UNIVERSITY OF THE FREE STATE
UNIVERSITEIT VAN DIE VRYSTAAT
YUNIVESITHI YA FREISTATA

UFS·UV
NATURAL AND AGRICULTURAL SCIENCES
NATUUR- EN LANDBOUWETENSKAPPE

Project Report

Testing the minimum inhibitory concentrations (MIC) of BLULYTE against *Serratia marcesens* ATCC strain and highly resistant strain with different contact times.

By:

Prof. R.R. Bragg B.Sc., B.Sc. (Hons), M.Sc., PhD.

Veterinary Biotechnology

Dept of Microbiology & Biochemistry

University of the Free State

P.O. Box 339

Bloemfontein

9300

Report date:

18 March 2021

Report No: 21BL001

Aims :

To evaluate the *in vitro* MIC of the product BLULYTE against the ATCC strain and highly resistant strain of *Serratia marcesens* with a 1 min, 5 min and 10 min. contact times.

Methods :

All techniques were conducted under conditions of Good Laboratory Practice.

A sample of product BLULYTE was supplied by BluLyte.

The bacterial strains were obtained from the bacterial culture collection of the Department of Microbial, Biochemical and Food Biotechnology. The bacteria were inoculated into 10 ml of Brain Heart infusion broth (BHI) and was incubated at 37C for 24 hours.

Samples were diluted according to the supplied instructions. All tests were performed in sterile distilled water. All tests were performed in sterile 96 well plates.

MIC tests were done by making two fold serial dilutions of the products as presented in the Tables below.

Once the dilutions were made, 10 µl of the 24-hour-old culture of the above bacteria were added to each of the tubes. A negative control tube, containing 100 µl sterile diluent was not inoculated with any bacteria. A positive control tube, which only contained 100 µl sterile diluent and no disinfectant (test material), was inoculated with 10 µl of the above bacterial cultures.

All of the tubes were mixed well and incubated at room temperature for the different contact times. Thereafter, 10 µl was removed from each well and used to inoculate the corresponding well containing 100 µl ml BHI in a new sterile 96 well plate. This was done in triplicate.

The plates containing the inoculated BHI were incubated for 24 hours at 37C. After the incubation time, the bacterial growth in each well was recorded, by observing the turbidity of the culture.

Results

Table 1: MIC for BLULYTE against the ATCC strain of *S. marcesens* in sterile diluent after a 1 min contact time.

Tube number	**Dilution**	**Bacterial growth**		
		1	2	3
1	100%	-	-	-
2	50%	+	+	+
3	25%	+	+	+
4	12.5%	+	+	+
5	6.30%	+	+	+
6	3.10%	+	+	+
7	1.60%	+	+	+
8	0.80%	+	+	+
9	0.4%	+	+	+
10	0.2%	+	+	+
Positive		+	+	+
Negative.		-	-	-

An MIC of 100% (undiluted) was found for product BLULYTE based on the product supplied after a 1 min contact time.

Table 2: MIC for BLULYTE against the ATCC strain of *S. marcesens* in sterile diluent after a 5 min contact time.

Tube number	**Dilution**	**Bacterial growth**		
		1	2	3
1	100%	-	-	-
2	50%	-	-	-
3	25%	-	-	-
4	12.5%	+	+	+
5	6.30%	+	+	+
6	3.10%	+	+	+
7	1.60%	+	+	+
8	0.80%	+	+	+
9	0.4%	+	+	+
10	0.2%	+	+	+
Positive		+	+	+
Negative.		-	-	-

An MIC of 25% (1 in 4 dilution) was found for product BLULYTE based on the product supplied after a 5 min contact time.

Table 3: MIC for BLULYTE against the ATCC strain of *S. marcesens* in sterile diluent after a 10 min contact time.

Tube number	**Dilution**	**Bacterial growth**		
		1	2	3
1	100%	-	-	-
2	50%	-	-	-
3	25%	-	-	-
4	12.5%	+	+	+
5	6.30%	+	+	+
6	3.10%	+	+	+
7	1.60%	+	+	+
8	0.80%	+	+	+
9	0.4%	+	+	+
10	0.2%	+	+	+
Positive		+	+	+
Negative.		-	-	-

An MIC of 25% (1 in 4 dilution) was found for product BLULYTE based on the product supplied after a 10 min contact time.

