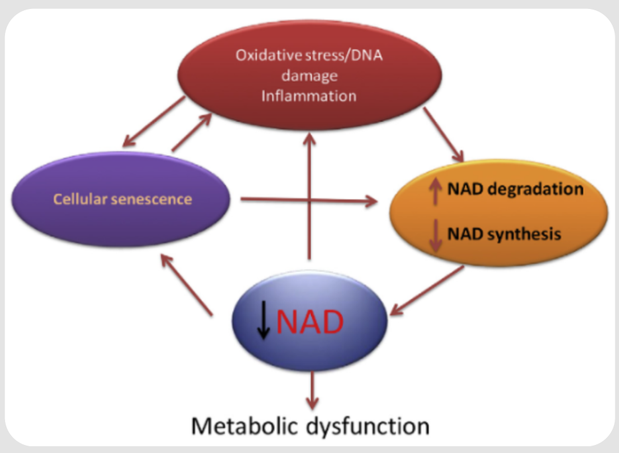
Evaluating the Potential of Isonicotnamide (INAM) as a Longevity-Promoting Chemical in *Saccharomyces cerevisiae*

Research Question:

What is the effect of Isonicotinamide on the chronological lifespan of three strains of *Saccharomyces cerevisiae*: BY4741 Wild Type, Bna7Δ mutant strain, and Swr1Δ mutant strain?

Aging in Mammals

Nicotinamide Adenine Dinucleotide (NAD) levels naturally diminish with age, causing an activity decline within sirtuins, which are histone deacetylases involved in numerous biological processes such as inflammation and metabolism regulation. Particularly within SIRT1, SIRT3, and SIRT6, declined sirtuin activity is linked with greater organ vulnerability to aging and aging-related diseases. NAD+ levels often decline due to damage from inflammation, malfunctioning DNA, and oxidative stress. Consequentially, the biological processes that depend on NAD+ are negatively impacted.



(Chini et al., 2017)

Aging in Yeast

Chronological Aging:

- Ethanol accumulates in cells, is converted to acetic acid, and induces apoptosis-like response
- Damaged mitochondria and oxidized proteins accumulate in cells, resulting in senescence

Replicative Aging:

- Damage is inherited by the mother cell and removed from the daughter cell
- Nuclear extrachromosomal rDNA circles and damaged mitochondria contribute to replicative senescence

Experiment Breakdown

<u>Growth Assay:</u>
Determine the effect of 0, 25, 50, 75, & 100 mM of INAM on physical yeast culture growth

<u>Aging CLS Assay:</u>
Determine the effect of 25 mM INAM on culture lifespan and viability over extended time period

Significant Growth Assay Result

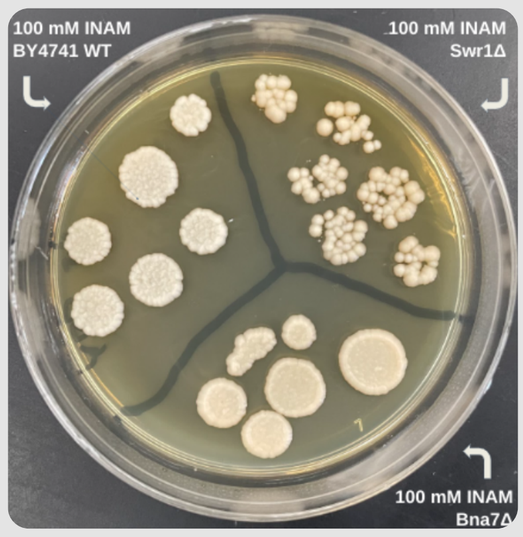


Figure 1: Swr1Δ Growth Inhibition at 100 mM INAM
Image collected and taken by researcher as part of the Growth Assay

