Inhibition of Rothia Species by Over-the-Counter Products and Bacterial Antagonists

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Abstract
The interaction between the human host micro biome and over the counter products has recently been investigated, with surprising results. Some over the counter items may negatively affect the health of the host, supporting the concept of the “hygiene hypothesis”, that is, that disease may be actually caused by the lack of beneficial commensal bacteria. Recent reports on the gluten metabolizing genus, Rothia, and a possible association with Celiac Disease beg the question, what happened to the Rothia? In this study inhibitory factors, such as, Over The Counter oral hygiene products and antagonistic bacteria were investigated and, in vitro, significantly inhibited the gluten metabolizing bacteria, possibly affecting human digestion and contributing to gluten sensitivity.

Keywords: Gluten oral bacteria, Rothia mucilaginosa and Streptococcus salivarius.

Introduction
The human body is host to trillions of microorganisms, including bacteria, molds, yeasts, viruses and archaea. In addition, the contribution of the microbiome to human health has become thoroughly established with roles such as educating the immune response, resisting pathogens, and digestion. As a result, the human microbiome project was designed to ascertain the microbial composition of the entire human body. Meanwhile, the oral microbiome has been extensively determined and reported in the literature. The current reported microbiome of the oral cavity region contains 619 taxa, derived from 13 phyla [1-4].

An additional 36,043 gene clones have been sequenced, identifying an additional 434 unique oral taxa that (after further validation) may be added to the database. Amongst the oral strains sequenced to date, two important gluten metabolizing species, Rothia mucilaginosa and Rothia aeria have been identified [4,5]. R. mucilaginosa and R. aeria are of the Rothia genus under the phyla Actinobacteria. R. aeria was named after its isolation from air in the Russian space laboratory Mir and is an oral inhabitant [6,7].

R. mucilaginosa is primarily found in the oral cavity but has been reported in the upper respiratory tract and also the duodenum [8-11]. Interestingly, mucosal damage in celiac disease is mostly found in this area of the gastro-intestinal system [12]. Oral micro-organisms that in vitro degrade dietary proteins may mean that they play an in vivo role in food metabolism. During mastication, ingested food is mixed with stimulated whole saliva and oral micro-organisms. This process accelerates food digestion while the bolus is still churning in the oral cavity [13]. For example, nitrate reducing bacteria have been described as being indispensable in the production of nitric oxide which regulates blood pressure and cardiovascular health and this further emphasize the importance of the oral microbiome in systemic health [14-16]. A favorable and potential source for gluten-degrading enzymes would be the micro-organisms inhabiting the human gastrointestinal tract. It is well reported that bacteria residing in and on the human body supply the host with numerous functions that are not encoded by the human genome [17]. For instance, bacteria that colonize the large intestine ferment starches that are resistant to mammalian digestive enzymes [18].

In addition, it has been reported that human breast milk contains a number of oligosaccharides that are only digested by gut bacteria, not the breast-feeding child [19,20]. Therefore, recent publications that report gluten-degrading bacteria as natural residents of the oral cavity are not surprising after all [21,22]. This discovery is also very significant, since the oral cavity represents the gateway to the gastrointestinal system in which gluten is mixed with the oral microorganisms in human saliva. The finding of gluten-degrading oral microbes then begs the questions, what are they susceptible to and what common source may reduce the glut genetic metabolizers or decrease their effectiveness of gluten processing, leading to gluten “sensitivity”?

Objective
The purpose of this study was to determine if there is any inhibition of beneficial oral biofilm species such as Rothia aeria, R. mucilaginosa...
and *R. dentocariosa*, *Streptococcus mutans* (pathogen-negative control) and also *Lactobacillus reuteri* strains (isolated from periodontal Probioptic) by Over The Counter (OTC) oral antimicrobials utilizing *in vitro* laboratory technique. The secondary objective was to determine the antagonism, if any, of the *Rothia* genus by *Streptococcus* species (*mutans* and *salivarius*) and known pathogens. *Rothia aeria* and *R. mucilaginosa* are reported to be important in the processing of gluten. Inhibition of these beneficial bacteria by OTC products, either directly or indirectly, would increase gluten sensitivity in patients. Beneficial bacteria may be indirectly inhibited by certain antagonistic bacteria that are relatively less sensitive to OTC products.

