

Detection of Extracellular Enzyme Activities in Various *Fusarium* spp.

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Thirty seven species of *Fusarium* were evaluated for their ability of producing extracellular enzymes using chromogenic medium containing substrates such as starch, cellobiose, CM-cellulose, xylan, and pectin. Among the tested species *Fusarium mesoamericanum*, *F. graminearum*, *F. asiaticum*, and *F. acuminatum* showed high β -glucosidase activity. Xylanase activity was strongly detected in *F. proliferatum* and *F. oxysporum*. Strong pectinase activity was also found in *F. oxysporum* and *F. proliferatum*. Amylase activity was apparent in *F. oxysporum*. No clear activity in cellulase was found from all the *Fusarium* species tested.

KEYWORDS: Chromogenic media, Extracellular enzymes, *Fusarium*

Fungi can produce diverse extracellular enzymes those are used to break down complex polysaccharides into simple sugars to be assimilated and used for growth and reproduction. These enzymes are useful for environmental and industrial applications such as food processing, brewery, biofuels, bioremediation etc. For those applications, exploration of new sources of useful fungal extracellular enzymes is demanded. The discomycete *Fusarium* is one of well-known fungi in agriculture and forestry as problematic plant pathogens and mycotoxin contaminating agents that can affect human and animal health (Booth, 1977; Nelson *et al.*, 1983). In fact, *Fusarium* is a large genus of filamentous fungi, and most of *Fusarium* species are harmless saprobes and relatively abundant members of the soil microbial community (Domsch *et al.*, 1980; Nwanma *et al.*, 1993). This ecological habitat of the fungus implies that *Fusarium* would be a useful resource of extracellular enzymes. However, information on the ability of producing enzymes in *Fusarium* spp. is rarely reported.

Culture collection is one of great resources where we can find new sources of extracellular enzymes. Currently diverse species of *Fusarium* are deposited and preserved in domestic fungal culture collections. The present study was carried out to find potent new sources of valuable fungal enzymes from *Fusarium* species. For this purpose, the ability of producing enzymes is evaluated with various *Fusarium* species using the plate screening methods with chromogenic substrates (Lee and Lee, 1997). This method is relatively straightforward and simply applicable tools for specific detection of polysaccharide degrad-

ing microorganisms (Castro *et al.*, 1995).

A total of 37 *Fusarium* species were obtained from Korean Agricultural Culture Collection (KACC, Suwon, Korea) and tested for their ability of extracellular enzyme activities in chromogenic media. The obtained fungi were maintained on potato dextrose agar (Difco, USA). Preculturing of the fungi were made either on PDA or on 2% malt extract agar (Difco, USA) at 25°C for 5 days. For the observation of fungal extracellular enzyme activity, the precultures were transferred onto the media containing each of 0.5% CM-cellulose (Sigma, USA), D-cellobiose (Sigma, USA), starch from potato (Sigma, USA), polygalacturonic acid (MP Biomedical, France), xylan from oat spelts (Sigma, USA) as enzymatic carbon source, 0.1% yeast nitrogen base (Difco, USA) as its fundamental nitrogen source, and 1.5% agar powder. 0.5% Congo red dyes (Teather and Wood, 1982) was used for chromogenic reaction due to its better performance in extracellular enzyme activity detection with various fungal species (Yoon *et al.*, 2007). After 5 days of culturing at 25°C, we could observe clear zone formed by reaction between enzyme and chromogenic substrates. For the clear zone measurement, cultured Petri plates were upside placed on white light box and photographed. The degree of extracellular enzyme activity was evaluated based on the size of formed clear zone. Clear zone size is divided into three groups. The first is strong activity group (defined as S at Table 1) that shows 2.5~4 cm of clear zone size. The second is moderate activity group (defined as M at Table 1) that shows 1~2.4 cm of clear zone size. The third is weak activity group that forms less than 1 cm of clear zone size and/or doesn't form clear zone (defined as N at Table 1). Mycelial growth was also measured simultaneously with

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Table 1. The ability of producing extracellular enzymes in *Fusarium* spp.

