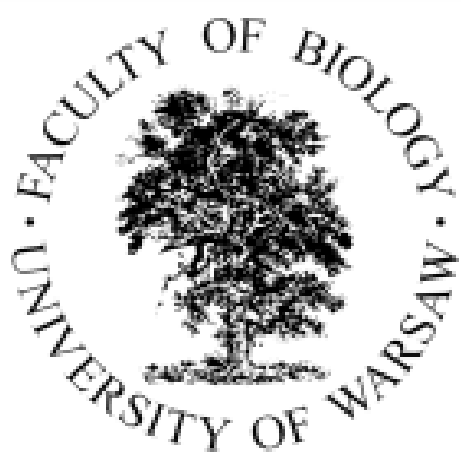




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Assessing the adhesion and invasiveness of *Prototheca* species towards murine keratinocytes

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Background and Aim

- *Prototheca* spp. are unicellular, chlorophyll-free microalgae widely distributed in the environment
- These microorganisms are the only known plants implicated in a range of clinically relevant opportunistic infections (protothecosis) in both humans and animals
- In particular, protothecal mastitis constitutes a major health and economic problem in dairy herds
- Our aim was to evaluate the ability and intensity of *Prototheca* spp. adhesion to murine skin cells

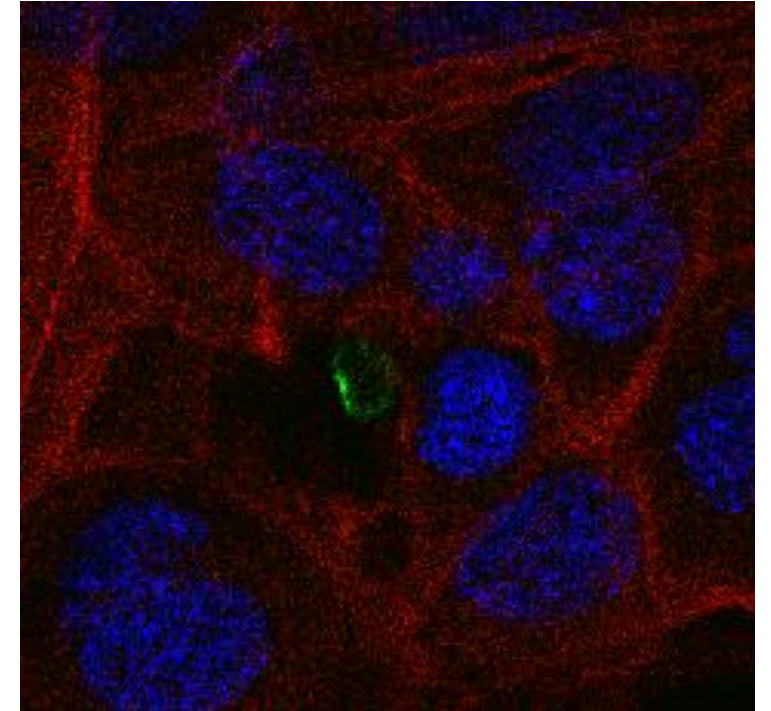


Fig. 1. *Prototheca bovis* immunostained with Alexa Fluor 488 inside of murine Kera-308 cell whose cytoskeleton was stained with phalloidin and DNA with Hoechst.



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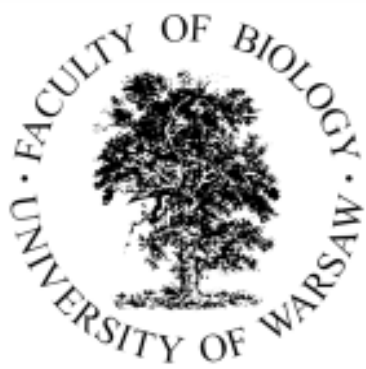
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Methods

