SUPPORTING INFORMATION

Antifungal activity of alpha-sarcin against *Penicillium digitatum*: proposal of a new role for fungal ribotoxins.

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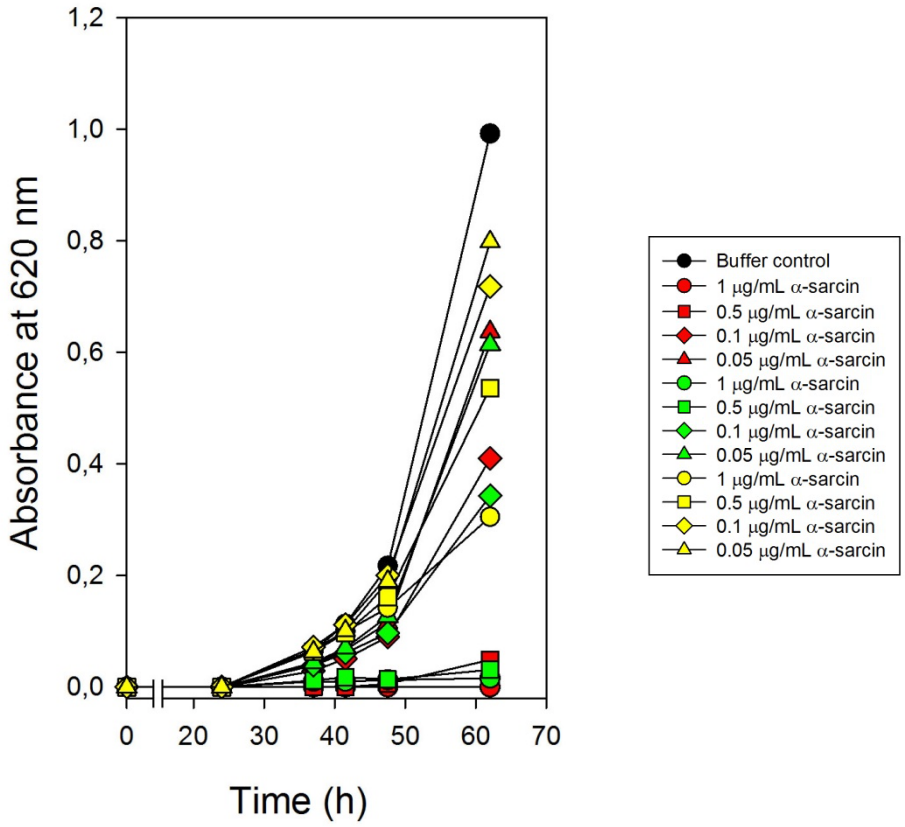
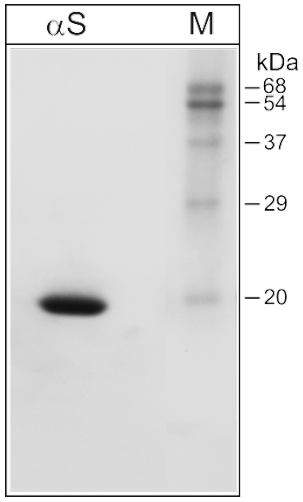
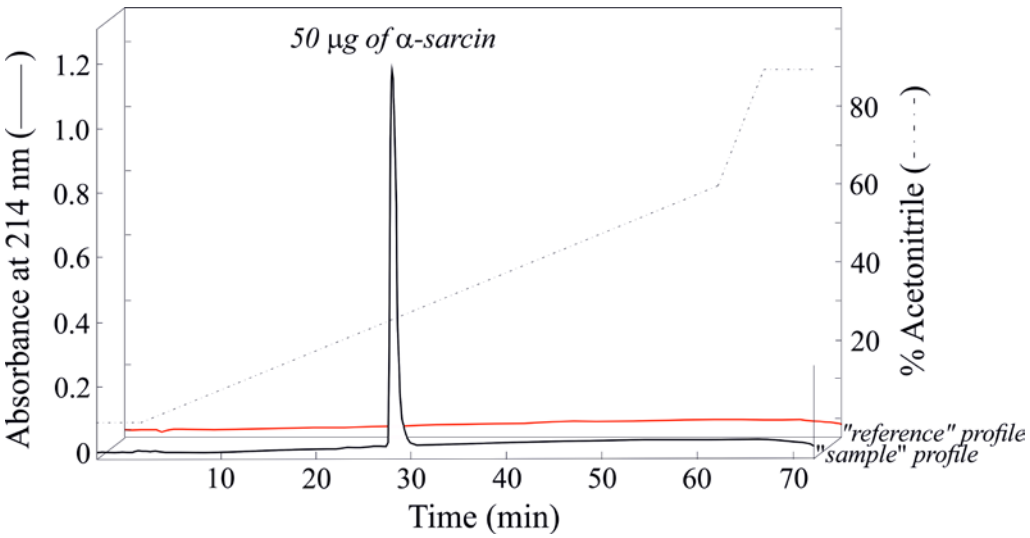


