Strain-dependent and indirect effect of *Stenotrophomonas maltophilia* on *Aspergillus fumigatus* in mixed biofilm

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Aspergillus fumigatus (Af) and *Stenotrophomonas maltophilia* (Sm) colonize the respiratory tract of immunocompromised patients or with chronic pulmonary diseases and form biofilms. We recently discovered an antibiosis effect of Sm on Af in an *in vitro* mixed biofilm realised at 37°C in RPMI + FBS medium. The bacteria inhibited the fungal growth and induced an increase of fungal cell wall thickness, which led to abnormal mycelium with highly branched hyphae. Our last study showed that all the Af strains were susceptible to Sm effects.

Is the fungal phenotype and inhibition in mixed biofilm dependent of Sm strains?

Methods

Planktonic culture of Sm strains: 24 h in LB medium at 25 or 37°C with agitation (200 rpm). Bacterial growth assessed by spectrophotometry (600 nm).

Formation of biofilms: polystyrene supports, in RPMI + FBS medium at 25 or 37°C for 24 h, without agitation

→ **Single inoculation** of Sm strains (10⁶ cells/mL). Biofilms biomass assessed by crystal violet coloration

→ **Co-inoculation** of Af 13073-GFP strain at 10⁵ conidia/mL with different strains of Sm (Tab 1) at 10⁶ or 10⁸ cells/mL. Assessed by CLSM (biofilm thickness and Af phenotype) and qPCR (Af quantification in single and mixed biofilms)

Isolates ID	Origin		Genogroup
Sm13637	Human	Mouth	6
Sm_6-1	Human	Sputum	6
Sm_6-2	Human	Catheter	6
Sm_6-3	Animal Dog	Trachea	6
Sm_2-1	Animal Horse	Trachea	2
Sm_2-2	Animal Horse	Trachea	2
Sm_D-1	Hospital environment	Endoscope	D
Sm_D-2	Hospital environment	Endoscope	D
Sm_D-3	Hospital environment	Gastroscope	D

Tab 1: *S. maltophilia* strains used to form mixed biofilm with Af13073

Results

Fig 1: planktonic and biofilm culture of Sm strains at 25 or 37°C (24 h)

Fig 3: Quantification of Af biomass in mixed biofilms and Af phenotype depending of Sm strains

Sm concentration in mixed biofilm = 10⁶ bacteria/mL

Flocculation of Sm_2-1 strain

Fig 2: Mixed biofilms thicknesses and Af phenotype depending of Sm strains used (37°C)

(37°C) Sm concentration in mixed biofilm = 10⁸ bacteria/mL

Strains	Planktonic growth	Biofilm growth	Inhibition of Af	Af modified phenotype
Genogroup 6	37°C	37°C	10 ⁶ bacteria/mL	10 ⁶ bacteria/mL
Genogroup D + Sm_2-2	25°C	25°C	10 ⁸ bacteria/mL	10 ⁸ bacteria/mL
Sm_2-1	25°C	37°C	10 ⁸ bacteria/mL	no

The antibiosis effect observed in our previous study between two human clinical strains of *A. fumigatus* and *S. maltophilia* seems dependent of bacterial strains. The difference of bacterial effect on fungi is dose-dependant and directly related to the fitness of the bacterial strains at 37°C. This effect is also dependent of other unknown factors, because Sm_2-1 doesn't induce any phenotype modification even with high concentration of bacteria (high growth at 37°C in biofilm condition). In further study, we will look for the molecules responsible of the antibiosis effects.

Conclusions & Future directions