Species name (KACC No.)	β -Glucosidase	Amylase	Xylanase	Pectinase
<i>F. acaciae-meamsii</i> (42144)	S	N	N	S
<i>F. acuminatum</i> (40531)	S	M	S	M
<i>F. anthophilum</i> (40235)	S	S	S	M
<i>F. asiaticum</i> (42185)	S	N	S	N
<i>F. austroamericanum</i> (42155)	S	N	M	N
<i>F. avenaceum</i> (41310)	S	N	M	N
<i>F. boothii</i> (42146)	S	N	M	M
<i>F. brasiliçum</i> (42152)	S	N	S	S
<i>F. caucasicum</i> (40528)	S	N	S	S
<i>F. cerealis</i> (41311)	S	N	M	S
<i>F. cortaderiae</i> (42150)	S	S	M	S
<i>F. culmorum</i> (42161)	S	S	S	M
<i>F. decemcellulare</i> (40856)	S	M	N	S
<i>F. dimerum</i> (40679)	S	N	N	N
<i>F. equiseti</i> (41037)	S	S	S	M
<i>F. fijikuroi</i> (41817)	S	N	M	M
<i>F. graminearum</i> (41044)	S	S	S	S
<i>F. incarnatum</i> (41315)	S	N	S	S
<i>F. lateritium</i> (41034)	S	N	N	S
<i>F. lunulosporum</i> (42158)	S	M	S	S
<i>F. meridionale</i> (42156)	S	N	N	N
<i>F. mesoamericanum</i> (42148)	S	N	M	S
<i>F. moniliforme</i> (40386)	S	S	S	M
<i>F. oxysporum</i> (42167)	M	S	S	S
<i>F. phaseoli</i> (42183)	S	S	S	S
<i>F. proliferatum</i> (40387)	S	S	S	S
<i>F. pseudograminearum</i> (42163)	S	N	M	N
<i>F. sambucinum</i> (41317)	S	N	N	N
<i>F. semitectum</i> (41036)	S	N	N	M
<i>F. solani</i> (41365)	S	S	S	S
<i>F. sp.</i> (42143)	S	S	S	S
<i>F. sporotrichioides</i> (41318)	S	S	M	M
<i>F. squiseti</i> (41033)	S	N	M	M
<i>F. subglutinans</i> (41319)	S	S	N	N
<i>F. tricinctum</i> (42097)	S	S	S	M
<i>F. verticillioides</i> (41321)	S	N	N	N
<i>F. virguliforme</i> (42166)	S	M	N	S

S: strong activity, M: moderate activity and N: weak or no activity.

the measurement of clear zone.

The results of activity detection of four different extracellular enzymes in 37 *Fusarium* species are given at Table 1. β -glucosidase, amylase, pectinase, and xylanase were detected but no cellulase was detected in the 37 species. Thirty-six of 37 *Fusarium* species tested showed strong activity of β -glucosidase, the enzyme that degrades D-cellobiose to glucose. Thus, the production of this enzyme looks popular in *Fusarium*. An example of this enzyme detection is in Fig. 1. Among these 36 species, strong activity was much apparent in *F. mesoamericanum*, *F. graminearum*, *F. asiaticum*, and *F. acuminatum*. In amylase detection, fourteen of 37 species showed strong activity. The strongest activity was detected in *F. oxysporum*. Amylase is the enzyme which degrades amylose in starch polymers. Fig. 2 shows few examples of amylase detection on chromogenic media. Regarding xyla-

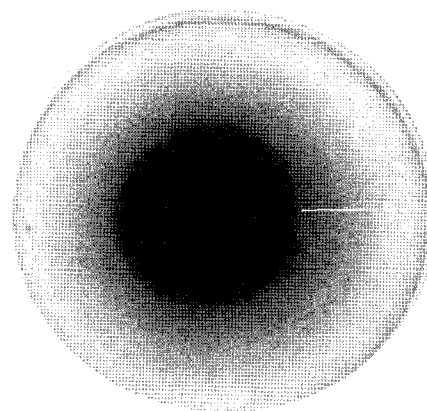


Fig. 1. An example of strong β -glucosidase activity shown chromogenic media by a *Fusarium* species. Bar indicates clear zone.

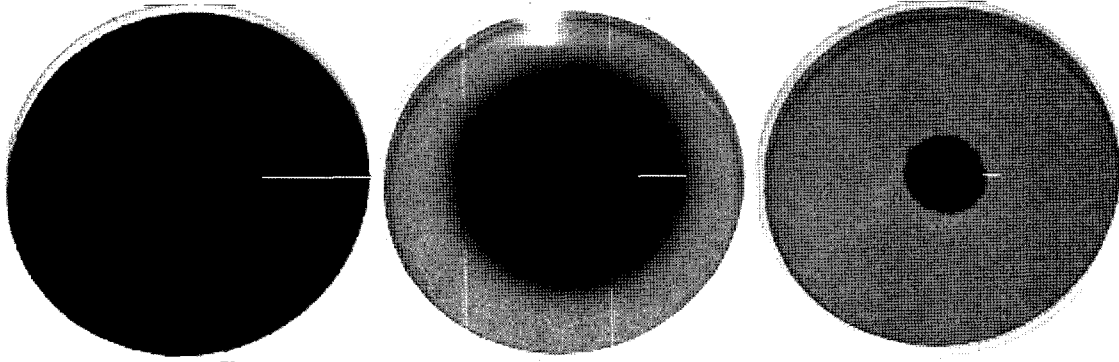


Fig. 2. Examples of amylose activities shown on chromogenic media by different *Fusarium* species. Bar indicates clear zones.

