Starving to Build: The Role of Polyphosphate Kinase 1 (PPK1) in the Development and Stress Response of *Myxococcus xanthus*

Faiyaz K. Hasan and Dean M. Fraga
Program in Biochemistry and Molecular Biology, The College of Wooster, Ohio

Background and Significance

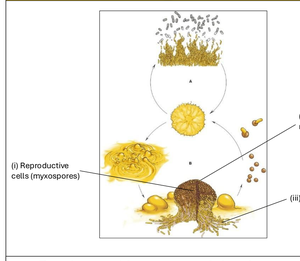


Figure 1: Multicellular life cycle of Myxococcus xanthus. (A) Vegetative growth in nutrient rich conditions where cells form swarms and engage in predation. (B) Development induced by starvation. Cell initiate developmental pathway with differentiated reproductive cells (myxospores) and environmentally resistant peripheral cells that protect the myxospores till sporulation by forming mounds called fruiting bodies. Some cells also undergo cell death through autolysis to provide nutrients for myxospores.[1]

- **Development is an energy intensive process and *M. xanthus* requires specialized energy buffering enzymes to accomplish proper fruiting body formation.**
- **Arginine Kinase (AK) and PPK1 are both energy buffering enzymes involved in the development pathway of *M. xanthus*.**

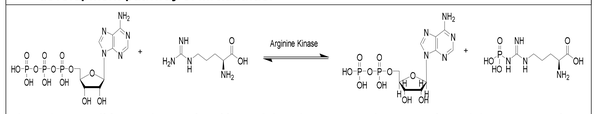


Figure 2: Reversible reaction catalyzed by Arginine Kinase converting ATP and L-arginine to ADP and N^{ω}-phospho-L-arginine in the forward direction.[2]

Figure 3: Reversible reaction catalyzed by Polyphosphate kinase 1 converting ATP into a linked chain of inorganic phosphates (polyphosphate) and ADP.

AK Loss Exhibits Phenotypic Variability

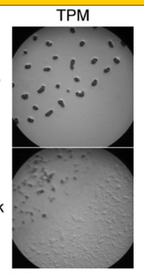


Figure 4: WT shows proper fruiting bodies while you can only see cell aggregations near the edge of the aliquot for the AK mutant after 72 hours of vegetative growth on TPM-agar plates.[3]

When subjected to rapid starvation, the AK null mutant (ΔAK) has an arrested developmental phenotype with faint weblike aggregates.[3] However this phenotype is unstable, and some ΔAK strains have exhibited the ability to partially rescue fruiting body formation.

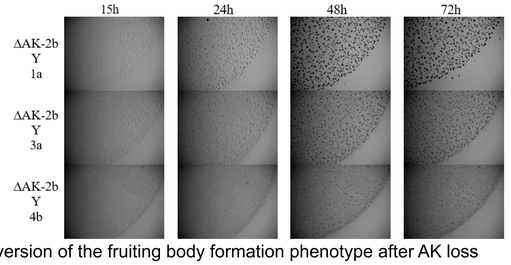


Figure 5: Technical replicates of a Δark mutant (ΔAK-2b) shows variability in FB development over time. At 72h, replicate 1a shows FB formation close to that of WT, while replicate 4b is arrested in development.[2]

Reversion of the fruiting body formation phenotype after AK loss suggests that there is compensation from another energy buffering gene, such as PPK1.

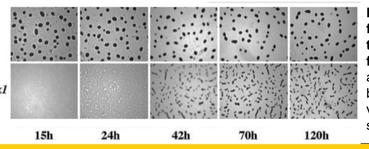


Figure 5: Fruiting body formation is delayed, and the morphology is distinct from WT. ΔPPK1 forms long and irregular shaped fruiting bodies and aggregation is only visible after 24 hours of starvation.[4]

Hypothesis & Research Objectives

Does PPK1 loss cause phenotypic variation in development and is it similar to the changes observed in ΔAK?

