Isolation, Bioassay and Safty Evaluation of Destruxins

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ABSTRACT

Destruxins（DTXs）can be produced by the green muscardine fungus ，Metarhizium anisopliae var．anisopliae in culture media. In this study，Czapek-Dox medium enriched with 0.5%bacto-peptone was used for shake flask production of destruxins.The inoculated medium in 500 mL flask was held at 27℃，agitated with a rotary shaker of 150rpm，incubated for 14-19 days（Liquide state fermentation，LSF).Plastic bag containing 200-300g of autoclaved polished-rice was inoculated and incubated at 28℃，24 hours l ight-period，for 14 days（Solid state fermentation，SSF）．After incubation，both cultures were subjected to extraction of destruxins by methylene chloride- methanol（95：5).The crude extract was run on HPLC（Column： waters 5C 18-AR，8×250mm）and separated with a linear gradient of acetonitrile：water（30：70） to acetonitrile：water（70：30）for 45 minutes．Six major peaks (6,15,19,23,25,33mins）were collected by HPLC．Compounds from different retention times were identified by FAB mass．Six destruxins（DA，DA2，DB，DMDB， DD，DE）were isolated from both cultures，but the major components were different．While，the total yields from LSF was considerably ligher than SSF． Comparsion of the biological activity of destruxins from LSF and SSF showed that the destruxins from SSF has a greater activity against Spodoptera exigua larvae than destruxins from LSF．The mortalities of DE， DA，DA2，DD，and DB against 3rd-instar larvae of Spodoptera exigua were 43，18.33，11.86，10，and 2.27％ respectively．Rats were orally dosed with Metarhizium anisopliae var．anisopliae spore suspensions．These suspensions（108spore/ mL)produced no adverse effects on the health of the test animals．There was no evidence of infection in tissues．85％recovery of Metarhizium anisopliae var．anisopliae spores was resulted from faces one day after dosing.Results also demonstrated no toxicity of destruxins（600mg/kg）to rats when administrated via oral route of exposure ．

Keywords : Metarhizium ，Destruxins，Isolation，Bioassay，Toxicity．

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