Supplementary Figure 1 Antifungal activity of α-sarcin against *Penicillium digitatum*. Antifungal activity of alpha-sarcin against *P. digitatum* was measured in a microtiter plate bioassay. Fungal growth was measured as an increase in absorbance at 620 nm. Conidia of *P. digitatum* were grown at 26 ºC in PDB medium in the absence (black circles) or the presence of different concentrations of α-sarcin added from the beginning (red symbols) or added after 24 h (green symbols) or 36 h of mycelial growth (yellow symbols). The symbols correspond to: 0.05 µg mL^{-1} α-sarcin (triangles), 0.1 µg mL^{-1} α-sarcin (diamonds), 0.5 µg mL^{-1} α-sarcin (squares) and 1 µg mL^{-1} α-sarcin (colored circles).

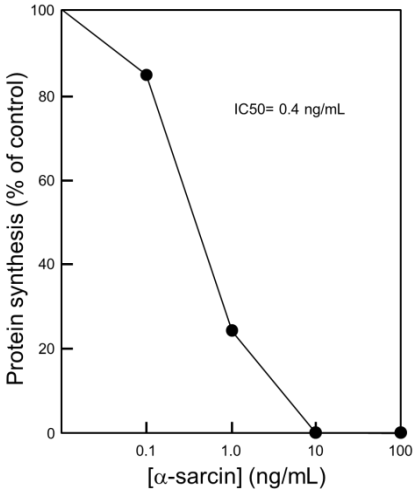
Supplementary Figure 2 Purity and specific activity of commercial α-sarcin. α-sarcin was purchased from Santa Cruz Biotechnology and the purity and specific activity were analyzed by SDS polyacrylamide gel electrophoresis, RP-HPLC, cell-free translation inhibition, and rRNA endonuclease activity in both cell-free and culture cell systems as indicated below.



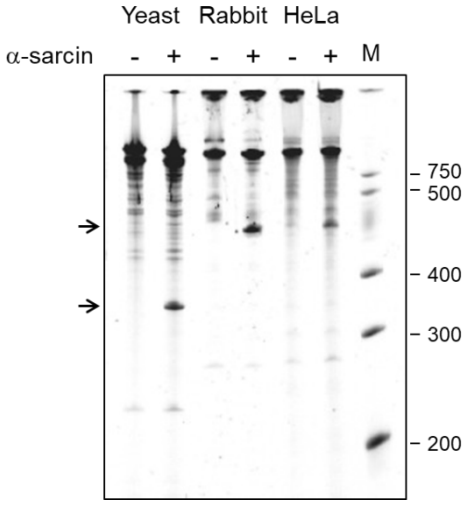
Supplementary Figure 2a Analysis of α-sarcin (αS) by SDS polyacrylamide gel electrophoresis. The protein (10 μg) was analyzed by SDS-PAGE in 12% gels and then stained with Coomassie brilliant blue. The numbers indicate the corresponding size of the standards (M) in kDa.



Supplementary Figure 2b HPLC elution profile of α-sarcin (50 μg) on a Breeze System from reversed-phase chromatography (RP-HPLC) using a C-4 column (4.6 × 250 mm, Phenomenex, Castel Maggiore, Bologna, Italy) as previously reported (Dosi et al., 2012).



Supplementary Figure 2c Effect of α-sarcin on protein synthesis. Translation assays were carried out using rabbit reticulocytes lysate as a cell-free system, as indicated in (Iglesias et al. 2015). Data represent the percentage of protein synthesis with respect to a control without α-sarcin. The data represent the mean of three duplicate experiments.



