Cellulose degradation and biofilm formation in the developmental life cycle of the cellulolytic actinomycete Thermobifida fusca

Almaris N Alonso, *University of Massachusetts Amherst*

Abstract

Actinomycetes have been used with enormous success in industrial processes; however, little is known about biofilm development by these filamentous microbes, and the presence of community development on insoluble cellulosic substrates such as cellulose. Cellulose is the most abundant biopolymer and renewable energy source on Earth, and its decomposition, which is carried out almost exclusively by microorganisms, is a key step in the cycling of carbon in the biosphere. It has long been known that cellulolytic bacteria may adhere to their insoluble substrate as it is degraded, although surprisingly little is known about microbial growth, colonization and community development on insoluble cellulosic substrates and non-nutritive surfaces. Previous investigations indicated that two Gram-positive cellulolytic soil bacteria, *Cellulomonas uda*, a facultative aerobe, and *Clostridium phytofermentans* , an obligate anaerobe, specifically adhered to nutritive surfaces forming a biofilm, but cells did not colonize non-nutritive surfaces. In this study is hypothesized that biofilm formation is a general strategy used by microbes in the degradation of insoluble substrates, and that it may serve as a means for microbes to secure a nutrient and persist in their environments. The objective of this study was to characterize biofilms produced by *Thermobifida fusca*, a Gram-positive cellulolytic actinomycete isolated from compost that rapidly degrades cellulose by means of a well-characterized extracellular cellulase system, and is a causative agent of Farmers Lung, the most common type of hypersensitivity pneumonitis. *T. fusca* was cultured with dialysis tubing as a nutritive surface for biofilm formation, and by using non-nutritive surfaces such as glass, plastic, metal and Teflon. ^ Dialysis tubing was colonized by *T. fusca* aleuriospores but not by mycelial pellets. Surface-attached growth, examined by confocal scanning laser and scanning electron microscopy revealed structures resembling biofilms with cells embedded in fibrous material suggestive of an exopolymeric (EPS) matrix. *T. fusca* cells possessed higher hydrophobicity than *C. uda* and *C. phytofermentans* cells implicating higher capacity to bind to surfaces. DNase1 inhibited biofilm formation when assayed on microtiter plates suggesting a role for extracellular DNA in *T. fusca* biofilm formation. Concanavalin-A bound to the EPS material of biofilms and mycelial pellets, indicating alpha-linked D-mannosyl and/or alpha-linked D-glucosyl residues. The carbohydrate content of biofilms and mycelial pellets increased during growth. *T. fusca* biofilm formation is reduced when lack or excess of nutrients such as; iron, nitrogen and salt. Robust biofilms were developed between pHs 7 and 9, whereas minimum biofilms were produced at pH 3 and 11. Cellulose degradation rate and *cel*E (endoglucanase E5) expression was similar for *T. fusca* biofilms and mycelial pellets. Also, results of this study indicate that in the life cycle of this actinomycete, cellulose is specifically colonized by aleuriospores, which germinate and degrade cellulose, ultimately developing into biofilms encased in a carbohydrate-containing EPS matrix, a hallmark of biofilm production. ^

Subject Area

Molecular biology|Microbiology

Recommended Citation