Table 4: MIC for BLULYTE against the highly resistant strain of *S. marcesens* in sterile diluent after a 1 min contact time.

Tube number	**Dilution**	**Bacterial growth**		
		1	2	3
1	100%	-	-	-
2	50%	+	+	+
3	25%	+	+	+
4	12.5%	+	+	+
5	6.30%	+	+	+
6	3.10%	+	+	+
7	1.60%	+	+	+
8	0.80%	+	+	+
9	0.4%	+	+	+
10	0.2%	+	+	+
Positive		+	+	+
Negative.		-	-	-

An MIC of 100% (undiluted) was found for product BLULYTE based on the product supplied after a 1 min contact time.

Table 5: MIC for BLULYTE against the highly resistant strain of *S. marcesens* in sterile diluent after a 5 min contact time.

Tube number	**Dilution**	**Bacterial growth**		
		1	2	3
1	100%	-	-	-
2	50%	-	-	-
3	25%	+	+	+
4	12.5%	+	+	+
5	6.30%	+	+	+
6	3.10%	+	+	+
7	1.60%	+	+	+
8	0.80%	+	+	+
9	0.4%	+	+	+
10	0.2%	+	+	+
Positive		+	+	+
Negative.		-	-	-

An MIC of 50% (1 in 2 dilution) was found for product BLULYTE based on the product supplied after a 5 min contact time.

Table 6: MIC for BLULYTE against the highly resistant strain of *S. marcesens* in sterile diluent after a 10 min contact time.

Tube number	**Dilution**	**Bacterial growth**		
		1	2	3
1	100%	-	-	-
2	50%	-	-	-
3	25%	-	-	-
4	12.5%	+	+	+
5	6.30%	+	+	+
6	3.10%	+	+	+
7	1.60%	+	+	+
8	0.80%	+	+	+
9	0.4%	+	+	+
10	0.2%	+	+	+
Positive		+	+	+
Negative.		-	-	-

An MIC of 25% (1 in 4 dilution) was found for product BLULYTE based on the product supplied after a 10 min contact time.

Table 7. Comparison of MIC results

Time	**ATCC strain**	**HRI**
1 min	Undiluted	Undiluted
5 Min	1 in 4 dilution	1 in 2 dilution
10 Min	1 in 4 dilution	1 in 4 dilution

Discussion

It can be seen from the above data that product BLULYTE, as supplied, was effective against the ATCC strain and the highly resistant strain of *Serratia marcesens* used in this experiment, even with only a 1 min contact time. A time dependent increase in efficacy can be seen for both strains, with an increased efficacy with increased time, up to a maximum of a 1 in 4 dilution or 25% of the supplied product. The MIC for BLULYTE was found to be a 1 in 4 dilution (25% concentration of the supplied product) against both strains can be seen after a 10 min contact time.

There appears to be some indication of resistance in the highly resistant strain as the product was only effective at a 1 in 2 dilution against the highly resistant strain with a 5 min exposure, while for the ATCC strain with a 5 min exposure a 1 in 4 dilution was effective.

This product did show 100% efficacy against both strains of bacteria used in this experiment, under these experimental conditions at all three contact times.

It must be stressed that the conditions used in this experiment were not harsh. The diluent used was sterile distilled water. Normally MIC tests are performed in diluent with 1% organic load and 300 ppm $CaCO_3$ – to simulate hard water conditions. Both of these factors can have an impact on the efficacy of a product, but from experience, this is not normally more than a 2 or 3 dilution factor difference.

Conclusion

It can be concluded from this experiment that the product BLULYTE was effective against the ATCC and the highly resistant strain of *Serratia marcesens* under these experimental conditions when used according to the recommendations. There appears to be some indication of possible resistance, but this will require additional research.

.

Signed this 18th day of March 2021