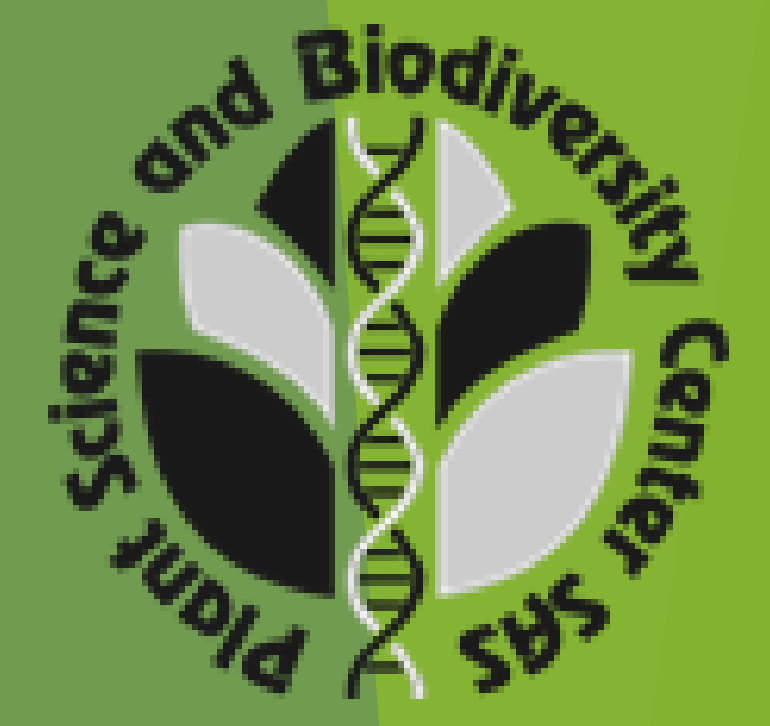


HYDROLYTIC ENZYMES OF CARNIVOROUS PLANTS AS A PROMISING ANTIFUNGAL AGENTS



Miroslav Rajnec¹ – Jana Libantová¹

¹Institute of Plant Genetics and Biotechnology, Plant Science Biodiversity Center v.v.i., SAS

Akademicka 2, 950 07 Nitra, Slovak Republic

Purified chitinase from the carnivorous plant *Drosera rotundifolia* and purified β -1,3-glucanase from *D. binata*, produced in a bacterial expression system, were tested for their potential antimicrobial effect against various filamentous fungi. Antifungal properties were evaluated against *Trichoderma viride*, *Alternaria solani*, *Rhizoctonia solani*, and *Fusarium poae*.

