WOOSTER
Program in Biochemistry and Molecular Biology

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Starving to Build

The Role of Polyphosphate Kinase 1 in Development and Stress Response of *Myxococcus xanthus*

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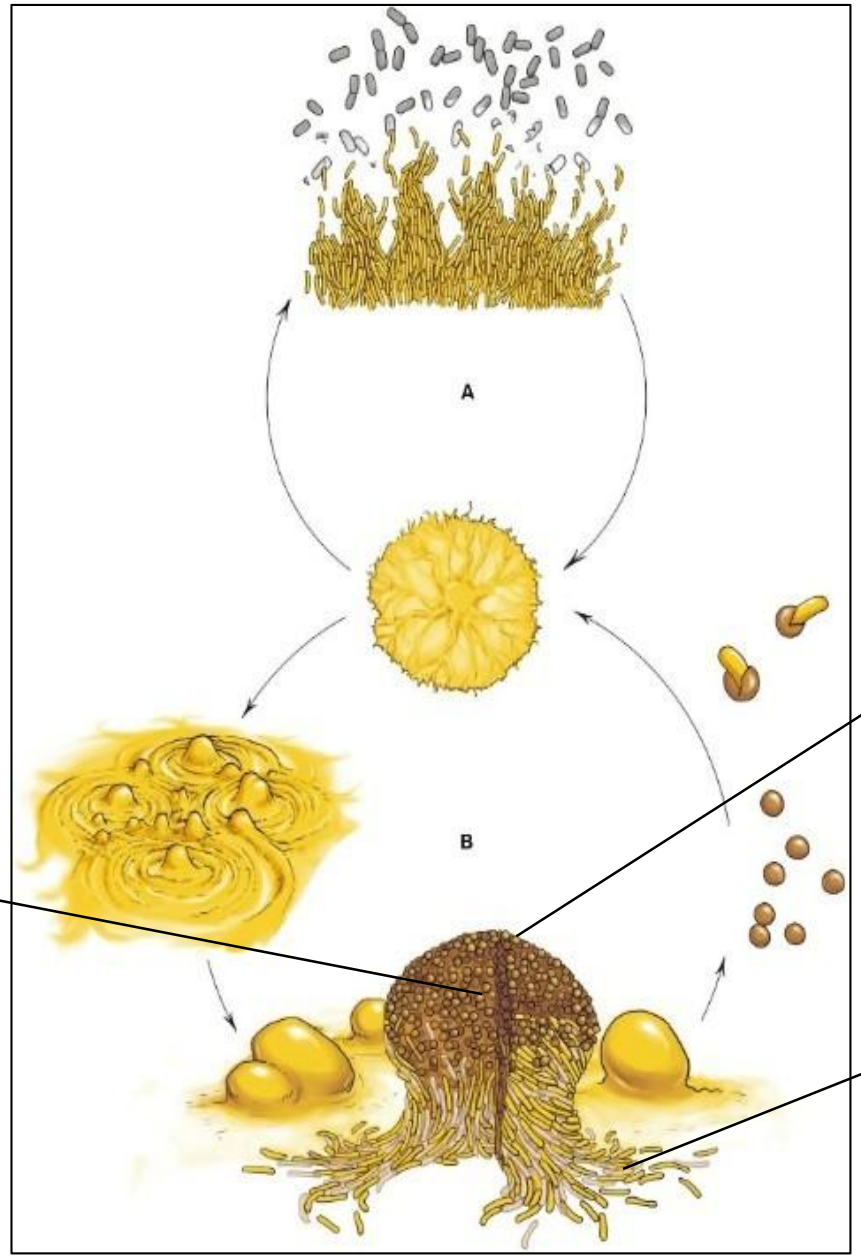
Biochemistry and Molecular Biology

Multicellular Life Cycle of *Myxococcus xanthus*



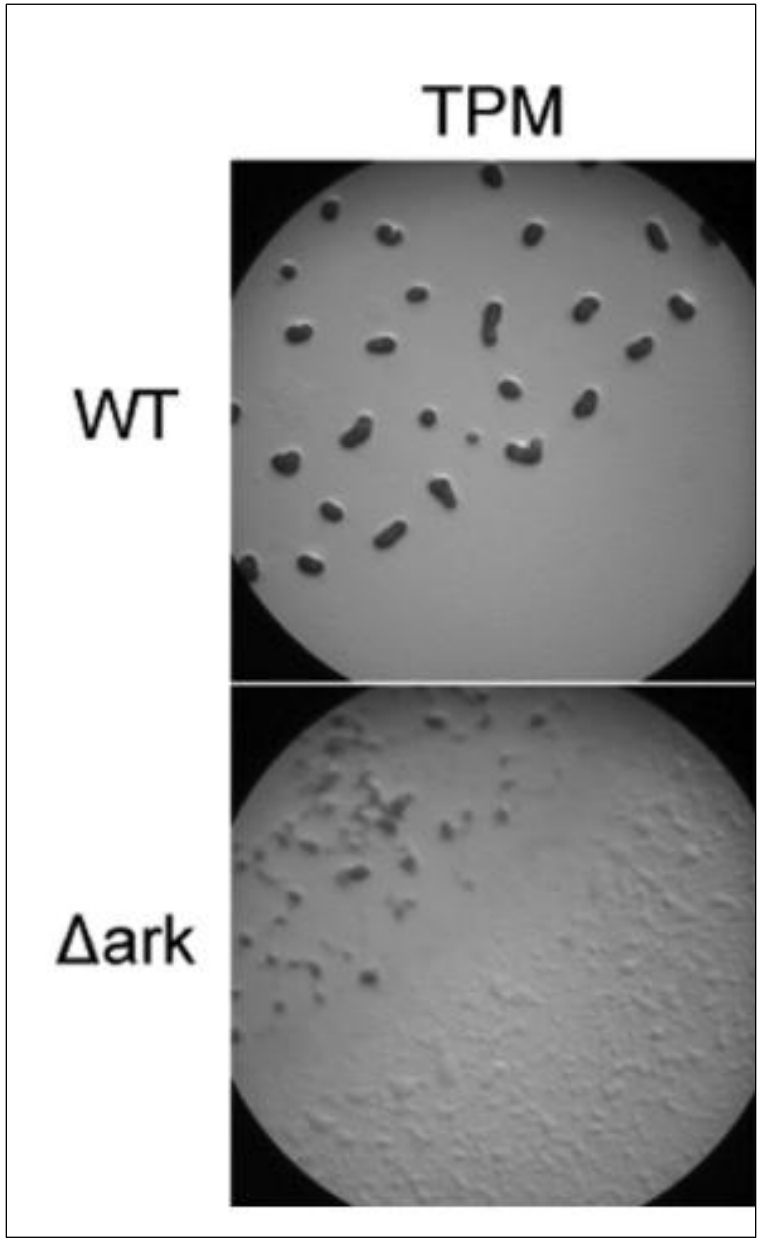
(i) Reproductive cells (myxospores)

