COMPARISON OF FIVE PROTEIN EXTRATION PROTOCOLS FOR *Pythium insidiosum*

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Abstract:

Pythiosis, is a life-threatening disease caused by *Pythium insidiosum*, and occurs mainly in tropical and subtropical countries. Several animal species are susceptible, including humans, however it has been observed great majority of the cases in equines. The disease has a difficult diagnosis and is frequently confused with zigomycosis, since the hyphae of the pathogen has the same aspect in hisological sections. Treatment is another challenge, since the pathogen does not respond well to antifungal therapy due to the lack of ergosterol in its cellular membrane. Studies on protein profiles of the pathogen are scarces and the present work aimed to standardize a protein extraction method by comparing five different protocols. The effectiveness of the protocol was evaluated by the quantification with Bradford method and also by SDS-PAGE. For extraction protocols it was employed the reagents PMSF (Phenylmethanesulfonyl fluoride), EDTA (Ethylenediaminetetraacetic acid), SDS (Sodium dodecyl sulfate), Tris-HCl and protease inhibitor (Sigma), all diluted in sterile distilled water. Some protocols were also evaluated with or without sonication. Protocol 1: PMSF and EDTA; protocol 2: protease inhibitor and EDTA; protocol 3: protease inhibitor, EDTA, SDS and Tris-HCl; protocol 4: the combination of protocol 2 with sonication; protocol 5: the combination of protocol 3 with sonication. The quantification showed that protocol 1 yield 0.1645 µg/µL; protocol 2 - 0.0912 µg/µL; protocol 3 - 6.8713 µg/µL; protocol 4 - 0.0891 µg/µL and protocol 5 - 8.3182 µg/µL. The SDS-PAGE showed only two bands in protocol 1 with size of 45 and 100 KDa. Protocol 2 revealed bands with 45, 60 and 100 KDa and protocols 3, 4 and 5 showed different band proteins ranging from 30 to 120 KDa, being more evident in protocol 3. We can conclude that the protocol with protease inhibitor, EDTA, SDS and Tris-HCl without sonication allowed the better results for protein extraction of *Pythium isidiosum*.

Keywords: *Pythium insidiosum*, pythiosis, protein extraction, SDS-PAGE

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