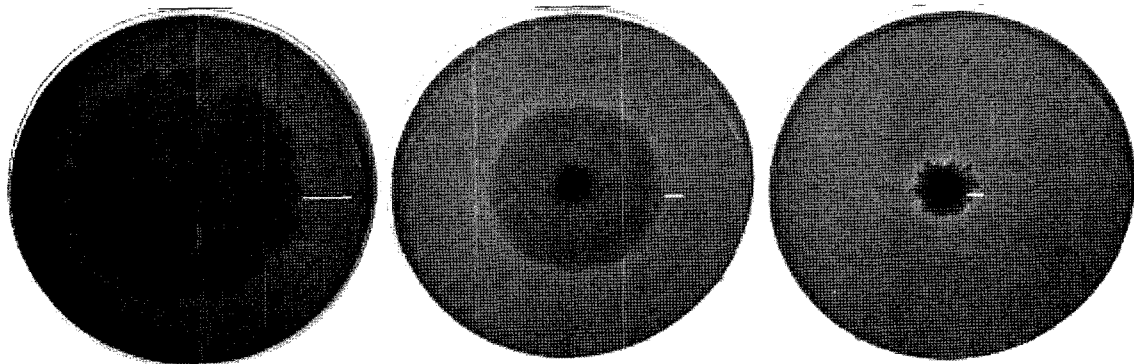


Fig. 3. Examples of xylanase activities shown on chromogenic media by different *Fusarium* species. Bar indicates clear zones.

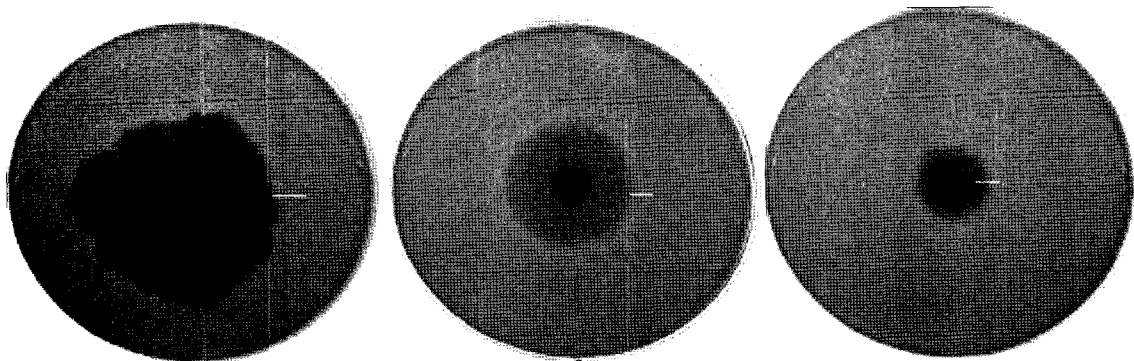


Fig. 4. Examples of pectinase activities shown on chromogenic media by different *Fusarium* species. Bar indicates clear zones.

nase detection, strong activity was found in 17 of 37 species. *F. proliferatum* and *F. oxysporum* produced strongest activity among the 17 species of strong activity pool. The patterns of colony growth and clear zone formed on chromogenic plates are shown in Fig. 3. Strong activity of pectinase (Fig. 4) was also detected from 17 of 37 species listed at Table 1. The strongest pectinase activity was also apparent in *F. oxysporum* and *F. proliferatum*.

When we compared each *Fusarium* culture for enzyme production ability, only *F. graminearum*, *F. phaseoli*, *F. proliferatum*, *F. solani*, and *F. sp.* showed that they have the strong ability of producing β -glucosidase, amylose, pectinase, and xylanase. On the contrary, *F. dimerum*, *F.*

meridionale, *F. sambucinum*, and *F. verticillioides* showed the least ability of extracellular enzyme production. These *Fusarium* species showed weak or no activity of amylose, pectinase, and xylanase.

Yoon *et al.* (2007) successfully detected cellulase in *F. solani* using the same plate assay methods based on chromogenic reaction. In this study we could not detect cellulase not only from *F. solani* but also from other *Fusarium* species. Considering that *F. solani* strains used for this study and that of Yoon's are different, the discrepancy in the results of cellulase detection in *F. solani* is likely from difference in strain properties. Since we could not detect cellulase from any of the species listed at Table 1, cellu-

lose is not common extracellular enzyme in *Fusarium*.

Overall, we have found that chromogenic media is also useful for the detection of extracellular β -glucosidase, amylase, pectinase, and xylanase in *Fusarium* spp. There were differences in the production of these enzymes among the tested 37 *Fusarium* species. Since most of the cultures used in this study are Korean origin, the results of our work provide would provide basic information on the biochemical properties of the *Fusarium* species deposited in KACC. Based on information of Table 1 that *F. oxysporum* produces strong pectinase activity and on information of the enzyme gene in GenBank database, Yoon (2007) easily cloned a gene encoding one of pectinases from *F. oxysporum* using PCR-based cloning approach. Since protein expression technology using protein expression vectors is developing, Yoon's results show that the use of DNA database and the fungal culture that demonstrated the ability of producing enzyme activity is synergistic for the work of a target enzyme and its gene. Thus, the finding of five *Fusarium* species that show strong activities in β -glucosidase, amylase, pectinase, and xylanase implies that these cultures deserve to further look to explore their potent values in industrial application.

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