

STUDY OF CRANBERRY FRACTIONS USE ON ORAL BACTERIA



REVIEW AND RESULTS

Tests conducted by the University of Bordeaux 2 Victor Segalen, Laboratory of cariogenic bacteria.

Antibacterial tests

Material and method:

- **Tested products:**

Exocyan cran1, Exocyan cran2, Exocyan cran10 ; these 3 extracts were tested at those concentrations: 2mg/mL, 5mg/mL and 10mg/mL.

- **Tested bacteria:**

Streptococcus mutans ATCC25175,
Lactobacillus rhamnosus ATCC 7469,
Porphyromonas gingivalis ATCC 33277,
Fusobacterium nucleatum ATCC 10953.

- **Environments of culture:**

S. mutans: Trypticase/soy broth and agar with blood
L. rhamnosus: Broth and MRS agar (Man, Rogosa and Sharpe)
P. gingivalis, *F. nucleatum*: Broth and WCA agar (Wilkins and Chalgren Anaerobe)

The specific environment of culture of each bacterial species containing extracts to be tested in the various concentrations, are sowed with an overnight culture, fitted to one DO of 0.1 (λ 650 nm).

A control is realized by adding some distilled water. The experiments are realized three times for each product and each bacterium.

After a culture of 24 hours at 37°C, an aliquot of 50 μ L of each test tube is sowed after dilution (10^{-4}) on agar culture specific of each species.

After a culture of 24 or 48 hours at 37°C in anaerobiosis, bacteria colonies are counted; an average of the 3 tests is made and the number of bacteria is expressed in log 10 UFC/mL (UFC = Unit Forming Colony).

The extract Exocyan cran2 could not be mixed in cell culture; an alcoholic solution was made which led to a lack of this product that was therefore unable to perform all the tests.

Results:

Number of bacteria expressed as log 10 UFC/mL

	<i>L.rhamnosus</i>	<i>Mutans S.</i>	<i>P.gingivalis</i>	<i>F.nucleatum</i>
Control	9.45	8.92	8.54	8.35
Exocyan cran1	9.47	8.63	8.47	8.49
Exocyan cran10	9.49	8.88	8.76	8.50

Extracts concentration: 2mg/mL

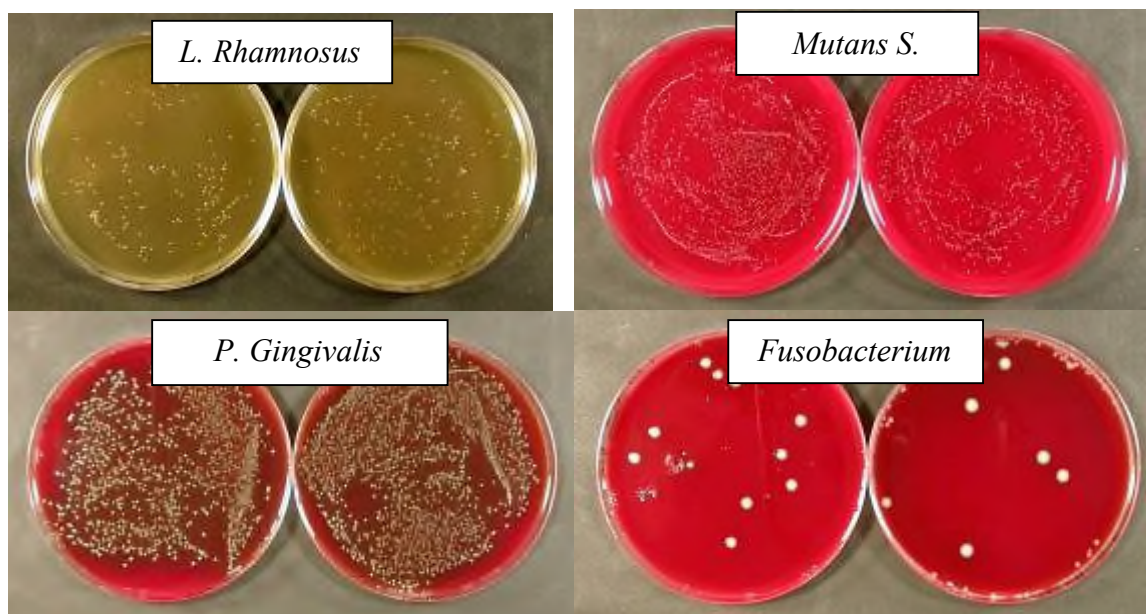
	<i>L.rhamnosus</i>	<i>Mutans S.</i>	<i>P.gingivalis</i>
Control	9.32	8.40	8.68
Exocyan cran1	9.31	8.48	8.62
Exocyan cran2	9.45	8.08	8.78
Exocyan cran10	9.47	8.10	8.54

