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Environmental Factors Affecting the Growth and Enzymatic Activity of Ceratocystis Radicicolathe Causal Agent of Black Scorch Disease on Date Palm

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Background

Ceratocystis radicicola is a soil-borne pathogen (Wingfield et al, 1993) causing sudden death of date palm (Phoenix dactylifera) in USA and South Africa (Bliss, 1941; Lind & Smit, 1999). It also has been reported to be associated with date palm in Qatar causing black scorch disease (Al-Naemi et al., 2014). Environmental factors such as poor hygienic conditions, stressed palms or senescent tree parts contribute to increase the incidence of black scorch in date palm (Laville, 1966).

Plant pathogens including fungi produce variety of enzymes that degrade plant cell wall. Fungi also secrete several molecular forms of hydrolases that attack the same substrate although they are different in isoelectric point and molecular weight. Extracellular enzymes secreted by fungi are able to macerate tissues and degrade cell wall components. Consequently, they must contain all enzymes related to the types of glycosidic linkages that are present in cell wall polysaccharides. The level of these enzyme activities correlates with the development of disease symptoms (Riou C et al., 1991).

Objectives

This study aimed to: (i) examine the influence of salinity and drought stresses on the growth of C. radicicola in vitro; (ii) study the enzymatic activities of xylanase, cellulase and pectinase in C. radicicola, (iii) examine the effect of

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various Carbon sources (sucrose, xylan, carboxymethyl cellulose and pectin) on C. radicicola enzyme activities; and (iv) investigate the effect of pH media on the fungal growth.

Methods

For salt stress, 4 mm disc of C. radicicola was cultured in PDA or PDB media with different concentrations of NaCl (0, 0.26, 0.43, 0.60, 0.86, 1.03, 1.20 and 1.37 M). The culture was incubated at 25°C for 7–21 days under dark conditions. Rate of relative fungal growth on PDA was recorded and given the following scales (–: No growth, 1+ very minimal growth, 2+: minimal growth, 3+: moderate growth, 4+: heavy growth and 5+: very heavy growth). The mate of fungal growth on PDB was harvested every weak and fresh and dry weight obtained. Hemocytometer was used for spore counting and the number of spores is divided by 5 and multiple to 104 to obtain the total number of spores/ml.

For drought stress, 4 mm disc of C. radicicola was cultured in PDA and PDB media with different percentages (0, 2, 4, 6, 8, 10, 20, 40 and 60%) of Poly Ethylene Glycol (PEG4400). The culture was incubated at 25°C for 8–10 days under dark conditions. The diameter of fungal growth on PDA was measured. The mate of fungal growth on PDB was harvested. Spores were counted by using hemocytometer.

In case of enzyme activity assay, Czapek media was prepared where the carbon source was substituted with 1% of the following: carboxymethyl cellulose (CMC), sucrose, pectin or xylan. To optimize the fungus growth, pH media was adjusted at different levels (5.5, 6, 6.5, 7, 7.5 and 8). In addition, standard Czapek broth media was used as a control. Four mm discs of C. radicicola were inoculated in czapek media and incubated at 25°C for 8–10 day. Cultures were centrifuged at 5000 rpm for 20 min. and supernatants were used to examine xylanase, cellulase and pectinase DNS – enzymatic activities. The enzymatic activity was calculated using the following equation; Enzyme activity = (standard factor × absorbance)/ time of incubation (min); whereas standard factor = (concentration (m mol/ml) of standard/absorbance at 540) × dilution factor. Spectrophotometer was used to measure the enzyme activity based on reduced sugar in the media.

Results

Results from salinity stress in vitro showed a clear growth of C. radicicola in PDB media with NaCl concentrations of 0.26, 0.43, 0.6, 0.86, 1.03 and 1.2 M during the first three weeks while no growth was occurred in PDB with 1.37 M. Radial growth of C. radicicola did not show any changes on PDA with 0.26, 0.43 and 0.6 M while fungal growth diameter decreased significantly under 0.86, 1.03 and 1.2 M concentrations to reach 4.9, 2.3 and 1.4 cm, respectively (Figure 1). Number of spores was decreased by increasing NaCl concentrations from 1.4 × 104 in control treatments to reach zero in 1.2 M.

Growth of C. radicicola was tested under physiological drought stress using different concentrations of PEG4400 (0, 2, 4, 6, 8, 10, 20, 40 and 60%). C. radicicola was able to survive under drought stress regimes up to 40% in the first seven days while it failed to grow at 60% of PEG4400.

Results of enzymatic activity assays showed that C. radicicola grow very well in czapek media supplemented with 1% both xylan and pectin as carbon sources while it showed weak growth in czapek media supplemented with 1% of CMC. On the other hand, C.radicicola did not grow in czapek media supplemented with 1% sucrose. Results also showed that both Xylanase and carboxymethyl cellulase had the highest enzymatic activities with 109 and 6.8 IU/ml, respectively when pectin was used as a carbon source at pH 8. Moreover, high pectinase activity was recorded (61 IU/ml) when pectin was used in the growth media at pH 7.5. When xylan was used as source of carbon, Xylanase showed high activity at pH 5. In standard czapek broth media, activities of xylanase and carboxymethyl cellulase enzymes increased by increasing pH while pectinase activity decreased.

Conclusions

To study the effects of salinity and drought stresses on the growth of C. radicicola in vitro, the fungus was exposed to different salt and drought stress conditions. Our results revealed that C. radicicola was able to survive and grow up to 1.2 M NaCl during the first

three weeks, while it could not grow at 1.37 M. Fungal growth diameter and number of spores were decreased by increasing NaCl concentration. Growth of C. radicicola was affected severly under physiological drought stress where it was not able to survive at 60% of PEG4400. Enzymatic activity assays showed that C. radicicola grow very well in czapek media supplemented with 1% both xylan and pectin as carbon sources while it showed weak growth in czapek media supplemented with 1% of CMC. On the other hand, C.radicicola did not grow in czapek media supplemented with 1% sucrose. Xylanase and carboxymethyl cellulase had the highest enzymatic activities when pectin was used as a carbon source at pH 8.