Inhibition of Autism Spectrum Disorder Associated Bacteria and *C. difficile* by Polyols

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Abstract

Objectives: To determine the effectiveness of erythritol and xylitol in the inhibition of gut bacteria possibly associated with Autism Spectrum Disorder (ASD) and *Clostridium difficile* Infection (CDI).

Methods: Seven bacterial strains associated with ASD, or with CDI and a control probiotic were tested for polyol inhibitory activity: *Clostridium histolyticum*, *Bacteroides vulgatus*, *Bifidobacterium longham*, and two strains each of *Clostridium bolteae* and *difficile*. Each strain was grown in brain heart infusion/sucrose media with polyol concentrations varying from 0% to 15% for erythritol and 0% -30% for xylitol. Growth of *Clostridium histolyticum* and *Bifidobacterium longham* was measured after 24 hours while all other strains were evaluated at 48 hours to permit additional growth. Optical density was measured using a spectrophotometer and the plates were read at 620 nm.

Results: All strains had results indicating polyol inhibition of growth. *Clostridium histolyticum* (Chis), *Bifidobacterium longham* (Blof), and both *Clostridium bolteae* (Cbol) strains showed reduced growth with increasing polyol concentration with an inflection point of about 4% for both xylitol and erythritol (complete or near complete inhibition relative to control wells). *Bacteroides vulgatus* (Bvol) grew very lightly in the BHI/sucrose. This strain has visible growth but very low OD values. Inhibition of growth with increasing polyol concentrations was observed but assessing the polyol inhibition break point was difficult with this strain.

Conclusions: Xyliol and erythritol at sufficient concentrations were able to inhibit the growth of bacterial strains that have been associated with the development of Autism Spectrum Disorder in recently published studies.

Keywords: Autism Spectrum Disorder, polyol, Bacterial strains, Optical density.

Abbreviations: ASD- Autism Spectrum Disorder, CDI - *Clostridium Difficile* Infection, Cbol - *Clostridium bolteae*.

Introduction

Polyols have been used for decades as a substitute for sucrose [1-4]. The most commonly used polyols for consumption are sorbitol, mannitol, xylitol, erythritol, maltitol, lactitol and isomalt [5]. Besides having fewer calories than regular sucrose, i.e., table sugar, polyols have other reported health benefits, especially in regards to oral health [6]. Well publicized studies showing the effectiveness of xylitol at reducing dental disease have been reported for decades, all with results demonstrating safety and effectiveness [7-9]. The well-publicized “Turku” and the “Belize” studies reported on the caries reduction by xylitol, with xylitol being more effective than sorbitol [10,11]. Xylitol chewing gums, toothpastes, lollipops, candies and mouth rinses are all part of a complete dental oral hygiene program [12].

Erythritol and xylitol are polyols that repeatedly have been demonstrated to possess anti-cariogenic and anti-periodontal disease properties [13]. Polyols (particularly the non-hexitol alditols or “sugar alcohols” erythritol and xylitol) have been found effective in inhibiting the transition to and maturation of biofilms from planktonic cells [14]. Xylitol clearly inhibits the formation of mixed species biofilms, in vitro [15].

Erythritol suppresses the maturation of biofilms and contributed to a healthier oral ecosystem [16]. Polyols can suppress the growth and virulence expression of mixed bacterial biofilms. Erythritol was the most effective polyol in suppressing the growth and organization of dental pathogens. Erythritol also exerted inhibitory effects on several pathways reduced growths through DNA and RNA depletion, attenuated extracellular matrix production and alterations of dipeptide acquisition and amino acid metabolism [17]. The bacteria associated with Autism Spectrum Disorder have been reported in the literature, with similar results independent of research institution and locality [18]. Autism Spectrum Disorder (ASD) has been linked to propionic acid producing bacterial species, such as, *Clostridia bolteae* and *C. histolyticum* [19-22].

Conversely the presence of Clostridia sporogenes could help protect against ASD by combining propionic acid with indole to produce 3-Indole Propionate, a neural protective metabolite, thereby neutralizing the epigenetic effect of propionic acid [23-25]. It has been theorized that the absence of *C. sporogenes* in the soil is related to the use of glyphosate, known by the trade name Roundup [18]. Absence of *C. sporogenes* in the soil and the environment could possibly shift the maternal microbiome, resulting in epigenetic changes in the fetus or...
infant. Bacteroides vulgatus also has been implicated in ASD as reported in the Frontiers in Microbiology by Coretti et al. [26].

