THE STUDIES ON THE ANTIMICROBIAL COMPOUNDS OF BACILLUS SUBTILIS V656

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ABSTRACT

THIS THESIS IS A STUDY OF THE UTILIZATION OF AGRICULTURAL AND MARINE WASTES BY MICROBES TO PRODUCE ANTIFUNGAL SUBSTANCE. THE ISOLATION, IDENTIFICATION OF FUNGICIDE PRODUCING MICROORGANISM AND THE PURIFICATION, CHARACTERIZATION OF FUNGICIDES WERE DESCRIBED. THE FUNGICIDE-PRODUCING MICROORGANISM WAS IDENTIFIED AS A STRAIN OF BACILLUS SUBTILIS AND NUMBERED AS V656. MAXIMUM INHIBITORY ACTIVITY (0.4 U/ML) FOR FUSARIUM OXYSPORUM WAS OBTAINED WHEN THE STRAIN WAS GROWN AEROBICALLY IN A MEDIUM CONSISTING OF 2% SHRIMP AND CRAB SHELL POWDER, 0.1% K2HPO4 AND 0.05% MGSO4?7H2O IN 100ML MEDIUM (PH 7.0) AT 30℃FOR 1 DAYS. THE FUNGICIDE WAS STABLE AT PH FROM 6 TO 9, BUT WAS NOT STABLE AT 100℃.THE INHIBITORY ACTIVITY INCREASED CORRESPONDING TO BACTERIAL GROWTH. THE FUNGICIDE WAS PURIFIED FROM THE CULTURE SUPERNATANT OF V656 BY AMMONIUM SULFATE FRACTIONATION AND DEAE-SEPHAROSE CL-6B COLUMN CHROMATOGRAPHY. THE PURIFIED ENZYMES (FⅠ,FⅡ) ESTIMATED BY SDS-PAGE AND HPLC HAVE A MOLECULAR WEIGHT OF 14KDA AND 17KDA. FⅠAND FⅡ BOTH SHOWED CHITINASE ACTIVITY AND INHIBITORY ACTIVITY. THE OPTIMUM PH AND OPTIMUM TEMPERATURE FOR CHITINASE, PH STABILITY AND 100℃ THERMAL STABILITY FOR INHIBITION, AND PIS OF FⅠAND FⅡ WERE (7, 40℃, 6-9, 3 MIN, 5.8) AND (6, 40℃, 5-9, 3 MIN, 5.3), RESPECTIVELY. THE ACTIVITY OF FⅠWAS ACTIVATED BY ZN2+, BUT STRONGLY INHIBITED BY CU2+. THE ACTIVITY OF FⅡ WAS ACTIVATED BY FE2+, BUT STRONGLY INHIBITED BY NA+. THE ACTIVITY OF BOTH ENZYMES WERE COMPLETELY INHIBITED BY THE PRESENCE OF ACETONE AND HG2+. THE CULTURE SUPERNATANT AND THE PURIFIRD ENZYMES WERE TESTED (FⅠ,FⅡ) FOR HYPHAL GROWTH, BOTH CAUSED ABNORMAL HYPHAL SWELLING ON THE TIP OF FUSARIUM OXYSPORUM. BESIDES FUSARIUM OXYSPORUM, THE FUNGICIDE FROM V656 DISPLAYED GOOD INHIBITION ON MANY OTHER PATHOGENIC FUNGI. THIS FUNGICIDE ALSO SHOWED EXCELLENT RESULT IN FIELD TEST.

Keywords : FUNGICIDE , CHITINASE , BIOCONTROL , SHRIMP AND CRAB SHELL POWDER , BACILLUS SUBTILIS.

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