- Type strains of three pathogenic (*P. bovis*, *P. ciferrii*, *P. wickerhamii*) and one non-pathogenic (*P. stagnora*) *Prototheca* species were used to infect murine keratinocyte cell line (Kera-308) at a MOI of 0.2
- After 4 hrs of incubation at 37°C in 5% CO₂, the exposed cells were washed with PBS, lysed in 0.5% saponin, plated on Sabouraud Dextrose Agar (SDA), and incubated for 72 hrs at 30°C before counting of CFU of cell-adherent algae
- The sum of CFU from culture medium and from the cell lysates constituted the total CFU
- The level of *Prototheca* spp. adhesion was calculated by determining the adhesion percentage, which was expressed as the CFU of cell-adhered *Prototheca* spp. relative to the total CFU of *Prototheca* spp.



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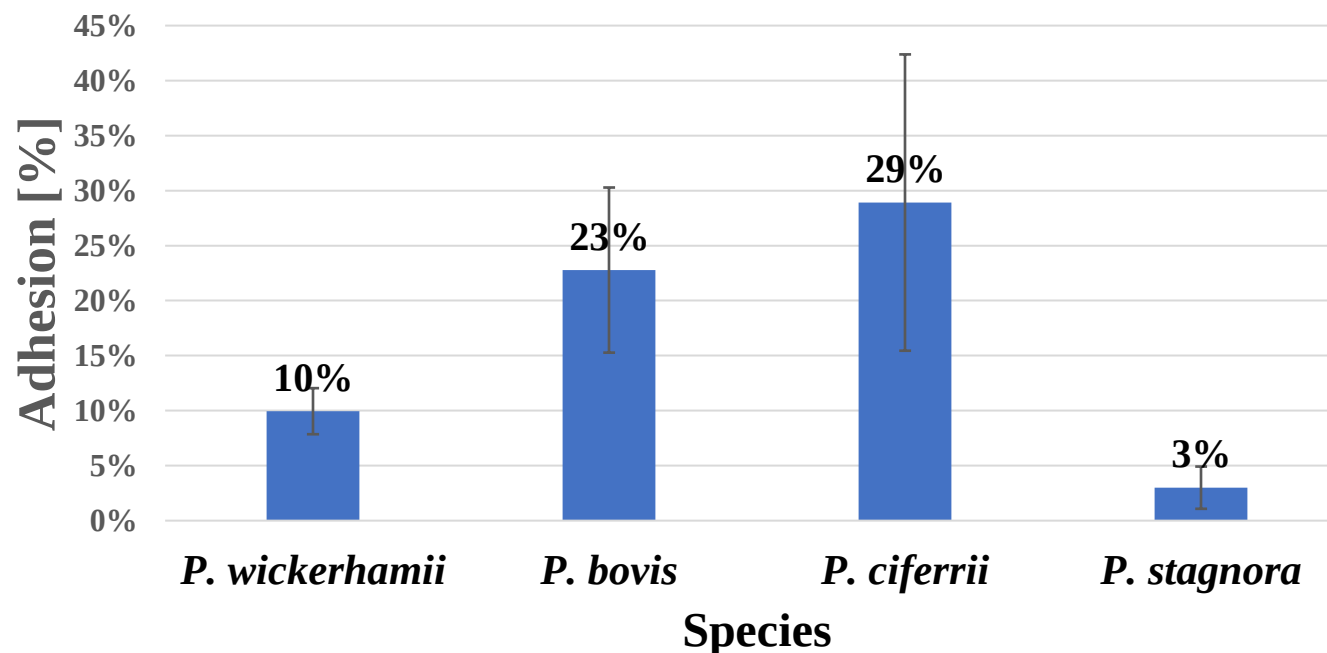
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Results

Adhesion of *Prototheca* spp. to the Kera-308 cells



- The mean adhesion percentages for pathogenic species were as follows: 10% – *P. wickerhamii*, 23% – *P. bovis*, 29% – *P. ciferrii* 29%
- *P. stagnora* showed the weakest adherence towards Kera-308 cells (3%)