- Test fruiting body formation via rapid starvation to observe phenotypic expression
- Grow WT, ΔAK, ΔAK-revertant (ΔAK-REV), and ΔPPK1 under salt and pH stress to determine if stress response is affected due to PPK1 loss
- Analyze ΔPPK1's genetic expression across different time-points of development to compare differential expression with WT, ΔAK, and ΔAK-REV.

Hypothesis: ΔPPK1 also shows phenotypic variability and adverse stress response suggesting functional overlap of PPK1 and AK in energy buffering of *M. xanthus*.

PPK1 Loss also Results in Fluctuation of Fruiting Body Morphology

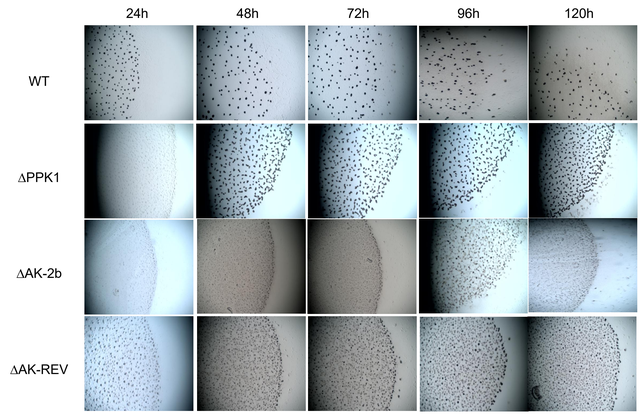


Figure 6: All strains (WT, ΔPPK1, ΔAK-2b, ΔAK-REV) were plated on starvation media (TPM) and allowed to develop for 120h. The revertant AK null mutant showed partial phenotypic recovery through FB formation while ΔAK-2b had irregular mounds suggesting some developmental progression. ΔPPK1 is delayed in aggregation but forms irregularly shaped fruiting bodies later in development, which is the phenotype recorded by Zhang et al. (2005).[4]

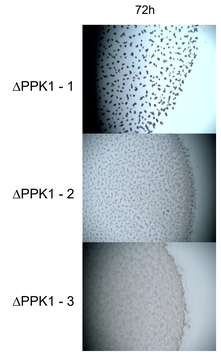


Figure 7: ΔPPK1 exhibits phenotypic variability across technical replicates. Although, ΔPPK1's ability to form proper fruiting bodies is hindered by the loss of PPK1, the extent to which it can aggregate, and form fruiting body-adjacent structures is highly variable.

- The phenotypic variability suggests that ΔPPK1 obtains some functional compensation for the loss of PPK1.
- This is similar to what has been observed with ΔAK-2b, suggesting that PPK1 and AK may compensate for the other's loss.[2]
- The delayed start to aggregate in ΔPPK1 also suggests that it struggles during early development and cannot commit to the developmental pathway as fast at WT.

Differentially Expressed Genes are Most Abundant at Spore Formation

Table 1. Genetic Profile throughout Development

Sample	Differentially Expressed Genes	Upregulated (LC˃>1)	Downregulated (LC˂<-1)
WT 0 vs 8	2329	1117	1053
WT 0 vs 32	2769	1431	1136
WT 0 vs 72	3145	1523	1348
ΔAK-2b 0 vs 8[2]	3815	1490	1605
ΔAK-2b 0 vs 32[2]	4090	1417	1556
ΔAK-REV 0 vs 8[2]	2702	1069	1242
ΔAK-REV 0 vs 32[2]	3812	1458	1683
ΔPPK1 0 vs 8	2593	1257	1138
ΔPPK1 0 vs 32	3414	1689	1457
ΔPPK1 0 vs 72	3719	1821	1577

*LC refers a log2FoldChange in genetic expression

- Differentially expressed genes (DEGs) increase throughout development, especially during spore formation and maturation (32-72 hours) across WT and all deletion mutants.
- ΔAK-2b has ~1.5x WT's DEGs at 8-hour and ~1.33x at 32-hour time points, indicating the importance of AK to variability.[2]
- ΔAK-REV has much lower increases in DEGs at both 8-hour and 32-hour time points compared to ΔAK-2b.
- ΔPPK1's DEGs were not very different throughout development from WT, suggesting that it has a more muted role in phenotypic variability than AK.

