

## ***In Vitro* Interactions of *Pseudomonas aeruginosa* with *Scedosporium* Species Frequently Associated with Cystic Fibrosis**

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## Inter-kingdom interactions between CF pathogens.

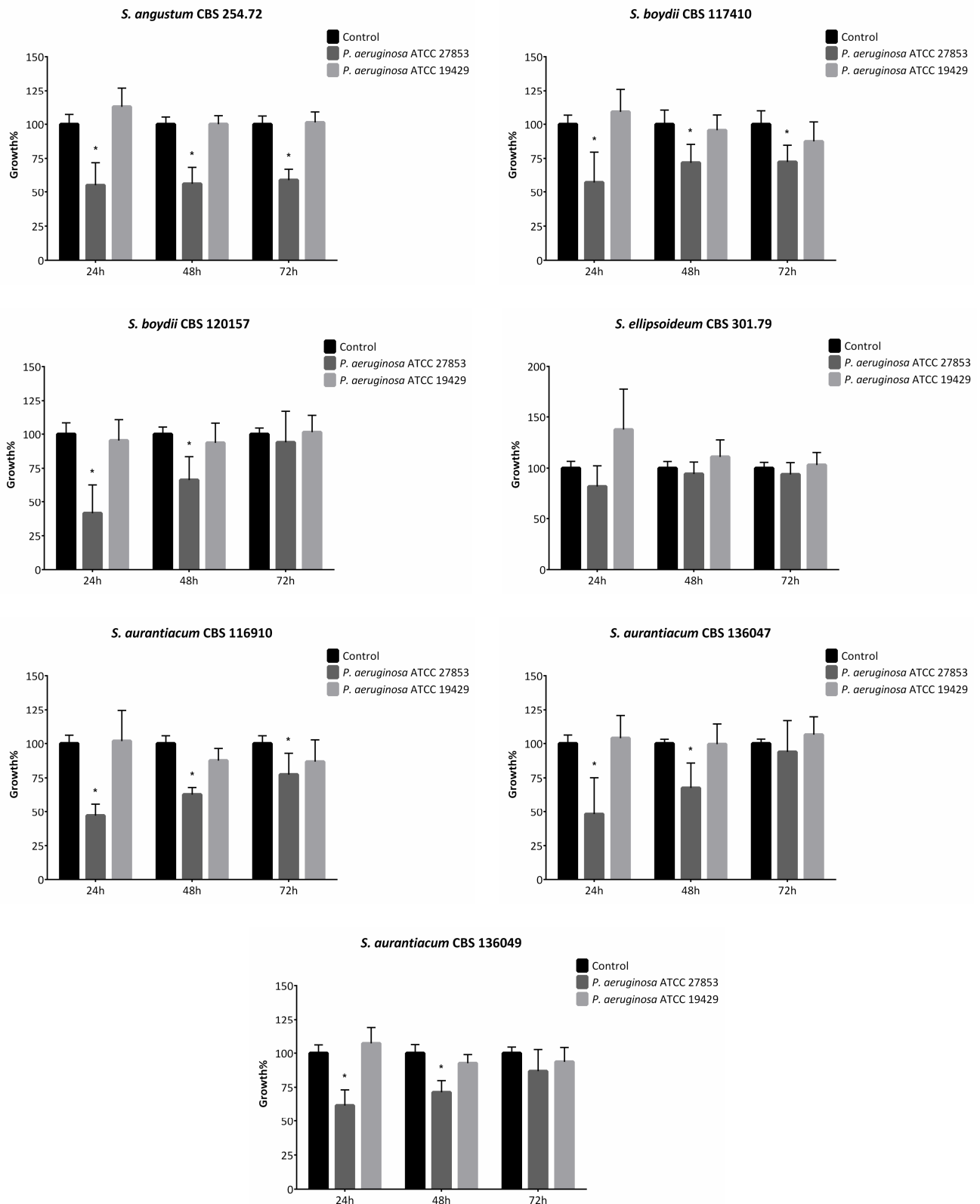
**Table 1S** Indirect inhibitory effect of bacterial diffusible molecules on the growth of *Scedosporium* spp. and *A. fumigatus* SZMC 23245 grown on RPMI-agar and on SCFM plates. While on RPMI-1640 agar mainly complete, on SCFM only partial inhibition zones were observed. Partial inhibition was read at the first point of significant reduce in growth (approximately 50-80% as judged by the naked eye). Results are the mean of three independent experiments.

Strain	<i>P. aeruginosa</i> ATCC 27853		<i>P. aeruginosa</i> ATCC 19429	
	Complete inhibition (RIA±SD)	Partial inhibition (RIA±SD)	Complete inhibition (RIA±SD)	Partial inhibition (RIA±SD)
<b>RPMI-agar</b>				
<i>S. angustum</i> CBS 254.72	+ (0.16±0.14)	+ (0.40±0.00)	-	+ (0.71±0.48)
<i>S. boydii</i> CBS 117410	+ (0.38±0.16)	-	+ (0.70±0.48)	+ (0.08±0.17)
<i>S. boydii</i> CBS 117432	+ (0.83±0.13)	-	-	+ (0.50±0.00)
<i>S. boydii</i> CBS 120157	+ (0.17±0.19)	+ (0.72±0.39)	-	+ (0.49±0.41)
<i>S. ellipsoideum</i> CBS 301.79	+ (0.24±0.26)	-	-	+ (0.65±0.47)
<i>S. aurantiacum</i> CBS 116910	+ (0.20±0.22)	-	-	+ (0.33±0.58)
<i>S. aurantiacum</i> CBS 136046	+ (0.40±0.11)	-	-	+ (0.06±0.11)
<i>S. aurantiacum</i> CBS 136047	+ (0.17±0.24)	+ (0.10±0.00)	-	+ (0.25±0.50)
<i>S. aurantiacum</i> CBS 136049	+ (0.53±0.05)	-	-	+ (0.07±0.15)
<i>S. apiospermum</i> SZMC 23374	+ (0.28±0.17)	-	-	+ (0.17±0.26)
<i>A. fumigatus</i> SZMC 23245	+ (0.75±0.23)	-	-	-
<b>SCFM</b>				
<i>S. angustum</i> CBS 254.72	-	+ (0.09±0.17)	-	-
<i>S. boydii</i> CBS 117410	-	+ (0.23±0.26)	-	-
<i>S. boydii</i> CBS 117432	-	-	-	+ (0.61±0.54)
<i>S. boydii</i> CBS 120157	-	+ (0.32±0.28)	-	+ (0.33±0,58)
<i>S. ellipsoideum</i> CBS 301.79	-	+ (0.17±0,29)	-	+ (0.33±0,58)
<i>S. aurantiacum</i> CBS 116910	-	+ (0.21±0.19)	-	+ (0.33±0,58)
<i>S. aurantiacum</i> CBS 136046	-	+ (0.72±0.34)	-	+ (0.33±0,58)
<i>S. aurantiacum</i> CBS 136047	-	+ (0.39±0.09)	-	-
<i>S. aurantiacum</i> CBS 136049	-	+ (0.57±0,18)	-	-
<i>S. apiospermum</i> SZMC 23374	-	+ (0.46±0.10)	-	+ (1.00±0.87)
<i>A. fumigatus</i> SZMC 23245	-	-	-	-

+, inhibition zone was observed; -, inhibition zone was not detected;