**Methods**

**Susceptibility Experiment**

Three colonies of *R. aeria*, *R. dentocariosa*, *R. mucilaginosa*, *S. mutans*, or *Lactobacillus* were obtained from isolation plates and grown in Mueller-Hinton media to a McFarland Standard of 0.5. Either Brucella agar plates, Rogosa agar, or Mueller-Hinton agar plates with 5% sheep blood were wholly spread to create a lawn with one cotton swab inoculation of chosen target bacteria. Five cotton discs were evenly distributed on the plate and 10 microliters of full strength OTC reagent was pipetted directly onto each corresponding disc. The plates were evaluated after 30 hours of growth at 36°C. Calipers were used to measure zones of inhibition in millimeters.

**Results**

Bacterial growths of all tested bacteria were inhibited by Crest ProHealth™, ACT™, Listerine SmartRinse™, and Chlorhexidine. *R. aeria* and *R. mucilaginosa* were also inhibited by Embrace™ varnish (Table 1).

| Reagent                   | *R. aeria* on blood agar | *R. dentocariosa* on blood agar | *R. mucilaginosa* on blood agar | *perio probiotic (Lactobacillus)* on blood agar | *S. Mutans* on blood agar |
|---------------------------|--------------------------|---------------------------------|---------------------------------|---------------------------------------------|--------------------------|
| Spry™ Xylitol Mouthwash™  | 0.0                      | 0.0                             | 0.0                             | 0.0                                         | 0.0                      |
| Crest Prohealth™          | 9.9                      | 12.12                           | 11.11                           | 14.16                                       | 14.10                    |
| ACT fluoride rinse™        | 10.10                    | 11.12                           | 14.14                           | 16.14                                       | 16.15                    |
| Listerine Smartrinse™     | 9.9                      | 10.11                           | 9.9                             | 14.14                                       | 14.12                    |
| Chlorhexidine (11.6% alcohol) | 13.12                   | 18.18                           | 13.12                           | 14.14                                       | 16.15                    |
| Listerine™ (27% Alcohol)  | 0.0                      | 0.0                             | 0.0                             | 0.0                                         | 0.0                      |
| Phosphate Buffered Saline (PBS) | 0.0                     | 0.0                             | 0.0                             | 0.0                                         | 0.0                      |
| 27% Alcohol               | 0.0                      | 0.0                             | 0.0                             | 0.0                                         | 0.0                      |
| Embrace™ varnish™ (has xylitol) | 8.9                    | 0.0                             | 12.12                           | 0.0                                         | 0.0                      |
| Spry™ Xylitol toothpaste gel | 0.0                      | 0.0                             | 10.12                           | 0.0                                         | 0.0                      |
| 50% Spry™ Xylitol toothpaste gel in PBS | -            | 0.0                             | 0.0                             | -                                           | -                        |
| Levoflaxacin (5 micrograms) | 30                       | 33                              | 30                              | 36                                          | 20                       |

Table 1: Susceptibility Experiment: The effect of over the counter oral hygiene products on oral bacteria.

Spry™ Xylitol Toothpaste Gel inhibited *R. mucilaginosa, L. reuteri*, a probiotic that inhibits many oral and pathogens, was significantly inhibited by OTC oral products, except the xylitol based. Xylitol based oral products did not inhibit the commensal *S. salivarius* nor the pathogens, *S. aureus, E. coli* and *P. aeruginosa* (Table 2).

| S. aureus | *S. salivarius* | E.coli | P. aeruginosa | VRE |
|-----------|-----------------|-------|---------------|-----|
| Spry™ Mouthwash | 0               | 0     | 0             | 0   |
| Embrace™ varnish | 0               | 0     | 0             | 0   |
| Spry™ Xylitol Gel diluted in PBS | 0       | 0     | 0             | 0   |
| PBS control | 0               | 0     | 0             | 0   |

Table 2: Susceptibility Experiment: The effect of OTC oral hygiene products on other bacteria of the human flora.

Xylitol based oral products do inhibit many oral pathogens and have been extensively used in dentistry for decades. Growth of *P. aeruginosa* was inhibited by *R. dentocariosa* and growth of *S. aureus* was inhibited by *R. mucilaginosa*. The zones of inhibition by the gluten metabolizers were demonstrably large. The inhibition of the beneficial gluten metabolizers and probiotic bacteria by OTC oral products may have been the result of fluoride concentration. An alcohol based product, Listerine™, did not greatly inhibit the gluten metabolizers (Figure 1).

