

Development of a postbiotic film to prevent dysbiosis

Mariana Rebelo, Cláudia Oliveira, Freni Tavaría(*)

Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina - Laboratório associado, Escola Superior de Biotecnologia, Porto, Portugal.

(*) ftavaria@ucp.pt

INTRODUCTION

Dental caries represents the most predominant infection worldwide among oral diseases, with more than 3.5 billion people experiencing it at least once in their lifetime. (1) Oral health is highly dependent on the equilibrium of the oral microbiota. Such microbiota, when balanced, has the function of protecting the oral cavity. (2) However, oral diseases, namely caries, appear when this community shifts and dysbiosis happens. (3) In dysbiosis, some members of the microbiota start an overgrowth process that originates the formation of biofilms. The main bacteria found in dental biofilms are *Streptococcus*, *Actinomyces*, *Prevotella*, *Porphyromonas*, *Tannerella*, and *Fusobacterium* spp. (2) Notably, bacteria in biofilm are usually more resistant than in the planktonic state. Recent studies have been focusing on preventive treatments capable of re-establishing the oralbiota equilibrium and modulating the microbiome. (2)

Taking this into consideration, probiotics, prebiotics, and postbiotics are promising strategies, considering consumer awareness of health as well. (1)

Probiotics can have a positive effect on the microflora, (4) control the inflammatory response, (5) modulate the innate and adaptive immune response, and inhibit the production of cytokines by pathogens. (2)

Recently, more research has been focusing on the functions of cell-free supernatants, especially to circumvent the disadvantages and limitations of probiotic use. (6, 7) Postbiotics are described as inactivated microorganisms or their cell components and metabolites, that promote health when administered. (5, 7)

The impregnation of ODF with probiotics or postbiotics should be considered as a potential alternative to target oral dysbiosis, aiming to prevent oral diseases, such as caries or periodontitis, as well as for its antibiofilm capacity, reducing not only the virulence of common oral pathogens, but also the risk of acquiring antibiotic resistance genes.

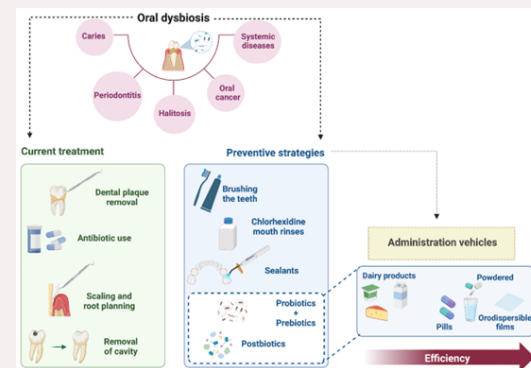


Figure 1 - Oral dysbiosis is the main factor contributing to the appearance of oral diseases. The current treatment is based on the removal of dental plaque, scaling and root planning for periodontitis, and cavity removal for caries. Recently, novel strategies have been gaining attention, namely the use of probiotics, prebiotics, and postbiotics. Several studies correlated the efficiency of these methods with the administration vehicles. Notably, dairy products are the least effective vehicles, and orodispersible films is the most effective.

METHODOLOGY

Lactiplantibacillus plantarum and *Lactocaseibacillus paracasei* were grown in MRS broth, centrifuged and filtered after 48h. The postbiotics obtained, CSF (cell-free supernatant), were diluted to different concentrations and co-incubated with *Streptococcus mutans*. The antimicrobial activity was assessed by plate counting. Additionally, the minimal inhibitory concentration and the time needed for *S. mutans* inhibition were evaluated. Antibiofilm capacity was determined by the crystal violet method. Finally, an orodispersible film based on polymers and plasticizers was developed to serve as an administration vehicle.

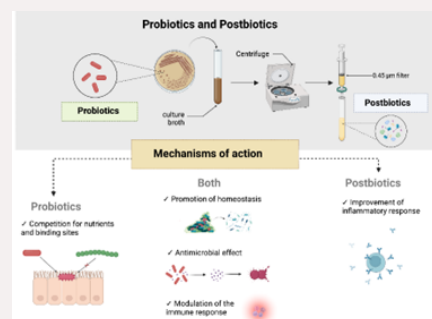


Figure 2 - Mechanism of action of probiotics and postbiotics. Postbiotics are product metabolites of probiotics, obtained after a cycle of centrifugation and a step of filtration with a membrane. They exert their functions as adjuvants of human health by different mechanisms, such as promoting homeostasis of oral microbiota, disrupting biofilm, and modulating the immune and inflammatory response. Probiotics also compete for nutrients and binding sites, which helps decrease pathobiont levels and produce molecules with antimicrobial effects. Notably, postbiotics also show antimicrobial effects, since they disrupt bacterial cell walls.