(ii) Environmentally resistant rod cells

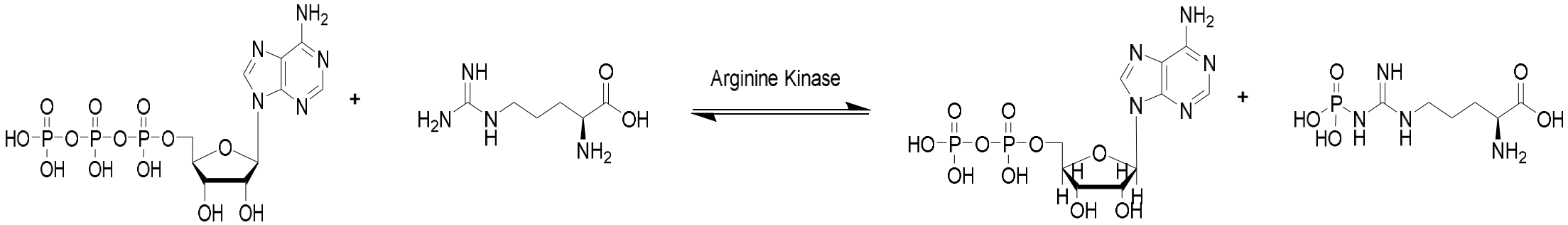
(iii) Autolysed cells

Figure adapted from Muñoz-Dorado et al. (2016) [CC-BY license].

Fruiting Body Formation upon AK Loss



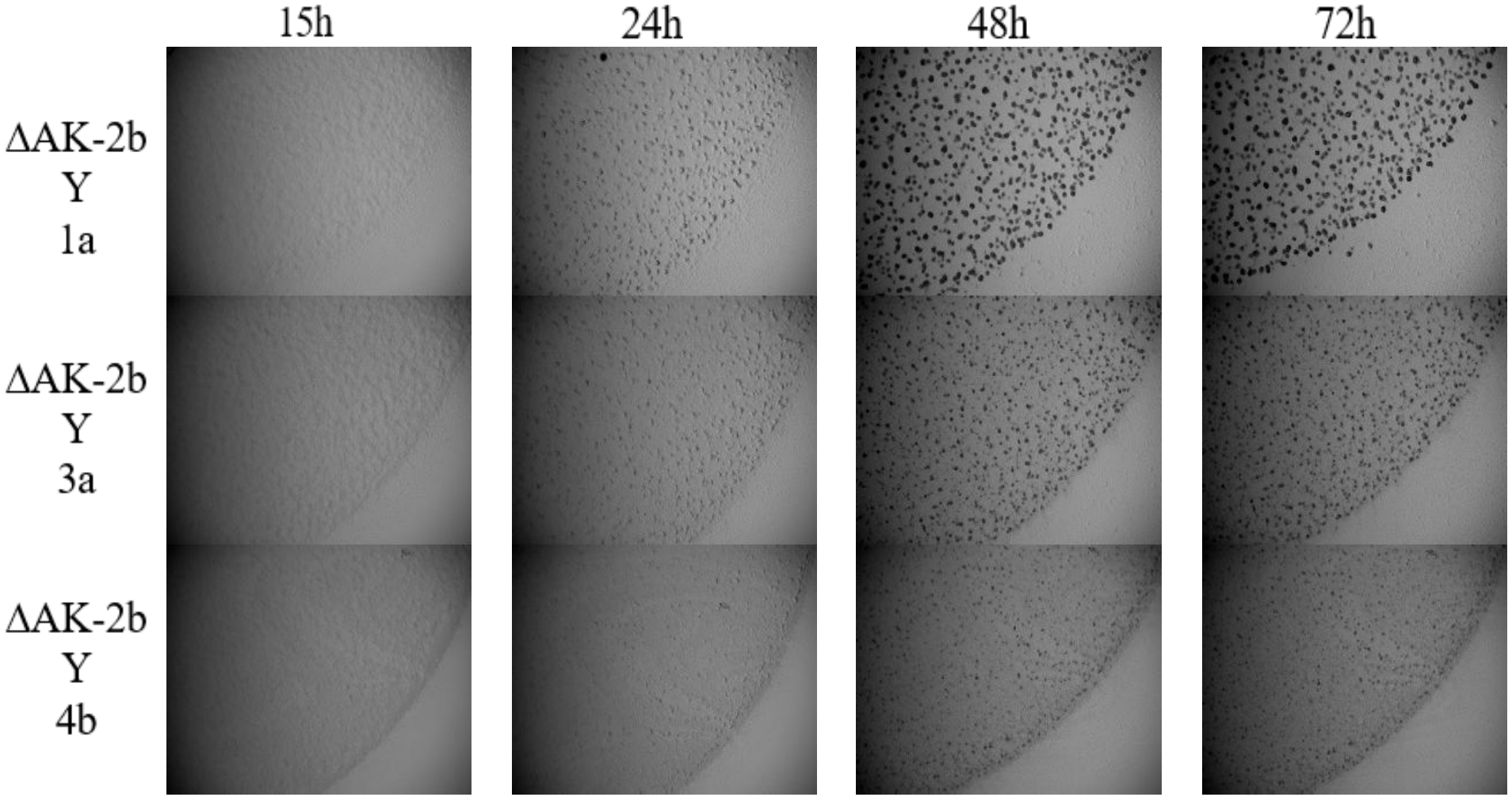
Energy Buffering Enzymes of *Myxococcus xanthus*



