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Research Article

EFFECT OF VARIOUS MEDIA TYPES ON THE RATE OF GROWTH OF *ASPERGILLUS NIGER*

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ABSTRACT
Aspergillus niger, a common soil borne fungus is industrially important and utilised in the fields of biotechnology and food microbiology. It is cultured enormously in large scale due to its positive significance also. In the present investigation, the rate of growth of *Aspergillus niger* has been compared in various media types viz. simple PDA (Potato Dextrose Agar), modified PDA (containing additional sucrose and peptone), CYA (Czapek's Dox + Yeast Extract Agar), and LCA (Lignocellulose Agar). The results showed that PDA is the most commonly used laboratory medium for fungi due to its good and balanced nutrient content. However, when optimised or modified by nutrients viz. sucrose and peptone, it gives better results of fungal growth rate. The LCA medium is however secondary while CYA has been considered as average medium for fungal growth rate.

INTRODUCTION
Aspergillus is a genus consisting of several hundred mold species found in various climates worldwide. *Aspergillus* was first catalogued in 1729 by the Italian priest and biologist Pier Antonio Micheli. *Aspergillus* species are highly aerobic and are found in almost all oxygen-rich environments, where they commonly grow as molds on the surface of a substrate, as a result of the high oxygen tension.
Aspergillus species are important in commercial microbial fermentations. For example, alcoholic beverages such as Japanese sake are often made from rice or other starchy ingredients (likemanioc), rather than from grapes or malted barley. Members of the genus are also sources of natural products that can be used in the development of medications to treat human disease.
Perhaps the largest application of *Aspergillus niger* is as the major source of citric acid; this organism accounts for over 99% of global citric acid production, or more than 1.4 million tonnes per annum.
Aspergillus niger is a haploid filamentous fungi and is a very essential microorganism in the field of biology. In addition to producing extracellular enzymes and citric acid, *A. niger* is used for waste management and biotransformations. The fungi is most commonly found in mesophilic environments such as decaying vegetation or soil and plants.
Genome sequencing of *A. niger* is important because of its involvement in producing citric acid as well as industrial enzymes, such as amylases, proteases, and lipases. The use of these enzymes are essential because of its importance for transformation to food enzymes. Other properties of this species include pathogens that cause the spoilage of food and production of secondary metabolites, such as aflatoxin, that are toxic. Metabolite production, involvement in food spoilage, and simply being a pathogen creates a great economic impact on the U.S. (roughly $45 billion on the U.S. economy alone). Understanding this economic importance as well as the effects it makes on the environment makes the genome sequencing of *A. niger* essential to biological applications. *A. niger* is easily isolated from common thing such as dust, paint, and soil. Commonly in labs, *A. niger* is isolated via chemostat cultures which can test positively or negatively for the fungi.
Aspergillus niger is an important industrial fungus that is widely used for the production of enzymes and metabolites, such as citric acid, but also as a host to produce heterologous proteins.
Aspergillus niger has been used to study fungal protein secretion, proteolysis, cell wall biosynthesis, cell morphology and polarity, degradation of plant (cell wall) polysaccharides, central carbon metabolism and

nutrient transport, both genetically and biochemically. Furthermore, as a soil saprobe with a wide array of hydrolytic and oxidative enzymes involved in the breakdown of plant lignocellulose, *A. niger* plays a significant role in the global carbon cycle. Finally, *A. niger* is an important model fungus for the study of eukaryotic protein secretion in general, the effects of various environmental factors on suppressing or triggering the export of various biomass degrading enzymes, molecular mechanisms critical to fermentation process development, and mechanisms involved in the control of fungal morphology.

MATERIALS AND METHODS

In the present investigation, *Aspergillus niger* was obtained from the soil of Mahatma Jyoti Rao Phoole Institution garden, New Sanaganer Road, Jaipur. This was cultured in four types of culture media viz. simple PDA, modified PDA, CYA and LCA, by inoculating 1 ml of the 10^{-3} serially diluted soil sample using spread plate technique. The inoculated media plates were incubated at 30°C. The colonies initiated as cream colored dots on 2nd day of inoculation. These white to pale yellow colonies quickly formed jet black conidia. Colonies were seen as perfectly round to oval to irregular in shape. On the 10th day mature colonies with spores were seen as structures in the form of numerous black dots. The elevation of colony appeared as raised. The colony margin was entire to undulate. Reverse of petriplate was white to pale yellow and growth produced radial fissures in the agar.

Slide preparation and microscopic observations were carried out to study the detailed structure of this fungus. After inoculation of soil sample the mixed culture or polyculture of various fungal species was obtained. Thus *Aspergillus niger* was identified by colony characteristics and microscopic observations. This study was based on the morphological (size/shape/colour) differences of fungal colony. Adhesive tape impressions for microscopic examination were taken from black powdery colonies on petridish. Tape was slightly pressed onto suspected fungal growth surfaces and mounted onto microscope slides. The stain solution cotton blue + lactophenol was applied directly to the tape mounted on the slide, and a cover slip was placed. This is a direct staining procedure followed by microscopic visualization of spore morphology. For the experiments showing rate of growth of *A. niger* in presence of various factors, the serially diluted (10^{-3}) soil sample was inoculated by spread plate method in 4 types of PDA media. The resultant fungi in various PDA were mixed polycultures. Amongst these *A. niger* colonies after identification were utilised for calculating growth rate. The calculation of growth rate as Colony forming Units (CFUs) per ml in every medium was carried out by the following formula:

CFUs/ml= number of colonies/ colony count x dilution factor

RESULTS AND DISCUSSION

It was observed that the hyphae of *Aspergillus niger* were septate and hyaline. Conidial heads were radiate initially, splitting into columns at maturity. The species was biseriate (vesicles produced sterile cells known as metulae that support the conidiogenous phialides). Conidiophores were long , smooth, and hyaline, becoming darker at the apex and terminating in a globose vesicle. Metulae and phialides covered the entire vesicle. Conidia were brown to black, very rough and globose.