**Figure 1:** Example of Inhibition of pathogen *P. aeruginosa* by gluten metabolizer, *R. dentocariosa*.
Discussion

In vitro results are not always applicable to the clinical situation. Indeed, the complexity of the human oral microbiome would make it difficult to predict a response to any oral intervention with certainty. The results of the present study are of a pilot nature, a negative finding would mean that there is little need for further investigation. However, in vitro studies are always necessary before progressing into more extensive, time consuming, and financially demanding clinical studies. The mere fact that OTC products, sometimes used ad libitum by patients, contribute to a reduction in beneficial bacteria should be a concern to all health practitioners. Of greater interest should be the extent of inhibition, as the zones of inhibition were quite significant in diameter. The average diameter of inhibition with an OTC product was 13 mm [Range: 5-18 mm] (Figure 2).

The mode of inhibition should be discovered, as it appears that the fluoride concentration of the OTC products may have been contributory. An alcohol based product was only inhibitory of the probiotic in this study, and not the gluten metabolizers. With de...

Another very important aspect of this study was the interaction between pathogenic and beneficial bacteria. The interaction, or rather, the inhibition of different bacterial species actually determines the health of the host and as such, is paramount in importance. The results were significant in that growth of Rothia species was inhibited by other bacteria. This suggests that if the oral flora equilibrium is changed by using OTC oral hygiene products, a domino effect can change the entire oral microbiome, which is the gateway to the digestive tract. The gastric microbiome is now recognized as a vital component of the host’s health, both mental and physical. Increased oversight concerning the over uses of anti-microbial, food preservatives that are also anti-microbial, and OTC products that inhibit commensal bacteria, is essential.

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Conclusion

Rothia and Lactobacillus species may be decreased in quantity by the overuse of oral antimicrobials. OTC products may alter the oral microbiome creating a situation less conducive for the survival of essential beneficial bacteria. The use of OTC products may decrease the enzymatic degradation of gluten containing foods by Rothia bacteria. This can possibly result in gluten sensitivity, thereby increasing the clinical prevalence of celiac disease. Further studies are required before any clinical implications may be concluded, but oral antimicrobials should be used only when necessary.

References

1. Camp JG, Kanther M, Semova I and Ravls JF. Patterns and scales in gastrointestinal microbial ecology (2009) Gastroenterol 136: 1989-2002. https://doi.org/10.1053/j.gastro.2009.02.075
2. Ley RE, Peterson DA and Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine (2006) Cell 124: 837-848. https://doi.org/10.1016/j.cell.2006.02.017
3. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, et al. The human microbiome project (2007) Nature 449: 804-810. https://doi.org/10.1038/nature06244

