Video Article

Gibberella zeae Ascospore Production and Collection for Microarray Experiments.

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Abstract

Fusarium graminearum Schwabe (teleomorph Gibberella zeae) is a plant pathogen causing scab disease on wheat and barley that reduces crop yield and grain quality. F. graminearum also causes stalk and ear rots of maize and is a producer of mycotoxins such as the trichothecenes that contaminate grain and are harmful to humans and livestock (Goswami and Kistler, 2004). The fungus produces two types of spores. Ascospores, the propagules resulting from sexual reproduction, are the main source of primary infection. These spores are forcibly discharged from mature perithecia and dispersed by wind (Francl et al 1999). Secondary infections are mainly caused by macroconidia which are produced by asexual means on the plant surface. To study the developmental processes of ascospores in this fungus, a procedure for their collection in large quantity under sterile conditions was required. Our protocol was filmed in order to generate the highest level of information for understanding and reproducibility; crucial aspects when full genome gene expression profiles are generated and interpreted. In particular, the variability of ascospore germination and biological activity are dependent on the prior manipulation of the material. The use of video for documenting every step in ascospore production is proposed in order to increase standardization, complying with the increasingly stringent requirements for microarray analysis. The procedure requires only standard laboratory equipment. Steps are shown to prevent contamination and favor time synchronization of ascospores.

Video Link

The video component of this article can be found at http://www.jove.com/video/115/

Protocol

1. Carrot agar (Klittich and Leslie, 1988) was prepared in 9-cm diameter Petri dishes.
2. A 2 mm cube of agar containing fresh mycelium grown on potato dextrose agar was applied to the centre of each Petri dish.
3. Cultures were grown at 25°C and a 12-h photoperiod for 96 hrs.
4. One ml of aqueous 2.5% Tween 60 was applied to the surface of the culture (Trail and Common, 2000) and spread across the culture with a plastic rod, by gently pressing.
5. Perithecia shooting ascospores developed 7-10 days after this induction treatment when kept in a high humidity environment at 25°C with the same photoperiod.
6. Spore collection is performed by changing the Petri plate lid, leaving perithecia to shoot for a limited time (for example 12 hrs) in the dark.
7. Sterile water is added to the lid, suspending ascospores by gently moving water on the surface.
8. Ascospores are collected in sterile Falcon tubes, washed and used for further analysis.

Discussion

The protocol presented here is based on previous procedures used for perithecial production for Fusarium spp. (Klittich and Leslie, 1988; Trail and Common, 2000). Standardization of the procedure by which large quantities of ascospores (sufficient for microarray analysis) were generated was essential for the reproducibility of the experiment. It has been reported that environmental factors, age and substrate differences can change the biological character of ascospores (Beyer and Verreet, 2005). Therefore the use of video may highlight small details in the way spores are produced and collected that should facilitate reproducibility. In particular the video of the procedure should improve the level of standardization among laboratories and facilitate the comparison of whole genome transcription studies which require ascospore production. The amount of RNA necessary for experiment procedure is relatively high so a large number of Petri dish should be processed synchronously. Video is a particularly suitable tool when it is necessary to implement whole genome transcriptional studies on new biological material, setting a standard for future experiments.

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