Tris-NAC ® (Tris-EDTA + N-acetylcysteine) activity against biofilm production, an *in vitro* study

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A biofilm is a structured consortium of bacteria embedded in a self-produced polymer matrix consisting of polysaccharides, protein and DNA. Bacterial and yeast biofilms cause chronic infections because they show increased tolerance to antibiotics, antifungals and disinfectant chemicals, as well as resisting phagocytosis and other components of the body's defense system.

The aim of the study was to assess the **efficacy** of a product containing N-acetylcysteine + Tris-EDTA (**Tris-NAC®** : ICF Srl, Palazzo Pignano, Italy) in *preventing the formation of biofilm* and *aiding its disintegration*.

MATERIALS AND METHODS

The study of biofilm growth inhibition, conducted by the Clever Bioscience Srl laboratory in Pavia (Italy), was evaluated using the microplate biofilm formation test against three microorganism strains: *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), *Malassezia pachydermatis* (DSM 6172). The same three microorganisms were used to study the formulation's action on the biofilm breakdown: for this study, the microorganisms were grown in microtiter plates, and the method to evaluate the growth of the biofilm is called Microtiter Dish Biofilm Formation Assay. Biofilm growth and production were assessed at two different times (8 hours and 24 h) for bacteria and at three different times for yeasts (8 h, 24 h, and 48 h).

- ***Pseudomonas aeruginosa*:**

Dilutions of ICF product											
1	2	3	4	5	6	7	8	9	10	11	12
1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096

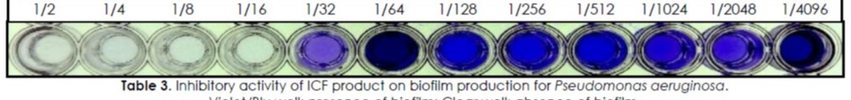

Table 3. Inhibitory activity of ICF product on biofilm production for *Pseudomonas aeruginosa*.
Violet/Blu well: presence of biofilm; Clear well: absence of biofilm.

- ***Staphylococcus aureus*:**

Dilutions of ICF product											
1	2	3	4	5	6	7	8	9	10	11	12
1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096

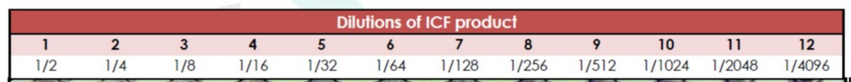

Table 6. Inhibitory activity of ICF product on biofilm production for *Staphylococcus aureus*.
Violet/Blu well: presence of biofilm; Clear well: absence of biofilm.

- ***Malassezia pachydermatis*:**

Dilutions of ICF product											
1	2	3	4	5	6	7	8	9	10	11	12
1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096

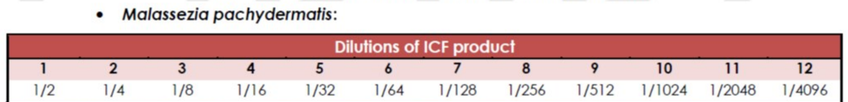

Table 9. Inhibitory activity of ICF product on biofilm production for *Malassezia pachydermatis*.
Violet/blu well: presence of biofilm; Clear well: absence of biofilm.

Subsequently, at different dilutions, the Tris-NAC® formulation was added to the same plates to evaluate the ability to desegregate the biomass during the 24 hours. The test was carried out in triplicate to obtain reliable results

RESULTS

The data show that **Tris-NAC ®** can disaggregate *Pseudomonas aeruginosa* biofilm both at 8 hours and at 24 hours. *Staphylococcus aureus* biofilm at 24 hours (in this case, at the 8th hour, the biofilm was still absent), and it was not able to completely disaggregate the *Malassezia pachydermatis* biofilm at 48 hours (at the 8th hour and at the 24th hour the biofilm was absent).

Furthermore, the formulation can *inhibit the biofilm formation* of all three microorganisms.

- ***Pseudomonas aeruginosa*:**

Time point 8 h

Dilutions of ICF product											
1	2	3	4	5	6	7	8	9	10	11	12
1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096

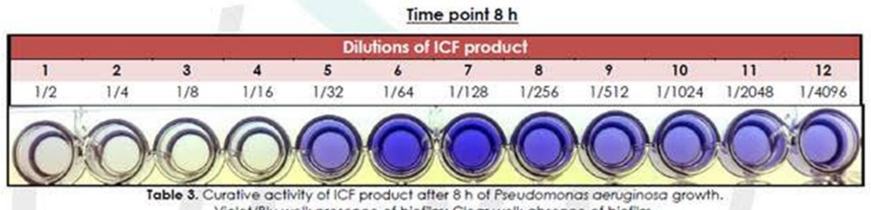

Table 3. Curative activity of ICF product after 8 h of *Pseudomonas aeruginosa* growth.
Violet/Blu well: presence of biofilm; Clear well: absence of biofilm.

Time point 24 h

Dilutions of ICF product											
1	2	3	4	5	6	7	8	9	10	11	12
1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096

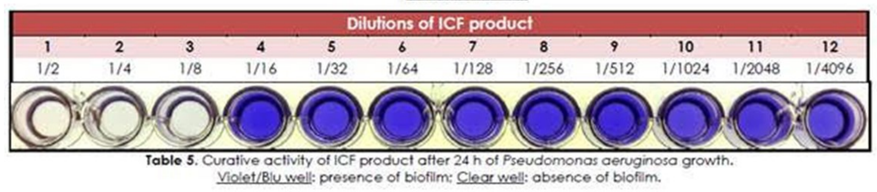

Table 5. Curative activity of ICF product after 24 h of *Pseudomonas aeruginosa* growth.
Violet/Blu well: presence of biofilm; Clear well: absence of biofilm.

- ***Staphylococcus aureus*:**

Time point 24 h

Dilutions of ICF product											
1	2	3	4	5	6	7	8	9	10	11	12
1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096

Table 8. Curative activity of ICF product after 24 h of *Staphylococcus aureus* growth.
Violet/Blu well: presence of biofilm; Clear well: absence of biofilm.

- ***Malassezia pachydermatis*:**

Time point 48 h

Dilutions of ICF product											
1	2	3	4	5	6	7	8	9	10	11	12
1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096

Table 11. Curative activity of ICF product after 48 h of *Malassezia pachydermatis* growth.
Violet/blu well: presence of biofilm; Clear blu well: partial disaggregation of biofilm.

CONCLUSIONS: The use of topical products, such as **Tris-NAC ®**, to prevent microbial biofilm formation is primary prevention in the management of skin and ear infections in dogs and cats. And the biofilm's breaking capacity makes the product useful as a therapy combined with other topical antimicrobials to treat diseases and avoid their chronicity and relapses.

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