STUDY OF POTATO DEXTROSE AGAR (PDA)

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ABSTRACT

Potato Dextrose Agar (PDA) is used for the cultivation of fungi. Potato Dextrose Agar (PDA) is a general purpose medium for yeasts and molds that can be supplemented with acid or antibiotics to inhibit bacterial growth. It is recommended for plate count methods for foods, dairy products and testing cosmetics. PDA can be used for growing clinically significant yeast and molds. Among the various species encountered *Aspergillusniger*, *A. fumigatus* were the dominant fungi in the library. Investigations by the petri plate exposure culture plate method helped us in determining the occurrence of fungal species in the air inside the selected library which also causes allergic diseases to human beings working therein. The fungi were also isolated from highly damaged, old and unreadable books. The colony diameter, culture characteristics (texture, surface and reverse colouration, zonation) and sporulation of selected test fungi were greatly influenced by the type of growth medium used. LCA exhibited comparatively higher mycelial growth in six test fungi, whereas all the ten isolates revealed heavy sporulation on this culture medium. Penicillium sp. and Acremoniumkiliense exhibited maximum colony growth on PDA, while Chaetomiumfunicola and Fusariumoxysporum showed highest growth on CYA medium. These results will be useful for fungal taxonomic studies.

Key words: Mycelialgrowth, sporulation, culture media. PDA

INTRODUCTION

Fungi grow on diverse habitats in nature and are cosmopolitan in distribution requiring several specific elements for growth and reproduction. In laboratory, these are isolated on specific culture medium for cultivation, preservation, microscopical examination and biochemical and physiological characterization. A wide range of media are used for isolation of different groups of fungi that influence the vegetative growth and colony morphology, pigmentation and sporulation depending upon the composition of specific culture medium, pH, temperature, light, water availability and surrounding atmospheric gas mixture (Northolt and Bullerman, 1982; Kuhn and Ghannoum, 2003; Kumara and Rawal, 2008). However, the requirements for fungal growth are generally less stringent than for the sporulation. Nowadays, fungal taxonomy is in a state of rapid flux, because of the recent researches based on molecular approaches, that is DNA comparisons of selected strains

either isolated locally or obtained from culture collection centre, which has changed the existing scenario of fungal systematic and often overturn the assumptions of the older classification systems (Hibbett, 2006). Different concepts have been used by the mycologists to characterize the fungal species, out of which morphological (phenetic or phenotypic) and reproductive stages are the classic approaches and baseline of fungal taxonomy and nomenclature that are still valid (Davis, 1995; Guarro et al., 1999; Diba et al., 2007; Zain et al., 2009). It seems evident that in near future, modern molecular techniques will allow most of the pathogenic and opportunistic fungi to be connected to their corresponding sexual stages and integrated into a more natural taxonomic scheme. Physical and chemical factors have a pronounced effect on diagnostic characters of fungi. Hence, it is often necessary to use several media while attempting to identify a fungus in culture since mycelial growth and sporulation on artificial media are important biological characteristics (St-Germain and Summerbell, 1996). Furthermore, findings for one species are not readily extrapolated to others, particularly for filamentous fungi, where significant morphological and physiological variations exist (Meletiadis et al., 2001). With these perspectives, the present study was undertaken to observe the influence of three different culture media on the mycelial growth, colony characters and sporulation patterns of ten dominant fungi isolated from decaying vegetable wastes.