Clostridia difficile (Cdif) is a gram-positive bacterium that is implicated in antibiotic-associated diarrhea. The relatively recent emergence of a newer hyper-virulent North American strain (NAP1) has been associated with the increase in incidence and severity of C. difficile infections (CDI) over the last decade [27]. Antibiotic overuse remains the leading risk factor for C. difficile infection. Several classes of antibiotics such as penicillins, cephalosporins, fluoroquinolones, and clindamycin have been implicated in causing CDI.

Besides antibiotic usage, other risk factors are reported to include advanced age, chemotherapy, use of proton pump inhibitors, chronic renal disease, chronic liver disease and malnutrition [28,29]. Treatment options include discontinuing the causative antibiotic and administering either vancomycin or fidaxomicin. Another option is fecal transplantation, the process in which feces from a person with the disrupted microbial balance are transplanted into the intestinal tract of a person with the disrupted microbial balance. This protocol has reported an 80% to 90% success rate in reducing the recurrence of C. difficile infections [30]. There remains some opposition to Fecal Transplantation Therapy due to the basic nature of the procedure and potential complications [31]. A simpler, safer and “cleaner” technique would be more appealing to patients and clinicians.

Materials and Methods

Bacterial isolates and media: C. bolteae and C. histolytica strains were kindly provided by Dr. Emma Allen-Verco PhD. (University of Guelph/Canada). B. vulgatus (8482) and B. longum (15707) were obtained from the American Type Culture Collection (ATCCC/Manassas Va.). C. difficile strains 5555 and 5557 were provided by Dr. Larry Kociolek MD (Lurie Children’s Hospital, Chicago, IL). All studies used a basal media of Brain Heart Infusion broth supplemented with 2% sucrose (BHI/Suc). Polysols were prepared separately at high concentrations in BHI/Suc for assay plate preparations. Xylitol was added to 60% (w/v) and Erythritol was prepared at 30% (w/v) in BHI/Suc. These polysol levels were the maximum achievable based on solubility. Final media preparations were sterilized and placed in an anaerobic chamber for at least 2 hours after preparation to cool and remain in a reduced state.

Assay Procedures

Assays were prepared in the anaerobic chamber. 96 well plates were employed with each test preparation in triplicate wells by adding 100 mcL of BHI/Suc at 2x concentration to all test wells. Bacterial preparations were made in BHI/Suc adjusted to a Macfarland standard concentration of 0.5. Final assay inocula of each strain with a further 1:100 fold dilution. 100 mcL of bacterial inocula was added to each test well with or without a polysol. Plates were incubated anaerobically for 24 or 48 hours and terminated when bacterial growth reached a visible level in control wells. Plate were then transferred to a plate spectrophotometer and read at 620 nm wavelength. Mean OD values for each well were calculated and OD values vs. polysol concentration were plotted.

Results

Seven strains were tested for polysol inhibitory activity C. histolyticum, B. vulgatus, C. bolteae (x2), C. difficile (x2), and Bifidobacterium longam. All strains grew to variable bacterial density levels. B. vulgatus had the poorest growth but still had measurable mean OD values to suggest polysol activity. Detailed OD values vs. polysol concentration are plotted as follows with relative inhibition inflection points (Figures 1-7).
Erythritol inhibits ASD bacteria at a lower concentration than xylitol. Both polyols were capable of significant inhibition of the ASD associated bacteria, in addition to the inhibition of antibiotic resistant C. diff strains. Erythritol may inhibit Bacteroides vulgatis better than xylitol but additional studies with a more optimal media for B. vulgatis need to be performed.

**Discussion**

Erythritol inhibits ASD bacteria at a lower concentration than xylitol. Both polyols were capable of significant inhibition of the ASD associated bacteria, in addition to the inhibition of antibiotic resistant C. diff strains. Erythritol may inhibit Bacteroides vulgatis better than xylitol but additional studies with a more optimal media for B. vulgatis need to be performed.

**Conclusion**

Xylitol and erythritol at sufficient concentrations were able to inhibit the growth of bacterial strains that have been associated with the development of ASD. Further research into the use of polyols for the treatment and possible prevention of ASD is recommended. Large clinical trials with patients that are correctly diagnosed with ASD then treated with xylitol supplementation and the resultant effects on behavior should be carefully explored. In addition, the uses of polyols to treat C. difficile infections also require clinical trials.

