Novel Interspecies Interactions of Oral Commensal *Corynebacterium* and Opportunistic Pathogen *Candida albicans*

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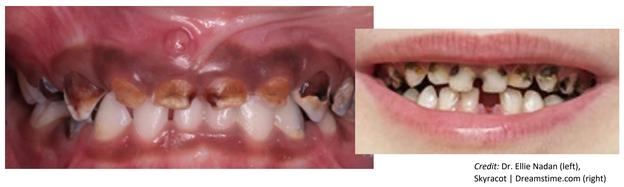
Introduction

- Dysbiosis in the mouth contributes to comprised oral and systemic health. (Figure 1)
- Polymicrobial diseases are resistant to common antibiotic therapies and disrupt host immune response. (Lamont & Hajishengallis, 2015)
- *Candida albicans (C. albicans)* is an opportunistic pathogen, forming a synergistic relationship with *Streptococcus mutans (S. mutans),* elevating plaque virulence.
- Herein we demonstrate the oral commensal *Corynebacterium durum*'s interspecies interaction with, and interruption of, the pathogenic *C. albicans/S. mutans* relationship.



Credit: Dr. Ellie Nadan (left), Skyracot | Dreamstime.com (right)

Figure 1: Severe Early Childhood Caries (s-ECC).

Materials & Methods

- Inoculation: *Streptococcus mutans* UA159 (anaerobic – 90% N2, 5% CO2, 5% H2), *Corynebacterium durum* JJ1 (aerobic – 5% CO2), *Candida albicans* ATC 14053 (aerobic) grown at 37ºC on Brain Heart Infusion Broth (BHI). For biofilm experiments, grown aerobically at 37ºC on BHI or 2.5% w/v Sucrose.
- Bacterial extracellular membrane vesicles (EMVs): Cultures inoculated (BHI) and agitated (180rpm) overnight, filtered (VivaSpin 20 Ultracentrifuge units), and concentrated.
- *Galleria mellonella* infection model: Larvae divided into groups of 10, cultures grown on BHI (OD > 2) then injected into *G. mellonella*. Post-injection larvae were kept at 37ºC, monitored for survival, and pigmentation changes were recorded over 7-day interval. (Figure 2)

Figure 2: Healthy (left) and deceased / pigmented (right) *Galleria Mellonella* infection model.

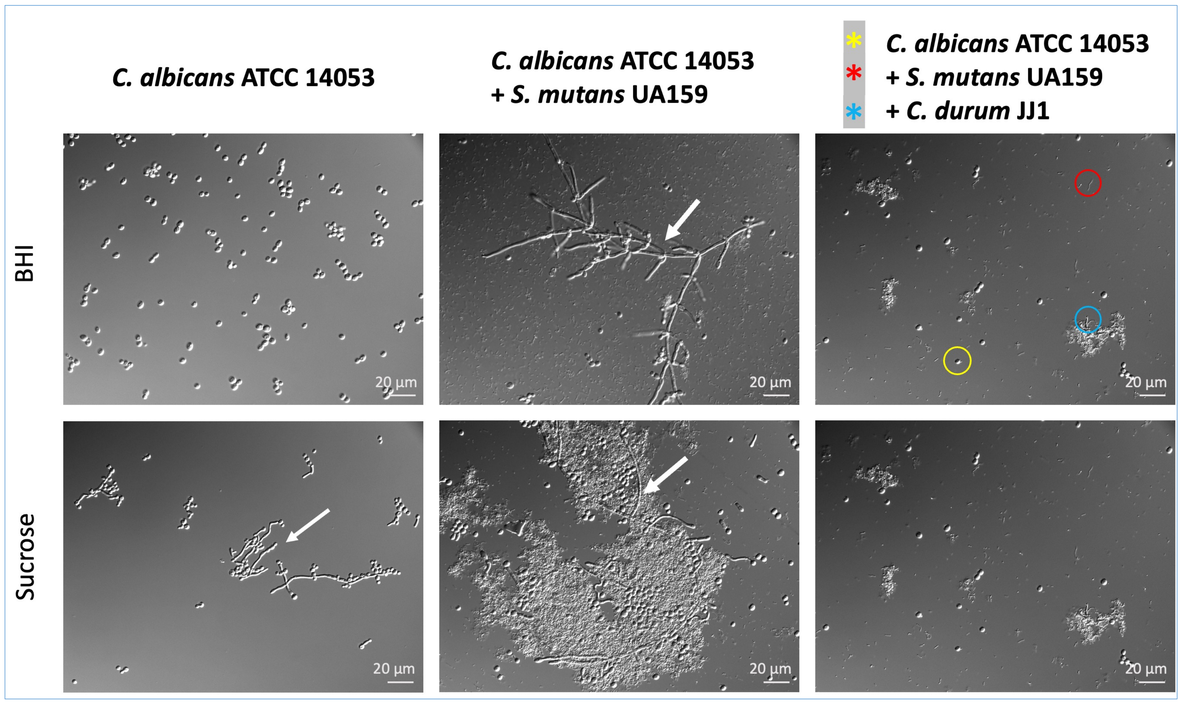


Figure 3: Interspecies interaction of *Candida albicans* (yellow), *Streptococcus mutans* (red), and *Corynebacterium durum* (blue) in BHI and Sucrose conditions.