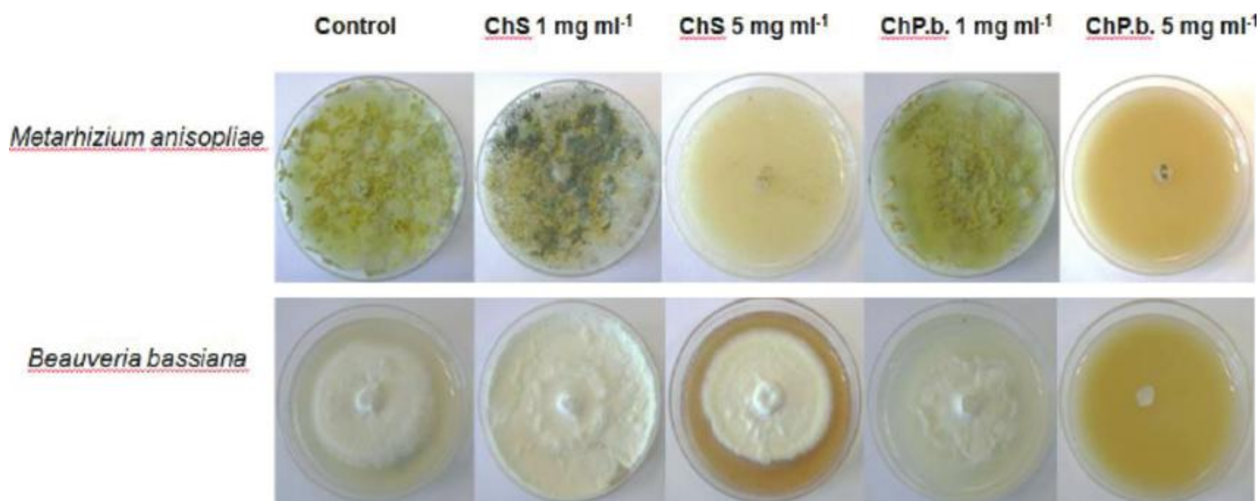

Fig 1: Growth of enthomopathogenic fungi on culture medium supplemented with shrimp chitosan and Pterophyllabeltrani chitosan. Check PDA medium as reference for basal growth and sporulation

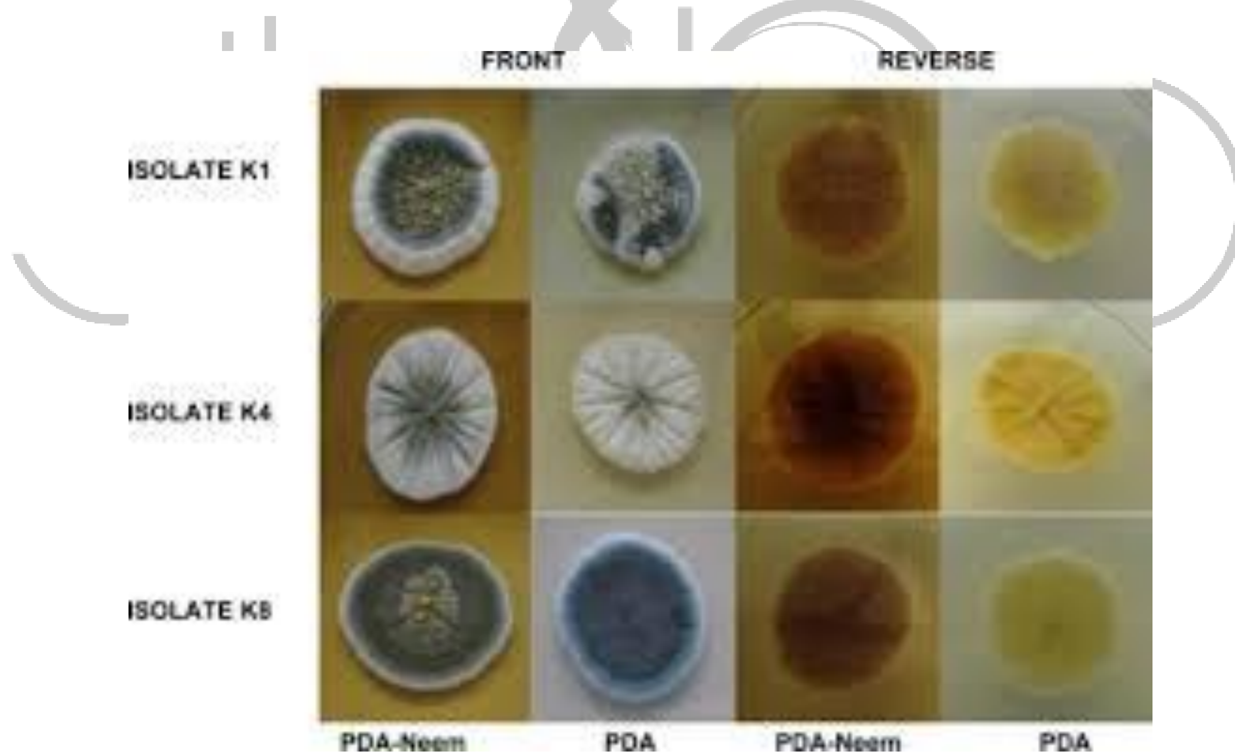

Fig 2: f2-ijms-9-1676: P. citrinum isolates (isolates K1, K4 and K8) grown on PDA and on PDA-NLE as described in 'Materials and Methods'.

