**Objectives:**

*Aspergillus fumigatus* (*Af*) and *Stenotrophomonas maltophilia* (*Sm*) are environmental saprophytic microorganisms that colonize the upper airways and can cause respiratory infections in humans. The treatment of infections caused by these organisms is particularly challenging due to multidrug resistance in *Sm* and the increasing incidence of azole resistance in *Af*. Additionally, *Af* and *Sm* are capable of forming mixed biofilms within the bronchial tract, further reducing their susceptibility to antimicrobial agents. Within these mixed biofilms, we have previously shown that *Sm* adheres to *Af* hyphae and negatively affects both *Af* growth and biofilm development. A deeper understanding of *Af–Sm* interactions is essential for the development of new therapeutic strategies. Galactosaminogalactan (GAG), a cell wall polysaccharide produced by *Af*, plays a crucial role in fungal adhesion to surfaces and is a key component of the extracellular matrix in both single-species and mixed-species biofilms.

In this study, we aimed to investigate: (i) the role of GAG in mediating adhesion between *Af* and *Sm*, and (ii) the effect of *Sm* on the expression of enzymes involved in GAG biosynthesis.

**Materials & Methods:**

We used a GAG-deficient *Af* mutant strain, *Af∆gt4c*, along with its parental strain *Af∆Ku80* (*Af* strain collection, Institut Pasteur). A total of 10⁵ conidia/mL were cultured for 15 hours in 12-well plates containing RPMI medium supplemented with 10% fetal bovine serum (FBS, Sigma). Two clinical *Sm* strains (genogroup 6) were grown overnight in LB broth, centrifuged, diluted 1:10 in sterile water, and stained with Syto9 (ThermoFisher®). The labeled Sm cells were then incubated for 3 hours with preformed *Af* hyphae.

In a second set of experiments, 24-well plates were coated overnight with purified GAG or a control mixture of chitin/galactomannan/β-1,3-glucan. After washing and blocking with 3% bovine serum albumin (BSA), Syto9-labeled *Sm* was incubated in the wells with or without the coated polysaccharides for 1 and 3 hours. Fluorescence images were captured using an Axiovert inverted microscope, and image acquisition was performed with Zeiss Zen 2012 software.

Total RNA was extracted after 24 hours of single (*Af*∆Ku80) or mixed biofilm (*Af*∆Ku80 + *Sm*, both clinical strains) formation using the Qiagen® AllPrep DNA/RNA kit. The expression levels of five genes involved in GAG biosynthesis were quantified, using *TEF1* as the housekeeping gene.

**Results:**

*Sm* adhered to hyphae of the GAG-producing strain *Af*∆Ku80 but not to the GAG-deficient mutant *Af*∆gt4c. Similarly, purified GAG supported *Sm* adhesion when coated onto well surfaces, whereas no binding was observed on wells coated with the control polysaccharide mixture. Furthermore, co-culture with Sm led to a significant upregulation of two GAG biosynthetic genes in *Af*∆Ku80: *ADG3*, encoding a GAG deacetylase, and *EGA3*, encoding a GAG-deacetylated hydrolase.

**Conclusions:**

Our findings indicate that GAG plays a pivotal role in mediating adhesion between *A. fumigatus* and *S. maltophilia* in mixed biofilms. Additionally, *S. maltophilia* appears to enhance GAG biosynthesis by upregulating key genes involved in its production, potentially increasing the deacetylated fraction of GAG and further stabilizing the biofilm structure.