Reversible reaction catalyzed by Arginine Kinase converting ATP and L-arginine to ADP and N^{ω}-phospho-L-arginine in the forward direction (Al-jarah, 2025).

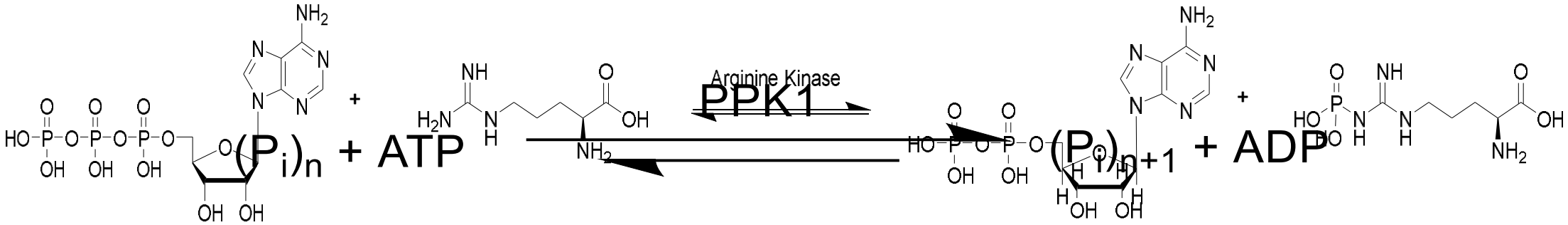
Bragg et al. (2012). Journal of Bacteriology, 194(10). https://doi.org/10.1128/JB.06435-11

Phenotypic Variability in the ΔAK mutant



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 (Al-jarah, 2025).*

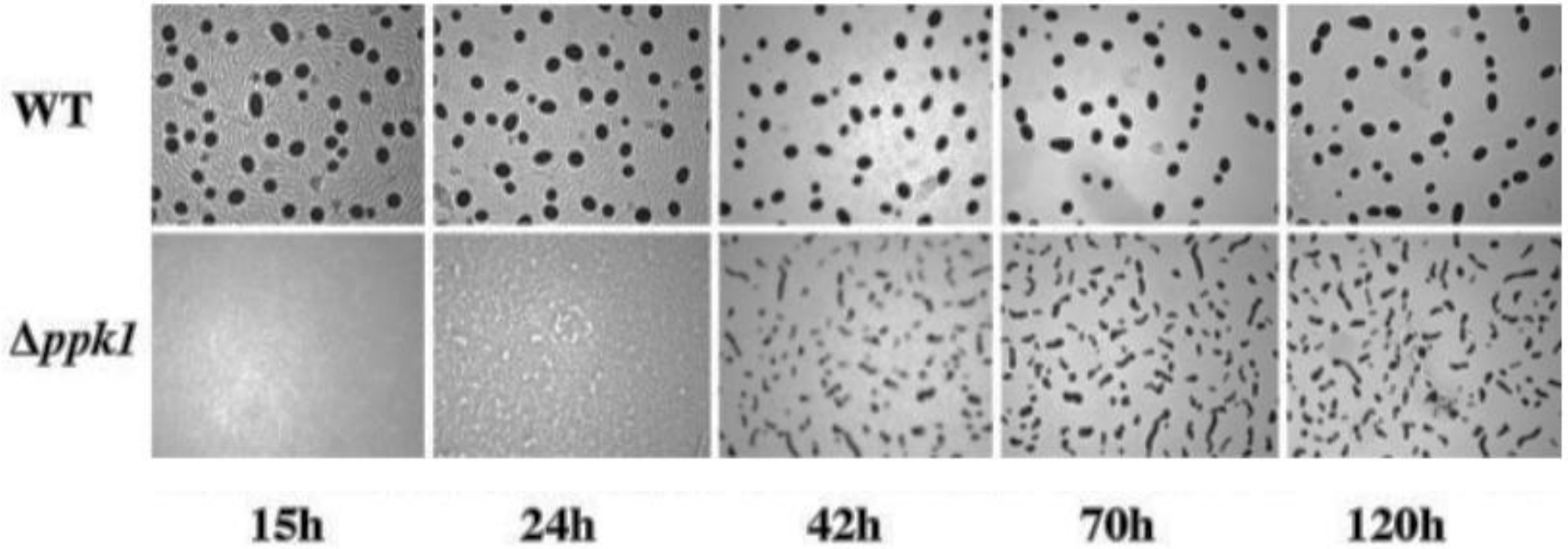
Energy Buffering Enzymes of *Myxococcus xanthus*



Reversible reaction catalyzed by Arginine Kinase converting ATP and L-Arginine into ADP and N-phospho-L-arginine (phosphagen) (AlFarah, 2025).