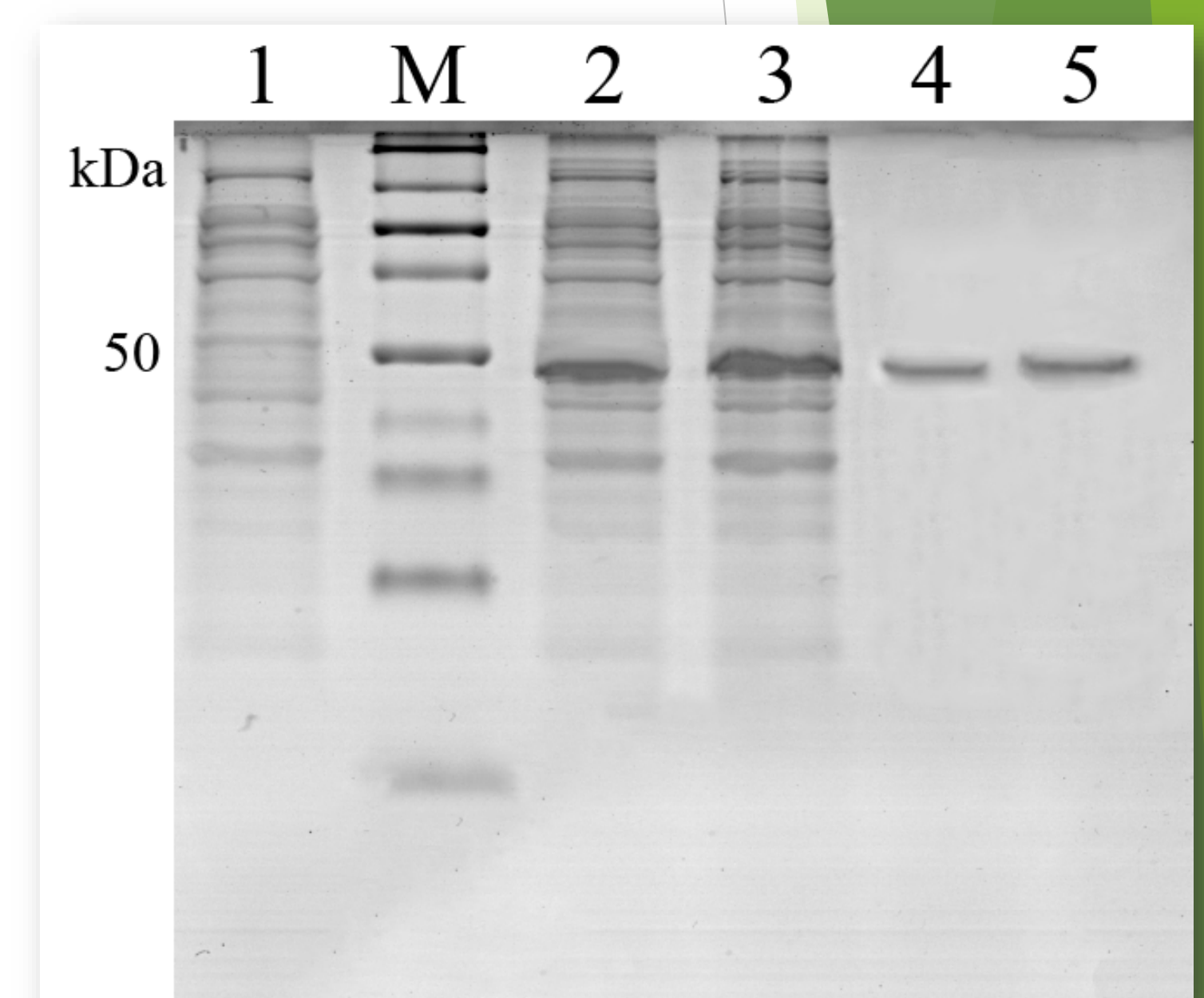
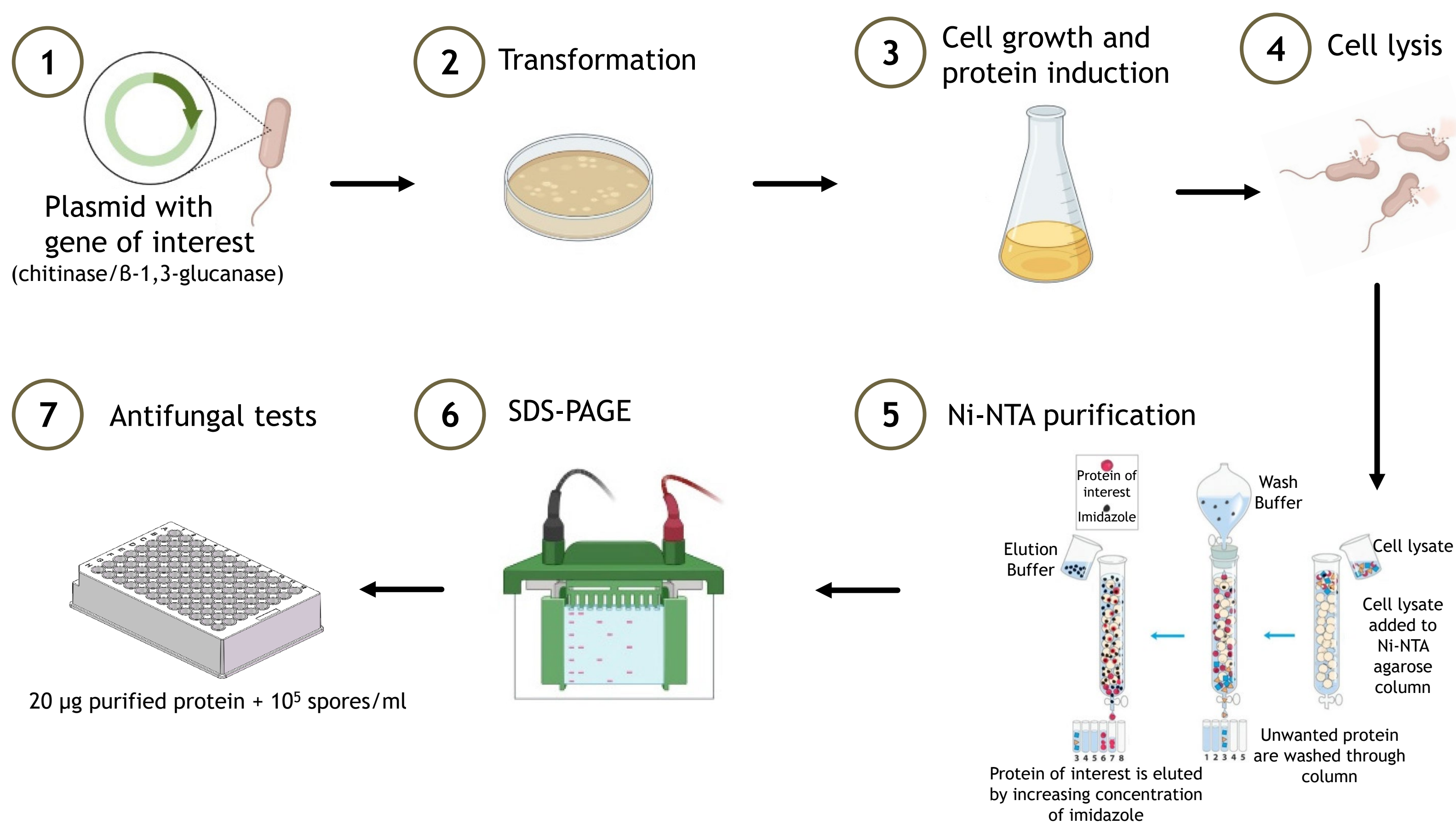