RESULTS

It was noted that there was no significant difference in the dissolution times of the 3 conditions tests (Figure 3), with all values being higher than 20min. After this, the pH was measured (Figure 5) and it could be observed that it dropped from 4,33 to 3,86 after impregnation with the postbiotics of *L. plantarum* ($p < 0,01$). However, when compared to the orodispersible film with postbiotics from *L. paracasei*, it was noted a pH value of 4,58 which is significantly higher than with the postbiotic from *L. plantarum* ($p < 0,001$).

The values for the contact angle can be observed in Figure 4. When compared to the control (54,6°), the orodispersible films of postbiotics from *L. plantarum* and from both bacteria are considerably different (39,7°, $p < 0,001$ and 44,7°, $p < 0,01$, respectively), meaning the orodispersible films increased their hydrophilicity after being impregnated with these postbiotics.

Another important factor considered was the film cytotoxicity (Figure 6), although there was no cytotoxicity noted in any of the conditions tested, all the orodispersible films with postbiotics had significantly lower viable cells after a 24 hour incubation period ($p < 0,05$).

Regarding the antimicrobial activity towards *S. mutans*, several assays were performed. Firstly, it was observed that the growth rate of the microorganism was significantly different in the presence of the postbiotic from *L. plantarum* ($p < 0,01$) (Figure 7). In the same way, the time-kill assay (Figure 8) performed with the different postbiotics demonstrated that after 2 hours the amount of *S. mutans* present in the sample drops significantly ($p < 0,05$).

Interestingly, when evaluating the minimal inhibitory volume (Figure 9) it was noted that a concentration of 10%(v/v) reduced significantly the growth of *S. mutans* overnight ($p < 0,01$).

Lastly, it is important to note that the postbiotics tested demonstrated antibiofilm capacity, (Figure 10), reaching almost 80% of biofilm inhibition in postbiotic concentrations higher than 60%.

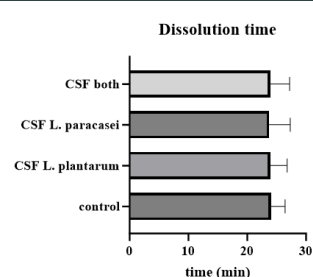


Figure 3 - Dissolution times, in minutes, of the orodispersible films impregnated with different postbiotics.

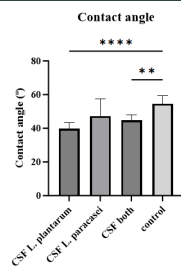


Figure 4 - Contact angles of the orodispersible films impregnated with different postbiotics.

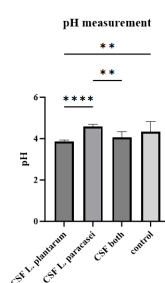


Figure 5 - pH values of the orodispersible films impregnated with different postbiotics.

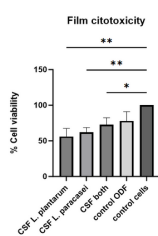


Figure 6 - Cytotoxicity values, based on the percentage of cell viability, of the orodispersible films impregnated with different postbiotics.

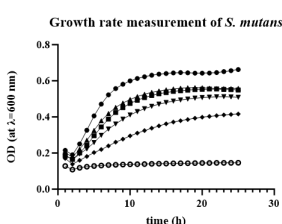


Figure 7 - Growth curve of *Streptococcus mutans* in co-culture with a postbiotic obtained from *Lactobacillus plantarum* in different concentrations.

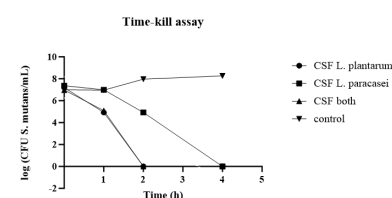


Figure 8 - CFU of *Streptococcus mutans* in co-culture with different postbiotic solutions obtained from *Lactobacillus plantarum* and *L. paracasei* over a period of 4 hours.

