



Screening and identification of *Aspergillus sclerotiorum* with activity against *Metschnikowia bicuspidata* and initial application on "milky disease" in *Eriocheir sinensis*

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Abstract

The Chinese mitten crab (*Eriocheir sinensis*), belongs to the Decapoda order, Eriocheiridae family, and *Eriocheir* genus. The mortality rate of diseased Chinese mitten crabs can reach 100 %, causing huge economic losses to the Chinese mitten crab industry. Biological antagonism is the phenomenon of growth inhibition between different microorganisms in the same ecological niche, which is prevalent within microbial communities. In this study, an antagonistic strain with a strong antagonistic effect on *M. bicuspidata* was screened from the soil, analyzed for morphological, molecular, physiological, biochemical characteristics, and identified as *Aspergillus sclerotiorum*. The effectiveness of *A. sclerotiorum* against the aquatic animal pathogen *M. bicuspidatum* was investigated for the first time in this study. The findings offer valuable insights for utilizing *A. sclerotiorum* in preventing and controlling "milky disease" in Chinese mitten crabs, thereby promoting the healthy development of the Chinese mitten crab aquaculture industry.

Methods

In this study, a fungus 2JHP001 with a strong antagonistic effect against *M. bicuspidata* was screened from soil samples by the double-layer plate method and the punching method. Subsequently, its morphological, molecular, physiological and biochemical characteristics were analyzed. The morphology of yeast after treatment with 2JHP001 crude extract was observed by SEM and TEM. The ethyl acetate extract was analyzed by UHPLC-MS. Finally, animal studies were conducted to prevent "milky disease".

Results

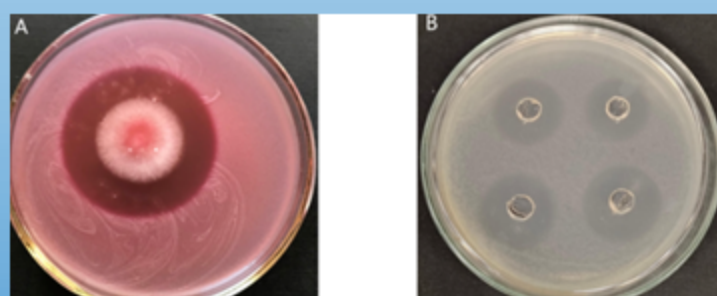


Fig. 1. Antagonist screening results. A: Preliminary screening results; B: Rescreening results.



Fig. 2. 2JHP001 Morphological features.

A: the front of the colony; B: the reverse of the colony; C: the sporulation structure (stained with Lactophenol Cotton Blue Staining Solution). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.

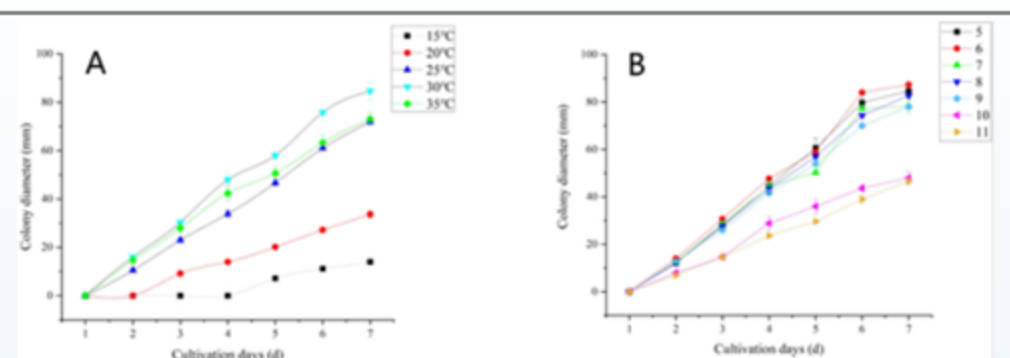


Fig. 3. Optimal growth conditions of 2JHP001. A: 2JHP001 Optimal Temperature; B: 2JHP001 Optimal pH