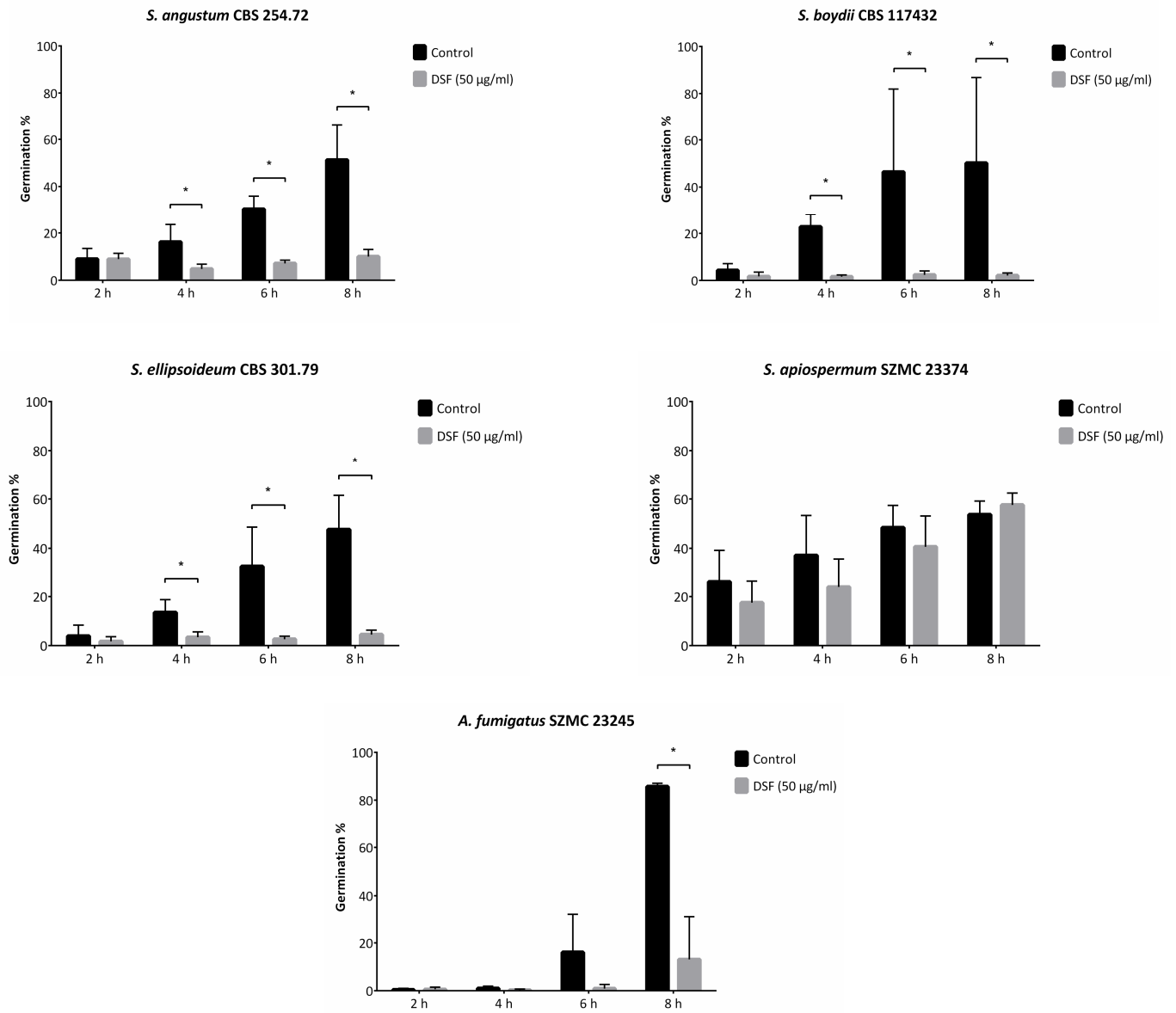
**Abbreviations:** ATCC, American Type Culture Collection; CBS, Centraalbureau voor Schimmelcultures; RIA, relative inhibitory activity (RIA = area of the inhibition zone/area of the related bacterial colony); SCFM, synthetic cystic fibrosis medium; SZMC, Szeged Microbiological Collection.

## Inter-kingdom interactions between CF pathogens.



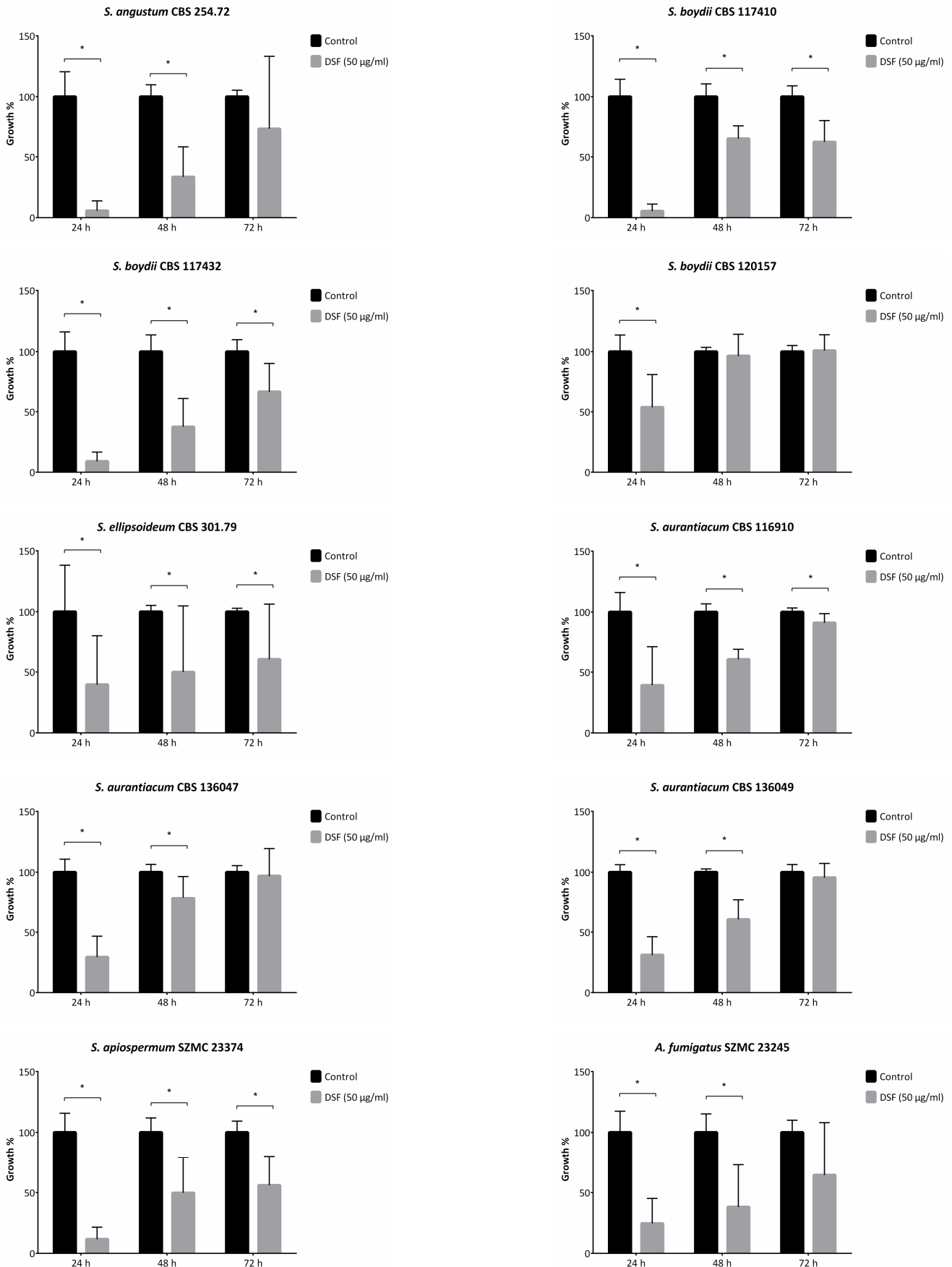
**Figure 1S** Indirect inhibitory effect of bacterial diffusible molecules on the growth of *Scedosporium* spp. grown in RPMI-1640 conditioned by *P. aeruginosa* strains. Results are the mean of three independent experiments with three individual replicates. Error bars indicate standard deviations. P values were calculated using unpaired t test, where  $p < 0.05$  was considered to be significant (indicated with asterisks).

