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Adaptation of *Salmonella* Typhimurium to triclosan

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Introduction

Salmonella sp. have an increasing importance in food-related disease-outbreaks, and combined with increasing resistance among *Salmonella*-species, this makes it very interesting to study in terms of development of resistance to antibiotics and possibly biocides.

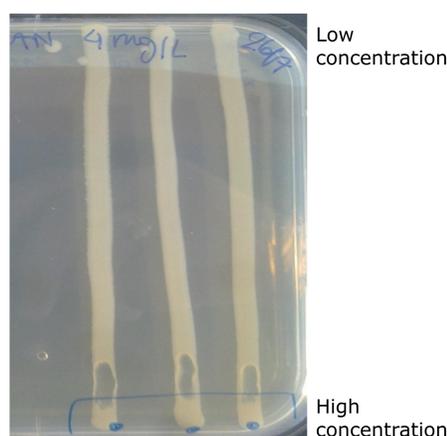
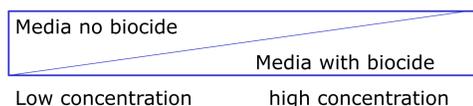
In recent years there have been increasing interest in examining the effect of biocides on pathogens and it has been hypothesized that prudent use of disinfectants can select for biocide resistant or tolerant strains.

Within the last 30 years there have been descriptions of bacteria developing resistance or tolerance towards biocides and in some cases cross-resistance to antibiotics. Recently there has been proof that in-use concentrations of disinfectants can select multidrug resistant mutants of *S. Typhimurium*.

Aim: To evaluate the possibility of adapting *Salmonella* Typhimurium to triclosan and examine the consequences regarding fitness, virulence and antibiotic resistance.

Adaptation

Gradient-plate assay



Strains

Salmonella Typhimurium 4/74 and *S. Typhimurium* isolated from fresh pork (provided by Karl Pedersen, DTU FOOD) named DTU3 were used in these studies.

Method

Subculture was done daily on gradient-plates

Start concentration: 1/2 MIC (1 mg/L)

After 2 days with growth to the highest concentration, the concentration was doubled.

Experiment was ended at concentration-maximum of 4048 mg/L as triclosan was no longer soluble.

MIC-values was determined using Müller Hinton broth and a two-fold dilution of triclosan. MBC values were determined as the concentration giving more than a 5 log-reduction of the inoculum.

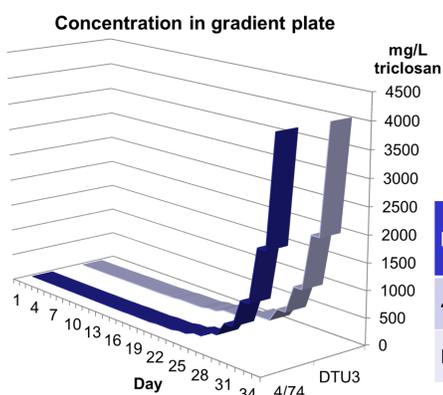
Results

Adaptation was possible for both strains.

The MIC-value was elevated around 1000 times. MBC-values were elevated above the 1000 mg/l usually considered lethal to *Salmonella*.

After 5 days subcultivation in media not containing triclosan the strains were still having the same MIC value.

Isolate	MIC start (broth)	MBC (start)	# sub	MIC end (broth)	MBC (end)
4/74	1-2 mg/l	>64 mg/l	36	1057 mg/l	4124 mg/l
DTU3	8 mg/l	>64 mg/l	36	4124 mg/l	8146 mg/l



Discussion and perspectives

It is possible to adapt *Salmonella* Typhimurium to the biocide triclosan in concentrations within in-use ranges. The adaptation is stable indicating it is caused by a mutation. The adapted strains are not impaired in growth, indicating that the adaptation has no fitness cost. Adaptation does not affect virulence more than repeated subcultivation does.

There seems to be a triclosan induced cross-resistance to some antibiotics. Mutations in *gyrA* can be responsible for fluoroquinolone resistance. Mutations in *GyrA* have previously been reported in triclosan resistant strains.

Mutations in or upregulation of *fabI* are often associated with triclosan resistance. Further analysis using Northern Blot will elucidate if that is the case with these strains, furthermore full genome sequencing will be done to identify the mutation(s) leading to triclosan resistance.

