

What growth source is superior for *Galderia*?

Investigating the optimal growth sources for *Galderia sulphuraria* growth and phycocyanin production.

BACKGROUND



Galderia sulphuraria is a thermoacidophilic red alga that grows optimally in pH 1-3 and temperatures exceeding 40°C. Its ability to thrive in both heterotrophic and mixotrophic conditions on a range of carbon sources makes it valuable in biotechnological applications, including wastewater treatment and pigment production.

G. sulphuraria produces **C-phycocyanin**, a rich blue water-soluble phycobiliprotein with applications in food, cosmetics, and pharmaceuticals. Demand for phycocyanin is increasing as a natural alternative to synthetic dyes, with the global market projected to reach **\$1.77 billion USD** by 2035^[1].

C-phycocyanin synthesis is strongly influenced by carbon availability and metabolic state, which brings forth the need to optimise growth conditions for maximal pigment yield in large-scale industrial production.

DISCUSSION

Our results showed significant differences in *G. sulphuraria* growth across media (**Type III RM-ANOVA**: $p < 0.001$, $D_{\text{endf}} = 40$, $F = 13.8534$). Pea protein sample exhibited highest growth. Exploratory pairwise comparisons found C exhibited significantly lower absorption than W in weeks 3 and 5 ($p < 0.01$, $df = 25.1$, $F = -3.853/-2.845$) which aligns with our prediction, and suggests wheat gluten (glucose) is the most efficient growth substrate for pigment production.

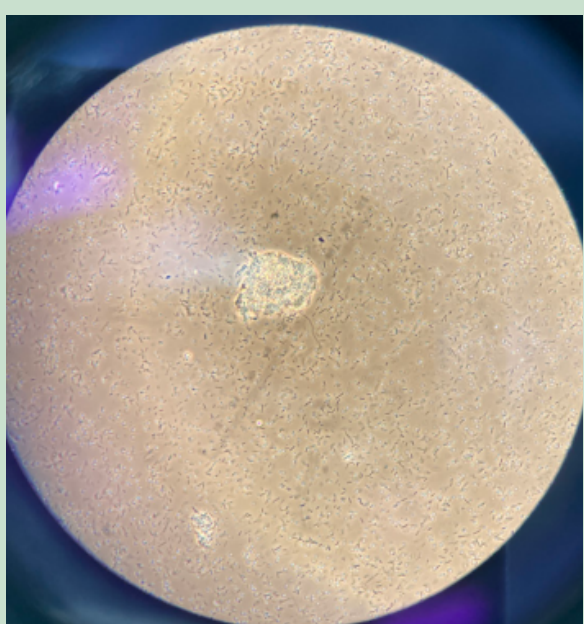


Fig. 3 (x40)
High concentration of unknown bacterial contaminant in W, week 3. *Galderia* also present, but appear to be outcompeted.

Limitations:

- **Experimental design:** Given small sample size ($N=3$), results from pairwise comparisons should be interpreted with caution. Further experimentation should use >5 replicates per group.
- **Contamination:** W consistently contained various microorganism contaminants (Fig.3). Therefore we cannot assume that wheat gluten's molecular properties solely aid in pigment production, because the presence of other organisms may play a role.
- **pH:** We did not ensure replicates were maintained at constant pH throughout, which may have resulted in unreliable readings. In future, use indicator strips to test each solution before stock culture addition.

Future experimentation:

- Further research is needed regarding the interactions between *G. sulphuraria* and other microorganisms, and its effect on pigment production.
- Measure for a longer time period (>6 weeks) to ensure all phases of the growth curve are met.
- Test under heterotrophic conditions by growing samples without light to ensure *G. sulphuraria* is exclusively using the carbon source for energy.
- Increase sample size for improved statistical power.

INTRODUCTION + AIMS

We tested the pigment production of *G. sulphuraria* strain SAG 107.79 on different growth substrates in 3 mixotrophic conditions, and 1 autotrophic control condition.

- **Aim** - To investigate the optimal energy supply medium for growth and pigment production of *Galderia sulphuraria*
- **Hypothesis** - *Galderia sulphuraria* will exhibit significantly lower signs of growth in C (autotrophic) vs S, WG, and P (mixotrophic) aligning with previous research^[2].

