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LETTER TO THE EDITOR

Evaluation and efficiency of curcumin against periodontal bacteria: an *in vitro* study

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To the Editor,

The effects of turmeric in the medical-scientific field are increasingly cited in literature. Turmeric has a long history as a medical herb within Ayurvedic medicine, and for its anti-inflammatory, antioxidant and antibacterial properties, it shows a therapeutic potential for various diseases (1-3). The aim of this study is to evaluate the effect of turmeric in the oral area, which has a very complex microbiome represented by 200 different organisms and 700 taxa. In recent years, there has been increasing interest in studies related to the oral microbiome which confirm the importance of oral health in the individual health. The correlations of the oral microbiome with other pathologies, such as fibrous cystic (fc), oral cavity tumors, esophageal tumors, lung tumors, prostate cancer, hematological tumors, colorectal cancer (CRC), rheumatoid arthritis and Alzheimer's disease, has been increasingly studied in the last decade, thanks also to the new analysis technologies.

The oral microbiome is something complex that interacts with the other microbiomes present in our body. The microbiome is made up of a sum of different microorganisms, and is a highly regulated ecosystem that involves different surfaces of the oral cavity (4, 5). Dysbiosis is an alteration of the homeostasis of the mouth, it can be determined by several factors such as oral hygiene, age, diet, smoking, hormonal changes and pathologies present in the body. In cases of dysbiosis, the balance of microbiome is interrupted leading to several alterations that may result in dental caries, gingivitis and periodontitis.

Gingivitis affects about 80% of the population and is due to the accumulation of bacterial plaque. The gold standard for its treatment is chlorhexidine. Chlorhexidine is a widely studied antiseptic, which has antimicrobial effects on gram-negative and gram-positive bacteria, fungi and viruses. In this study, the quantitative and qualitative profile of a sample of bacterial plaque were traced through molecular analysis. The aim of this study is to demonstrate how turmeric can be a possible bacteriostatic and bactericidal modulator in the oral cavity.

MATERIALS AND METHODS

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the CAST (Center for Advanced Studies and Technology)

Key words: curcumin; turmeric; oral health; bacteria; periodontal disease

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of Chieti. The patient was selected from the dental clinic of Chieti University. The patient gave consent for the study of the extracted genetic material.

Molecular genetic test

The molecular genetic test for the identification of the 11 bacterial species of periodontal-pathogenic bacteria (*aggregatibacter actinomycetemcomitans AA, porphyromonas gingivalis PG, prevotella intermedia PI, tannerella forsythia TF, treponema denticola TD, parvimonas micra PM, fusobacterium nucleatum FN, campylobacter rectus CR, eubacterium nodatum EN, eikenella corrodens EC, capnocytophaga CSP*) is highly specific (Fig. 1) (6). The test used I-Dent® plus11 (Hain Lifescience - Bruker) and provides data on the quality and quantity of 11 periodontal-pathogenic species and on their affiliation with "bacterial complexes". The test results represent an optimal control for an individual therapy for the support of mechanical therapy and possible antibiotic therapy. The test draws a threshold line between non-surgical periodontal treatment and antibiotic therapy. The threshold concentrations are:

AA	(+)	corresponding to $< 10^3$
PG	+	corresponding to 10^4
TF	+	corresponding to $< 10^5$
TD	+	corresponding to $< 10^5$
PI	+	corresponding to $< 10^5$
PM	++	corresponding to $< 10^6$
FN	+++	corresponding to $> 10^7$
CR	++	corresponding to $< 10^6$
EN	++	corresponding to $< 10^6$
EC	+++	corresponding to $> 10^7$
CS	+++	corresponding to $> 10^7$

DNA extraction of collected samples from bacterial plaque were taken using sterile paper cones. The cones were placed in a 1.5 ml tube, avoiding possible causes of contamination. A magnetic stir bar was added to the 5% Chelex 100 Resin solution in 10 mM Tris pH 8.4/8.5 and shaken on a magnetic stirrer. During the stirring, 100 µl of Chelex solution were removed using a 1000 µl pipette and then incubated for 15 min at 60°C in an ultrasonic bath and for 15 min at 100-105°C in a thermal block. After vortexing the lysate for 30 s, it was centrifuged for 1 min at 12,000 rpm in a benchtop centrifuge, and 2.5 µl of the supernatant was used for the amplification reaction. Two amplification reactions were prepared for each sample using the kit (AM-A1, AM-A2 and AM-B).