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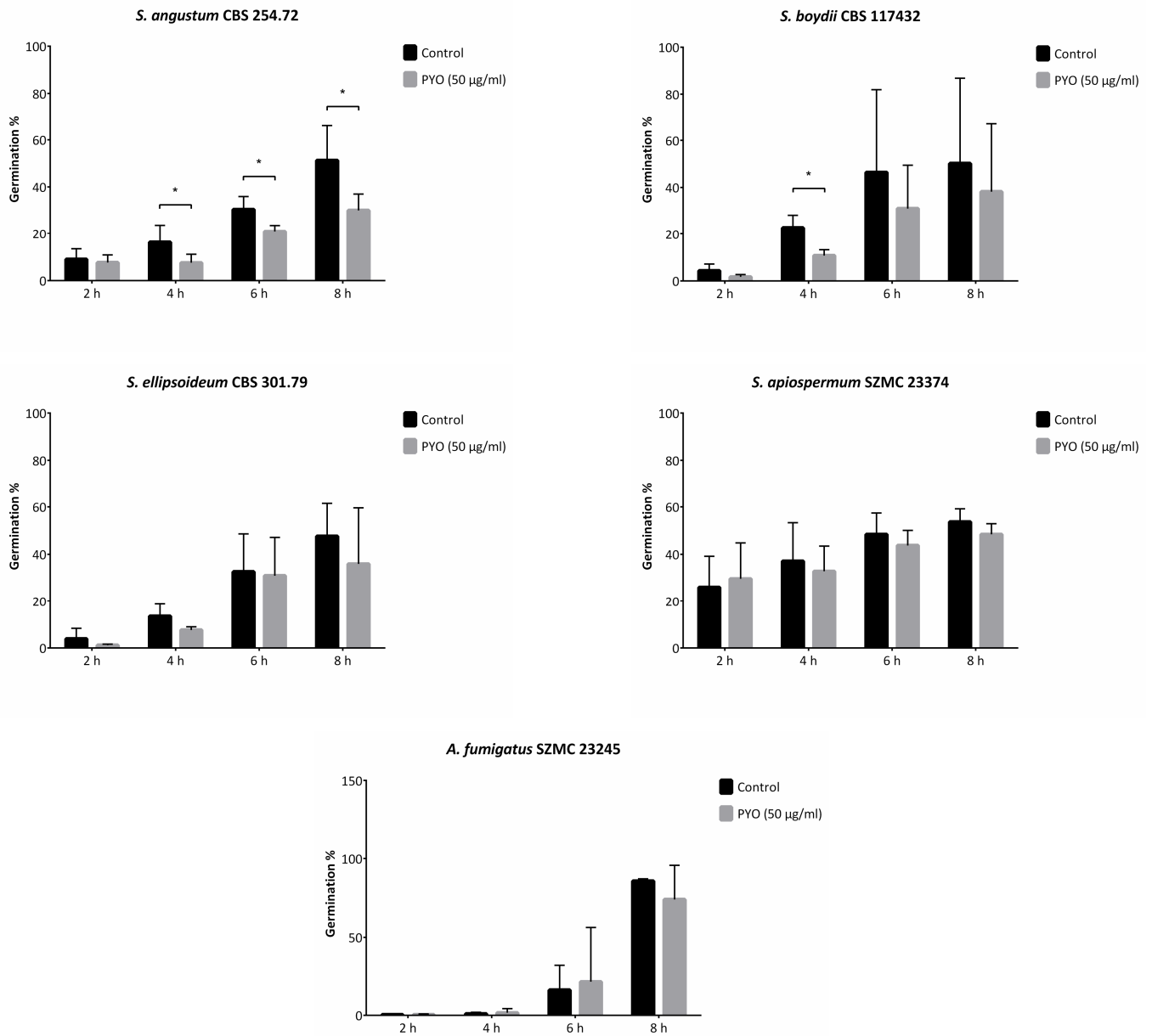
**Figure 2S** Germination ability of conidia collected from *Scedosporium* and *A. fumigatus* isolates in the presence of 50 µg/ml DSF. Results are the mean of three independent experiments. Error bars indicate standard deviations. P values were calculated using unpaired t test, where  $p < 0.05$  was considered to be significant (indicated with asterisks).

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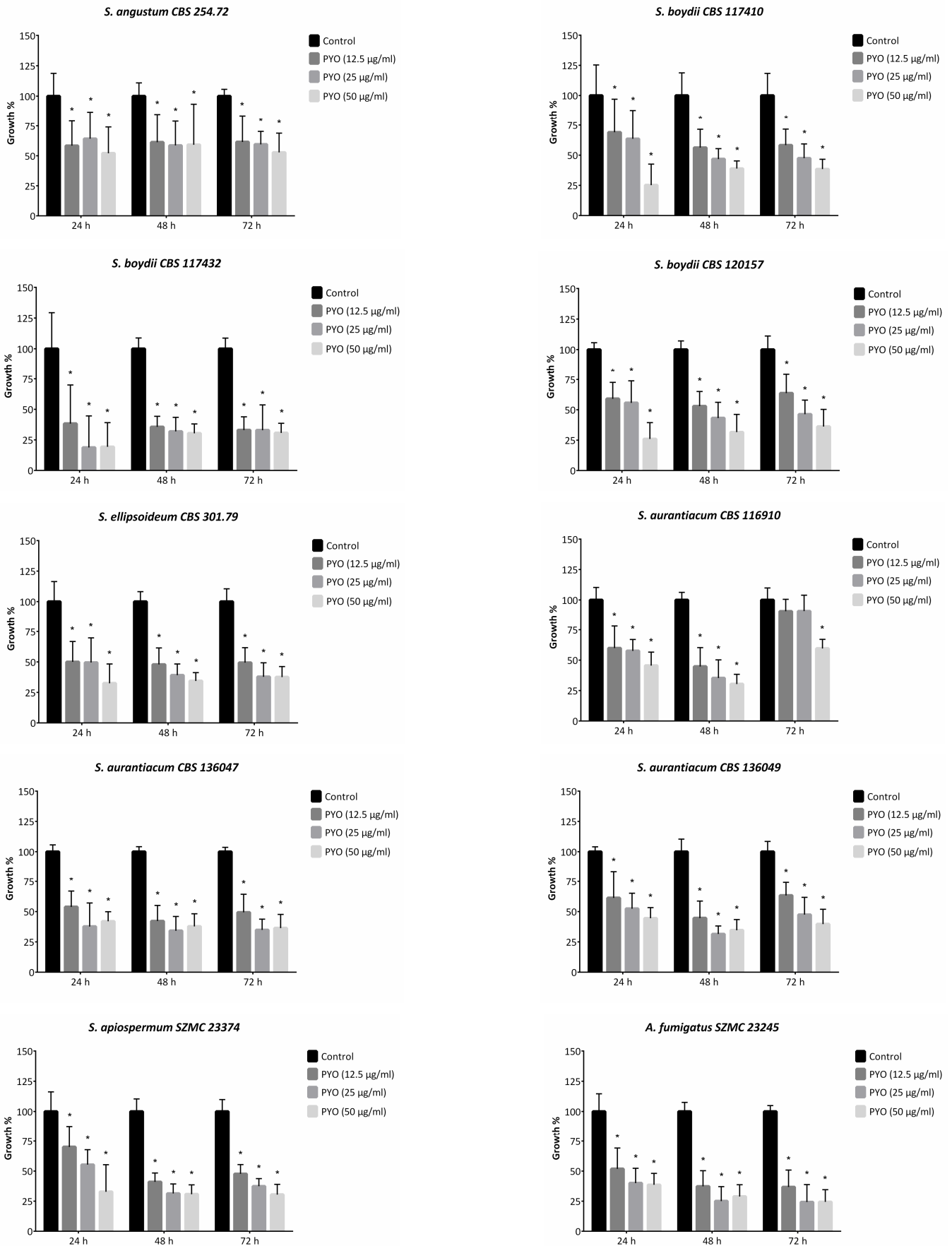
**Figure 3S** Growth rate of *Scedosporium* and *A. fumigatus* isolates in the presence of 50 µg/ml DSF. Results are the mean of three independent experiments with three individual replicates. Error bars indicate standard deviations. P values were calculated using unpaired t test, where  $p < 0.05$  was considered to be significant (indicated with asterisks).

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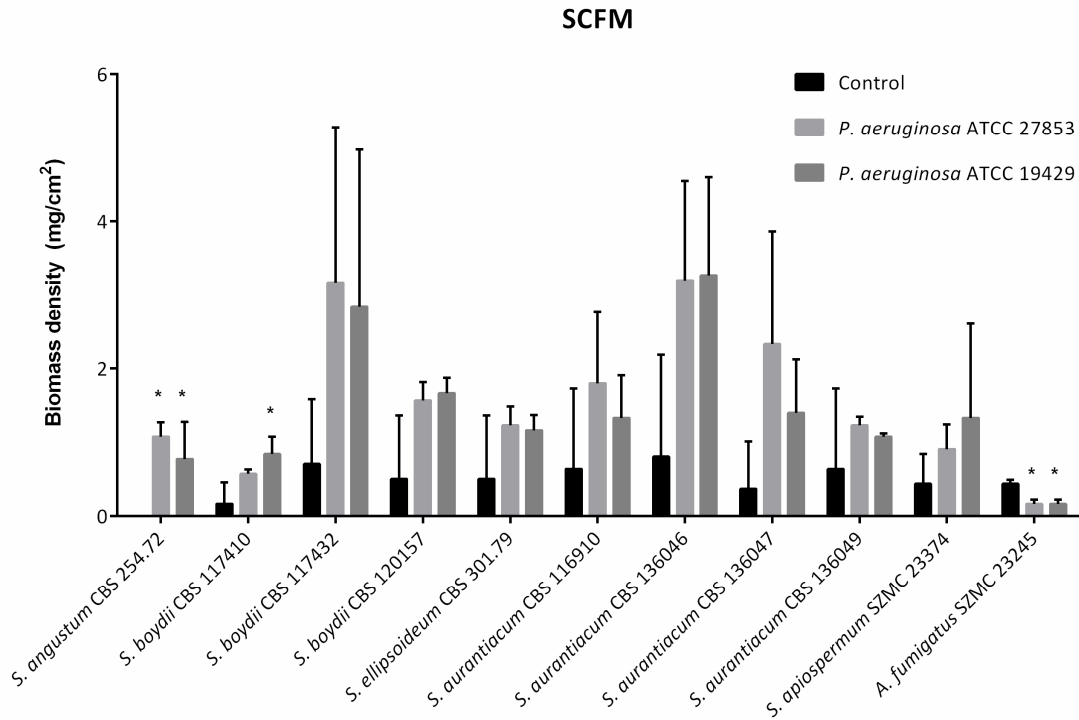
**Figure 4S** Germination ability of conidia collected from *Scedosporium* and *A. fumigatus* isolates in the presence of 50 µg/ml pyocyanin. Results are the mean of three independent experiments. Error bars indicate standard deviations. P values were calculated using unpaired t test, where  $p < 0.05$  was considered to be significant (indicated with asterisks).