Bragg et al. (2012). Journal of Bacteriology, 194(10). https://doi.org/10.1128/JB.06435-11

Fruiting Body Formation upon PPK1 Loss



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 (Zhang et al., 2005).*

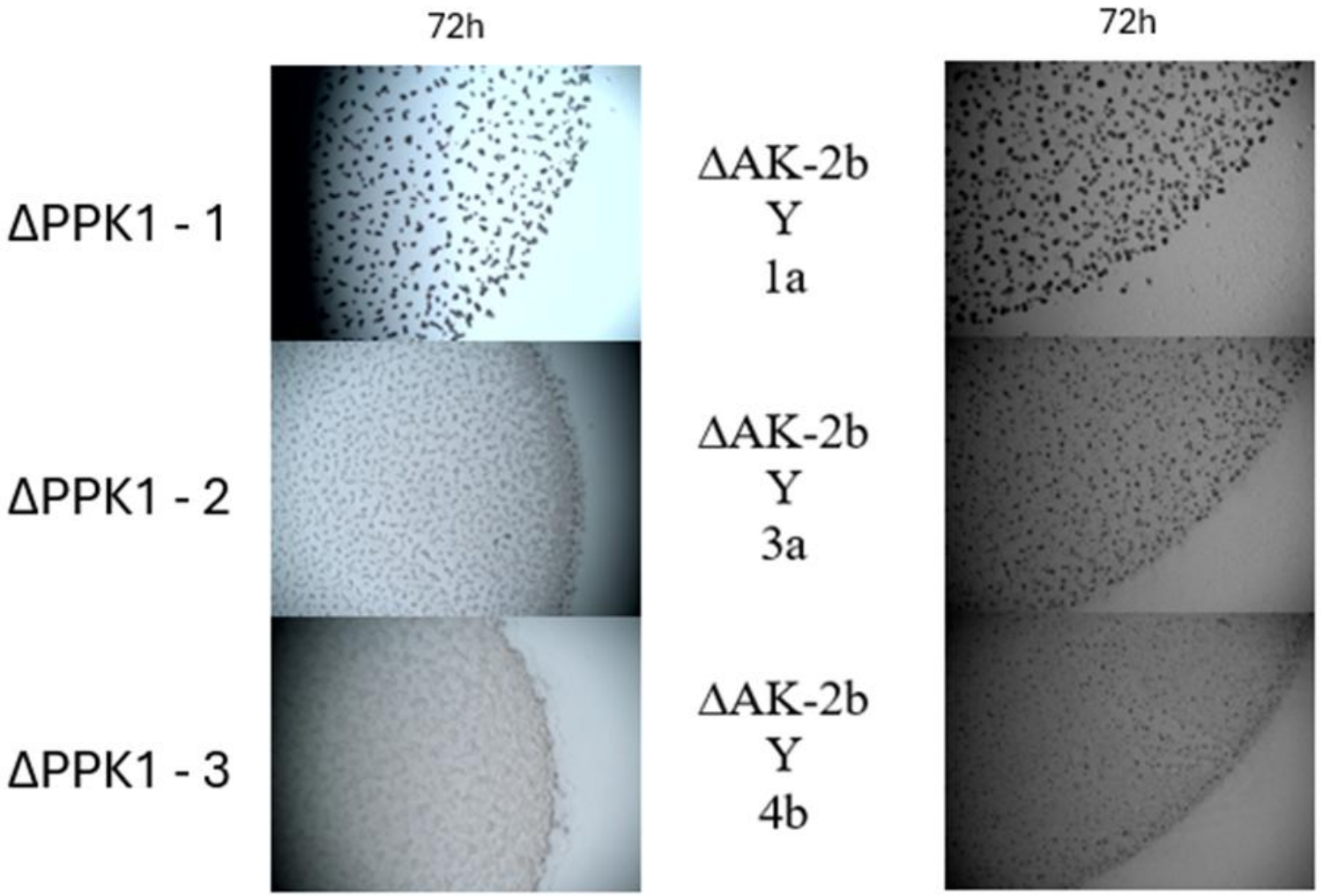
Research Question and Hypothesis

Does ΔPPK1 show phenotypic variation in development and is it similar to the morphological changes observed in ΔAK?

- Development Assay
- Stress Response Assays
    - Salt stress
    - pH stress
- RNA-sequencing

