Analysis of Probiotic Candidates' Suppression of *Candida albicans* Mediated Inflammation

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Abstract

Purpose: *Candida albicans* has been linked to oral diseases ranging from dental caries to oral cancer. It appears to enhance the cariogenicity of the dental pathogen *Streptococcus mutans*, and it's also associated with pro-inflammatory changes in the oral microbiota. The goal of this project was to test probiotic candidates, previously isolated from children with a caries-free history and partially screened for properties related to caries prevention, for inhibition of *C. albicans* and suppression of inflammation.

Methods: *C. albicans* was grown in the presence of probiotic candidates in addition to health and disease associated controls to evaluate inhibition of *C. albicans* and to determine their influence on hyphae formation. Candidates were assayed using quantitative polymerase chain reaction (QPCR) to quantify relative expression of potential oncogenesis-related inflammatory cytokines, transcription factors/genes and enzymes in co-cultures with two squamous cell carcinoma cell lines and epithelial cell models.

Results: None of the candidate strains tested had the ability to inhibit the growth of *C. albicans*; however, four of nine candidates displayed the ability to reduce hyphae formation or coexist with *Candida* without inducing increased hyphae formation indicating minimal or reduced stress response from *C. albicans*. QPCR revealed two candidates, A2 and A3, significantly decreased expression of BCL3, BIRC3, JUN, IFIT3, SERPINE1, MMP-1, IL-6 and IL-8 compared to cultures of *C. albicans* alone.

Conclusion: Certain bacterial strains from children with a caries free dental health history have the potential to suppress chronic inflammation which may aid in the prevention of oral disease associated with *C. albicans*.

Background & Objectives

- *Candida albicans* has been linked to oral diseases ranging from dental caries to oral cancer
- Probiotic strains may directly inhibit the growth of oral pathogens or indirectly promote oral health in other ways
- Aim 1: Testing for the inhibition of oral pathogen growth in the presence of candidate probiotic strains obtained from children with a caries-free dental health history
- Aim 2: Testing for *Candida albicans'* stress response in the presence of candidate probiotic strains
- Aim 3: Testing effects of candidate probiotic strains on expression of pro-inflammatory cytokines as well as carcinogenesis promoting genes and transcription factors in cell co-cultures
- Hypothesis: Bacterial strains isolated from children with a caries free history will be a source of probiotic candidates that inhibit the growth of oral pathogens, suppress chronic inflammation, or reduce expression of carcinogenesis promoting genes and transcription factors

Materials and Methods

Strain Preparation

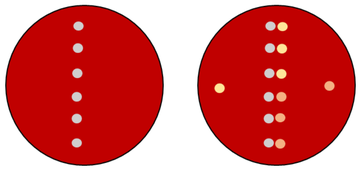
Culture:

- Previously isolated bacterial strains from children with caries free history
- Blood agar plates

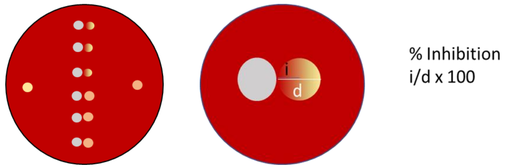
Suspension:

- PBS buffer
- Spectrophotometer to measure turbidity
- OD = 0.100 A +/- 0.005
- 7µl inoculum

Day 1: Initial Inoculation Day 2: Secondary Inoculation



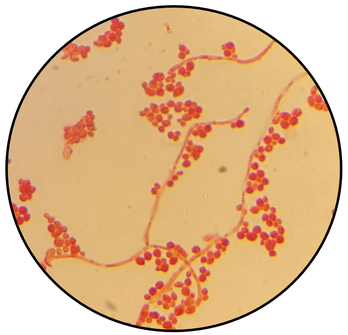
Day 3: Results Zone of Inhibition Measurement



% Inhibition
i/d x 100

C. albicans cultured in the presence of probiotic strains

- Stained and observed under microscope at 1000x
- Observe change in hyphae levels compared to control