**References**

1. Horecker BL, Lang K, Takagi Y. International symposium on metabolism, physiology and clinical uses of pentoses and pentitols (1969) Springer-Verlag, Berlin, Germany.
2. Sipple HL, McNutt KW. Sugars in Nutrition (1974) Academic Press, New York, USA.
3. Hefferen JJ, Koehler HM. Foods, Nutrition and Dental Health (1981) Pathotox Publishers, Park Forest South, IL, USA.
4. Rugg-Gunn J. Sugarless, the Way Forward: Proceedings of an International Symposium (1991) Elsevier Applied Science, London, UK.
5. Rice T, Zannini E, Arendi EK, Coffey A. A review of polysols - biotechnological production, food applications, regulation, labeling and health effects (2019) Crit Rev Food Sci Nutr Pp: 1-18. https://doi.org/10.1080/10408398.2019.1625859

6. Scheibe OB, Fejerskov. Xylitol in caries prevention: what is the evidence for clinical efficacy? (1998) Oral Dis 4.

7. Edgar WM. Sugar substitutes, chewing gum and dental caries - a review (1998) British Dental Journal 184: 29-32.

8. Mandel D. Caries prevention – current strategies, new directions (1996) J American Dental Association 127: 1477-1488.

9. Trahan L. Xylitol: a review of its action on mutants streptococci and dental plaqu - its clinical significance (1995) The International Dental Journal 45: 77-92.

10. Scheinin A, Mäkinen KK, Kalevi Y. Turku sugar studies. V. Final report on the effect of sucrose, fructose and xylitol diets on the caries incidence in man (1976) Acta odontologica Scandin 34: 179-216. https://doi.org/10.3109/00016357608997711

11. Mäkinen KK, Bennett CA, Hujol PP. Xylitol chewing gums and caries rates: a 40-month cohort study (1995) J Dent Res 74: 1904-1913. https://doi.org/10.1177/00220345950740121501

12. Cannon ML, and Peldyak JN. The prevention and treatment of neural arterial gingival simplex (2019) Dental Res Manag 3: 32-37. https://doi.org/10.33805/dt272-6978.123

13. Sánchez MC, Romero-Lastra P, Ribeiro-Vidal H, Llama-Palacios A and Figueroa E. Comparative gene expression analysis of planktonic Porphyromonas gingivalis ATCC 33277 in the presence of a growing biofilm versus planktonic cells (2019) Clin Infection BM Microbiol 19: 58. https://doi.org/10.1186/s12866-019-1423-9

14. Badet C, Furiga A and Thébaud N.Effect of xylitol on an in vitro model of oral biofilm (2008) Oral Health Prev Dent 6: 337-341.

15. Janus MM, Volgenant CMC, Brandt BW, Buigs MJ, Keijser BJF, et al. Effect of erythritol on microbial ecology of in vitro gingivitis biofilms (2017) J Oral Microbiol 9: 1. https://doi.org/10.1080/20029727.2017.1337477

16. Hashino E, Kubonishi MA, Alghamdi SA, Yamaguchi M, Yamamoto R, et al. Erythritol alters microstructure and metabolic profiles of biofilm composed of Streptococcus gordonii and Porphyromonas gingivalis (2013) Mol Oral Microbiol 28: 435-451. https://doi.org/10.1111/omi.12037

17. Janakiram C, Deepan Kumar CV and Joseph J. Xylitol in preventing dental caries: a systematic review and meta-analyses (2017) J Nat Sci Biol Med 8: 16-21. https://doi.org/10.4103/0976-9668.198344

18. Argou-Cardozo I and Zeidán-Chulil F. Clostridium Bacteria and Autism Spectrum Conditions: A Systematic Review and Hypothetical Contribution of Environmental Glyphosate Levels (2018) Med Sci (Basel) 6: 29. https://doi.org/10.3390/medsciences06020029

19. MacFabe DF, Cain DP, Rodriguez-Capote K, Franklin AE, Hoffman JE, et al. Neurobiological effects of intraventricular propionic acid in rats: possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders (2007) Behav Brain Res 176: 149-169. https://doi.org/10.1016/j.bbr.2006.07.025

20. Shultz SR, MacFabe DF; Ossenkopf KP, Scratch S, Whelan I, et al. Intracerebroventricular injection of propionic acid, an enteric bacterial metabolic end-product, impairs social behavior in the rat: implications for an animal model of autism (2008) Neuropharmacology 54: 901-911. https://doi.org/10.1016/j.neuropharm.2008.01.013

