α-Synuclein: A Non-Lethal Interaction with Gram-Positive Bacteria

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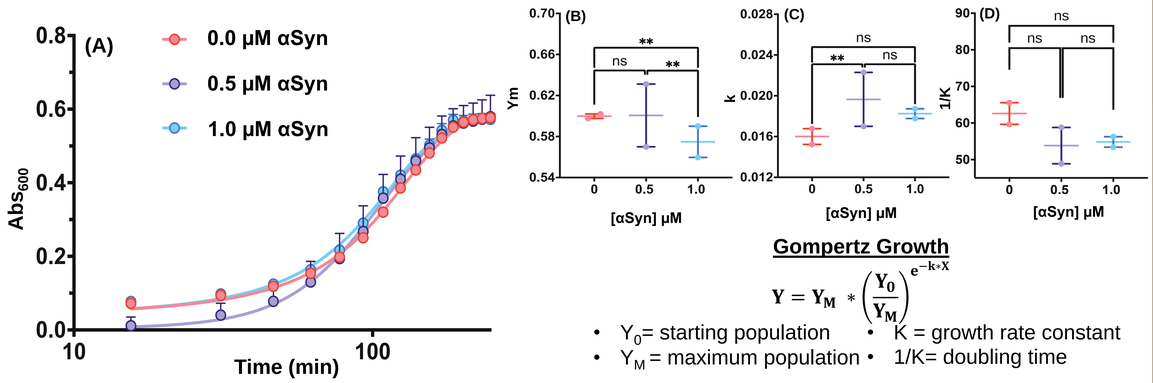
αSyn does not impact *Enterococcus faecalis* planktonic growth



Gompertz Growth

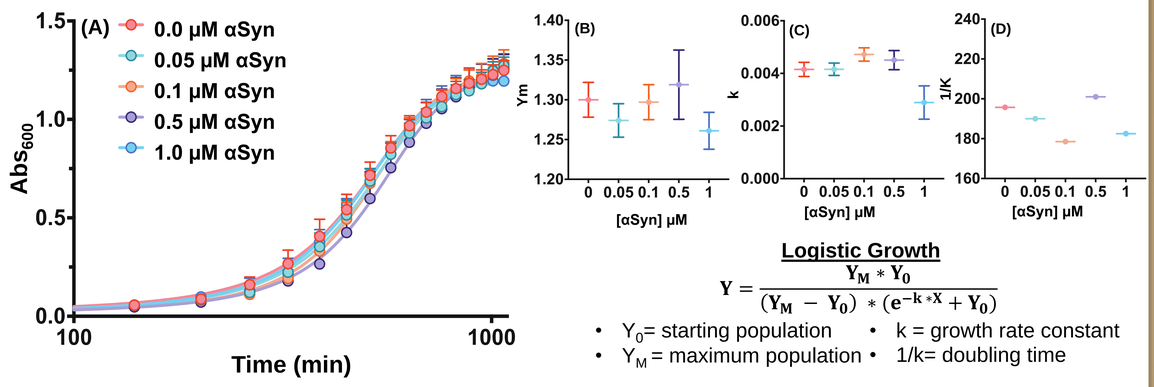
$$Y = Y_M * \left(\frac{Y_0}{Y_M}\right)^{e^{-k*X}}$$

- Y_0= starting population
- K = growth rate constant
- Y_M = maximum population
- 1/K= doubling time

Fig 1. *E. faecalis* planktonic growth curve: (A) *E. faecalis* planktonic growth measured over time in the presence of varying [αSyn]. Conditions: Brain heart infusion media (BHI), 37 °C, (*n* = 5 biological, 3 technical replicates; error bars are + SEM). Data fitted to Gompertz growth eqn using non-linear regression, straight line **(B), (C),** and **(D),** Parameters for Gompertz determined from fits. Data are shown as mean ± std dev. Data compared using 2-way ANOVA (** = p < 0.005).

- ***E. faecalis* growth was monitored over time in the presence of varying αSyn concentrations**
- **αSyn did not have a biologically significant impact on lag time, growth rate, doubling time, or maximum bacteria population**

αSyn does not impact *Streptococcus mutans* planktonic growth



Logistic Growth

$$Y = \frac{Y_M * Y_0}{(Y_M - Y_0) * (e^{-k*X} + Y_0)}$$

- Y_0= starting population
- k = growth rate constant
- Y_M = maximum population
- 1/k= doubling time

Fig 2. *S. mutans* planktonic growth curve: (A) *S. mutans* planktonic growth measured over time in the presence of varying [αSyn]. Conditions: BHI, 37 °C, (*n* = 5 biological, 1 technical replicate; error bars are + SEM). Data fitted to logistic growth eqn using non-linear regression, straight line **(B), (C),** and **(D),** Parameters for logistic determined from fits. Data are shown as mean ± std dev. Data compared using Welch's t test (** = p < 0.005).