METHODS

Potato Dextrose Agar with Chlortetracycline is recommended for the microbial enumeration of yeast and mold from cosmetics.[8,9,12] Potato Dextrose Agar with Chloramphenicol is recommended for the selective cultivation of fungi from mixed samples. Potato Dextrose Agar consists of potato infusion and dextrose. Potato infusion provides a nutrient base for luxuriant growth of most fungi. Dextrose serves as a growth stimulant. The incorporation of tartaric acid (TA) in the medium lowers the pH to 3.5, thereby inhibiting bacterial growth. Chloramphenicol acts as a selective agent to inhibit bacterial overgrowth of competing microorganisms from mixed specimens, while permitting the selective isolation of fungi. In *Standard Methods for the Examination of Dairy Products*, the American Public Health Association (APHA) recommends Potato Dextrose Agar with TA (Tartaric Acid) for determining yeast and mold counts from butter, margarine and related products.[8]

The U.S. Food & Drug Admistration (FDA) recommends Potato Dextrose Agar with Chlortetracycline for the evaluation of yeast and mold from cosmetic products.[12] Storage: Upon receipt, store at 2-8°C away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing. The expiration dating on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended quality control incubation times.Refer to the document "Storage" on the Hardy Diagnostics Technical Document website for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials,

and for recommendations for the management of exposure to infectious disease, refer to CLSI document M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" on the Hardy Diagnostics Technical Document website for more information.Refer to the document SDS Search instructions on the Hardy Diagnostics website for more information.

For cat. nos. W93, W96 and W96R.

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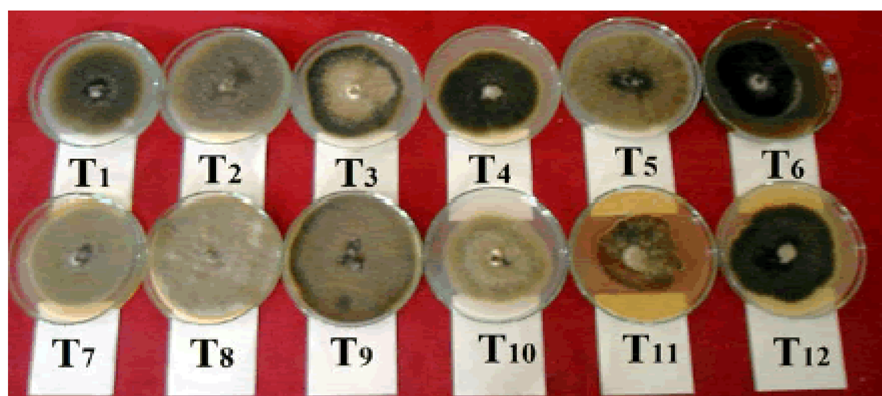
This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*



T1: Asthana and Hawker's Agar; T2: Corn Meal Agar; T3: Czapek's Dox Agar; T4: Glucose Peptone Agar; T5: Hansen's Agar; T6: Host Leaf Extract Agar; T7: Malt Extract Agar; T8: Oat Meal Agar; T9: Potato Dextrose Agar; T10: Richard's Agar; T11: Sabouraud's Agar; T12: Waksman Agar.

Figure 1: Growth of *A. solani* on different solid media.

Fig 3: Evaluation of media for fungi growth

Sterilize all biohazard waste before disposal.Refer to the document "Precautions When Using Media" on the Hardy Diagnostics Technical Document website for more information.

Refer to the document SDS Search instructions on the Hardy Diagnostics website for more information.Specimen Collection: Consult listed references for information on specimen collection.[1-5] When using Potato Dextrose Agar with TA (Tartaric Acid), Potato Dextrose Agar with Chlortetracycline or Potato Dextrose Agar with Chloramphenicol, refer to listed references for the appropriate method of use and interpretation.[2,4,6-12] Consult listed references for information regarding the interpretation of growth of fungal species.[2-12] It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

Refer to the document "Limitations of Procedures and Warranty" on the Hardy Diagnostics Technical Document website for more information.Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided. Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

