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New Antimicrobial Peptides as Potential Candidates in the Control Growth of *Botrytis cinerea*

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Introduction

Antimicrobial peptides (AMP) are small-polycationic molecules present in the innate immune response [1]. There are currently more than 3000 experimentally reported AMP [2]. Particularly, temporins, a small AMP family present in frogs, have been discovered as potential candidates for antimicrobial activity. These peptides serve to protect the frog against invasion by a variety of pathogenic micro-organisms and represent a component of the innate immunity of these organisms [3,4]. Within this arsenal of defensive peptides, some members of the temporin family have been described [5]. Until now, temporins have only been used in the control of bacteria and some yeasts. In the present work, the inhibition of the phytopathogenic fungus *Botrytis cinerea* growth by temporins for their potential application in the control of crops with agronomic interest.

Results and Discussion

Peptides were synthesized on solid phase using Fmoc/tBu chemistry and the purities afterwards were analyzed by RP-HPLC and MALDI MS. The study was carried out testing different concentrations of the peptides in Petri dishes with potato dextrose agar medium (PDA, Oxoid). PDA plates were inoculated with 1 µL of the spore suspension and incubated in the dark for 3 days. The fungus growth diameter on the plate was measured and the percentage of inhibition was calculated by comparison with a control with pure dimethyl sulfoxide (DMSO). Three peptide concentrations were tested: 10, 50 and 100 µg/mL, and each test was performed in triplicate.

In a first *screening*, 11 AMP were tested. These peptides were chosen from different peptide data bases [6-8], with different amino acid sequence and length (between 4 and 14 amino acids). Best results were obtained with peptides SLLSLIRKLIT and FASLLGKALKALA, with 42% and 50% fungal growth inhibition, respectively. Both peptides had the Ser-Leu-Leu sequence and a high percentage of hydrophobic amino acids in their structure.

From these results, a second screening in databases was carried out searching for AMP with these properties. As a result, we found predominately temporins, with the Ser-Leu-Leu sequence and hydrophobic amino acids. Afterwards, ten simple-to synthesize temporins were selected from the data base, with less than 20 amino acid long and without oxidizable residues (cysteine, tryptophan, methionine). Those temporins were used for the second *screening* using the same conditions. A fungus growth inhibition between 40% and 60% was observed for 7 peptides at a concentration of 100 µg/mL (Table 1). In general, temporins inhibited fungus growth with a high percentage, except for temporin E, that only inhibited in a 31%. The other 3 temporins did not show growth inhibition (data not shown). Particularly, 55% and 57% of inhibition was observed with temporin B and 1CSb respectively. Both peptides were highly hydrophobic peptides with a high content of Leucine and Isoleucine in their structure and with no net charge.

Table 1. Percentage of Botrytis cinerea growth inhibition using temporins at different peptide concentrations.

Peptide	*Sequence*	*Peptide concentration (μg/mL)*		
		10	*50*	*100*
Temporin E	VLPIIGNLLNSLL	14.4	11.0	31.6
Temporin B	LLPIVGNMMKSLL	25.7	40.2	54.8
Temporin A	FLPLIGRVLSGIL	21.7	38.6	47.8
Temporin1CSb	FLPIIGKLLSGLL	30.9	48.6	57.4
Temporin CGa	FLPILGNLLNGLL	14.5	30.1	42.2
Temporin G	FFPVIGRILNGIL	10.4	44.4	46.4
Temporin PRb	ILPILGNLLNSLL	0.8	23.3	49.4

These results showed that temporins are promising candidates for *Botrytis cinerea* growth control. All peptides that inhibited fungal growth present similar properties as hydrophobicity, amino acid composition, length, and net charge. These findings indicate that other members of temporin family could be potential candidates for fungus growth control. However, only few of these peptides have been proved as antifungal alternatives, so this is an unexplored field. Additional research in temporin activities and efficiency are necessary to incorporate more suitable alternatives in the control of this and other phytopathogenic fungi.

Moreover, synergic activity can be considered for growth control. Temporin B and 1csb could be used as a template to make rational modifications that may eventually improve antifungal activity. Temporins are a large family that have not been fully studied yet and have been gaining importance in antimicrobial studies in recent years [9]. The need for new and more effective antibiotic and antifungal agents makes necessary the discovery and application of new drugs based on natural peptides such as AMP.

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