METHODS

1. Sample Preparation

The following components were aseptically pipetted into their respective tube:

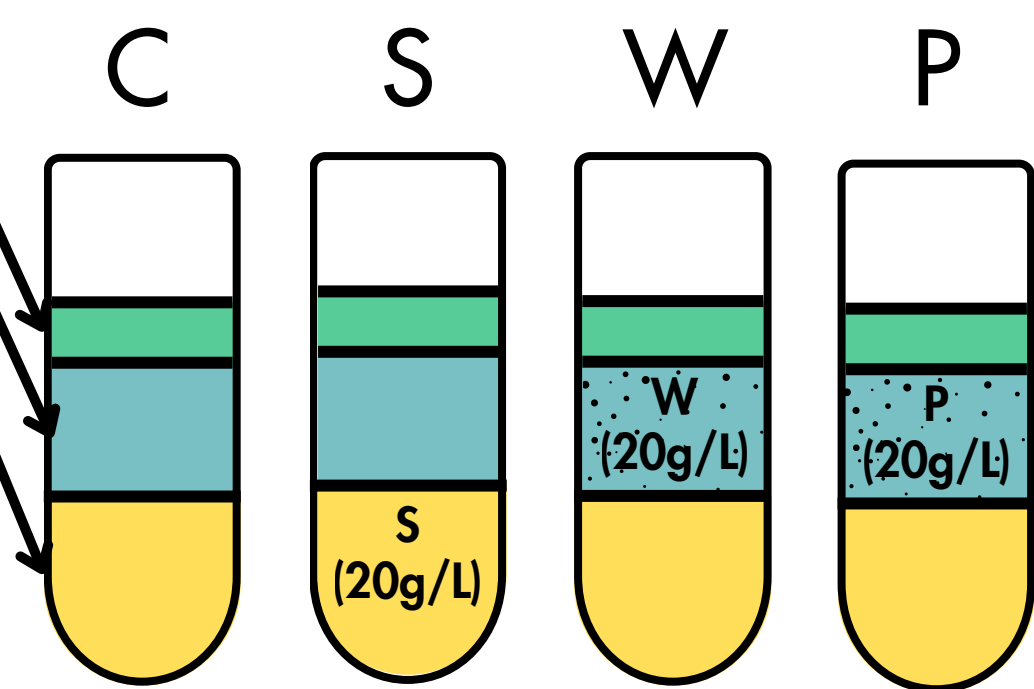
- **C** - 5 ml MS + 5ml dH₂O + 0.1ml stock culture
- **S** - 5ml **sucrose** MS medium (20g/L) + 5ml dH₂O + 0.1ml stock culture
- **WG** - 5 ml MS + 5ml solution containing 0.1g dissolved **wheat gluten** powder (20g/L) + 0.1ml stock culture
- **P** - 5 ml MS + 5ml solution containing 0.1g dissolved **pea protein** powder (20g/L) + 0.1ml stock culture

Test tubes were vortexed for 10s between each step. 3 replicates per condition were prepared and incubated at 37°C. Observations and quantitative measurements were recorded at 1 week intervals for 5 weeks.

G. sulphuraria culture

5ml dH₂O

5ml MS medium

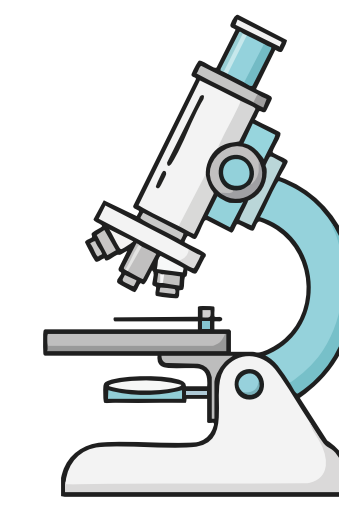


Measurements

- **Pigment optical density** - 1ml of culture from each replicate was added to a cuvette and placed in a spectrophotometer set to 680nm
- **Dry Biomass** - 1ml of each replicate was centrifuged at 4000RPM for 15 mins. Pellets were collected + weighed.

