THE UNIVERSITY OF ARIZONA
Water & Energy Sustainable Technology Center

Surface Time-Kill Test of Stainless Steel Coupons Coated with RS Disinfectant against *Candida auris* (ATCC #MYA-5000)

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Company: SRFC_BIO, Inc.
Test Organism: *Candida auris* (ATCC #MYA-5000)
Test Materials: Control (uncoated) and coated stainless steel coupons
Contact Times: 0, 1, 3, 10, 30 minutes

MATERIALS AND METHODS

Preparation of stainless steel coupons

1. Stainless steel coupons (2" × 2") were washed gently with soap and water and then wiped three times with a 70% ethanol solution to remove any dust / debris.
2. A volume of 0.1 ml of RS Disinfectant (SRFC_BIO, Inc.) was placed onto the surface of each of the test stainless steel coupons and spread evenly using a sterile pipette tip. The coated coupons were then placed into separate sterile petri dishes and placed into a Class II Biosafety Cabinet for 24 to 26 hours prior to the start of the test to allow the coating to dry and adhere to the coupon surfaces.
3. Control coupons (not coated with the RS Disinfectant) were likewise washed with soap and wiped with ethanol before they were also placed in sterile petri dishes in a Class II Biosafety Cabinet for 24 to 26 hours prior to the start of the test.

Antimicrobial efficacy test protocol

1. A culture of *Candida auris* (ATCC #MYA-5000) was prepared on the day before testing by inoculating one colony into 100 ml of tryptic soy broth (TSB) and incubation overnight at 37°C.
2. On the test date, the yeast cells were diluted in TSB to obtain a density of ~1×10^7 colony-forming units (CFU) per ml.
3. All control and test stainless steel (type 304) coupons measured 2" × 2". Control and test coupons were inoculated with 0.1 ml of the test yeast cell suspension (containing ~1.0×10^6 CFU) and the suspension was spread evenly over the coupon surface using a sterile pipette tip.
4. The coupons were placed in sterile petri dishes in sealed Tupperware chambers with moist paper towels and incubated at room temperature (22 ± 2°C) to prevent drying of the microbial solution.
5. Triplicate samples from the control coupons were collected immediately upon inoculation to determine the baseline yeast concentration at time (t) = 0 minutes. The coupons were swabbed to recover the yeast cells and the swabs placed into separate 1-ml volumes of Letheen neutralizing broth. The samples were vortexed for 30 seconds and then the swabs were aseptically removed and discarded.
6. The samples were then 10-fold serially diluted and inoculated onto tryptic soy agar (TSA) plates using the spread plate method. The plates were incubated for 24 hours at 37°C and the colonies enumerated.
7. All other control and test coupons were held at room temperature in the sealed Tupperware chambers (22 ± 2°C at a relative humidity of ~95%). At t = 1, 3, 10, and 30 minutes, triplicate samples of the remaining test (coated) coupons were sampled in the manner described previously and assayed on TSA plates as before. Triplicate control coupons were also sampled after 30 minutes to determine the stability of the test organism under the test conditions.
8. Colonies were counted, and the levels of CFU recovered per coupon determined. The data were reported as the logarithmic reduction using the formula $-\log_{10} (N_t / N_0)$, where N_0 was the concentration of surviving *C. auris* at t = 0 minutes and N_t was the concentration of *C.*

auris in the sample collected at time = *t* (i.e., 1, 3, 10, or 30 minutes).

Neutralization verification test

1. To confirm that the RS Disinfectant was sufficiently neutralized by placement in Letheen neutralizing broth, a neutralization verification test was performed. A volume of 1.0 ml of the RS Disinfectant was added to two tubes containing 9.0 ml of Letheen neutralizing broth. For the controls, a volume of 1.0 ml of phosphate buffered saline (PBS) was added to two tubes containing 9.0 ml of Letheen.
2. The solutions were mixed and allowed to sit for five minutes and then a volume of 0.1 ml of an overnight culture of *C. auris* (ATCC #MYA-5000) was added to all four tubes resulting in a final concentration of approximately 1.0×10^6 CFU/ml in each tube. The solutions were mixed again and then allowed to sit for 10 minutes at room temperature (22 ± 2°C).
3. Ten-fold serial dilutions of the neutralized solutions were assayed as described previously. If the solution was completely neutralized by the Letheen neutralizing broth, it was expected that there would be no or very little reduction in the recovered yeast numbers in comparison to the controls with no disinfectant added. The neutralization may be considered valid when the difference between the *C. auris* recovery between the controls and neutralized antimicrobial solutions is ≤ 0.50 $\log_{10}$.

RESULTS

Table 1. $\log_{10}$ reduction of *Candida auris* (ATCC #MYA-5000) in neutralization verification test. The neutralization by Letheen neutralizing broth was considered valid if the average reduction was ≤ 0.50 $\log_{10}$.

Treatment	Exposure Time (minutes)	Replicate	$\log_{10}$/ml Recovered	$\log_{10}$ Reduction	Average $\log_{10}$ Reduction ± SD	Neutralization Validated?
None (Controls)	10	1	6.30	N/A	N/A	N/A
		2	6.36			
RS Disinfectant	10	1	6.33	0.00	0.00 ± 0.00	YES
		2	6.38	0.00		

N/A = not applicable
SD = standard deviation

Table 2. Log_{10} reduction of *Candida auris* (ATCC #MYA-5000) on stainless steel coupons with and without coating with RS Disinfectant after 1, 3, 10, and 30 minutes of exposure. The test was conducted at room temperature (22 ± 2°C) at a relative humidity of ~95%.

Exposure Time (minutes)	Sample	Control Coupons (No coating)		Test Coupons (Coated w/ RS Disinfectant)	
		Log_{10} Reduction*	Average Log_{10} Reduction* ± SD	Log_{10} Reduction*	Average Log_{10} Reduction* ± SD
1	A	ND		0.81	0.73 ± 0.36
	B			0.34	
	C			1.05	
3	A	ND		1.25	1.11 ± 0.19
	B			0.9	
	C			1.2	
10	A	ND		1.14	1.46 ± 0.74
	B			0.95	
	C			2.31	
30	A	0.15	0.20 ± 0.08	2.11	2.03 ± 0.44
	B	0.29		1.56	
	C	0.17		2.42	

* Initial Inoculum (in 0.1 ml) = 1.27×10^6 CFU
SD = standard deviation
ND = not determined

DISCUSSION

The RS Disinfectant was successfully neutralized at the concentration used during the test with the Letheen neutralizing buffer. Therefore, the reductions observed in the *C. auris* during the test may be considered valid.

Reductions in *C. auris* were observed in all the samples exposed to the RS Disinfectant in comparison to the numbers recovered from the control stainless steel coupons both initially ($t = 0$ minutes) and control coupons at the end of the test ($t = 30$ minutes). The reductions observed were statistically significant ($P \leq 0.05$) after 3 minutes and 30 minutes of exposure to the RS Disinfectant-coated coupons in comparison to both sets of controls (i.e., at 0 and 30 minutes), but were not significant after 1 minute and 10 minutes. This was due to the higher level of variability between the samples after 1 and 10 minutes (standard deviations of 0.36 and 0.74) relative to the reductions observed. For instance, a comparable standard deviation was also observed with the samples after 30 minutes (standard deviation of 0.44), but the reductions were higher relative to this level of variability.