Citation: Mark LC, Kabat B, Yopev R, Jantra L, Muhammad A, et al. Inhibition of Rothia species by over-the-counter products and bacterial antagonists (2020) Edelweiss Appli Sci Tech 4: 5-8.
4. Chen T, Yu WH, Izard J, Baranova OV, Lakshmanan A, et al. The Human Oral Microbiome Database: a web accessible resource for investigating oral microbe taxonomic and genomic information (2010) Database (Oxford) 2010: baq013. https://doi.org/10.1093/database/baq013
5. Dewhurst FE, Chen T, Izard J, Paster BJ, Tanner AC, et al. The human oral microbiome (2010) J Bacteriol 192: 5002-5017. https://doi.org/10.1128/jb.00542-10
6. Wei G, Zamkhchari M, Dewhurst F, Schuppan D, Oppenheim F, et al. Isolation and Characterization of Gluten Degrading Bacteria From the Oral Cavity (2011) Gastroenterol 140: S-641. https://doi.org/10.1016/j.gaster.2011.06.056-5
7. Li Y, Kawamura Y, Fujiwara N, Naka T, Liu H, et al. Rothia aeria sp. nov., Rhodococcus baikourensis sp. nov. and Arthrobacter rassicus sp. nov., isolated from air in the Russian space laboratory Mir (2004) Int J Syst Evol Microbiol 54: 827-835. https://doi.org/10.1099/ijs.0.02828-0
8. Kazor CE, Mitchell PM, Lee AM, Stokes LN, Loesche WJ, et al. Diversity of bacterial populations on the tongue dorsa of patients with halitosis and healthy patients (2003) J Clin Microbiol 41: 558-563. https://doi.org/10.1128/CMI.41.2.558-563.2003
9. Collins MD, Hutson RA, Baverud V and Falsen E. Characterization of a Rothia-like organism from a mouse: description of Rothia nasimurium sp. nov. and reclassification of Stomatococcus mucilaginosus as Rothia mucilaginosa comb. Nov (2000) Int J Syst Evol Microbiol 50 Pt 3: 1247-1251. https://doi.org/10.1099/ijs.0.02971-0
10. Zaura E, Keijser BJ, Huse SM and Crielard W. Defining the healthy “core microbiome” of oral microbial communities (2009) BMC Microbiol 9: 259. https://doi.org/10.1186/1471-2180-9-259
11. Ou G, Hedberg M, Horstedt P, Baranov V, Forsberg G, et al. Proximal small intestinal microbiota and identification of rod-shaped bacteria associated with childhood celiac disease (2009) Am J Gastroenterol 104: 3058-3067. https://doi.org/10.1111/j.1572-0241.2009.30673.x
12. Bonamico M, Mariani P, Thanesi E, Ferri M, Nenna R, et al. Patchy villous atrophy of the duodenum in childhood celiac disease (2004) J Pediatr Gastroenterol Nutr 38: 204-207. https://doi.org/10.1097/00055176-200402001-00019
13. Kodukula K, Faller DV, Harpp DN, Kanara I, Pernokas J, et al. Gut microbiota and salivary diagnostics: The mouth is salivating to tell us something (2017) BioRes open access 6: 123-132. https://doi.org/10.1089/biores.2017.0020
14. Hyde ER, Andrade F, Vaksman Z, Parthasarathy K, Jiang H, et al. Metagenomic analysis of nitrate-reducing bacteria in the oral cavity: implications for nitric oxide homeostasis (2014) PLoS one 9: e88645. https://doi.org/10.1371/journal.pone.0088645
15. Koopman JE, Buijs MJ, Brandt BW, Keijser BJ, Crielard W, et al. Nitrate and the origin of saliva influence composition and short chain fatty acid production of oral microcosms (2016) Microbial Ecol 72: 479-492. https://doi.org/10.1007/s00248-016-0775-z
16. Kapijl V, Haydar SM, Pearl V, Lundberg JO, Weitzberg E, et al. Physiological role for nitrate-reducing oral bacteria in blood pressure control (2013) Free Radical Biol Med 55: 93-100. https://doi.org/10.1016/j.freeradbiomed.2012.11.013
17. Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, et al. Metagenomic analysis of the human distal gut microbiome (2006) Science 312: 1355-1359. https://doi.org/10.1126/science.1124234
18. Hooper LV, Midvind T and Gordon JI. How host-microbial interactions shape the nutrient environment of the mammalian intestine (2002) Annu Rev Nutr 22: 283-307. https://doi.org/10.1146/annurev.nutr.22.011602.092259
19. Kirmiz N, Robinson RC, Shah IM, Barile D and Mills DA. Milk glycans and their interaction with the infant-gut microbiota (2018) Annu Rev Food Sci Techno 19: 429-450. https://doi.org/10.1146/annurev-food-030216-030207
20. Moosavie S, Atakora F, Miliku K, Sepehrli S, Robertson B, et al. Integrated analysis of human milk microbiota with oligosaccharides and fatty acids in the CHILD cohort (2019) Frontiers Nutri 6: 58. https://doi.org/10.3389/fnut.2019.00058
21. Helmerhorst EJ, Zamakhchari M, Schuppan D and Oppenheim FG. Discovery of a novel and rich source of gluten-degrading microbial enzymes in the oral cavity (2010) PLoS One 5: e13264. https://doi.org/10.1371/journal.pone.0013264
22. Tian N, Fuller L, Jeffler DA, Kelly CP, Hansen J, et al. Salivary gluten degradation and oral microbial profiles in healthy individuals and celiac disease patients (2017) App Environ Microbiol 83: e03330-16. https://doi.org/10.1128/aem.03330-16