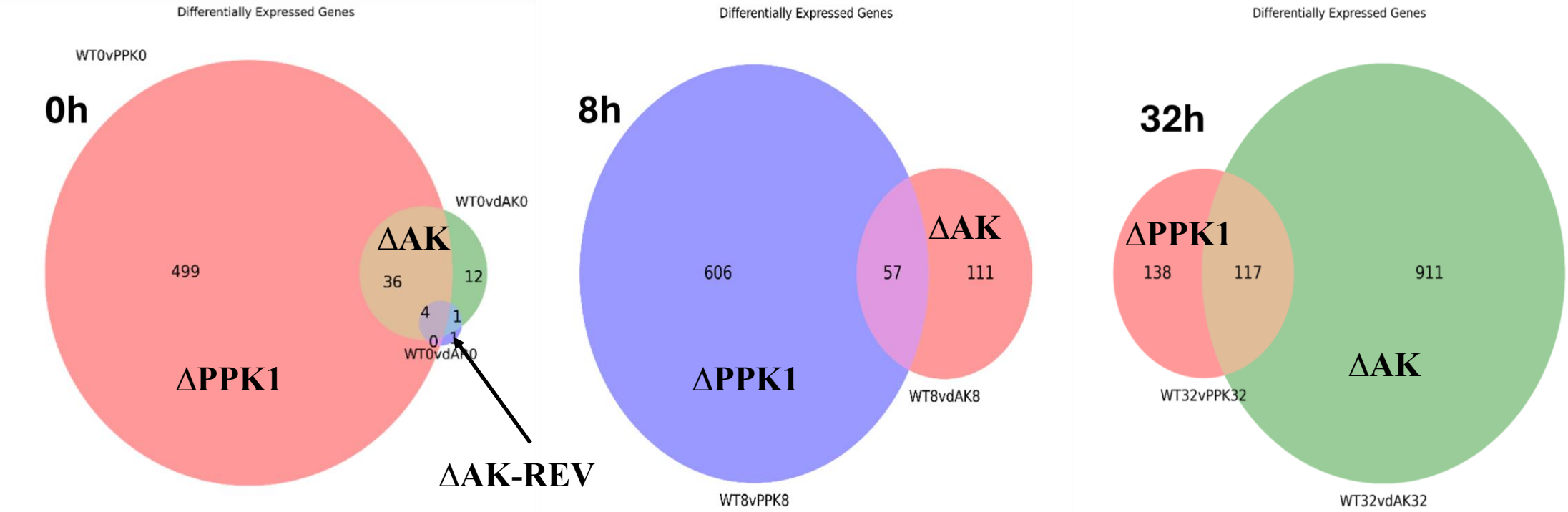
Hypothesis: ΔPPK1 also shows phenotypic variability and adverse stress response suggesting functional overlap of PPK1 and AK in energy buffering of *M. xanthus*.

ΔPPK1 Exhibits Phenotypic Variability



- **ΔPPK1 exhibits phenotypic variability across technical replicates.**
- **The variability is similar to that observed by Al-jarah (2025) in ΔAK-2b**
- **Although the morphology of partially rescued FB are slightly different.**

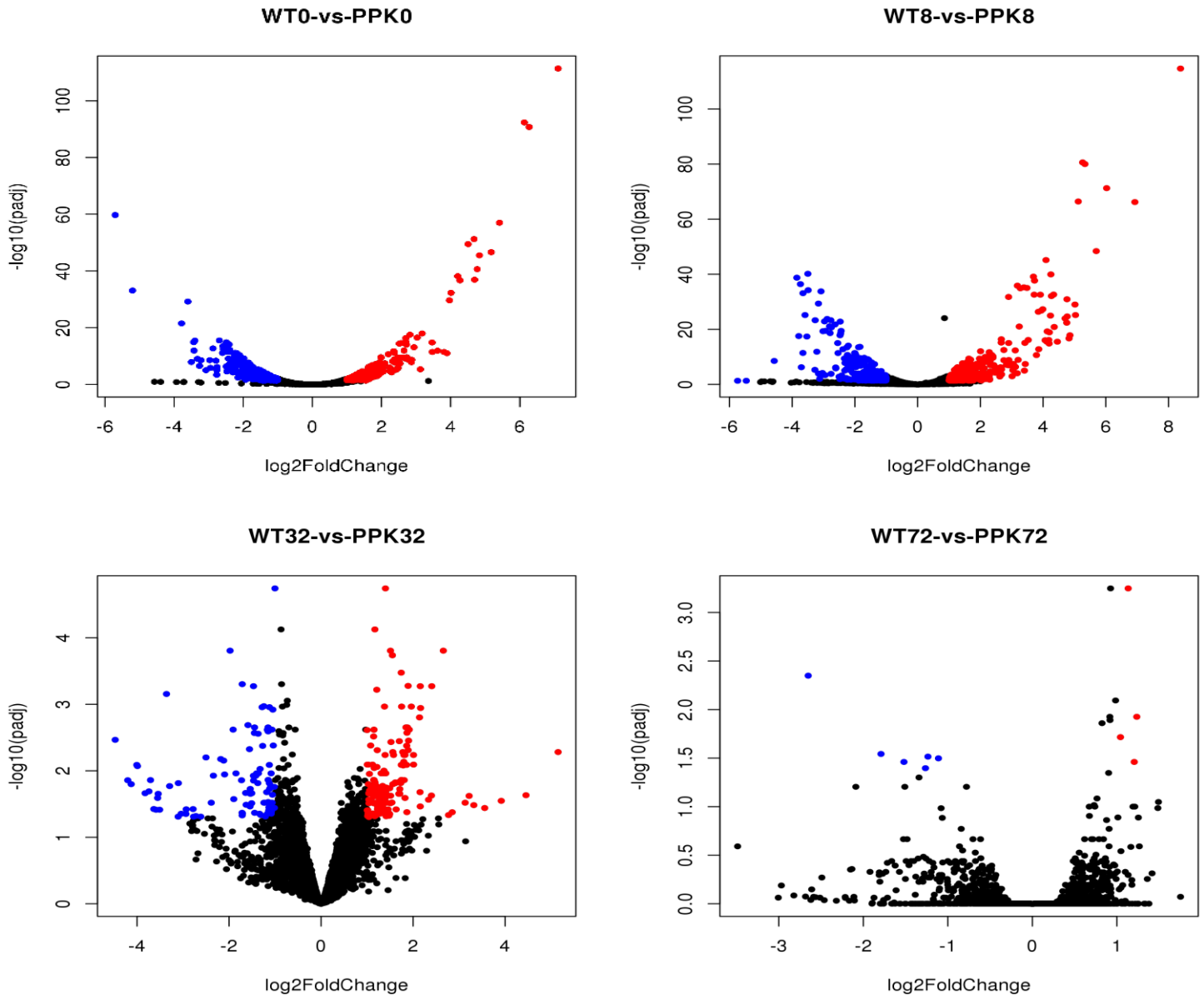
Gene Expression Profiles of *M. xanthus* Mutants Relative to WT



