the minimum inhibitory concentration (MIC) for the peptide PT13 against that particular strain. The serial dilution of peptide PT13 (2–256 μg/mL) in mullerhinton broth medium was used to find the MIC as the lowest concentration that caused complete inhibition of growth. The minimum inhibitor concentrations (MICs, in mg/L) were determined by the micro-dilution technique according to CLSI guidelines [15]. A control was used without treating with PT13 to calculate the IC50 inhibitory value of PT13 against *S.aureus* strains.

2.4. Proteomic expression profile of S aureus under influence of PT13 (In-solution digestion followed by LC-MS/MS analysis)

As concluded in from our previous study, PT13 had significant inhibitory effect against *S. aureus.* The whole proteomic expression profile of *S. aureus* under influence of PT13 treatment was obtained and compared with the untreated control. One of representative *S. aureus* strain was harvested in 5 ml of nutrient broth for up to 4–6 h at 37 °C overnight with 200 rpm on a rotary shaker. Following that 16 μM of PT13 (IC50 value) was added in one of the samples and another was used as a control without any treatment. Once the mid-log exponential

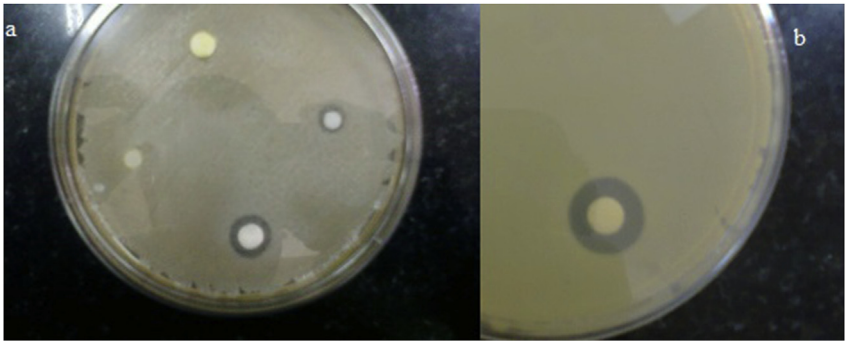

Fig. 1. a: Antimicrobial susceptibility testing of Crude extract from *Populus trichocarpa* (8–10 mm), **b:** Purified peptide activity (14 mm).