- ***S. mutans* growth was monitored over time in the presence of varying αSyn concentrations**
- **αSyn did not have a biologically significant impact on lag time, growth rate, doubling time, or maximum bacteria population**

- **~1 million people live with Parkinson's disease (PD) in the US. There are no disease-modifying treatments available.**
- **αSyn is associated with PD disease pathologies.**
- **Since evidence suggests gut and oral microbiome health influences PD pathology and αSyn is an antimicrobial agent, we examined the interaction between αSyn and gut/oral bacteria.**
- **Using methods to measure both planktonic and biofilm growth, we found αSyn differentially impacts gram positive bacteria.**
- **Contrary to previous results, we show αSyn can increase membrane integrity and biofilm formation in bacteria.**

αSyn differentially affects *E. faecalis* and *S. mutans* biofilm formation

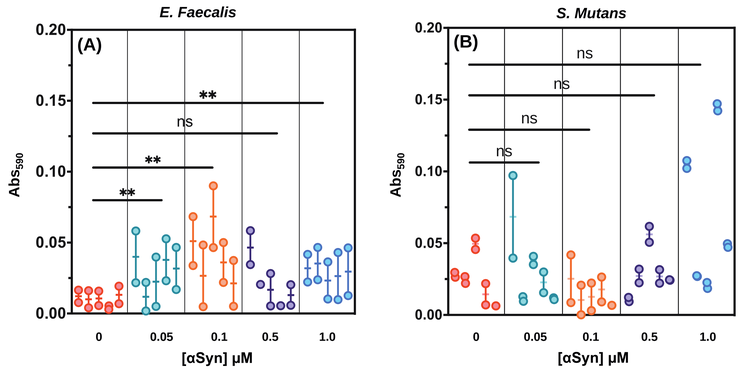
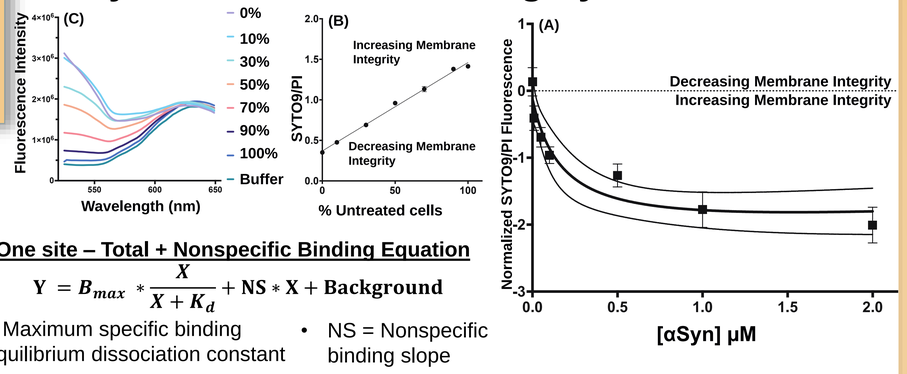


Fig 3. Biofilm Formation: (A) *E. faecalis* biofilm formation in BHI media with varying concentrations of α-synuclein following incubation at 37 °C for 48 hours. N = 5 biological replicates; 2 technical replicates. Error bars are ± SEM. (** significant at the level of p < 0.05) **(B)** *S. mutans* biofilm formation in BHI media with varying concentrations of α-synuclein following incubation at 37 °C for 48 hours. N = 5 biological replicates; 1 technical replicate. Error bars are ± SEM.

- **Biofilm formation was evaluated following incubation for 48 hours in varying αSyn concentrations.**
- **αSyn increased the biofilm formation in *E. faecalis*, but had no significant impact on biofilm formation in *S. mutans*.**

αSyn alters membrane integrity of *E. faecalis*



One site – Total + Nonspecific Binding Equation

$$Y = B_{max} * \frac{X}{X + K_d} + NS * X + Background$$

- B_{max} = Maximum specific binding
- K_d = Equilibrium dissociation constant
- NS = Nonspecific binding slope

Fig 4. *E. faecalis* SYTO9/Propidium Iodide Fluorescence Assay: (A) Normalized *E. faecalis* SYTO 9/PI fluorescence at varied concentrations of α-synuclein. Data fit to one site binding – total and nonspecific binding (straight line) with 95% confidence interval (dashed line). N = 1 biological replicate with 3 technical replicates. **(B)** E. faecalis emission spectrum after addition of SYTO 9 and Propidium Iodide (PI) into varying ratios of untreated cells:cells treated with isopropanol. **(C)** Standard curve of SYTO 9/PI with percent of untreated cells

- **SYTO 9 and Propidium Iodide (PI) fluorescence was observed at varying αSyn concentrations and normalized to fluorescence of increasing ratio of isopropanol treated cells.**
- **Increased SYTO 9/PI fluorescence ratio indicates increased membrane integrity of E. faecalis when exposed to α-synuclein**

References

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2. G. A. O'Toole, Microtiter Dish Biofilm Formation Assay. J. Vis. Exp. (2011), doi:10.3791/2437.
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FUTURE DIRECTIONS

- **We observed that α-synuclein is not bactericidal to the Gram-positive bacteria *Enterococcus faecalis* and *Streptococcus mutans* and our SYTO9/PI fluorescence assay demonstrated a positive correlation between α-synuclein concentration and increased SYTO 9:PI fluorescence, indicating enhanced bacterial viability.**
- **Given the relevance of α-synuclein to Parkinson's disease pathogenesis, future studies could explore how α-synuclein's effects on bacterial membrane integrity and biofilm formation alter host-microbe interactions and contribute to neurodegenerative processes in the context of Parkinson's disease**

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