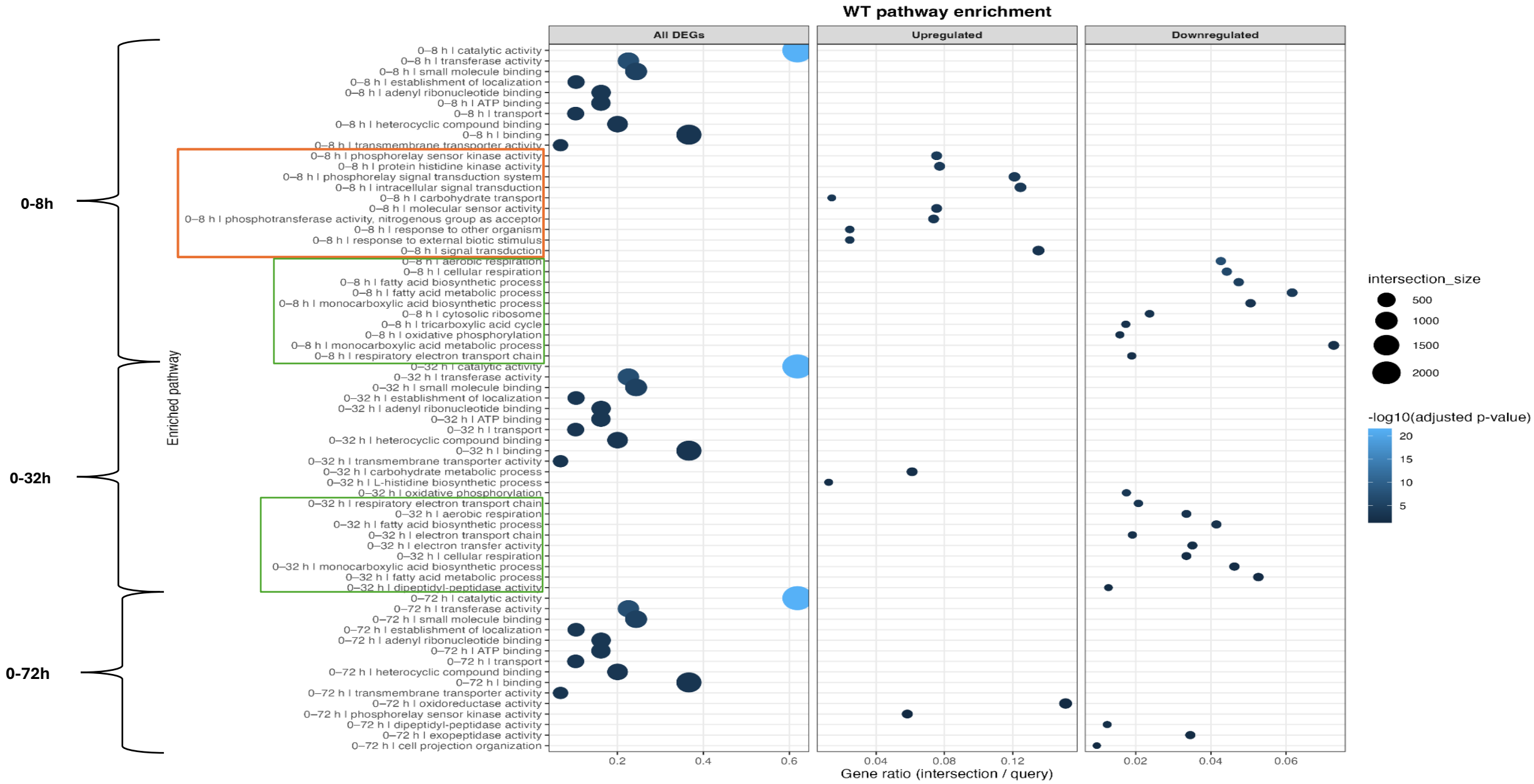
ΔPPK1 is the most different from WT at 8h while ΔAK-2b diverges the most at 32h.