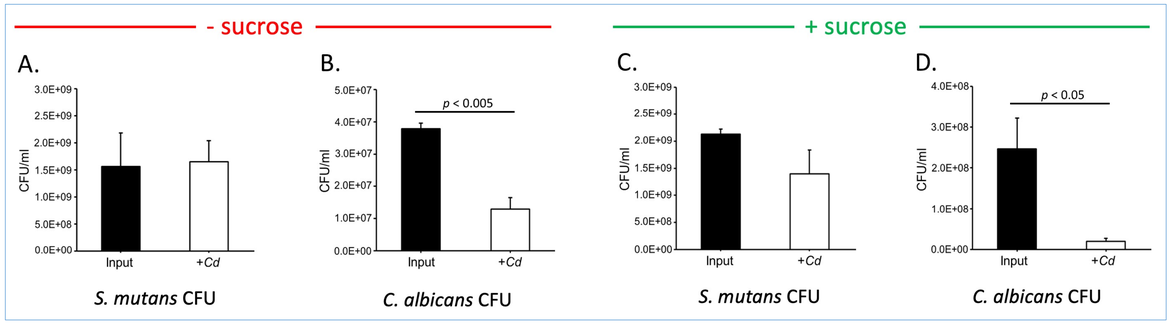


Figure 4: Impact of *C. durum* on *C. albicans* and *S. mutans* in BHI and Sucrose conditions compared to single species. Colony forming units (CFUs) determined based on optical density after serial dilution, n=3 biological replicates.

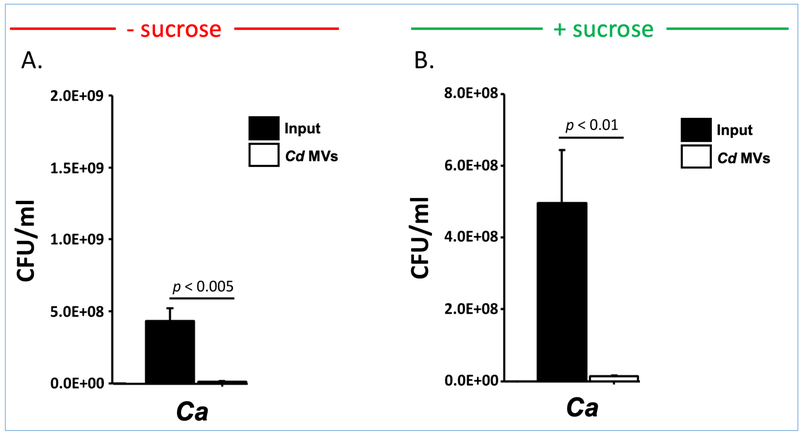


Figure 5: Interference of *C. albicans* (*Ca*)/*S. mutans* interaction with 100µL (approx. 1.5×10^{-11}) of *C. durum* extracellular membrane vesicles (EMVs). Colony forming units (CFUs) of *C. albicans* based on optical density after serial dilutions, n=3 biological replicates.

Results

- ***C. durum* inhibits *C. albicans* hyphae** when co-cultured with *S. mutans*. Hyphae is necessary for biofilm formation and indicative of plaque virulence. (Figures 3, 4)
- **Extracellular membrane vesicles** from *C. durum* are **responsible for interference** with *C. albicans*. (Figure 5)
- *C. durum* significantly **reduces *C. albicans* virulence in an *in vivo* model**. (Figure 6)

Conclusions

- Oral diseases are caused by dysbiotic host environments including the virulent *C. albicans/S. mutans* interaction.
- Some *Corynebacterium* species release fatty acids in EMVs, these abolish *C. albicans* hyphae formation, necessary for biofilm formation and pathogenesis.
- Supplying *Corynebacterial* species or their EMVs might be a **viable option to restore health to those suffering from polymicrobial diseases** such as severe Early Childhood Caries (s-ECC).

Figures 4-6: Adapted from Treerat et. al. *in preparation*

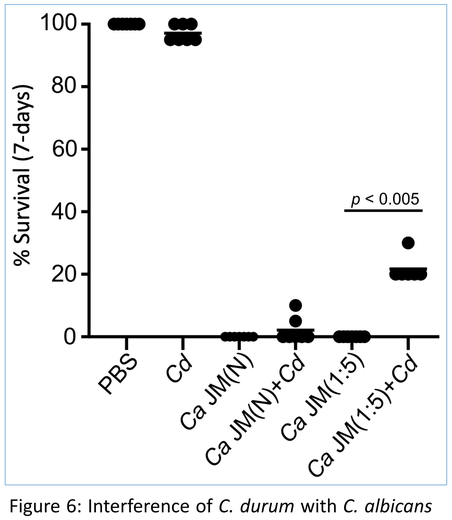


Figure 6: Interference of *C. durum* with *C. albicans* virulence in an *in vivo Galleria Mellonella* infection model. 10 larvae per condition tracked over 7-days, mean ± SD plotted, (N) signifies undiluted solution.

References

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This research was funded by NIH-NIDCR grant DE021726, DE028252, and R25 DE032536. This poster was printed by UO Libraries.