Fig.1 Process of transformation of cells, expression and purification of protein of interest, subsequent detection on polyacrylamide gels and evaluation of antifungal properties „in vitro“

Fig.2 SDS-PAGE analysis of recombinant chitinase and β -1,3-glucanase from crude protein extracts and after Ni-NTA purification. Lane 1 – crude protein extract of non-induced *E. coli* BL21-CodonPlus(DE3)-RIL cells, lane 2 – expressed recombinant chitinase in crude protein extract of induced cells, lane 3 – expressed recombinant β -1,3-glucanase in crude protein extract of induced cells, lane 4 – purified recombinant chitinase, lane 5 – purified recombinant β -1,3-glucanase, lane M – Spectra™ Multicolor Broad Range Protein Ladder (ThermoFisher Scientific)

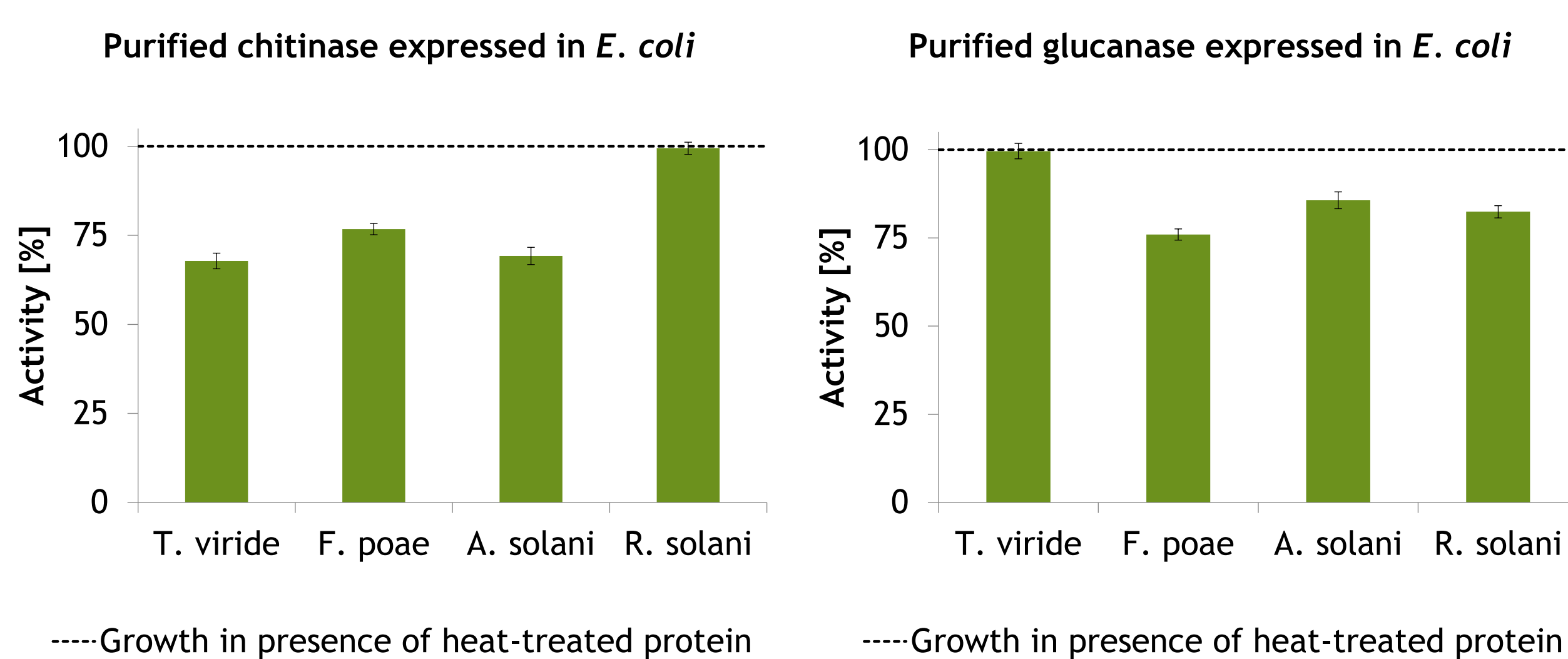


Fig.3 Antifungal effect of purified chitinase and β -1,3-glucanase on the growth of selected filamentous fungi

After successful expression and subsequent purification on Ni-NTA agarose, which was confirmed by the detection of expressed transgenic proteins by SDS-PAGE, antifungal activity was investigated under “in vitro” conditions. Purified chitinase significantly inhibited the growth of *F. poae* (23,3%), *A. solani* (30,8%), and *T. viride* (32,2%), recombinant β -1,3-glucanase showed significant inhibition in the case of *F. poae* (24,1%), *A. solani* (14,3%) and *R. solani* (17,6%).

Based on obtained data for the inhibition effect of tested purified chitinase and β -1,3-glucanase in “in vitro” conditions, there is promising potential of incorporation of these transgenes with aim of improving crop resistance to fungal pathogens.