Differential Expression Governs Early Developmental Phenotype for ΔPPK1

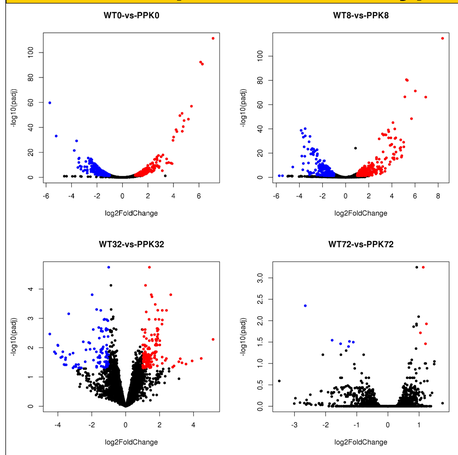


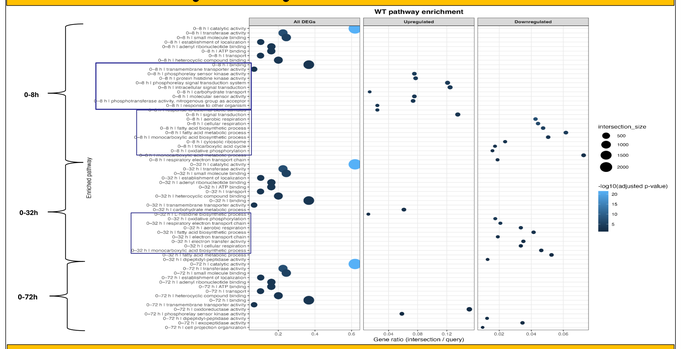
Figure 8: WT and ΔPPK1 are most significantly different 8 hours into development. Volcano plots show significantly upregulated (red dots) and downregulated (blue dots) genes. Early development has very significant differentially expressed genes but by 32h, the significance and count of differentially expressed genes drops drastically. By 72h, there are only a handful of significant differentially expressed genes. The genes which showed unsignificant change in expression are depicted with black dots.

Table 2. Genetic Profile Differences across *M. xanthus* strains

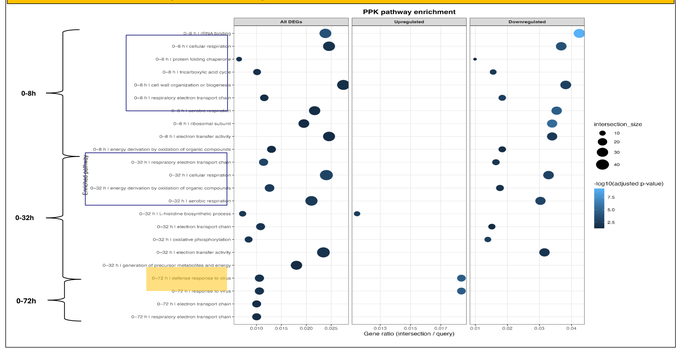
Sample	Differentially Expressed Genes	Upregulated (LC˃>1)	Downregulated (LC˂<-1)
WT vs ΔAK-2b 0[2]	39	39	0
WT vs ΔAK-2b 8[2]	87	70	11
WT vs ΔAK-2b 32[2]	2567	767	469
WT vs ΔAK-REV 0[2]	0	0	0
WT vs ΔAK-REV 8[2]	6	5	1
WT vs ΔAK-REV 32[2]	40	17	11
WT vs ΔPPK1 0	546	217	279
WT vs ΔPPK1 8	673	328	288
WT vs ΔPPK1 32	256	153	86
WT vs ΔPPK1 72	10	3	6

*LC refers a log2FoldChange in genetic expression

Pathway Analysis of *M. xanthus* WT



Pathway Analysis of *M. xanthus* ΔPPK1



PPK1 Loss has Negligible Effect on Stress Response

Table 3. Generation doubling times (hours) of *M. xanthus* strains under 0.2M NaCl stress