Rate of Growth of Aspergillus Niger in Simple PDA Medium

Amongst fungal polyculture, spiral patterns of growth were sometimes encountered in the colonies of A. niger. Such patterns arised from various causes. For example an endogenous rhythm of sporulation in *Aspergillus niger* produced a colony which forms an Archimedes spiral. It looked like a black powdered dusting has been left on PDA. The fungus started to develop in small circular patterns on the media and appeared as black spotty dots . On PDA, the radially expanding colonial growth form of the fungal mycelium was most evident, extending from an inoculum, on, within and sometimes above the substrate, forming a near spherical three-dimensional colony.

Rate of Growth in Modified /Optimised PDA Containing Sucrose+Peptone

At high nutrient level PDA+dye+sucrose+peptone) , the *Aspergillus niger* colonies formed thick layers due to the high nutrient influx. There appeared roughening in the colony interface at relatively high nutrient levels. At high nutrient level, hyphae were produced densely inside the colony. With increasing nutrient content, colony shapes became similar to the compact morphology. Increasing nutrient content increased colony and mycelial density.At low nutrient level, the colonies of *Aspergillus niger* were seen in the form of thin mycelial layers. Hyphae were created homogeneously inside the colonized area.

Dense *Aspergillus* colonies were observed upto 10^{th} day of inoculation. As the nutrient content in PDA depleted upto 10^{th} day, sporulation triggered. Hence on the 10^{th} day fungal colonies exhibited maximal spores.

During development, *A. niger* extended its hyphae into the medium. The various forms and patterns of fungal growth were referred to as a phenomenon of biological self-organization. Each colony was seen as a uniform multicellular structure developing radially by growth and branching of mycelium. The colony was able to consume substrate (carbon source, i.e. sucrose) and to produce diffusible metabolites which could suppress other microbial development viz. bacteria. Hence fungal colony initiation led to suppression of bacterial growth due to production of mycotoxins (aflatoxins) in the PDA.The final stage of fungal morphogenesis was observed as the formation of spores. Different growth patterns were seen arising in colonies of *A. niger* while they were cultivated on PDA+sucrose+peptone.

Rate of Growth in Czapek's Dox + Yeast Extract Agar (CYA)

It was observed that poor or moderate fungal sporulation occurred in CYA. Okunowo *et al.*,(2010) also observed least sporulation and minimum mycelia growth of a fungus on Czapek' s Dox agar which may be due to the presence of chloride ion in the test medium. Thus Czapek's dox agar suppresses the fungal sporulation due to presence of chloride ions.

Rate of Growth in Lignocellulose Agar (LCA)

In the present study, *Aspergillus niger* showed heavy fruiting bodies formation in LCA. Osono and Takeda, (1999) stated that LCA because of its low glucose content suppresses the overgrowth of fast growing species. Thus the fast growing *Aspergillus niger* in nature is suppressed by LCA medium in comparison to PDA. However, the sporulation was enhanced as compared to CYA. Hence this medium can be used for fungal identification.

Table 1: Growth rate of *Aspergillus niger* as CFUs/ml in various types of nutrient media

S. No.	Media type	Colony characteristics	Growth rate as CFUs/ml
1.	Simple PDA	velvety texture, white mycelium with typical black spores, Yellow reverse of petriplate, zonation is Heavily furrowed on the reverse, Heavy sporulation	$10x10^3$
2.	PDA+Sucrose+Peptone	Texture dense velvety, mycelium entirely covered by black spores, yellow to orange reverse of petriplate, extremely furrowed zonation on the reverse, sporulation triggered	$22x10^3$
3.	Czapek's Dox + Yeast Extract Agar (CYA)	Powdery texture, mycelium White with black spores , reverse of petriplate Yellow, zonation as single concentric ring at periphery and radial furrow at the centre, sporulation moderate	$7x10^3$
4.	Lignocellulose Agar (LCA)	Powdery texture, Hyaline mycelium with black spores , reverse of petriplete was Colourless, zonation was light and concentric, Heavy sporulation	$8x10^3$

CONCLUSIONS

The media components are an important criteria for fungal culture and study, along with important physiological parameters that lead to maximum sporulation in fungi (Kim *et al*., 2005; Saxena *et al*., 2001; Saha *et al*., 2008). In the present investigation, type of culture media and their chemical compositions significantly affected the mycelial growth rate and conidial production in *Aspergillus niger*. PDA is one of the most commonly used culture media because of its simple formulation and its ability to support mycelial growth of a wide range of fungi. Several workers stated PDA to be the best media for mycelial growth (Xu *et al*., 1984; Maheshwari *et al*., 1999; Saha *et al*., 2008). *Aspergillus niger* has been seen to thrive best on PDA, which is rich in nutrients, thus encouraging the mycelial growth and sporulation. Moreover, the variations in colour of spores, especially among *Aspergillus* spp. are one of the main criteria used widely for their identification and taxonomic placement (St-Germain and Summerbell, 1996) which seems to be mainly attributed to the constituents of a medium.

Thus, our findings revealed that different types of culture media differentially influenced the growth, colony character and sporulation of the test fungi viz. *Aspergillus niger*. Out of four test media employed in the present study, PDA+sucrose+peptone (modified PDA prepared by us) was found to be most suitable for heavy sporulation, secondly PDA reproduced most visible colony morphology. It has been concluded that instead of using any single culture medium addition of nutrients in suitable culture medium viz. optimisation would be more appropriate for increased spore formation and rate of growth in fungal spp.

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