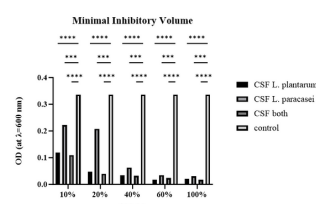


Figure 9 - Minimal inhibitory volume of the different postbiotics obtained from *L. plantarum* and *L. paracasei* co-cultured with *S. mutans* in different concentrations.

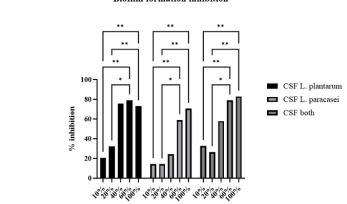


Figure 10 - Percentage of biofilm inhibition of *S. mutans* of the different postbiotics in different concentrations, co-cultured with *S. mutans*.

CONCLUSION

- 1- This study offered an overview of the potential of postbiotics to prevent oral dysbiosis, focusing on their antimicrobial and anti-biofilm activity.
 - 2- The postbiotics showed antimicrobial action against *S. mutans*.
 - 3- Orodispersible films with postbiotics from *L. plantarum* and *L. paracasei* displayed antibiofilm activity against *S. mutans* biofilm.
 - 4- The impregnation of postbiotics changed the physical characteristics of the orodispersible films, even though it has not precluded its use.
- Given the obtained results, orodispersible films impregnated with postbiotics should be considered as a potential alternative to target oral dysbiosis.

ACKNOWLEDGMENTS

Figure 1 and Figure 2 were created using Biorender.com. The authors acknowledge Biorender's support. The authors would like to thank the staff of the CBQF for their assistance in this project. This work was supported by National Funds from Fundação para a Ciência e a Tecnologia (FCT) through the project [UIDB/50016/2020]. CSO was supported by the Be@t - Textile Bioeconomy Project (Investment TC-C12-i01 - Sustainable Bioeconomy [02/C12-i01/202]) through an individual Junior Researcher contract.

REFERENCES

- (1) Hernández P, Sánchez MC, Llama-Palacios A, Ciudad MJ, Collado L. Strategies to Combat Caries by Maintaining the Integrity of Biofilm and Homeostasis during the Rapid Phase of Supragingival Plaque Formation. *Antibiotics*. 2022;11(7):880. <https://doi.org/10.3390/antibiotics11070880>
- (2) Urvashi, Sharma D, Sharma S, Pal V, Lal R, Patil P, Grover V, Korpole S. Bacterial Populations in Subgingival Plaque Under Healthy and Diseased Conditions: Genomic Insights into Oral Adaptation Strategies by *Lactobacillus* sp. Strain DISK7. *Indian journal of microbiology*. 2020;60(1):78-86. <https://doi.org/10.1007/s12088-019-00828-8>
- (3) Sánchez MC, Alonso-Español A, Ribeiro-Vidal H, Alonso B, Herrera D, Sanz M. Relevance of Biofilm Models in Periodontal Research: From Static to Dynamic Systems. *Microorganisms*. 2021;9(2):428. <https://doi.org/10.3390/microorganisms9020428>
- (4) Qiu W, Zhou Y, Li Z, Huang T, Xiao Y, Cheng L, Peng X, Zhang L, Ren B. Application of Antibiotics/Antimicrobial Agents on Dental Caries. *BioMed research international*. 2020;2020:5658212. <https://doi.org/10.1155/2020/5658212>
- (5) Zhao R, Hu H, Wang Y, Lai W, Jian F. Efficacy of Probiotics as Adjunctive Therapy to Nonsurgical Treatment of Peri-Implant Mucositis: A Systematic Review and Meta-Analysis. *Frontiers in pharmacology*. 2021;11:541752. <https://doi.org/10.3389/fphar.2020.541752>
- (6) Jastrzab R, Graczyk D, Siedlecki P. Molecular and Cellular Mechanisms Influenced by Postbiotics. *International journal of molecular sciences*. 2021;22(24):13475. <https://doi.org/10.3390/ijms222413475>
- (7) Scott E, De Paeppe K, Van de Wiele T. Postbiotics and Their Health Modulatory Biomolecules. *Biomolecules*. 2022;12(11):1640. <https://doi.org/10.3390/biom12111640>