Broth culture of dental plaque

For the growth of an aliquot of periodontal bacteria a solution consisting of: urea broth enriched with mineral salts and yeast extract was used. The bacteria were collected directly from the patient using a sterile paper cone and placed in the culture broth and subsequently in the orbital incubator at 37°C overnight.

Preparation of the agar media

For the preparation of the agar media, 10 cm Petri dishes containing LB broth 1.5% agar were used. In particular, in 800 ml of distilled water were dissolved by stirring for 3 h at room temperature, 10 g of tryptone, 5 g of yeast extract and 10 g of NaCl. Subsequently the solution was brought to pH 7.2 dispensed in 100 ml autoclaved bottles for 1 h at 121°C 1.5 atm. For the solid culture plates, liquid LB was complemented by adding 1.5% of agar, in turn dissolved in a microwave oven.

Preparation of curcumin solution on media for evaluation in vitro

Firstly, 0.8 g of turmeric root was dissolved in 40 ml of 100% ethanol and left to stir overnight at 37°C. The following day the insoluble residue was left to settle and the ethanolic part containing curcumin was diluted in an equal volume (40 ml) tris mM, and DTA 1 mM,

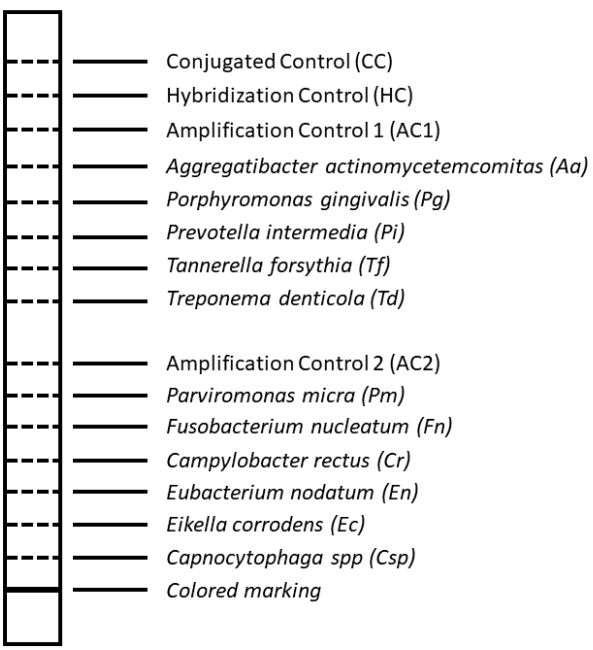


Fig. 1. *data strip*

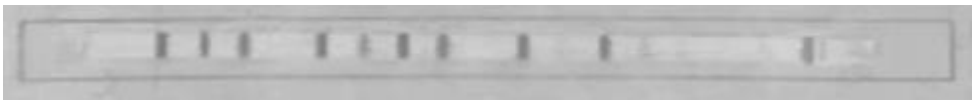
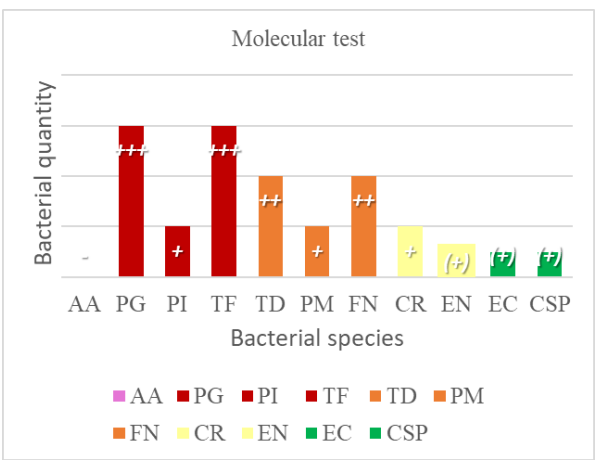