Extracts concentration: 5 mg/mL

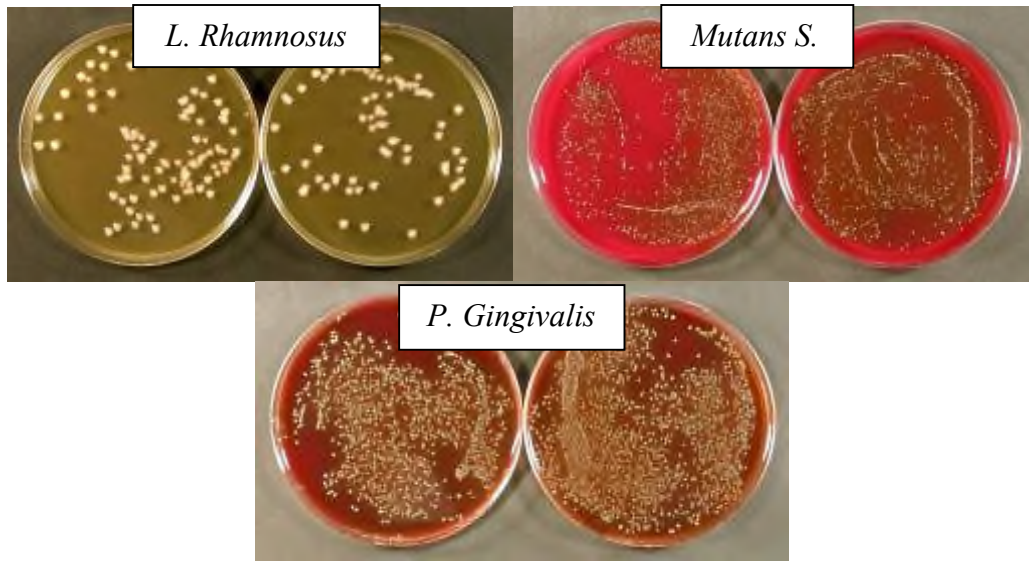
	<i>L.rhamnosus</i>	<i>Mutans S.</i>	<i>P.gingivalis</i>	<i>F.nucleatum</i>
Control	9.43	8.63	8.63	6.02
Exocyan cran1	9.48	7.78	8.75	0
Exocyan cran2	9.39	7.28	8.52	5.74
Exocyan cran10	9.25	5.70	7.7	0

Extracts concentration: 10 mg/mL

EXOCYAN CRAN 1 (2mg/mL)