Observations

- **Sample colour** by eye
- **Light Microscopy** - to assess cell population dynamics

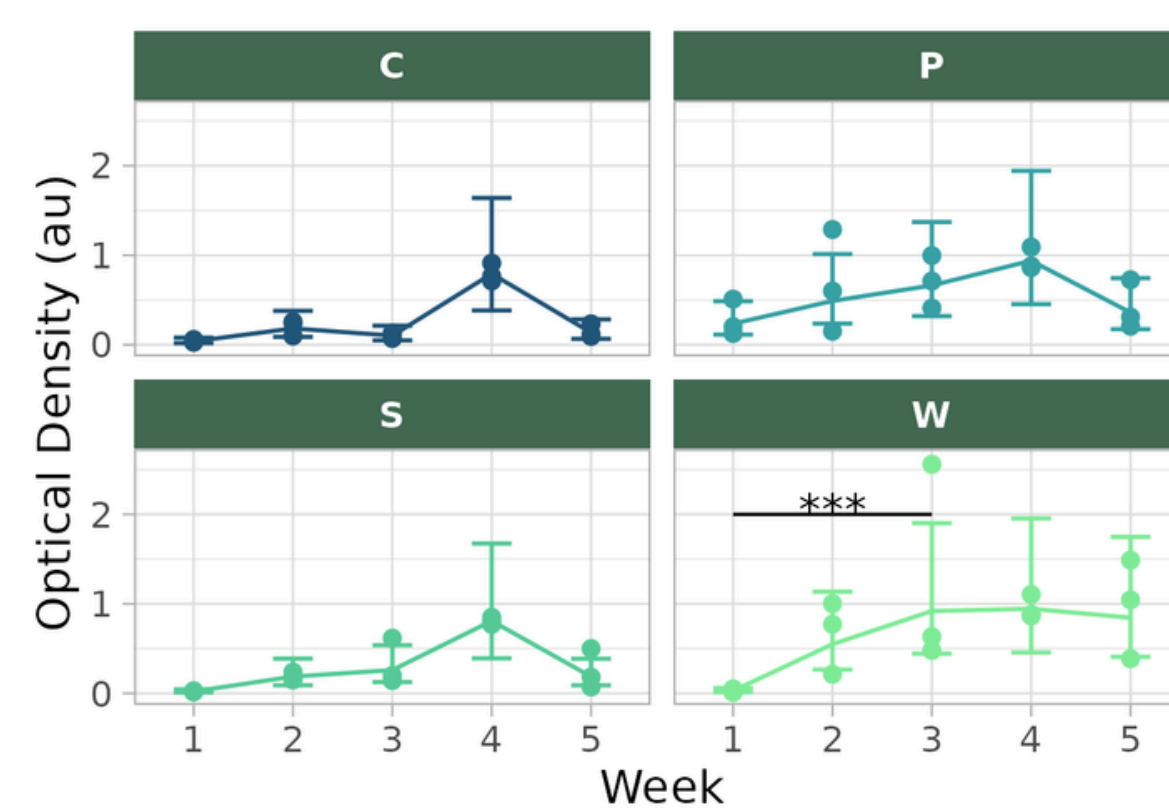


2. Statistical Analysis:

- Quantitative data was analysed on R using a **mixed effects model**.
- Absorbance variability appeared high in WG treatment, likely due to a single high-leverage observation in week 3 and small sample size.
- To adjust for variability between individual replicates, **Type III RM-ANOVA** hypothesis tests were used. TukeyHSDs were used post-hoc for pairwise comparison.

