Novel peptide-based control measures against the rice fungal pathogen *Pyricularia oryzae*

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Introduction

The filamentous fungus *Pyricularia oryzae* is the main causal agent of the rice blast disease, which accounts for 10-30% yield losses per year globally (Talbot, 2003). The disease is worldwide distributed in more than 85 countries, including Italy and Vietnam (Fig. 1; Talbot, 2003), and causes typical leaf spot eye shaped symptoms characterized by large, spreading lesions with a necrotic center and a chlorotic margin (Fig. 1; Wilson and Talbot, 2009).

Figure 1: Left: global distribution of rice blast disease. Right: typical blast disease symptoms on rice leaves.

P. oryzae has been recently ranked as the most important fungal pathogen of crops (Fig. 2; Dean et al., 2012).

Rank	Fungal pathogen
1	<i>Magnaporthe oryzae</i>
2	<i>Botrytis cinerea</i>
3	<i>Puccinia</i> spp.
4	<i>Fusarium graminearum</i>
5	<i>Fusarium oxysporum</i>

Figure 2: Most important fungal pathogens of crops worldwide.

New antimicrobial peptides, analogs of the natural peptaibol trichogin GA IV (tric), have been synthesized (De Zotti et al., 2009; De Zotti et al., 2012) and tested *in vitro* against several *P. oryzae* strains from different geographic origin. Trichogin GA IV is the main component of the mixture of peptide congeners produced by *Trichoderma longibrachiatum* as part of its defense mechanism against other microorganisms (Battaglia et al., 2013). As all peptaibols, its sequence is characterized by the presence of the non-coded residue Aib (α -aminoisobutyric acid), a C-terminal 1,2-aminoalcohol (Leucinol, Lol) and an acylated N-terminus (1-Octanoyl). All peptide sequences are reported in Table 1.

Table 1: Primary structure of tested trichogin analogs.

Abbreviation	Sequence
tric	1-octanoyl-Aib-Gly-Leu-Aib-Gly-Gly-Leu-Aib-Gly-Ile-Lol
K2	1-octanoyl-Aib-Lys-Leu-Aib-Gly-Gly-Leu-Aib-Gly-Ile-Lol
K259G6	1-octanoyl-Aib-Lys-Leu-Aib-Lys-Gly-Leu-Aib-Lys-Ile-Lol
K56	1-octanoyl-Aib-Gly-Leu-Aib-Lys-Lys-Leu-Aib-Gly-Ile-Lol
K5	1-octanoyl-Aib-Gly-Leu-Aib-Lys-Gly-Leu-Aib-Gly-Ile-Lol
K6	1-octanoyl-Aib-Gly-Leu-Aib-Gly-Lys-Leu-Aib-Gly-Ile-Lol

Results & Discussion

Synthesis

All analogs were synthesized by SPPS using a cost-effective strategy with Oxyma Pure / DIC (diisopropylcarbodiimide) as activating agents. All peptides were obtained in high yield and crude purities.

Conformational analysis

Several spectroscopic techniques were applied to shed light on the 3D-structure preferably adopted by the synthesized analogs in solution. Our Circular Dichroism (CD) analysis showed that all peptides mainly assume a right-handed 310-helical conformation in a membrane-mimicking environment (Fig. 3).

Figure 3: CD spectra of selected analogs in aqueous SDS 100mM (peptide concentration: 0.1 mM).

Antifungal activity

The synthesized peptides have been screened *in vitro* against two different strains (AP and Guy11) of the rice fungal pathogen *P. oryzae*. *In vitro* germination and growth tests conducted in microtiter plates inoculating conidia in potato dextrose medium in presence of peptides showed significant differences in the biocidal activity among the synthesized peptaibols. Although the assay revealed some differences in efficacy between strains, all analogs showed a strong inhibitory effect against *P. oryzae* at 50 μ M concentration.

Microscopy

The effect of the most active peptides on fungal conidia was also verified by optical microscopy analysis. In particular, the most effective peptides resulted able to inhibit spore germination and caused in fungal cells morphological modifications probably involving vacuolation and membrane damages.

Conclusions

We exploited a cost-effective, solid-phase synthetic strategy to afford trichogin GA IV analogs with high yield and purity. The conformational studies showed that the analogs adopt a 310-helical structure regardless the sequence modifications. Preliminary tests have highlighted that these peptaibol analogs are non-toxic against plant cells.

Our *in vitro* screening and microscopic analysis have allowed to identify several peptide sequences very effective in inhibiting *P. oryzae* spore germination and fungal growth. The most effective sequences are currently being tested *in vivo* on rice leaves to verify their possible efficacy in protecting rice from the blast disease.

References

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