Venn diagrams generated using Python

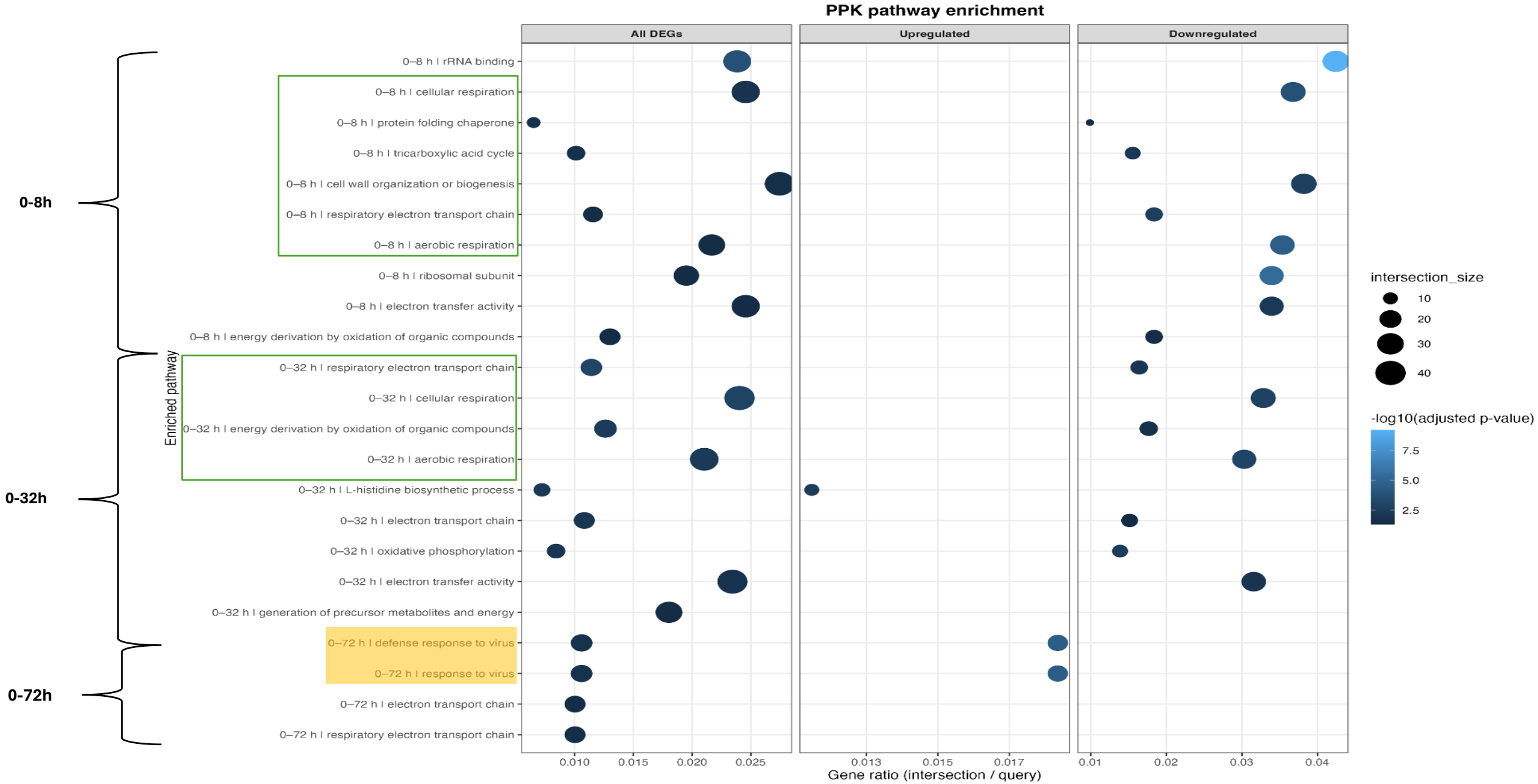
Differential Gene Expression in ΔPPK1



Pathway Analysis of *M. xanthus* WT



Pathway Analysis of *M. xanthus* ΔPPK1

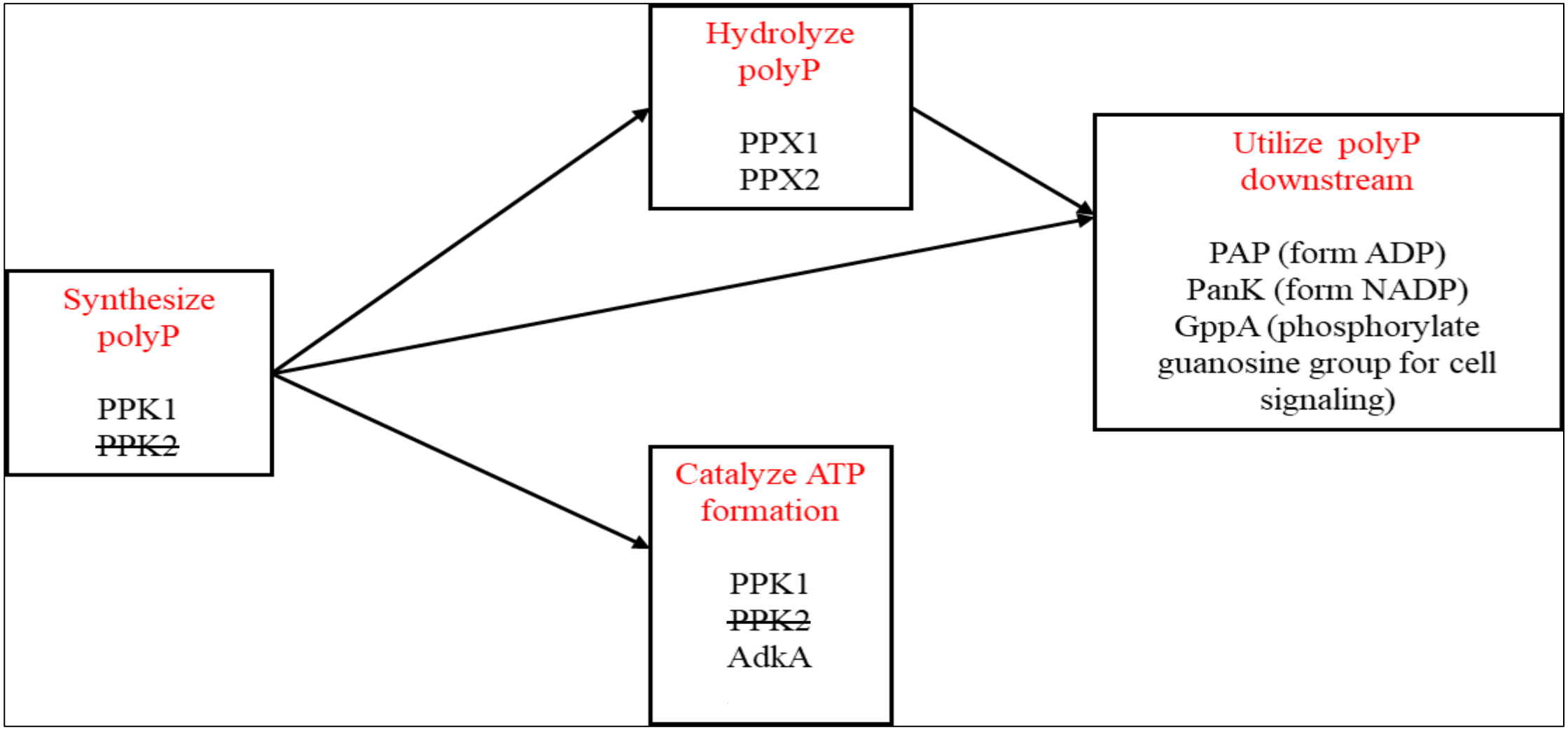


Key Takeaways

- ΔPPK1 demonstrated phenotypic variability like ΔAK-2b, but with differing morphology
- ΔPPK1 and ΔAK-2b had limited overlap in differential gene expression even though both strains exhibited arrested phenotype during development
- PPK1 is likely to play a role in early development as opposed to AK, which is highly expressed around sporulation (~ 32h)
- AK is not upregulated in ΔPPK1 and PPK1 is not upregulated in ΔAK-2b or ΔAK-REV

