Comparative studies on biofilm development by *Aspergillus niger* on polyester sheet and muslin cloth

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Abstract

Filamentous fungi are naturally adapted to adhere on the surfaces in submerged cultures. Cell adhesion plays a vital role in biofilm development in submerged cultures. The objective of the present study is to evaluate the growth rate of *Aspergillus* on the polyester sheet and muslin cloth with and without solid support in submerged cultures. The growth of *A. niger* was observed to be high in polyester sheet when compared with muslin cloth.

Keywords: *Aspergillus niger,* Biofilm, Surface adhesion fermentation, Polyester sheet, Pellets

Introduction

A biofilm is an aggregate of microorganisms in which cells adhere to each other and to a surface. These adherent cells are frequently embedded within a self-produced matrix of extracellular polymeric substance. A large number of studies have been performed targeted at the bacterial biofilms. However, little attention has been paid to medically relevant fungal biofilms. Filamentous fungi are used in biotechnological processes due to their metabolic versatility and to their capability for secreting enzymes, proteins and other important industrial metabolites. Fungal spore adhesion depends on both its rough surface and adhesive substances that form a pad between spore and support. *A niger* is better to know in the form of pelletized growth under submerged cultivation, pellets consists of an outer shell of growing hyphae and an inner mass of non growing mycelium (W1) (Troung et al. 2004). Pellets are highly entangled dense masses of hyphae (Domingues et al.2000; Karns et al.1995).

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It has also been reported in literature that biofilm fermentation produces higher enzyme yields than SmF with lower biomass yields. Biofilm fermentation depends upon surface adhesion, hence a new fermentation category named as surface adhesion fermentation (SAF) was established and first introduced by Gutieroz correa and villenea (Villenea and Guiterrez –Correa 2006). The present paper evaluate and compare the growth rate of *Aspergillus niger* on polyester sheet and muslin cloth like solid support and with no support in lactose based media.

Materials and Methods

All the chemicals and reagents were purchased from Himedia, India. *Aspergillus niger* NCIM777 strain was procured from National Chemical Laboratory (NCL) Pune. Fungal spores from a stock, kept at 4^0 C in 20% (v/v) glycerol. *Aspergillus* cultures were grown on PDA slants at 28^0 C for 4 days. Slants were maintained at 4^0C and subcultured at monthly intervals.

Batch experiments were performed in 250 ml Erlenmeyer flasks containing 100 ml of production media containing (g/l) Urea, 0.3; $(NH_4)SO_4$, 1.4; KH_2PO_4, 2.0; $CaCl_2.2H_2O$, 0.4; $MgSO4.7H_2O$, 0.3; Peptone, 1.0; Tween-80, 0.2; $FeSO_4.7H_2O$, 0.005; $MnSO_4.7H_2O$, 0.0016; $ZnSO_4.7H_2O$, 0.0014; $CoCl_2.6H_2O$, 0.02 with lactose 10, as carbon source. Liquid media was adjusted to an initial pH 5.0 and sterilized for 15 min at 120°C. Inocula of 4 ml culture broth having 0.56 g/l cell dry weight in production media was used. Biofilm formation of *Aspergillus niger* NCIM 777 was studied by adding sterilized muslin cloths and polyester sheet. Muslin cloth with 3 holes and 9 holes of 0.5 cm diameter each was added to the first two Erlenmeyer flasks. Muslin cloth without holes was added to the third flask. Polyester sheet was added to the fourth flask and fifth flask without any solid support. Fermentation was carried out in all five conical flasks on a rotatory shaker at 180 rpm at 30°C for 3 days.

Results and Discussions

Table 1, describes the growth of *Aspergillus niger* in lactose based media with and without solid support. Heavy growth of *A. niger* was observed with muslin cloth with 3 hole when

compared with muslin cloth with 9 hole or without hole. This result might be due to the fact that muslin cloth with hole has

Table 1: Growth of *Aspergillus niger* as biofilm and pellets nature

Support materials	Growth and Production media	Fermentation time(hr)	Growth of *Aspergillus niger*	Nature of growth
Muslin cloth without hole	Lactose based	96	Very light growth	Biofilm
Muslin cloth with 3 hole	Lactose based	96	Good growth	Biofilm
Muslin cloth with 9 hole	Lactose based	96	Light growth	Biofilm
Polyester sheet	Lactose based	96	Very heavy growth	Biofilm
Without solid support	Lactose based	96	Heavy growth	Big pellets

the capability for better aeration and mass transfer of the nutrients which required for microbial growth. But small biofilm has been observed in muslin cloth with 9 hole, may be due to loose support.

Heavy growth has been observed with polyester sheet solid support. Polyester sheet acts as a fine support for *A.niger*, *when* compared to muslin cloth. It provides a better solid, thick scaffold for fungal biofilm growth under the agitating condition. Polyester sheet with rough textures also provides a favorable condition for the biofilm development, due to better adhesion property.

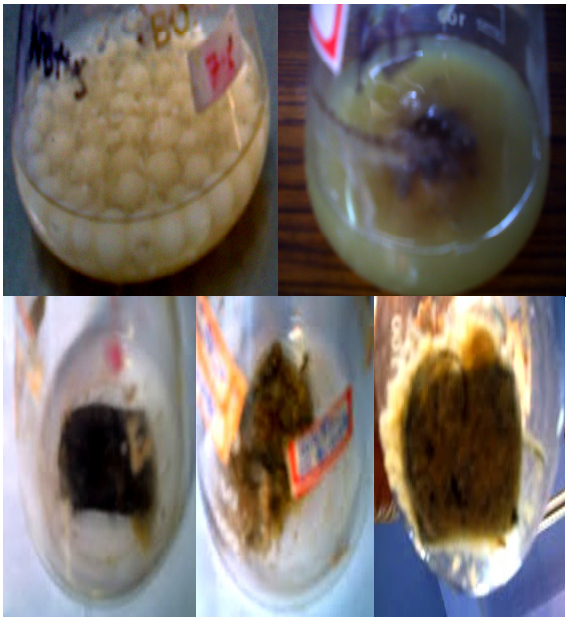

Fig 1-5: Pelleted form of growth, Biofilm growth on muslin cloth, Polyester sheet without biofilm, Polyester sheet with biofilm (front view), Polyester sheet with biofilm (backview) respectively.

Fig 1 represents the growth of *A.niger* in the form of big pellets whereas Fig 2 reveals biofilm development on muslin cloth. Fig 3-5 describes the *A.niger* growth in the form of biofilm under polyester support.

Conclusion

The present study confirms the efficiency of polyester sheet in Surface adhesion fermentation when compared with muslin cloth using *Aspergillus niger* NCIM 777 strain.

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