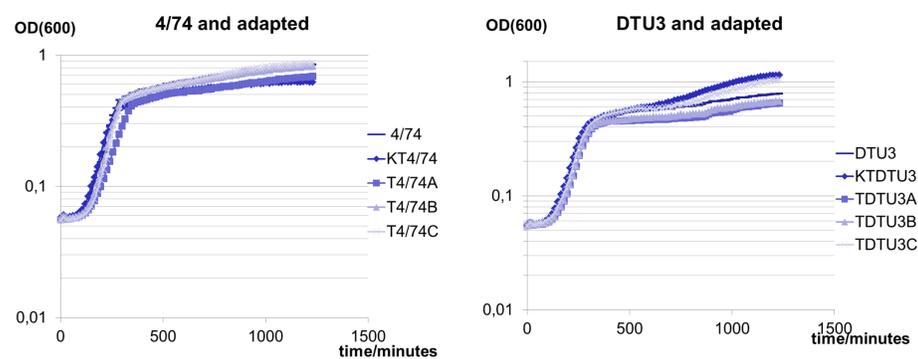
Fitness and Virulence

Method

Growth was evaluated from growth-curves obtained using Bioscreen C in a volume of 195 µl MHB and a 5 µl inoculum of an overnight-culture adjusted to OD(600) = 0,05

Results

Growth of wild-type and adapted strains are similar.

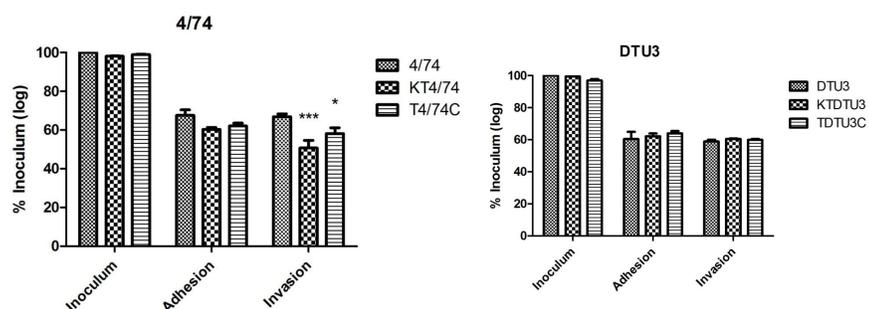


Method

Virulence was evaluated in vitro using INT407 epithelial cells. Adhesion and invasion was evaluated after 30 minutes.

Results

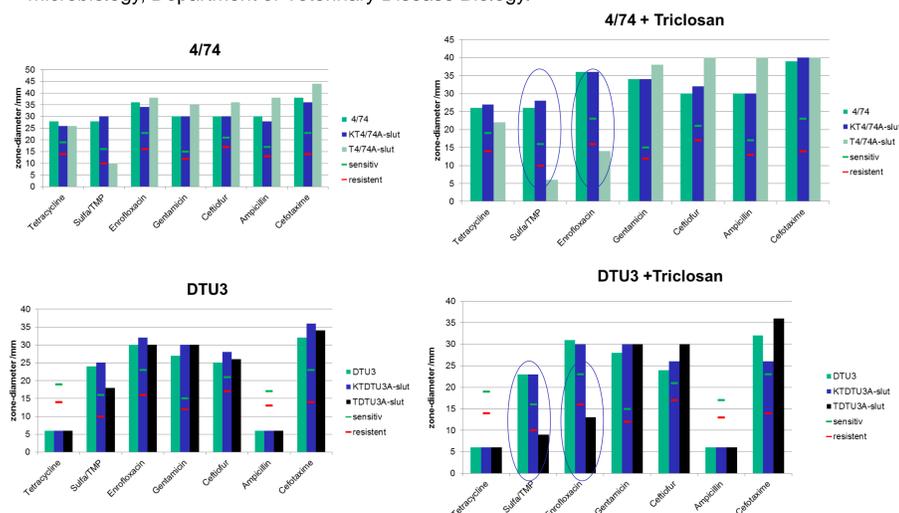
There were no difference in adhesion between wild-type and adapted strains. Only adapted strains of 4/74 showed reduced invasion, but the control-strain which have been subcultivated as many times as the adapted strains was more impaired regarding invasion.



Antibiotic resistance

Method

Antibiotic resistance was evaluated by disk diffusion on Müller Hinton agar with and without triclosan (1 mg/L for non-adapted and 1000 mg/L for adapted). Interpretation into resistant, intermediate and sensitive was done according to the cut-off values used by the section of microbiology, Department of Veterinary Disease Biology.



Results

As can be seen from the diagrams above, the adapted strains show resistance towards sulfamethoxazole/trimethoprim and enrofloxacin when tested on plates containing triclosan.