Fig. 2. *Strip of molecular test*

pH 7.8 in order to have a solution of 1% curcumin and 50% of ethanol. The agar plates supplemented with 100 μL of the curcumin solution were subsequently used, pre-adsorbing curcumin on the LB agar surface; after spreading, the plates were incubated for 30 min before bacterial sowing. Adequate controls of the LB agar plates were set up in control for the standard growth of the bacterial preparation. The experiment was conducted in triplicate for the analysis of the plaque. At the end of the preparation of the plates, an aliquot of the bacteria was plated with sterile loop, leaving them in an overnight incubator at 37°C.

RESULTS

The patient had 10 out of 11 bacterial species present in his oral cavity (Figs. 2, 3). More bacterial clusters were involved. Some bacterial strains exceeded the threshold value for antibiotic treatment



- = <10^4 (exception AA: 10^3)
(+) = 10^4 (exception AA: 10^3)
+ = <10^5 (exception AA: 10^4)
++ = <10^6 (exception AA: 10^5)
+++ = >10^7 (exception AA: 10^6)

Fig. 3. *Analysis of periodontal bacteria*

Table I. *Control group plates and added plate of 0.01% curcumin*

	Plate 1	Plate 2	Plate 3	Average
Control Group	28	32	40	33.3
Curcumin 0.1%	18	19	30	22.3
% bacterial inhibition	35.71%	40.63%	25%	33.03%

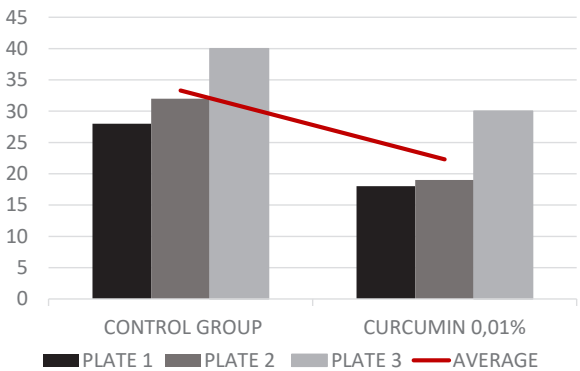


Fig. 4. *Bar graphs of control group and added plate of 0.1% curcumin*

as PG, TF and TD. These bacteria had a high pathogen load. After this analysis, the patient, was treated with mechanical therapy (scaling and root planing). The bacterial plaque sample was amplified with the described techniques, after conducting an *in vitro* study in triplicate. In the plates with the 0.01% curcumin solutions an inhibition of bacterial growth could be observed (Table I and Fig. 4) due to the properties of the curcuminoid polyphenols.

DISCUSSION

Numerous studies have been conducted on curcumin, and a PubMed research with the word

"curcumin" highlighted 15,326 articles. The properties of turmeric have been largely studied with findings also in the oral cavity. Mouthwashes, topical gels, subgingival irrigations, and sealants have been tested, and turmeric has also been used for the treatment of precancerous lesions (7, 8). In all these experiments, its properties have been demonstrated (9-11). Our intention was to also demonstrate its antibacterial properties. In this preliminary study we demonstrated that a 0.01% curcumin solution, prepared as previously described, can produce a bacteriostatic effect. Further studies are needed to observe a possible modulation of some specific bacterial strains, repeating a PCR analysis after the addition of the curcumin solution and repeating the PCR analysis several times in order to compare any bacterial modulations in the different phases of bacterial plaque formation in the oral cavity.

Turmeric, with its properties, could represent a valid support for the modulations of the dysbiosis of the oral microbiome. The *in vitro* result could anticipate the effect *in vivo*, however further in depth studies are needed to better understand the dynamics of curcumin as antibacterial and bactericidal mediator of the oral cavity.

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