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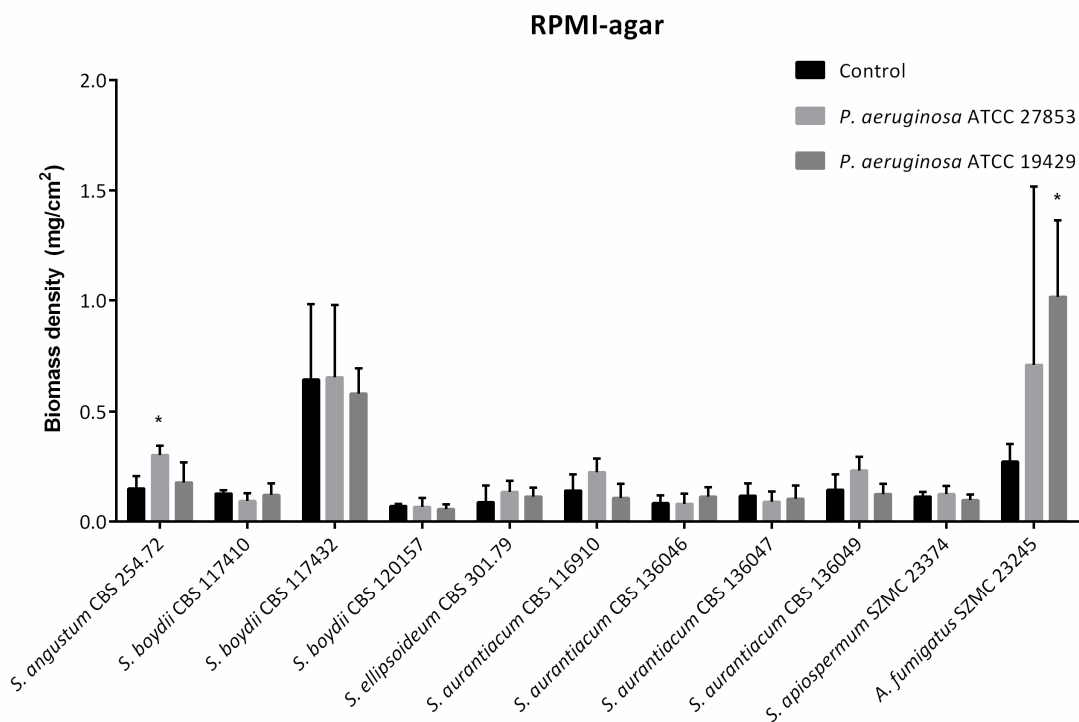


**Figure 5S** Growth rate of *Scedosporium* and *A. fumigatus* isolates in the presence of 50 µg/ml pyocyanin. Results are the mean of three independent experiments with three individual replicates. Error bars indicate standard deviations. P values were calculated using unpaired t test, where  $p < 0.05$  was considered to be significant (indicated with asterisks).

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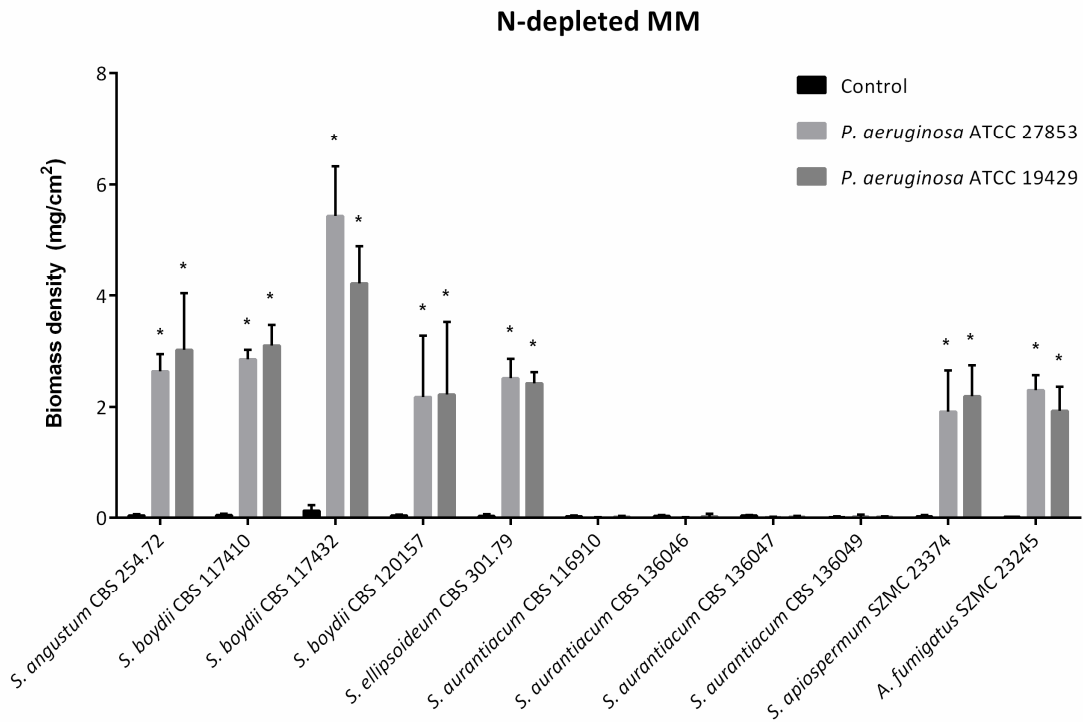
**Figure 6S** The biomass densities of *Scedosporium* and *Aspergillus fumigatus* isolates grown on synthetic cystic fibrosis medium (SCFM) in the plate-in-plate tests in absence (control) and in the presence of *Pseudomonas aeruginosa* strains ATCC 27853 and 19429. Results are the mean of three independent experiments. Error bars indicate standard deviations. P values were calculated using unpaired t test, where  $p < 0.05$  was considered to be significant (indicated with asterisks).



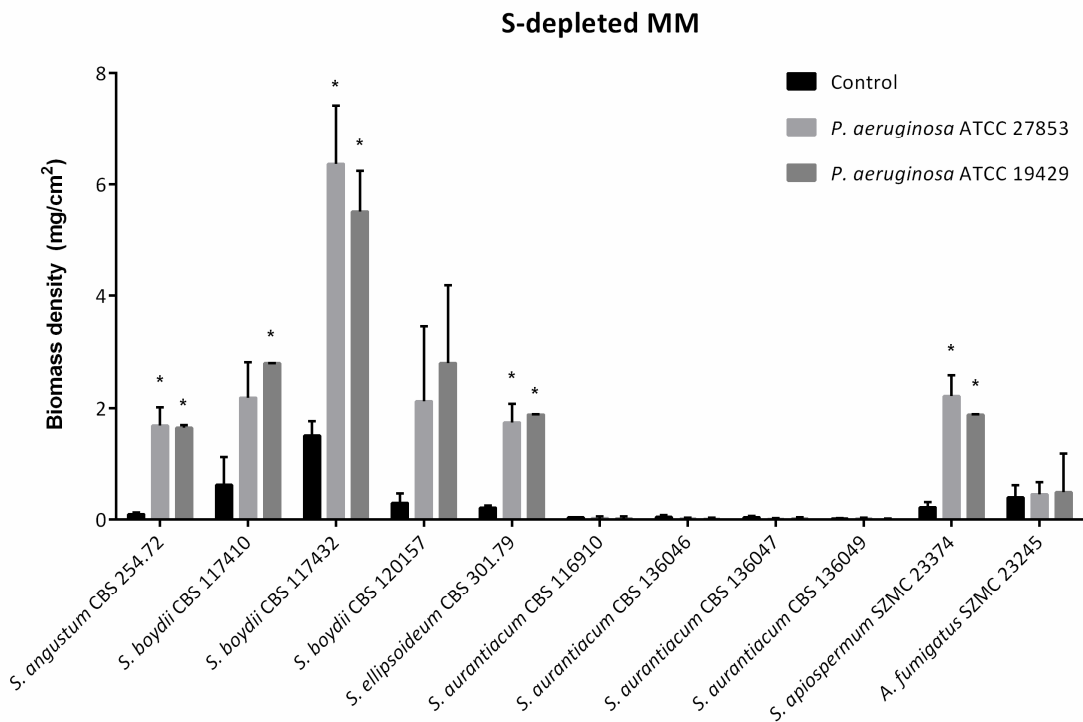
**Figure 7S** The biomass densities of *Scedosporium* and *Aspergillus fumigatus* isolates grown on RPMI-agar in the plate-in-plate tests in absence (control) and in the presence of *Pseudomonas aeruginosa* strains ATCC 27853 and 19429. Results are the mean of three independent experiments. Error bars indicate standard deviations. P values were calculated using unpaired t test, where  $p < 0.05$  was considered to be significant (indicated with asterisks).