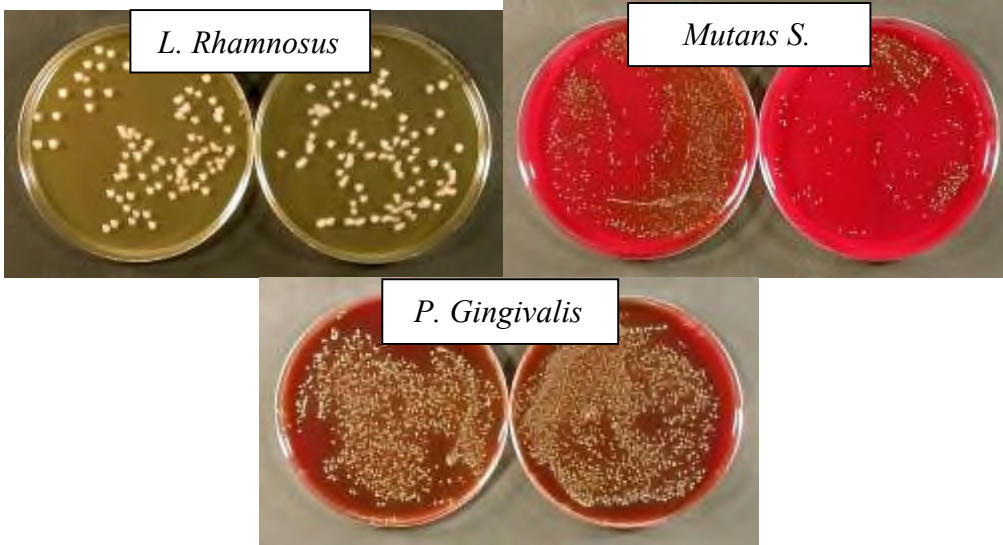
EXOCYAN CRAN 1 (5mg/mL)



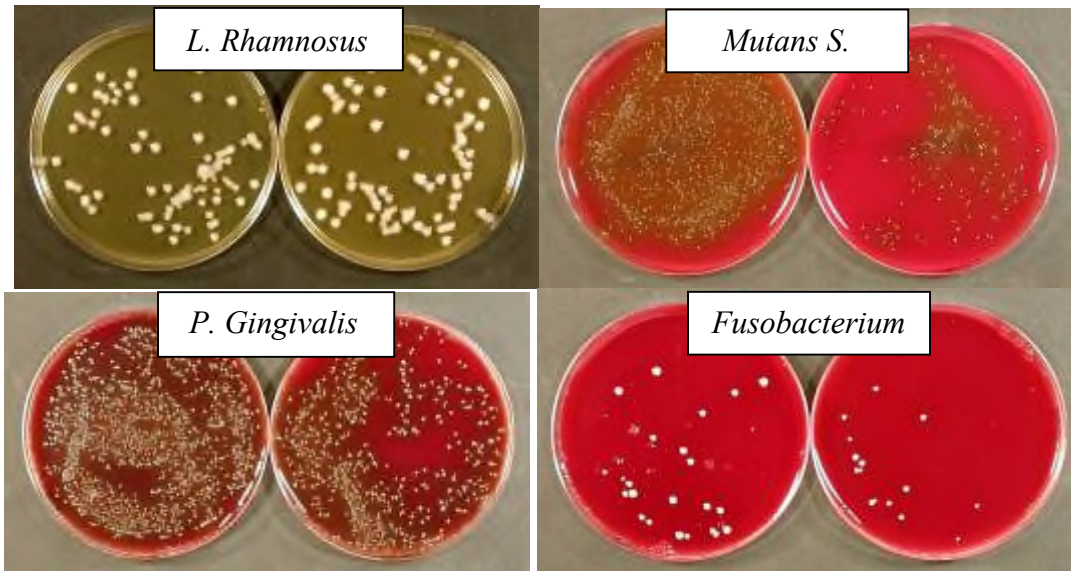
EXOCYAN CRAN 1 (10mg/mL)



EXOCYAN CRAN 2 (5mg/mL)