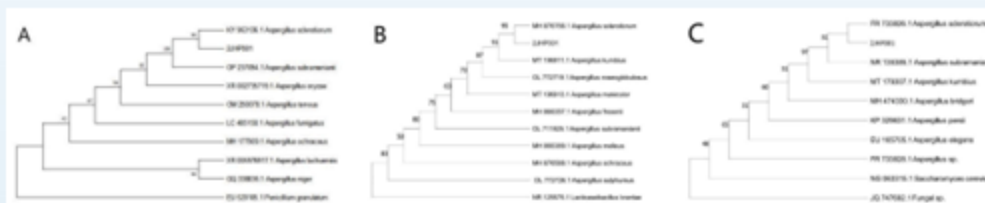


Fig. 4. Phylogenetic tree of 2JHP001. A: 2JHP001 18S rDNA (GenBank accession no. OR064077.1) phylogenetic tree; B: 2JHP001 26S rDNA (GenBank accession no. OR064173.1) phylogenetic tree; C: 2JHP001 ITS rDNA (GenBank accession no. OR056350.1) phylogenetic tree.

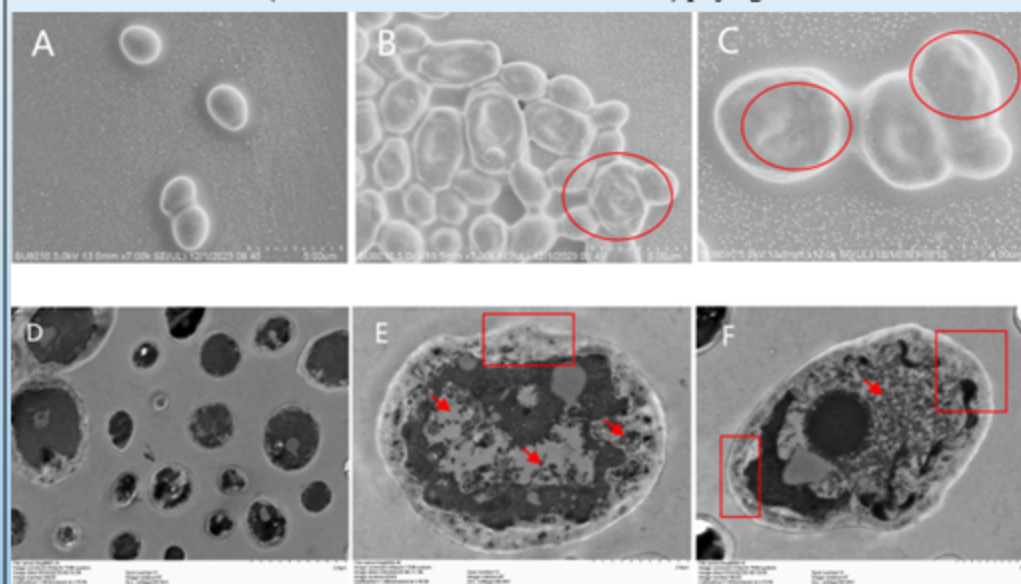


Fig. 5. Electron microscopy observation of yeast. Scanning Electron Microscope A: control; B-C: yeast treated with 2JHP001 crude extract; Transmission Electron Microscopy D-F: Yeast treated with 2JHP001 crude extract. (○) Yeast cells indentation and atrophy; (□) Yeast cell walls and membrane destruction. (×) Yeast cell organelles damage

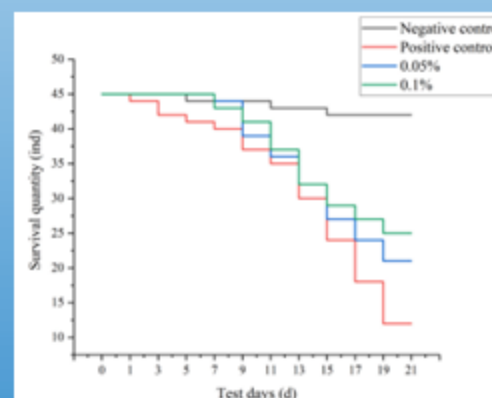


Fig. 6. Survival quantity of *Eriocheir sinensis*

Conclusion

A strain of *A. sclerotiorum* 2JHP001 with high antagonistic activity against *M. bicuspidata* was screened from soil, which has the potential to be applied in the prevention and control of "milky disease" in Chinese mitten crabs.