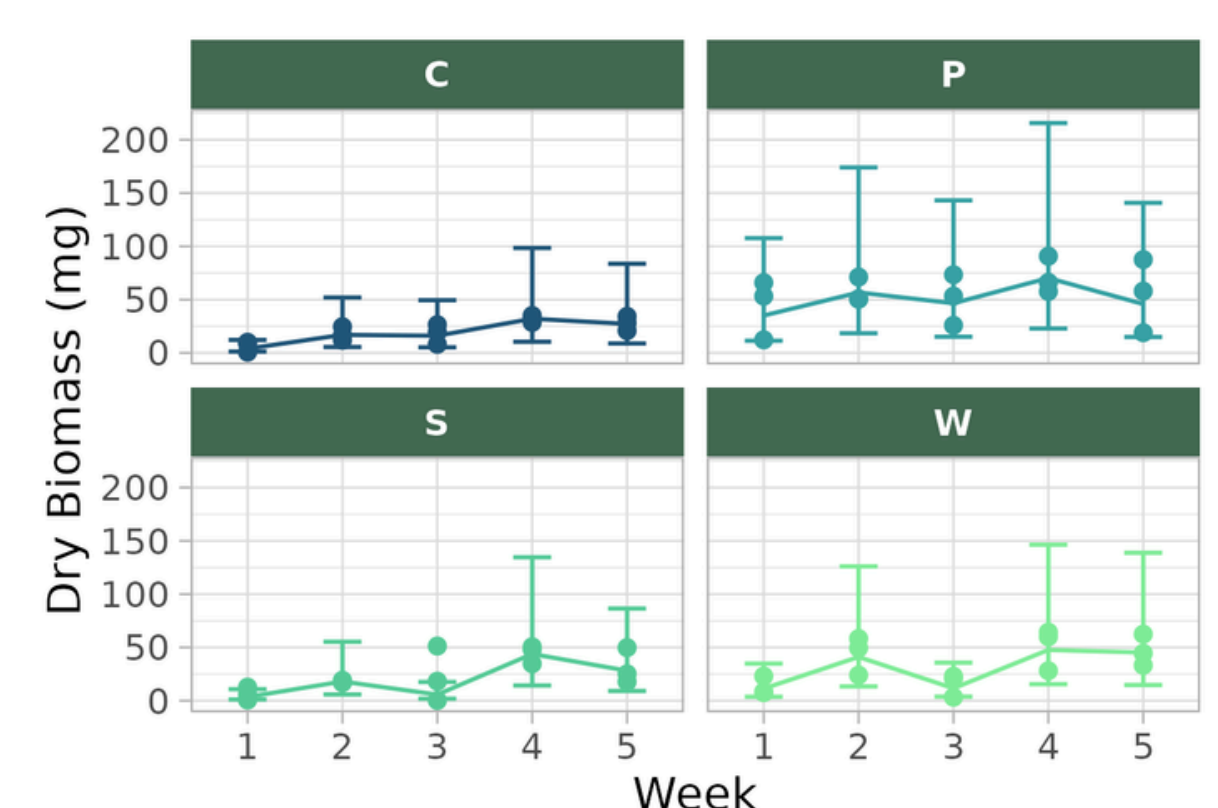
RESULTS

Fig. 1 Change in mean pigment absorbance over 5 weeks. Error bars indicate 95% CI.



- **Pigment absorbance** significantly increased week 1-4 for all groups ($p < 0.0001$, $T = -6.883$, $df = 8$)
- **W** exhibited fastest pigment production rate weeks 1-3, ~ 0.0238 au/day.

Fig. 2 Change in mean dry biomass over 5 weeks, error bars indicate 95% CI.



- No significant change in biomass between weeks on average

REFERENCES

[1] Choudhury, N.R. (2025). Phycocyanin Market Size and Share Forecast Outlook 2025 to 2035. [online] Futuremarketinsights.com, Delaware: Future Market Insights Inc., pp.1-300. Available at: <https://www.futuremarketinsights.com/reports/phycocyanin-market#thankyou> [Accessed 7 Dec. 2025].

[2] Abiusi, F., Trompeter, E., Hoenink, H., Wijffels, R.H. and Jansen, M. (2021). Autotrophic and mixotrophic biomass production of the acidophilic *Galdieria sulphuraria* ACUF 64. *Algal Research*, [online] 60(102513), pp.1-10. doi:<https://doi.org/10.1016/j.algal.2021.102513>.

Table 1. TukeyHSD Post-hoc pairwise comparison of nutrient groups. Dry Biomass of P significantly $>$ than C, S, and W across all 5 weeks.

Contrast	Estimate	SE	df	t.ratio	p.value	
Control - Pea	-35.27	5.98	8	-5.897	0.0016	**
Control - Sucrose	-4.15	5.98	8	-0.695	0.8964	
Control - Wheat	-13.57	5.98	8	-2.270	0.1845	
Pea - Sucrose	31.11	5.98	8	5.203	0.0036	**
Pea - Wheat	21.69	5.98	8	3.628	0.0276	*
Sucrose - Wheat	-9.42	5.98	8	-1.575	0.4421	