C. albicans exhibiting yeast and hyphae forms

QPCR for three carcinogenesis promoting proteins associated with *C. albicans*

- BCL1, ECE1, ADH1

Co-culture with probiotic candidate strains and/or *C. albicans* and/or cancer cells

- SCC 193, 180 & GMSMK cell lines

QPCR

- Direct-zol™ RNA MicroPrep kit from Zymo Research
- Primers for specific cytokines, transcription factors and genes associated with chronic inflammation and/or oropharyngeal cancer
  - ATF3, BCL3, BICR3, JUN, IFIT3, SERPINE1, MMP-1, IL-1b, IL-6, IL-8

Results

Aim 1 & 2:

Candidate strain effects on C. albicans growth, hyphae formation and gene expression

Test Strain	Inhibition (Y/N)	ZOI %	CA Hyphae Change	CA Co-Aggregation	BCR1	ECE1	ADH1
H2	N	0	+	49%			
H3	N	0	+	58%		+	
H4	N	0	+	53%			
H5	N	0	+	67%			+
A1	N	0	Same	49%	-		
A2	N	0	-	52%	-	-	
A3	N	0	-	55%	-		
A4	N	0	-	64%			
A5	N	0	-	65%			
SM	N	0	Same	56%	-		
SSG	N	0	+	53%	-		

+ means increased significantly
- means decreased significantly

Aim 3

Candidate strain effects on *C. albicans* influence on human cell line gene expression in co-culture

	CA	A2	A3	CA+A2	CA+A3
ATF3	1+	2-	2-	2-	2-
BCL3	1+			1-,2-	1-,2-
BIRC3					
JUN	1+	2-	1+,2-	1+,2-	1+,2-
IFIT3	1+	2-	2-	2-	2-
SERPINE1	1+	2-	2-	2-	2-
MMP-1	1+	1+,2-	1+,2-	1+,2-	2-
IL-1b	1+	2-	2-	2-	2-
IL-6	1+	2-	2-	2-	2-
IL-8	1+	2-	2-	2-	2-

1 means comparing to control group (cell line basal expression)
2 means comparing to Candida group (CA)
+ means increased significantly
- means decreased significantly

Conclusions

- Although none of the candidates inhibited the growth of *C. albicans*, some reduced hyphae formation of *C. albicans* which may indicate a reduced stress response in the presence of probiotic candidates A2, A3, A4, and A5.
- Candidates A2 and A3 suppressed *C. albicans* induced gene expression in SCC and GMSMK cell line co-cultures (8/10 factors suppressed expression) which supports the hypothesis
  - This may be beneficial in helping deflect or prevent *C. albicans* effects in promoting inflammation and cancer development
- No strain gave the desired results in every test and therefore we suggest the most effective strategy may be to use a combination of strains with varying beneficial phenotypes

References

1. Allaker RP, Stephen AS. Use of Probiotics and Oral Health. *Curr Oral Health Rep.* 2017; 4(4):309-318. doi:10.1007/s40496-017-0159-6
2. Ayuningtyas NF, Mahdani FY, Pasaribu TAS, et al. Role of *Candida albicans* in Oral Carcinogenesis. *Pathophysiology*. 2022;29(4):650-662. Published 2022 Dec 7. doi:10.3390/pathophysiology29040051
3. Chen C, Méndez E, Houck J, et al. Gene expression profiling identifies genes predictive of oral squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev.* 2008;17(8):2152-2162. doi:10.1158/1055-9965.EPI-07-2893
4. Hoppe T, Kraus D, Novak N, et al. Oral pathogens change proliferation properties of oral tumor cells by affecting gene expression of human defensins. *Tumour Biol*. 2016;37(10):13789-13798. doi:10.1007/s13277-016-5281-x
5. Xiao J, Huang X, Alkhers N, et al. Candida albicans and Early Childhood Caries: A Systematic Review and Meta-Analysis. *Caries Res*. 2018;52(1-2):102-112. doi:10.1159/000481833

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