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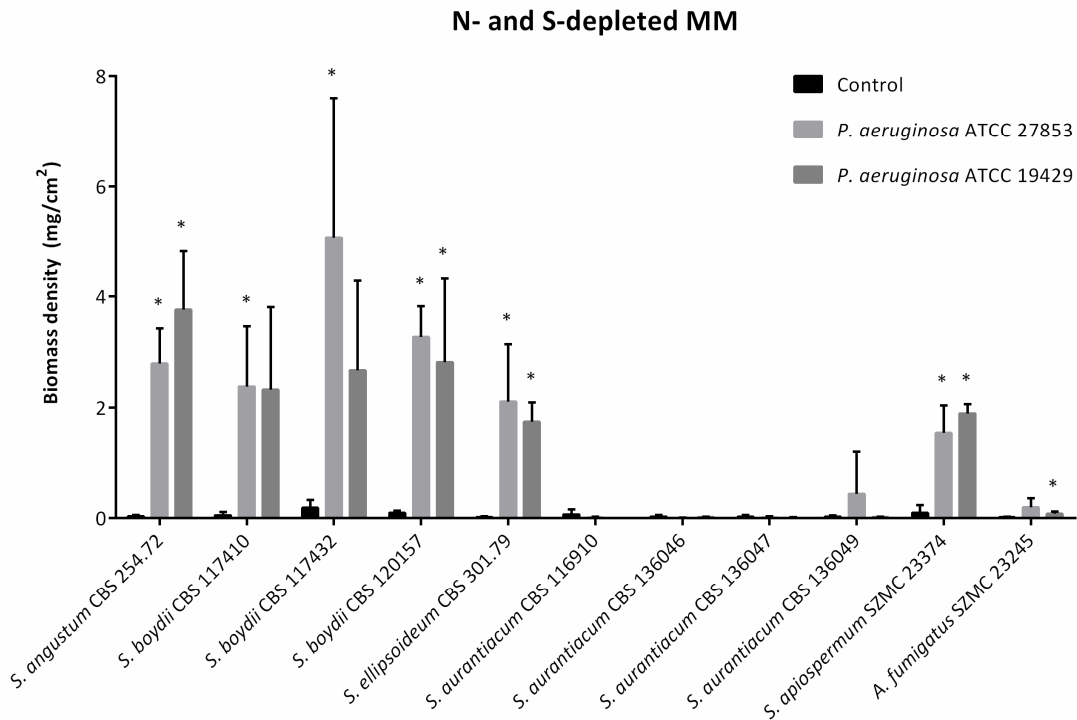


**Figure 8S** The biomass densities of *Scedosporium* and *Aspergillus fumigatus* isolates grown on nitrogen-depleted minimal medium (N-depleted MM) in the plate-in-plate tests in absence (control) and in the presence of *Pseudomonas aeruginosa* strains ATCC 27853 and 19429. Results are the mean of three independent experiments. Error bars indicate standard deviations. P values were calculated using unpaired t test, where  $p < 0.05$  was considered to be significant (indicated with asterisks).

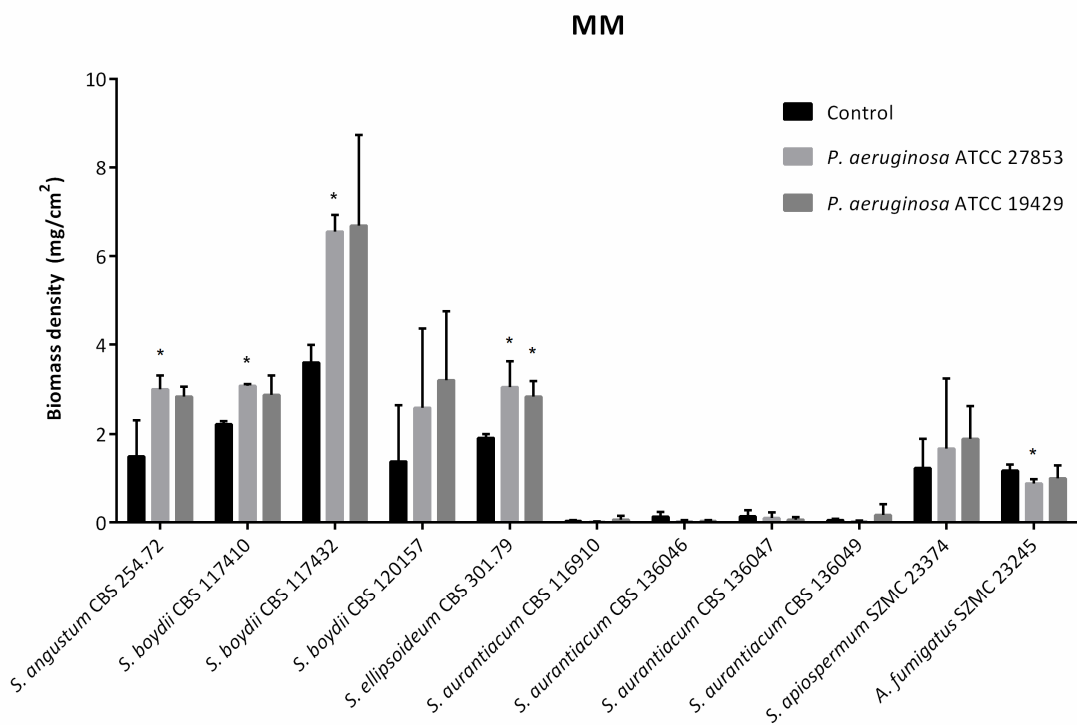


**Figure 9S** The biomass densities of *Scedosporium* and *Aspergillus fumigatus* isolates grown on sulfur-depleted minimal medium (S-depleted MM) in the plate-in-plate tests in absence (control) and in the presence of *Pseudomonas aeruginosa* strains ATCC 27853 and 19429. Results are the mean of three independent experiments. Error bars indicate standard deviations. P values were calculated using unpaired t test, where  $p < 0.05$  was considered to be significant (indicated with asterisks).

## Inter-kingdom interactions between CF pathogens.



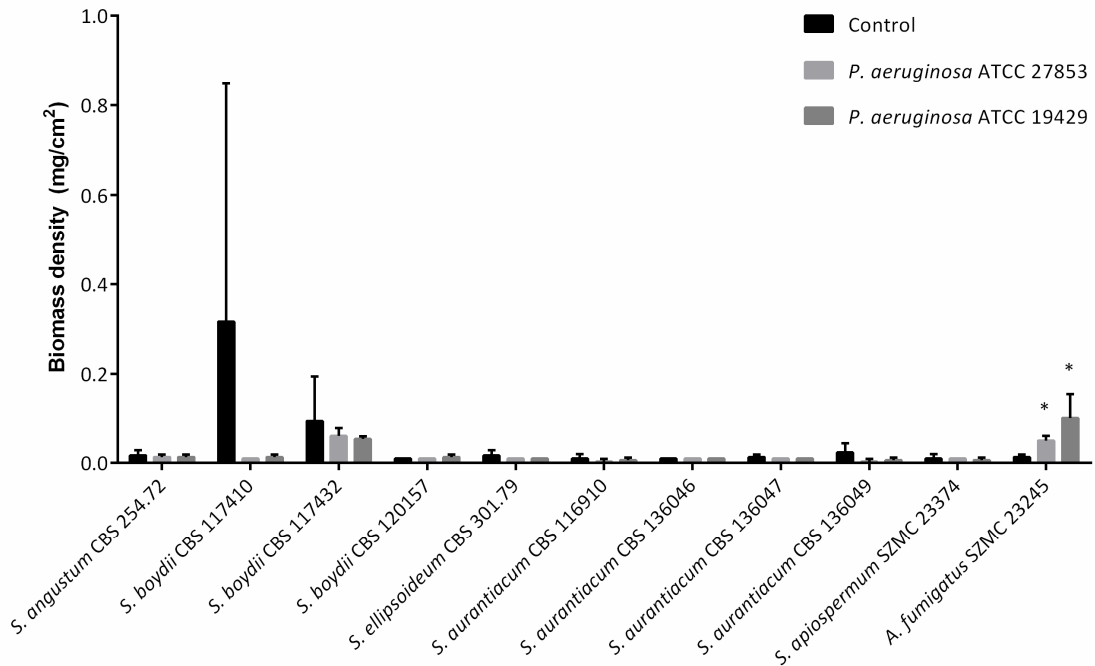
**Figure 10S** The biomass densities of *Scedosporium* and *Aspergillus fumigatus* isolates grown on nitrogen- and sulfur-depleted minimal medium (N- and S-depleted MM) in the plate-in-plate tests in absence (control) and in the presence of *Pseudomonas aeruginosa* strains ATCC 27853 and 19429. Results are the mean of three independent experiments. Error bars indicate standard deviations. P values were calculated using unpaired t test, where  $p < 0.05$  was considered to be significant (indicated with asterisks).