M. xanthus strains	Control	0.2M NaCl
WT	7.8 ± 1.1	13.9 ± 2.3
ΔPPK1	6.6 ± 1.4	16.6 ± 5.3
ΔAK-2b	8.2 ± 2.3	14.9 ± 3.5
ΔAK-REV	8.9 ± 1.8	13.5 ± 2.5

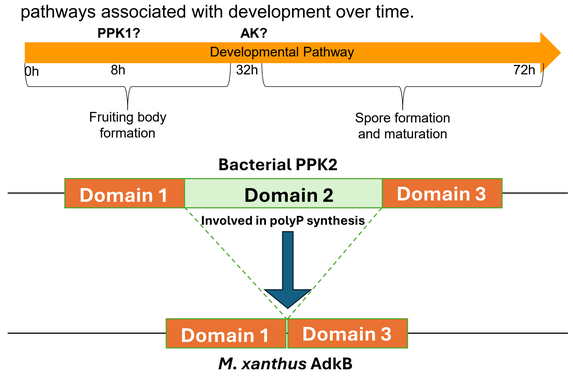
Table 4. Generation doubling times (hours) of *M. xanthus* strains due to pH 5.5 transient shock

M. xanthus strains	pH 7.6 (control)	pH 5.5
WT	6 ± 3	7 ± 3
ΔPPK1	7 ± 3	7 ± 2
ΔAK-2b	7 ± 3	7 ± 2
ΔAK-REV	7 ± 2	8 ± 2

- ΔAK-2b and ΔPPK1 do take longer to grow under salt stress than WT but the variability of those results are too high to attribute the growth defect solely to salt stress.
- No strains show any significant growth defect under transient low pH stress.
- These results contradict ΔAK-2b's stress response data found in the literature, particularly for pH stress, even though the WT patterns are largely consistent with Bragg et al. (2012).[3\]
- Oxidative stress was also attempted but all the *M. xanthus* strains were extremely sensitive to H_2O_2, even at very low concentrations (<5mM) and could not survive.

Future Research and Experiments

- Create a double mutant of PPK1 and AK to see if there is complete loss of fruiting body formation or if phenotypic instability persists.
- Analyze the RNA-sequencing data across different time-points and use previous ΔAK-2b and ΔAK-REV RNA-seq data to establish the pathways associated with development over time.



Acknowledgements

- Henry J. Copeland Fund for Senior Independent Study, The College of Wooster
- Carl O. Schultz Endowment for Conference Travel
- STEM Succes Initiative, The College of Wooster
- APEX Travel Grant, The College of Wooster
- Wooster Local Chapter of the A.C.S.

References

[1]Muñoz-Dorado, J., Marcos-Torres, F. J., García-Bravo, E., Moraleda-Muñoz, A., & Pérez, J. (2016). Myxobacteria: Moving, Killing, Feeding, and Surviving Together. *Frontiers in Microbiology*, *7*. https://doi.org/10.3389/fmicb.2016.00781
[2]Al-jarah, Shahad, "Does Arginine Kinase Bias the Switch? Examining if an Arginine Kinase Deletion Impacts Successful Fruiting Body Formation in Tan and Yellow Phase Changing Myxococcus xanthus" (2025). *Senior Independent Study Theses*. Paper 11348. https://openworks.wooster.edu/independentstudy/11348
[3]Bragg, J., Rajkovic, A., Anderson, C., Curtis, R., Van Houten, J., Begres, B., Naples, C., Snider, M., Fraga, D., & Singer, M. (2012). Identification and Characterization of a Putative Arginine Kinase Homolog from Myxococcus xanthus Required for Fruiting Body Formation and Cell Differentiation. *Journal of Bacteriology*, *194*(10), 2668–2676. https://doi.org/10.1128/JB.06435-11
[4]Zhang, H., Rao, N. N., Shiba, T., & Kornberg, A. (2005). Inorganic polyphosphate in the social life of Myxococcus xanthus: Motility, development, and predation. *Proceedings of the National Academy of Sciences*, *102*(38), 13416–13420. https://doi.org/10.1073/pnas.0506520102