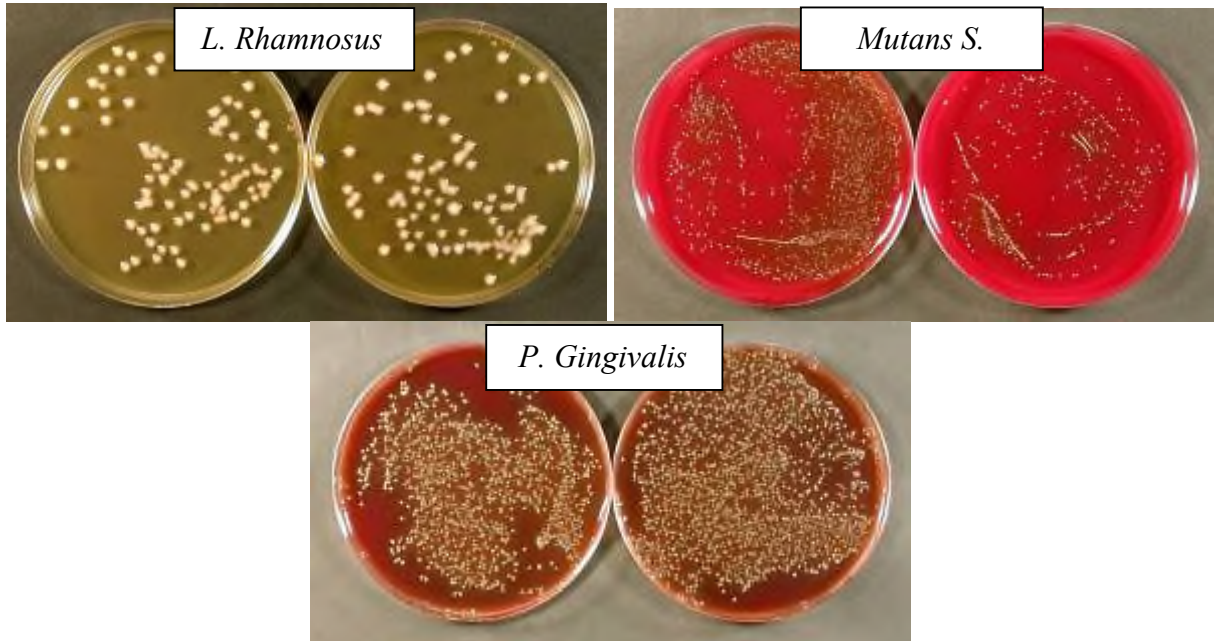
EXOCYAN CRAN 2 (10mg/mL)



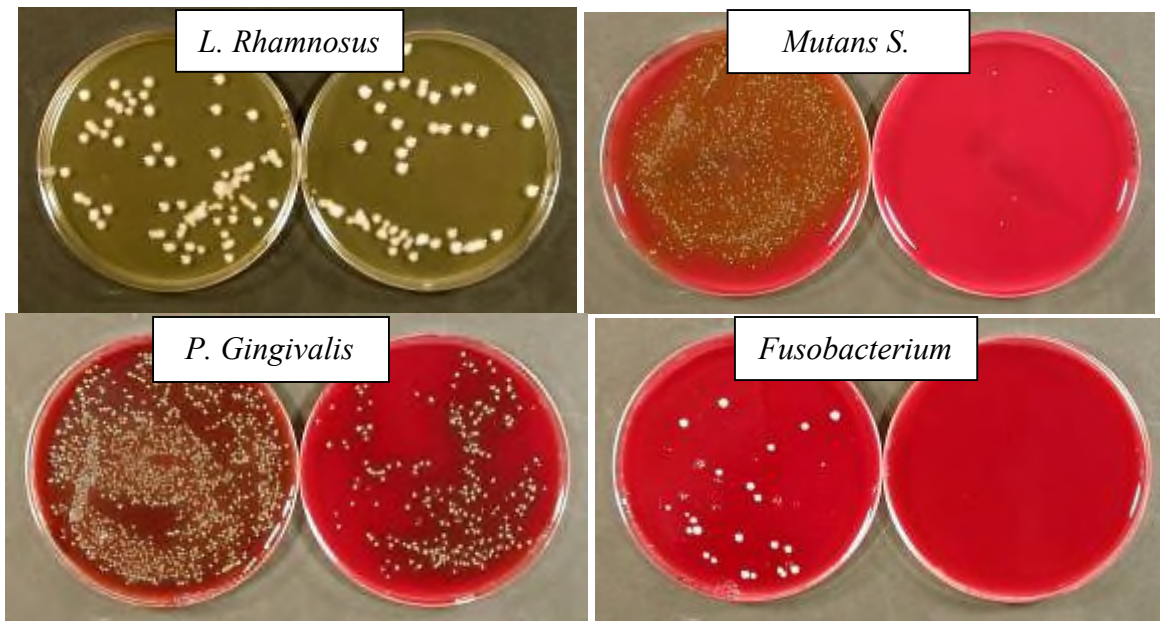
EXOCYAN CRAN 10 2(mg/mL)