**Figure 11S** The biomass densities of *Scedosporium* and *Aspergillus fumigatus* isolates grown on minimal medium (MM) in the plate-in-plate tests in absence (control) and in the presence of *Pseudomonas aeruginosa* strains ATCC 27853 and 19429. Results are the mean of three independent experiments. Error bars indicate standard deviations. P values were calculated using unpaired t test, where  $p < 0.05$  was considered to be significant (indicated with asterisks).

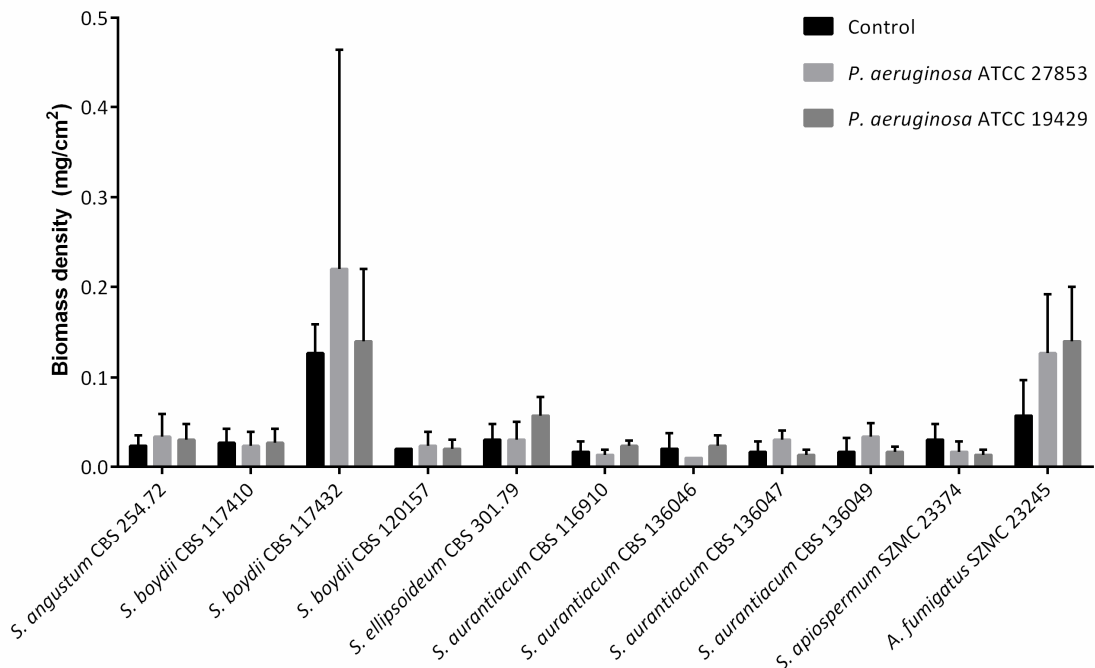
## Inter-kingdom interactions between CF pathogens.

### C- and N-depleted MM



**Figure 12S** The biomass densities of *Scedosporium* and *Aspergillus fumigatus* isolates grown on carbon- and nitrogen-depleted minimal medium (C- and N-depleted MM) in the plate-in-plate tests in absence (control) and in the presence of *Pseudomonas aeruginosa* strains ATCC 27853 and 19429. Results are the mean of three independent experiments. Error bars indicate standard deviations. P values were calculated using unpaired t test, where  $p < 0.05$  was considered to be significant (indicated with asterisks).

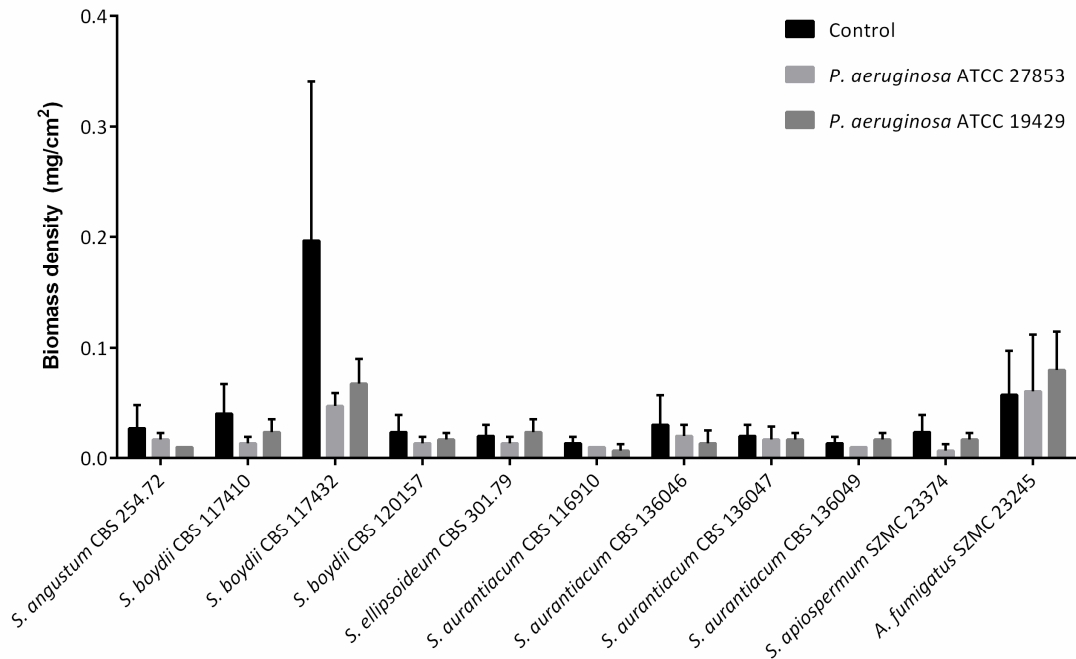
### C-depleted MM



**Figure 13S** The biomass densities of *Scedosporium* and *Aspergillus fumigatus* isolates grown on carbon-depleted minimal medium (C-depleted MM) in the plate-in-plate tests in absence (control) and in the presence of *Pseudomonas aeruginosa* strains ATCC 27853 and 19429. Results are the mean of three independent experiments. Error bars indicate standard deviations. P values were calculated using unpaired t test, where  $p < 0.05$  was considered to be significant (indicated with asterisks).

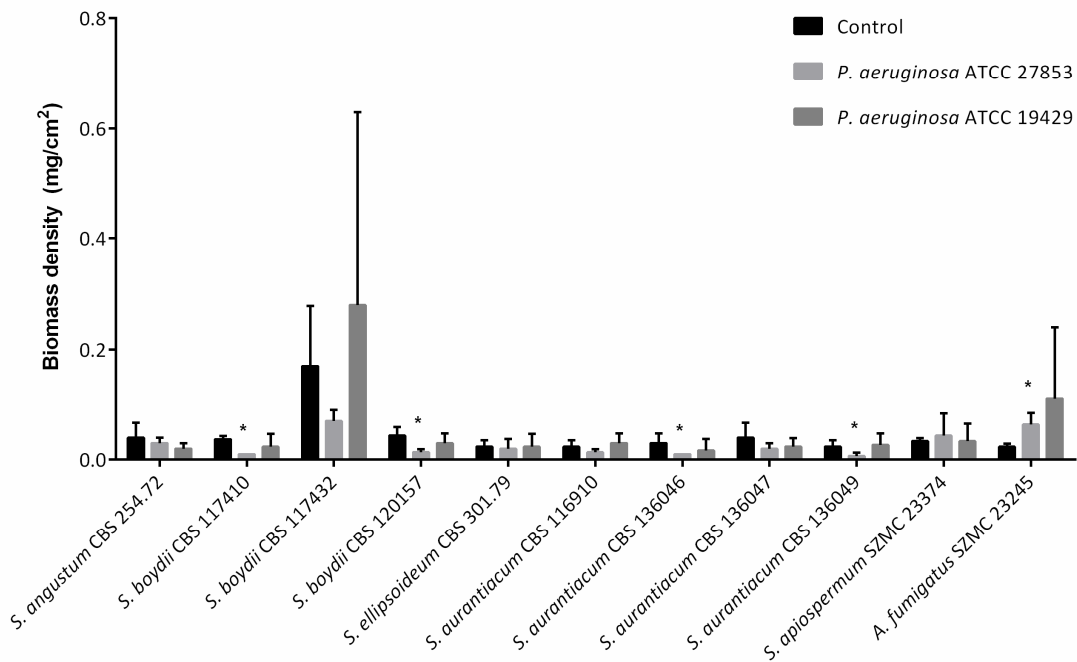
## Inter-kingdom interactions between CF pathogens.

### C- and S-depleted MM



**Figure 14S** The biomass densities of *Scedosporium* and *Aspergillus fumigatus* isolates grown on carbon- and sulfur-depleted minimal medium (C- and S-depleted MM) in the plate-in-plate tests in absence (control) and in the presence of *Pseudomonas aeruginosa* strains ATCC 27853 and 19429. Results are the mean of three independent experiments. Error bars indicate standard deviations. P values were calculated using unpaired t test, where  $p < 0.05$  was considered to be significant (indicated with asterisks).