CLS Aging Assay Results

Data collected by researcher as part of the CLS Aging Assay

Seven Total Mean Absorbance Readings (600 nm)
3/04/23 - 3/29/23

	3/04	3/07	3/10	3/14	3/21	3/23	3/29
Plain YPD Only	0.54	0.54	0.54	0.61	0.69	0.69	0.69
25 mM INAM YPD Only	0.79	0.64	0.90	0.86	1.09	1.06	1.00
WT Strain + Plain YPD	0.72	1.41	1.83	3.14	2.28	3.24	1.58
Swr1 Strain + Plain YPD	0.63	1.50	2.05	3.28	3.13	1.24	2.99
Bna7 Strain + Plain YPD	1.10	0.58	1.71	2.80	2.61	3.02	1.21
WT Strain + 25 mM INAM YPD	1.23	0.89	1.89	3.17	3.33	3.18	3.15
Swr1 Strain + 25 mM INAM YPD	0.84	1.89	1.77	2.69	3.19	2.89	1.58
Bna7 Strain + 25 mM INAM YPD	1.31	2.06	1.51	2.82	2.99	3.19	2.82

Figure 2: Absorbance reading results of the three yeast strains exposed to either plain YPD or 25 mM INAM YPD

T-Test

WT vs WT + 25 mM INAM
P = 0.486119401

Swr1Δ vs Swr1Δ + 25 mM INAM
P = 0.9909415938

Bna7Δ vs Bna7Δ + 25 mM INAM
P = 0.2758389141

P < 0.05, so NOT statistically significant

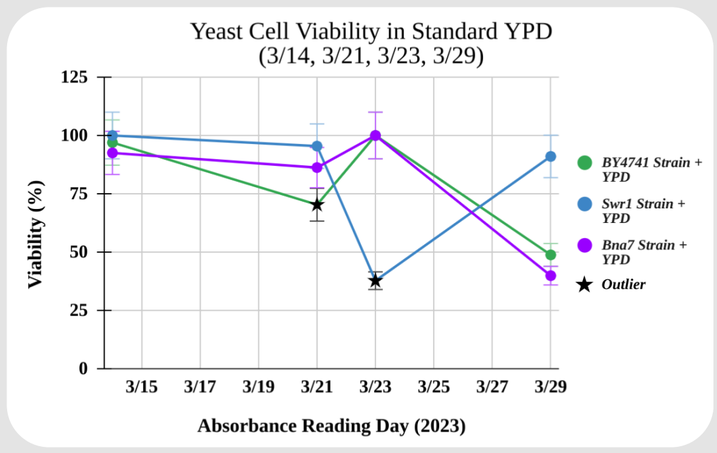


Figure 3: The decline in viability for all three strains grown in plain YPD

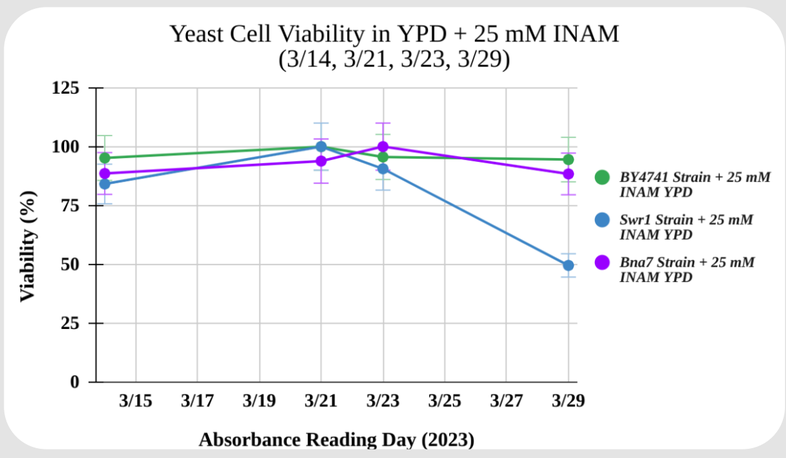


Figure 4: The decline in viability for all three strains grown in 25 mM INAM YPD

Conclusion

25 mM INAM exposure has been ruled out as a statistically significant promoter of yeast cell viability. However, since the growth assay suggests that the influence of INAM is dose-dependent as shown by the inhibition of the Swr1Δ strain solely at higher concentrations (100 mM), further work with several additional concentrations and strains is necessary to truly determine its greater potential for future longevity studies.

References

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2. Kalita, A., Hesles, E. E., Power, L. N., Wang, D., Singh, P. K., & Smith, J. S. (2021). Isonicotinamide extends yeast chronological lifespan through a mechanism that diminishes nucleotides. https://doi.org/10.1101/2021.07.11.451986
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