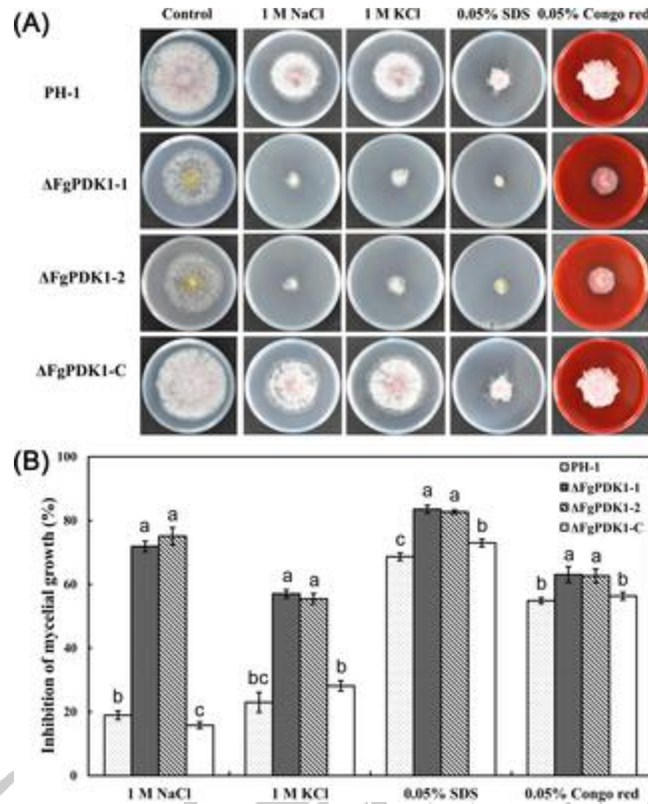


Fig 4: media for fusarium

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificates of analysis (CofA) available from Hardy Diagnostics Certificates of Analysis website. In addition, refer to the following documents on the Hardy Diagnostics Technical Document website for more information on QC: "Introduction to Quality Control" and "Finished Product Quality Control Procedures," or see reference(s) for more specific information.Potato Dextrose Agar with TA, Potato Dextrose Agar with Chlortetracycline and Potato Dextrose Agar with Chloramphenicol should appear slightly opalescent, and light amber in color, with no precipitate, chips, or debris. The new technique is not only reliable, it is very inexpensive and easy to use in any laboratory with few resources. CIAT uses this method to store about 500 cultures of insect pathogenic fungi and 1000 cultures of plant pathogenic fungi and bacteria. Evaluations of purity, pathogenicity and virulence were performed on fungi stored between 5 to 10 years. With a few

exceptions the fungus was recovered easily and with the same characteristics of pathogenicity and virulence it had when first stored. This technique has been successfully implemented in other institutions with great results. Research studies at CIAT are adapting this methodology to work with bacteria and viruses.