### C-, N- and S-depleted MM

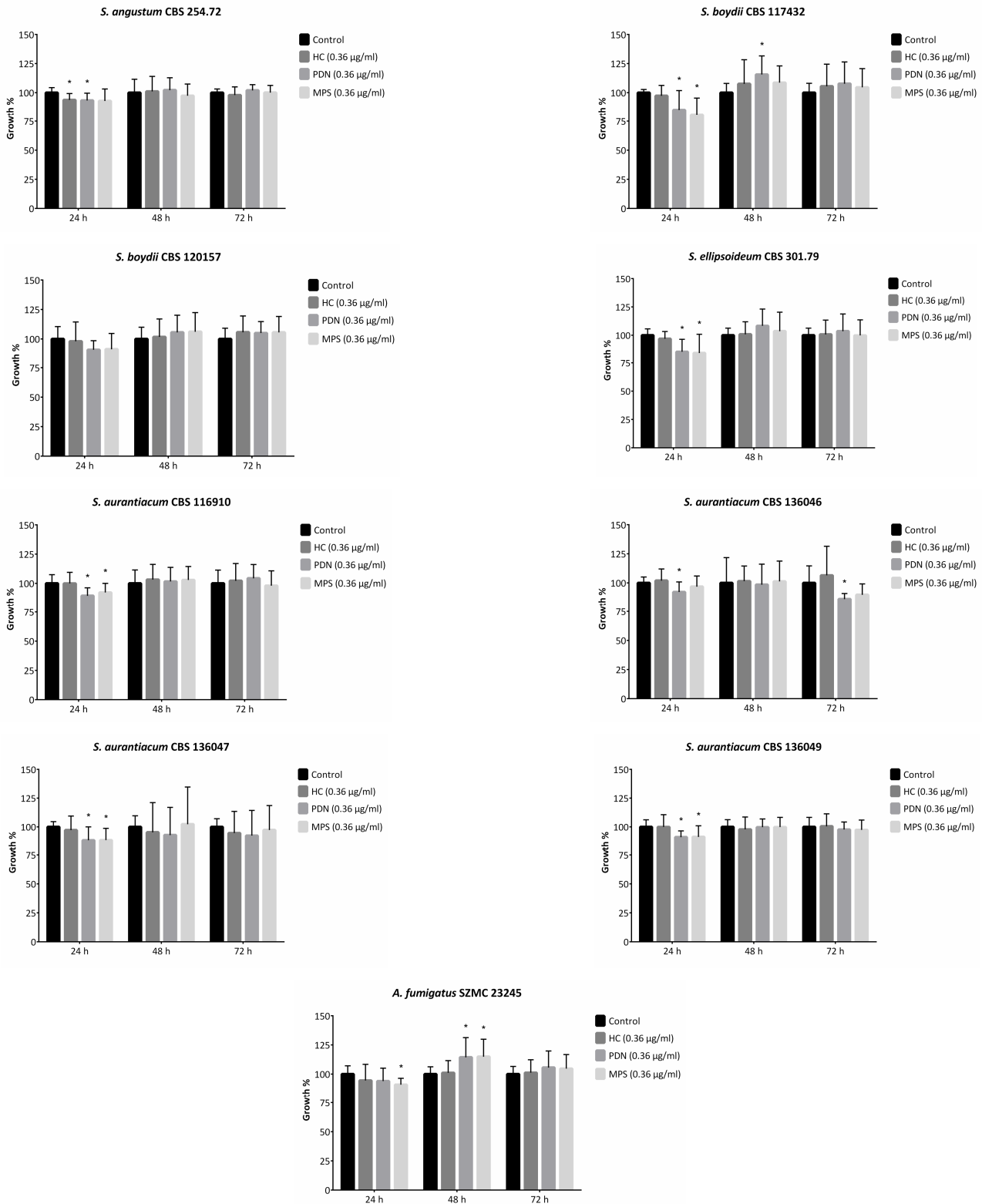


**Figure 15S** The biomass densities of *Scedosporium* and *Aspergillus fumigatus* isolates grown on carbon-, nitrogen and sulfur-depleted minimal medium (C-, N- and S-depleted MM) in the plate-in-plate tests in absence (control) and in the presence of *Pseudomonas aeruginosa* strains ATCC 27853 and 19429. Results are the mean of three independent experiments. Error bars indicate standard deviations. P values

### **Inter-kingdom interactions between CF pathogens.**

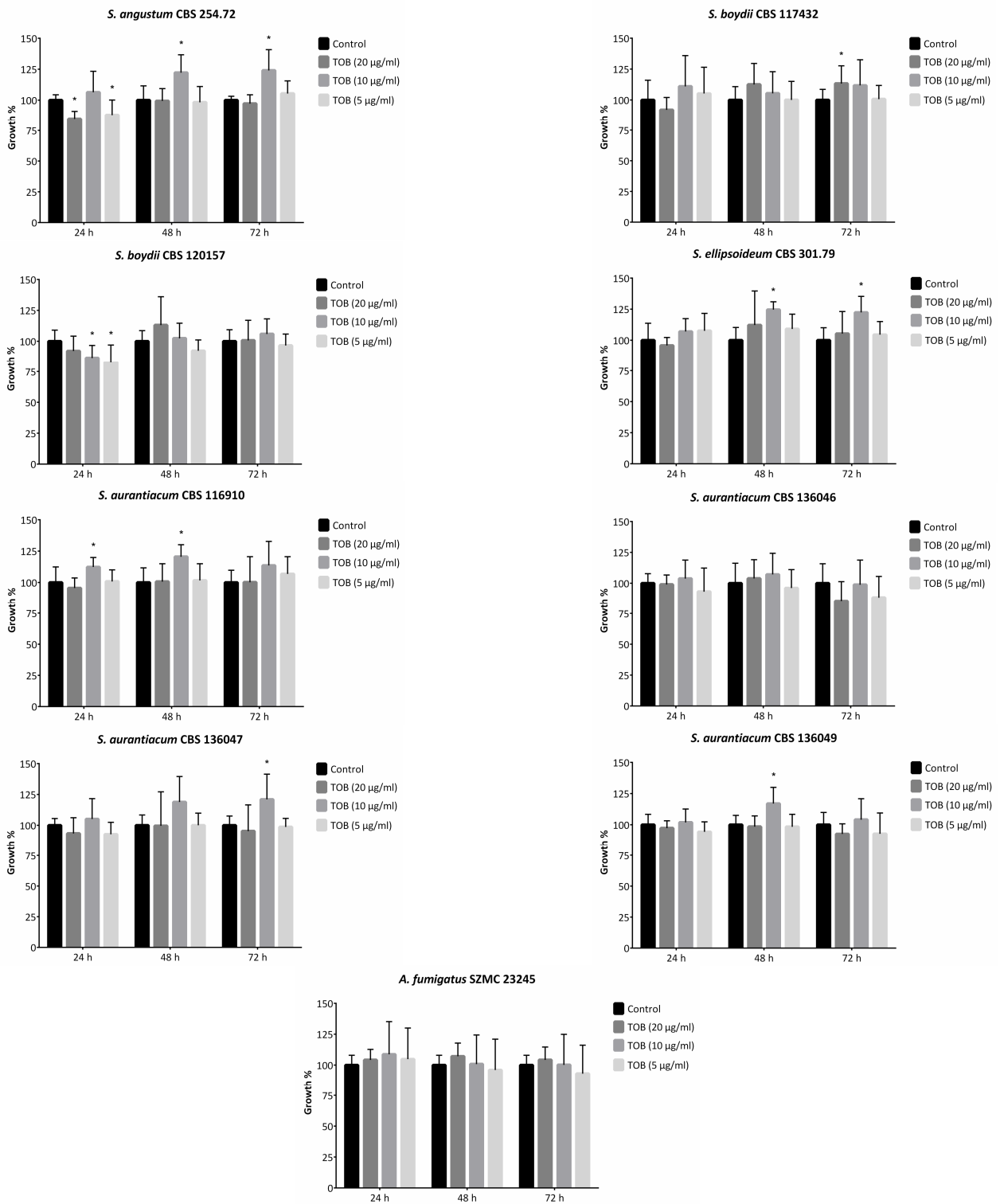
were calculated using unpaired t test, where  $p < 0.05$  was considered to be significant (indicated with asterisks).