EXOCYAN CRAN 10 5(mg/mL)



EXOCYAN CRAN 10 (10mg/mL)



Products are deemed significantly efficient (bacteriostatic) with a decrease of colonies amount of 1 log vs control and antibacterial if there is a total inhibition of bacterial culture.

Discussion:

The three extracts have a bacteriostatic effect on *Mutans Streptococci* at 10 mg/mL concentrations.

On *Porphyromonas Gingivalis*, only Exocyan cran10, at a 10 mg/mL, has a bacteriostatic action.

Fusobacterium Nucleatum is the most sensitive bacterium tested, at 10 mg/mL, Exocyan cran1 and Exocyan cran10 have antibacterial action, Exocyan cran2 has a bacteriostatic activity.

Lactobacillus Rhamnosus growth is not modified by any of the extracts, whatever the concentration is.

Adhesion tests

Material and method:

It is here about the method of Hamada and Torii (1978)

- **Tested bacterium:** *Streptococcus mutans* ATCC 25175
- **Environment of culture:** TS broth (5 ml) enriched in sucrose (1%)

Broth TS enriched in sucrose, containing extracts in various tested concentrations, are sowed with 0.25 mL of an overnight culture of *S. mutans*, fitted to one DO of 0.1 (λ 650 nm).

Controls are achieved with distilled water.

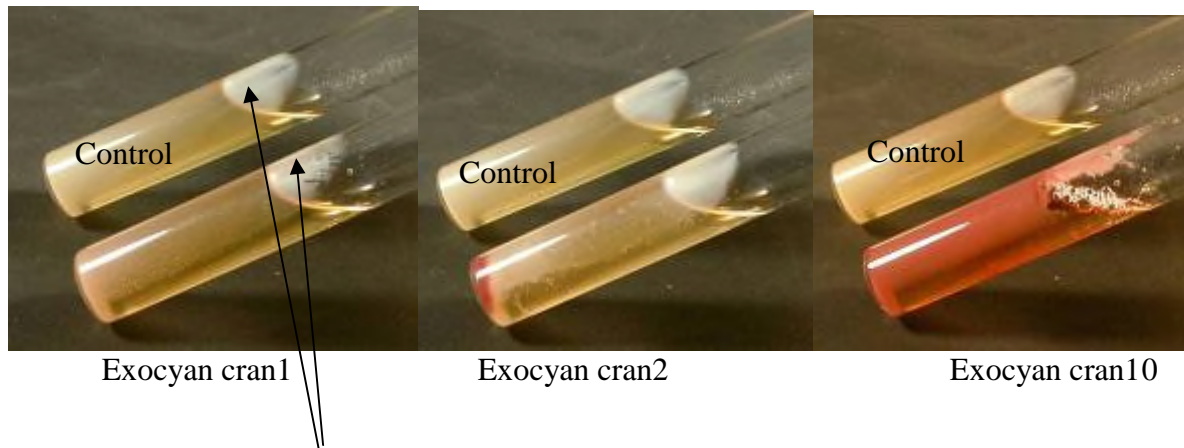
Test tubes are put in culture on an oblique portoir (angle of 30°) at 37°C during 24 hours.

The experiments are realised 3 times for each product and each bacterium. A biofilm forms on the test tube wall. Then a score is attributed according to the result; 0: absence of biofilm, 4: thick and very sticky biofilm.