21. Shultz SR, MacFabe DF, Martin S, Jackson J, Taylor R, et al. Intracerebroventricular injections of the enteric bacterial metabolic product propionic acid impairs cognition and sensorimotor ability in the Long-Evans rat: further development of a rodent model of autism (2009) Behav Brain Res 200: 33-34. https://doi.org/10.1016/j.bbr.2008.12.023

22. MacFabe DF, Cain NE, Boon F, Ossenkopf KP and Cain DP. Effects of the enteric bacterial metabolic product propionic acid on object-directed behavior, social behavior, cognition, and neuroinflammation in adolescent rats: Relevance to autism spectrum disorder (2011) Behav Brain Res 217: 47-54. https://doi.org/10.1016/j.bbr.2010.10.005

23. Rose S, Bennuri SC, Davis JE, Wynne R, Slattery JC, et al. Butyrate enhances mitochondrial function during oxidative stress in cell lines from boys with autism (2018) Translational Psychiatry 8: 42. https://doi.org/10.1038/s41373-017-0089-z

24. Wikoff WR, Andóta A, Liu J, Schultz PG, Lesley SA, et al. Metabolomics analysis reveals large effects of gut microbiota on mammalian blood metabolites (2009) Proceedings of the National Academy of Sciences of the United States of America, USA 106: 3698-3703. https://doi.org/10.1073/pnas.0812874106

25. Parthasarathy A, Cross PJ, Dobson R, Adams LE, Savka MA, et al. A Three-Ring Circus: Metabolism of the three proteogenic aromatic amino acids and their role in the health of plants and animals (2018) Front Mol Biosci 5: 29. https://doi.org/10.3389/fmolb.2018.00029

26. Cozetti L, Paparo L, Riccio MP, Amato F, Cuomo M, et al. Gut microbiota features in young children with autism spectrum disorders (2018) Frontiers in microbiology 9: 3146. https://doi.org/10.3389/fmicb.2018.03146

27. See I, Mu Y, Cohen J, Beldavs ZG, Winston LG, et al. NAPI strain type predicts outcomes from Clostridium difficile infection (2014) Lancet Infect Dis 14: 1389-1400.

28. Khanaf N, Vanhems P, Barbut F, Luxemburger C, CDI01 Study group, et al. Factors associated with Clostridium difficile infection: A nested case-control study in a three year prospective cohort (2017) Anaerobe 44: 117-123.

29. Arriola V, Tischendorf J, Musuza J, Barker A, Rozelle JW, et al. Assessing the risk of hospital-acquired clostridium difficile infection with proton pump inhibitor use: a meta-analysis (2016) Infect Control Hosp Epidemiol 37: 1408-1417.

30. Kassam Z, Lee CH, Hunt RH. Review of the emerging treatment of Clostridium difficile infection with fecal microbiota transplantation and insights into future challenges (2014) Clin Lab Med 34: 787-798.

31. van Beurden YH, de Groot PF, van Nood E, Nieuwdorp M, Keller JJ, et al. Complications, effectiveness, and long term follow-up of fecal microbiota transplantation treatment in recurrent Clostridium difficile infection (2017) European gastroenterology journal 5: 868-879. https://doi.org/10.1177/2050646616678099

32. Macfabe DF. The role of enteric bacterial metabolites in mitochondrial dysfunction in autism – from animal models to human population. Microb Ecol Health Dis. 2013; 2863-2868.

33. Midvedt T. The gut: a triggering place for autism – possibilities and challenges (2012) Microb Ecol Health Dis. 23. https://doi.org/10.3402/mehd.v23i0.18982

34. Mangioli F, Iaino G, Franceschi F, Fagiulini S, Gasbarrini G, et al. Gut microbiota in autism and mood disorders (2016) World J Gastroenterol 361-368. https://doi.org/10.3748/wjg.v22.i36.1417

35. Horvath K, Papadimitriou JC, Rabszyn A, Drachenberg C, Tildon JT. Gastrointestinal abnormalities in children with autistic disorder (1999) J Pediatr 135: 559-563.

36. Dengate S and Ruben A. Controlled trial of cumulative behavioural effects of a common bread preservative (2002) J Paediatrics and child health 38: 373-376. https://doi.org/10.1046/j.1440-1770.2002.00009.x

37. Kang DW, Adams JB, Coleman DM, Pollard EL, Maldonado J, et al. Long-term benefit of Microbiota Transfer Therapy in autism symptoms and gut microbiota (2019) Scientific reports 9: 5821. https://doi.org/10.1038/s41598-019-42183-0

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