



# Compensatory adaptation to the loss of biological fitness associated with the acquisition of fusidic acid resistance in *Staphylococcus aureus*



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## Introduction

Antibiotic resistance due to chromosomal mutations causing structural modifications in the cellular target of the drug, such as fusidic acid resistance, is often associated with a fitness burden for the resistant bacteria. As shown recently, certain amino acid exchanges in elongation factor G (EF-G) of *Staphylococcus aureus*, such as H457Y, cause resistance to fusidic acid. Interestingly, clinical *S. aureus* isolates showing the substitution H457Y frequently harbour additional amino acid exchanges within EF-G (e.g. S416F) which do not contribute to fusidic acid resistance. The aim of the present study was (i) to analyse the biological costs of the fusidic acid resistance-mediating mutation H457Y and (ii) to investigate whether the mutation S416F is able to compensate for this fitness burden.

## Material and Methods

The influence of mutational changes within EF-G on the biological fitness of *S. aureus* was analysed by measuring growth kinetics as well as by means of fitness assays and coagulase activity assays, using isogenic recombinant *S. aureus* strains carrying either the wild-type EF-G gene (*fusA*) or a mutant *fusA* derivative (S416F, H457Y or H457Y/S416F) on a multicopy plasmid. Three separate experiments were performed for each assay at least. The fitness of the fusidic acid-resistant (*fusR*) *fusA* mutants H457Y and H457Y/S416F in comparison to the fusidic acid-susceptible (*fusS*) strain carrying the wild-type *fusA* gene was determined by pairwise competition experiments. For this purpose, 10 ml 2xYT-medium were inoculated with equal amounts of the *fusR* mutant and the *fusS* strain and incubated at 37°C for 16 hours. The number of viable cells was counted at the beginning and the end of the experiment on YT-agar containing either no fusidic acid or 1 µg/ml of this drug. The number of generations (G) of the competing strains was calculated as described by Billington *et al.*:  $G = (\log B - \log A) : \log 2$ , with A being the number of CFU/ml at time 0 and B the number of CFU/ml at the end of the culture period.

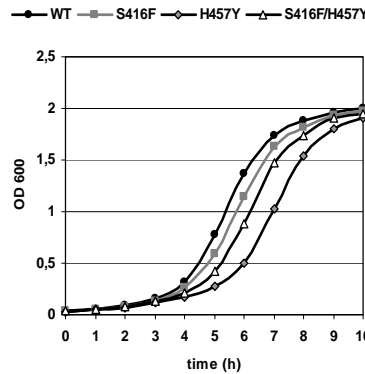


Figure 1: Growth kinetics of recombinant strains. Experiments were performed in triplicate. Results of one experiment are shown exemplarily.

Table 1: Comparison of growth of recombinant strains after 6 h

<i>S. aureus</i> strain with EF-G mutation	OD <sub>600</sub> ratio of EF-G mutant vs Wild-type <sup>a</sup>
S416F	0.86 ± 0.04
H457Y	0.37 ± 0.06
S416F/H457Y	0.64 ± 0.09 <sup>b</sup>

<sup>a</sup> Results are expressed as means ± SEM of three experiments.  
<sup>b</sup> P < 0.05 compared with the results for H457Y

Table 2: Relative fitness (F) of *fusR* mutants

analysed strains	F <sup>a</sup>
H457Y : WT	-0.75 ± 0.17
S416F/ H457Y : WT	-0.19 ± 0.09 <sup>b</sup>

WT, wild-type

<sup>a</sup> Results are expressed as means ± SEM of three experiments.  
<sup>b</sup> P < 0.05 compared with the results for H457Y:WT

Table 3: Coagulase activity of recombinant strains

time (h)	WT	H457Y	S416F	S416F/H457Y
1	0	0	0	0
1,5	3+	0	2+	0
2	4+	2+	4+	3+
2,5		3+		4+
3		4+		

WT, wild-type

Accordingly, the relative fitness (F) of the *fusA* mutants was determined as follows:

$$F = G_{\text{fusR-strain}} : G_{\text{fusS-strain}}$$

Coagulase activity of recombinant *S. aureus* strains was analysed using BBL™ Coagulase Plasma (Becton Dickinson) corresponding to the recommendations of the manufacturer.

## Results

The fusidic acid resistance-mediating mutation H457Y in EF-G caused a marked impairment of the biological fitness whereas the amino acid exchange S416F reduce the biological fitness of the bacteria only slightly when present individually. The strain expressing the EF-G derivative with the double mutation H457Y/S416F, however, grew significantly faster (Figure 1 and Table 1), showed enhanced fitness in competition with the wild-type (Table 2) and exhibited a higher coagulase activity (Table 3) than the strain harbouring the single exchange H457Y.

## Discussion

In conclusion, the data presented in this report provide evidence at the molecular level that the deleterious effects of fusidic acid resistance-mediating exchanges within EF-G of *S. aureus* can be reduced considerably by specific compensating mutations in this target protein. This compensatory adaptation most likely plays a significant role in the stabilization of resistant bacteria within a given population.

Hence, bacteria often do pay a metabolic price, such as reduced growth rate, reduced invasiveness, or loss of virulence for the acquisition of drug resistance in the short term, but their adaptation to the physiological cost is likely to foster stable maintenance of resistance in the long term. Extrapolation of these findings make it tempting to speculate that resistance will never disappear completely because there is no evolutionary disadvantage to being resistant once adaptation has taken place.