Supplementary Figure 2d rRNA endonuclease activity of α-sarcin on S30 from yeast and rabbit reticulocytes lysates cell-free systems and HeLa cells. rRNA endonuclease activity was assayed as indicated in (Iglesias et al. 2015; 2017). Each lane contained 3 μg of RNA isolated from either untreated (-) or treated samples (+). The arrows indicate the RNA fragment released as a consequence of the endonuclease activity of α-sarcin. Numbers indicate the size of the standards (M) in nucleotides

Materials and Methods

Materials

The sources of the chemicals used in this work have been indicated previously (Citores et al. 2016) and most of them were obtained from Sigma-Aldrich. The strain of *Penicillium digitatum* was isolated in our laboratory and typified by the Spanish Type Culture Collection (CECT), Valencia, Spain. α-sarcin was purchased from Santa Cruz Biotechnology and showed to be pure and fully active (Supplementary Figure 2). RNA Century-Plus Markers were from Ambion.

Antifungal activity measurements

Growth inhibition assays of α-sarcin against *P. digitatum* were performed in 96 well microtiter plates. Conidia of *P. digitatum* (100 spores/well) obtained as indicated (Citores et al. 2016) were incubated at 26 ºC in 150 µL PDB medium in the presence of different concentrations of α-sarcin. Fungal growth was monitored spectrophotometrically using a microtitre plate reader (ELISA reader Multiskan) and microscopically (Motic AE31 inverted Microscope) after 24, 37, 41, 47, 62, 66, and 68 h of incubation. The mean results ± S.D. of three experiments performed in triplicate are reported. Data were analysed by ANOVA test.

Mycelium for RNA and DNA extraction was prepared from cultures grown in 6 well plates containing 1.7 mL PDB medium inoculated with 3000 spores in the absence or the presence of 0.25 µg mL^{-1} α-sarcin. The plates were incubated at 26 °C. After the growth of *P. digitatum* for 4 days, the mycelium was harvested by filtration through filter paper under vacuum, extensively washed with sterile water, weighted and stored at -80 °C. The experiments were carried out with ten wells.

rRNA endonuclease activity on *P. digitatum* ribosomes

Preparation of the 30000 xg (S30) supernatants from *P. digitatum* was performed as described elsewhere (Iglesias et al. 2016). The rRNA endonuclease activity of α-sarcin was assayed in 100 µL samples of S30 supernatants from *P. digitatum*, which were incubated with 3 µg α-sarcin for 1h at 30 ºC. After treatment, the RNA was extracted with phenol (Iglesias et al. 2017). RNA samples were separated on a 5% (w/v) urea-polyacrylamide gel, and stained with Gel Red (Biotium, Inc.) and visualized with an ultraviolet lamp using a Gel Doc XR system (Bio-Rad) (Iglesias et al. 2017).

Ribosome inactivation analysis in *P. digitatum* cultures

RNA from *P. digitatum* grown in the presence of α-sarcin was obtained from 30 mg of mycelium ground in a ceramic mortar with liquid nitrogen, using the RNA Plant Minikit (Qiagen), according to the company's procedure. RNA samples (3 µg) were separated on a 5% (w/v) urea-polyacrylamide gel, and stained with Gel Red (Iglesias et al. 2017).

DNA fragmentation analysis in *P. digitatum* cultures

The DNA from *P. digitatum* grown in the presence of α-sarcin, was obtained from 0.5 g of mycelium ground in a ceramic mortar with liquid nitrogen, then 20 mg was transferred to an Eppendorf tube and suspended in 100 µL of 1M sorbitol containing 0.1 M EDTA pH 7.4, 0.1% (v/v) 2-mercaptoethanol and 100 units lyticase. After incubation at 30 ºC for 1 h the DNA was isolated following the instructions of the Genomic Prep Cells and Tissue DNA Isolation Kit (GE Healthcare). DNA (2 µg) electrophoresis was carried out in 1.8% (w/v) agarose gels using TBE buffer (0.089 M Tris, 0.089 M boric acid, 2 mM EDTA, pH 8.0) at 50 V for 4 h. DNA was stained for 20 min with Gel Red and visualized with an ultraviolet lamp.

Other procedures

DNA fragmentation analysis of COLO 320 (human colon adenocarcinoma) were performed as described elsewhere (Citores et al. 2016; Iglesias et al. 2016). Protein concentrations were determined using the spectrophotometric method of Kalb and Bernlohr (Kalb and Bernlohr 1977).

References

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