Results:

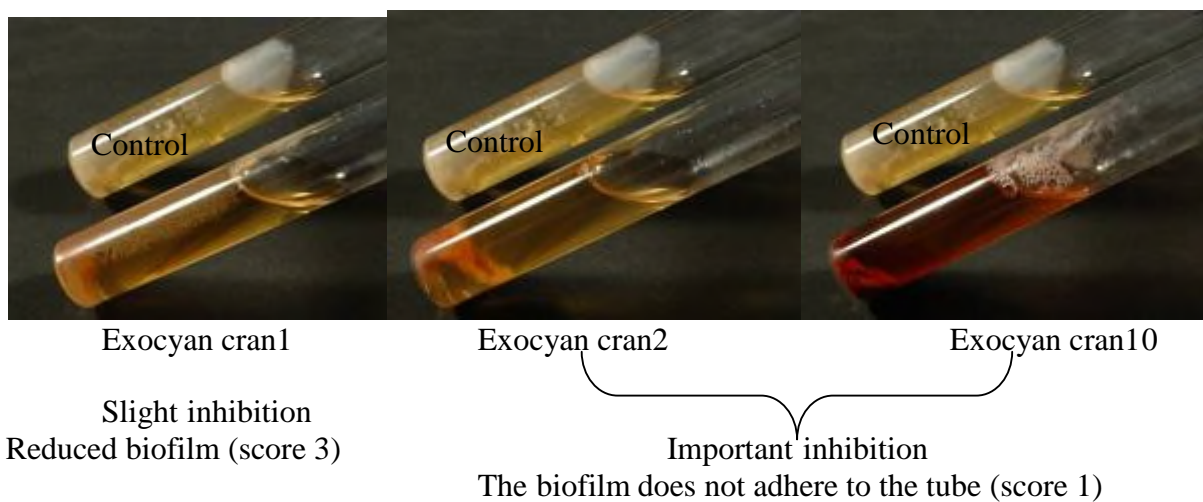
Total or partial inhibition (scores of 0 to 3) of *Mutans Streptococci* is considered as a positive result.

2mg/mL concentration



No inhibition, the biofilm is clearly visible, thick and strongly adheres to the test tube (score 4)

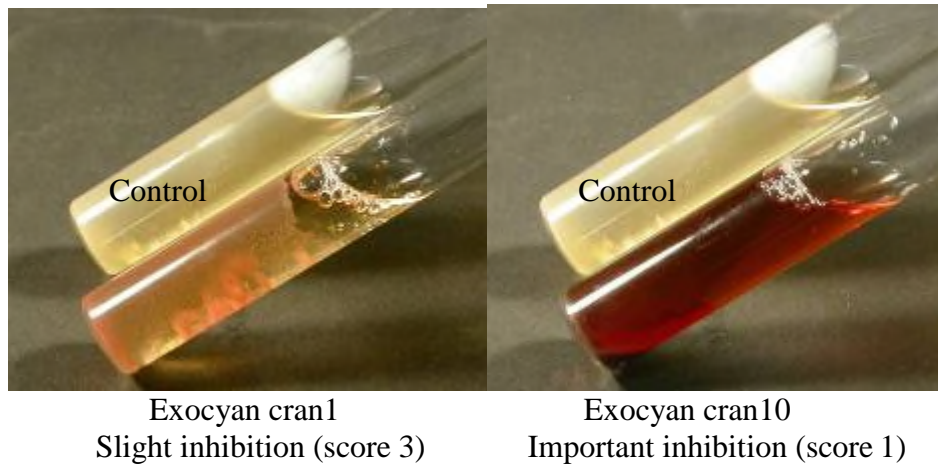
5mg/mL concentration



Slight inhibition
Reduced biofilm (score 3)

Important inhibition
The biofilm does not adhere to the tube (score 1)

10mg/mL concentration



Discussion:

An inhibition is obtained with all three extracts with 5 mg/mL concentrations, and with Exocyan cran1 and 10 at 10 mg/mL concentration (Exocyan cran2 not tested). However, Exocyan cran1 has lower inhibition efficiency (score 3) compared to the 2 other extracts (score 1).