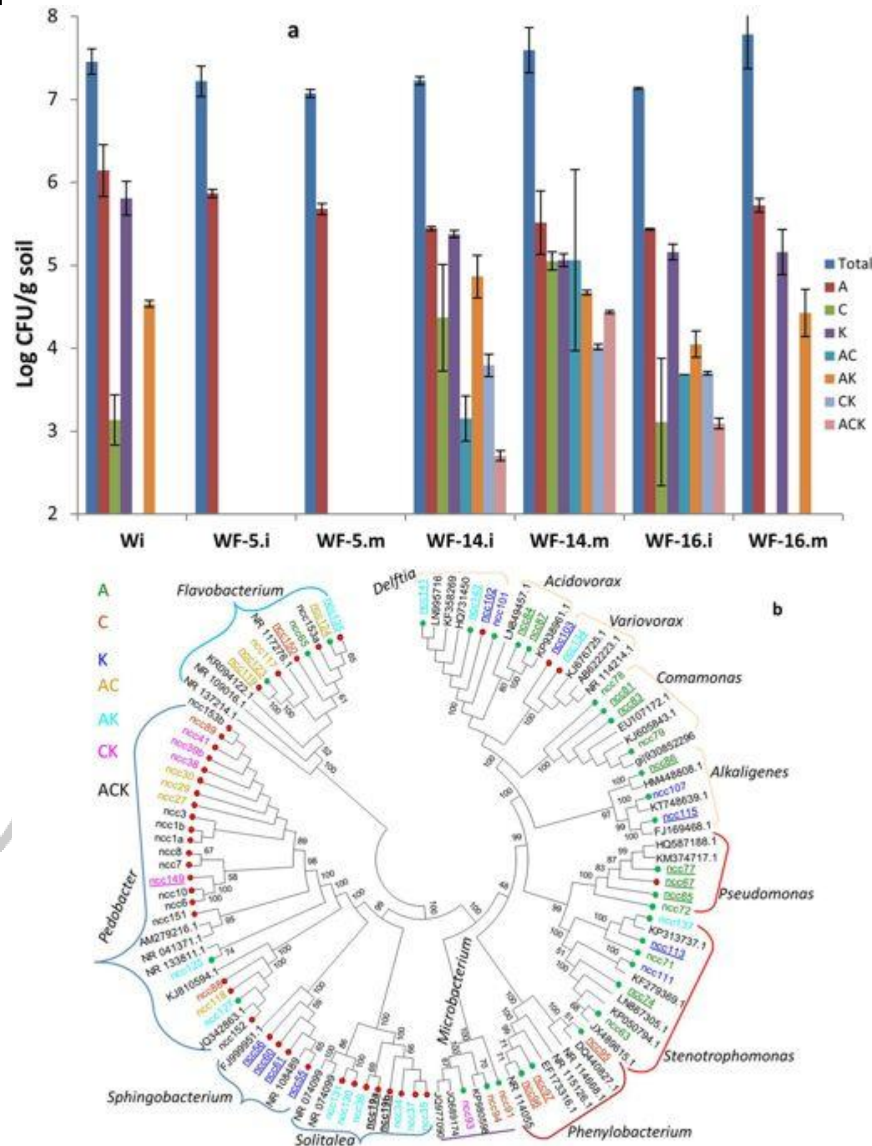


Fig 5: Cultivable ARB from the waste water W source affected or not by fungal colonization in pre-sterilized soil microcosms, sampled from inoculation point (i) and respective fungal migration front (m) (**a**), and their phylogenetic analyses (**b**). Blue bars are total bacteria on R2A without any antibiotic while A, C and K represent ampicillin, ciprofloxacin and kanamycin respectively, applied in medium alone or in combination. Error bars represent the standard deviations (n = 3). W corresponds to microbial inoculum originating from sludge/water of waste water treatment plant, while F-5, F-14, and F-16 represent the three selected fungal strains NFC-5, NFC-14, and NFC-16, respectively. *No bacteria were detected at migration point (Wm) for non-fungal treatments. In **'b'** the strains with underline codes are isolated from m-point and others from i-point; Red and green nodes of the tree represent the M and W source respectively; while the text colors represent different antibiotics used to isolate these strains. The tree is constructed via neighbor joining method considering Maximum Composite Likelihood model with Bootstrap replicates of 1000, and bootstrap values equal to or greater than 50 are shown.

Conclusion

The first step is to isolate the fungus into pure culture. A tiny pinch of the fungus is taken directly from the insect or plant host and added to a Petri dish containing culture media. If the identity of the fungus is known, selective culture media might be available; if not, general media such as PDA (Potato Dextrose Agar) can be used for the initial isolation. Lactic Acid or Chloramphenicol can be added to any standard medium in order to reduce contamination by bacteria. After a pure culture has been obtained, the fungus is grown for 5 to 10 days. Now the storage process can be initiated.Culture collections are expensive to support, as they require special equipment and continuous attention in order to maintain fungal cultures without losing their pathogenicity or virulence. Two examples of these collections are the USDA-ARS Collection of Entomopathogenic Fungal Cultures, with more than 5500 cultures of over 350 species of fungi from 900 hosts. The International Entomopathogenic Bacillus Centre in the Institute Pasteur has nearly 3500 strains of *Bacillus thuringiensis*, the most important bacterium used in biocontrol. Cultures are typically either freeze-dried in a process called lyophilization, or stored in liquid nitrogen at ultra-low temperatures. Both techniques require intense labor and expensive equipment.

At the International Center of Tropical Agriculture (CIAT, in Colombia), expenses were reduced with a novel, reliable and cheap technique. The dry filter paper technique was developed by RosalbaTobon and Ximena Aricapa in the early 1980s and can be used for preservation of cultures of insect pathogenic and plant pathogenic fungi as well as many molds.

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