## Inter-kingdom interactions between CF pathogens.



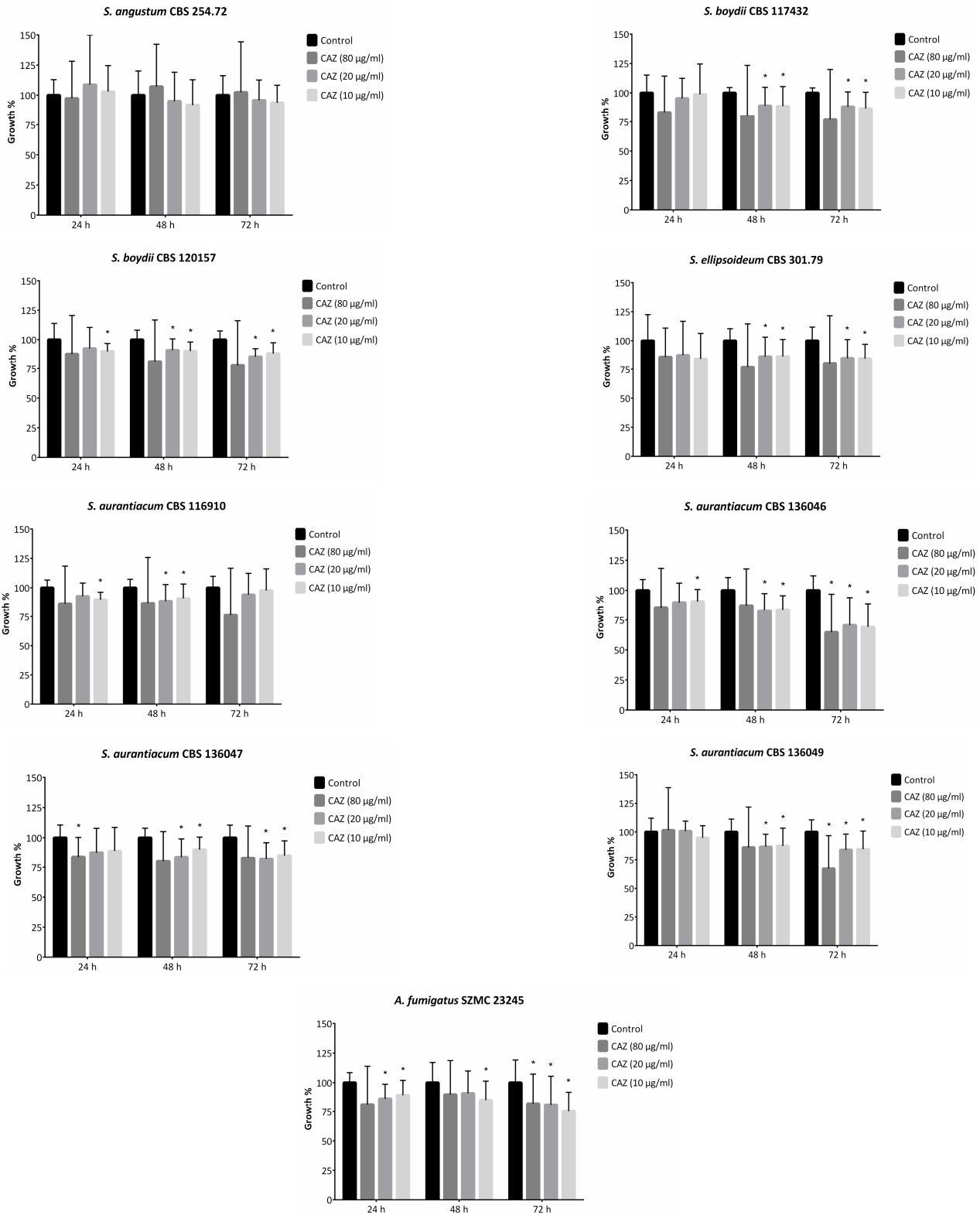
**Figure 16S** Growth rate of *Scedosporium* and *A. fumigatus* isolates in the presence of  $10^{-6}$  M (approx.  $0.36 \mu\text{g/ml}$ ) corticosteroid compounds (*i.e.*, hydrocortisone, HC; prednisone, PDN; methylprednisolone, MPS). Results are the mean of three independent experiments with three individual replicates. Error bars indicate standard deviations. P values were calculated using unpaired t test, where  $p < 0.05$  was considered to be significant (indicated with asterisks).

## Inter-kingdom interactions between CF pathogens.



**Figure 17S** Growth rate of *Scedosporium* and *A. fumigatus* isolates in the presence of tobramycin (TOB). Results are the mean of three independent experiments with three individual replicates. Error bars indicate standard deviations. P values were calculated using unpaired t test, where  $p < 0.05$  was considered to be significant (indicated with asterisks).

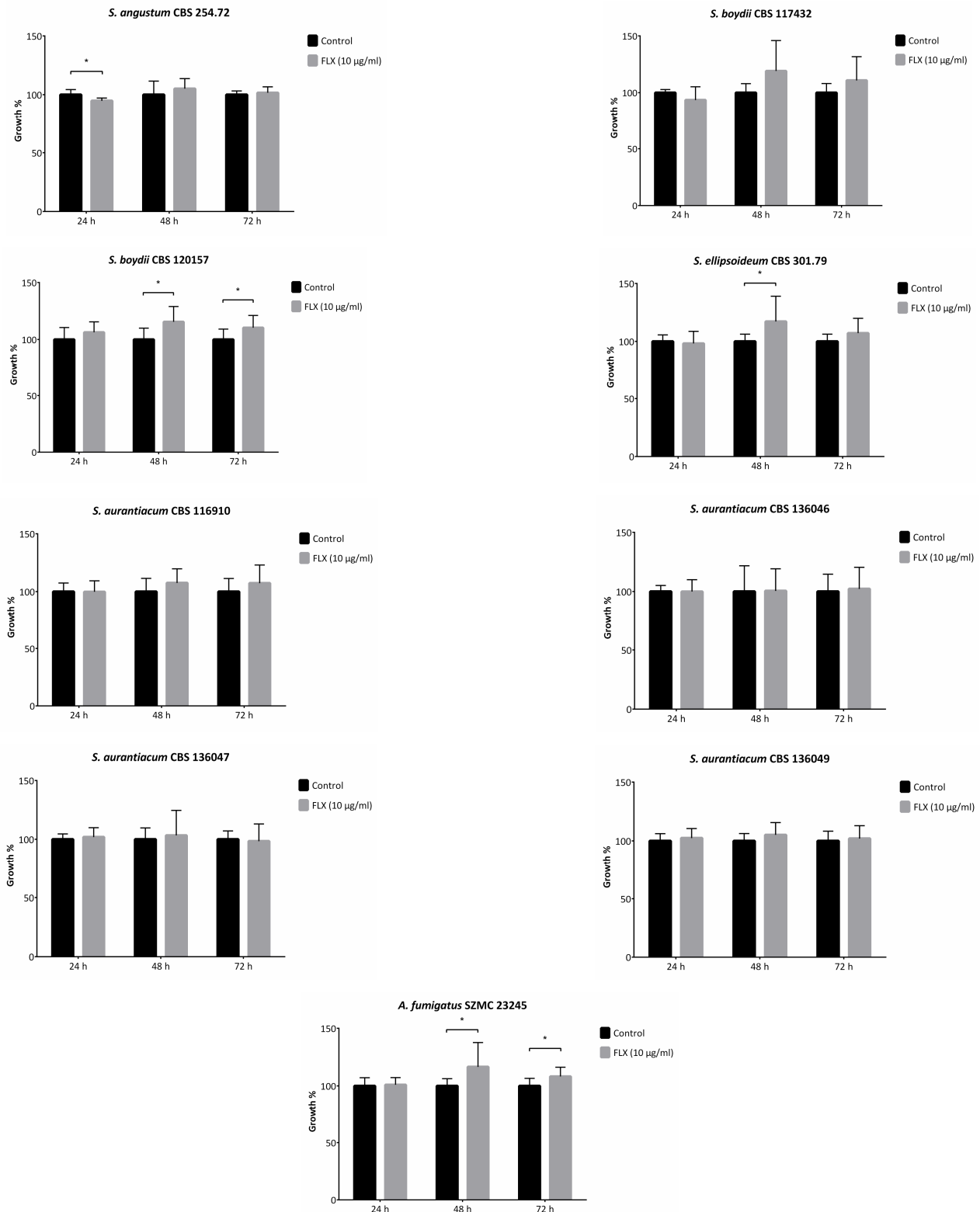
## Inter-kingdom interactions between CF pathogens.



**Figure 18S** Growth rate of *Scedosporium* and *A. fumigatus* isolates in the presence of ceftazidime (CAZ). Results are the mean of three independent experiments with three individual replicates. Error bars indicate standard deviations. P values were calculated using unpaired t test, where  $p < 0.05$  was considered to be significant (indicated with asterisks).



## Inter-kingdom interactions between CF pathogens.



**Figure 19S** Growth rate of *Scedosporium* and *A. fumigatus* isolates in the presence of 10 µg/ml flucloxacillin (FLX). Results are the mean of three independent experiments with three individual replicates. Error bars indicate standard deviations. P values were calculated using unpaired t test, where p<0.05 was considered to be significant (indicated with asterisks).