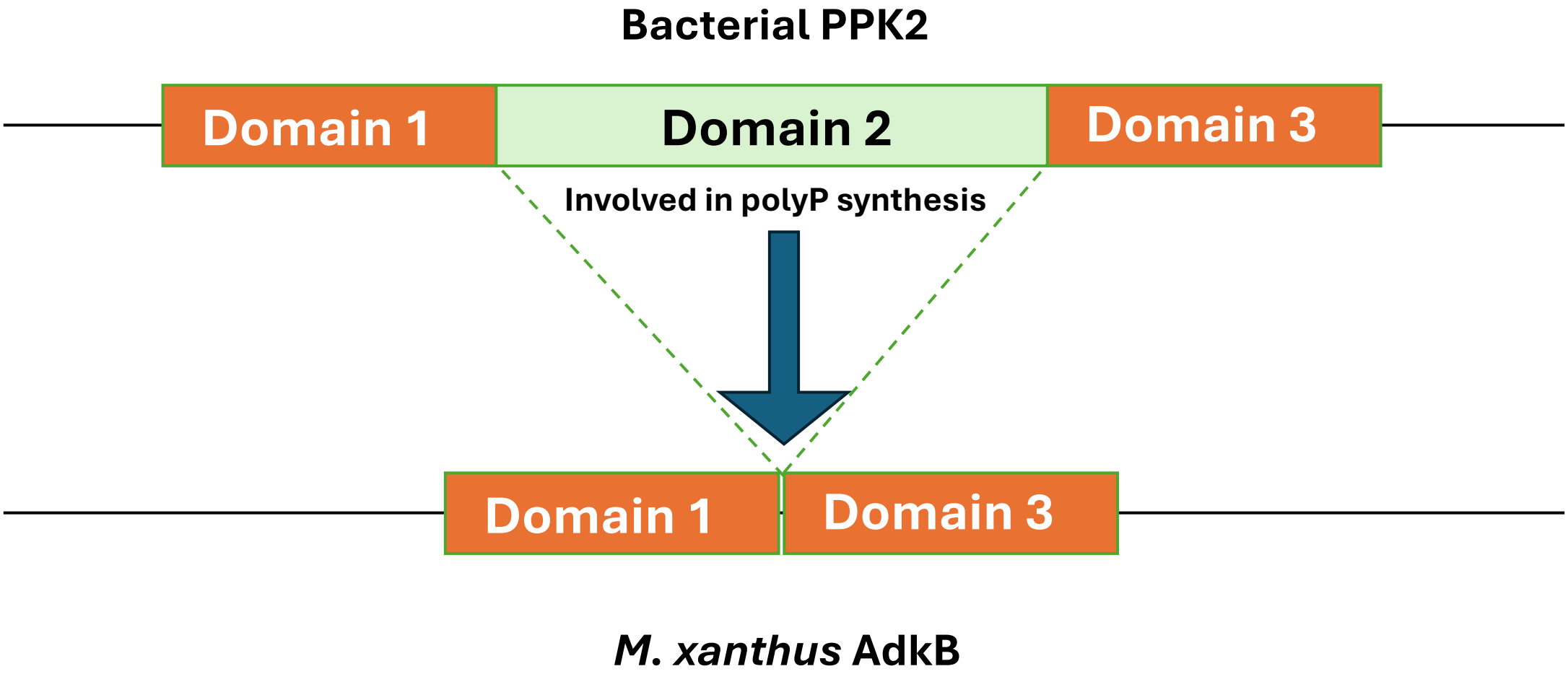
What does this mean?

Polyphosphate: A Multifunctional Substrate



PolyP chemistry is needed in many cellular functions. Specialized enzymes exist for most functions. *Important functions like synthesis and hydrolysis have more than one specialized enzyme.*

Evolutionary Causes for Phenotypic Variability



Future Directions

- **Construct ΔAK ΔPPK1 double deletion mutant to study fruiting body phenotype, stress response, and changes in gene expression**
- ***Myxococcus stipitatus* PPK2 could be inserted into the *M. xanthus* ΔAK-2b genome to test if it can stably rescue fruiting body formation**

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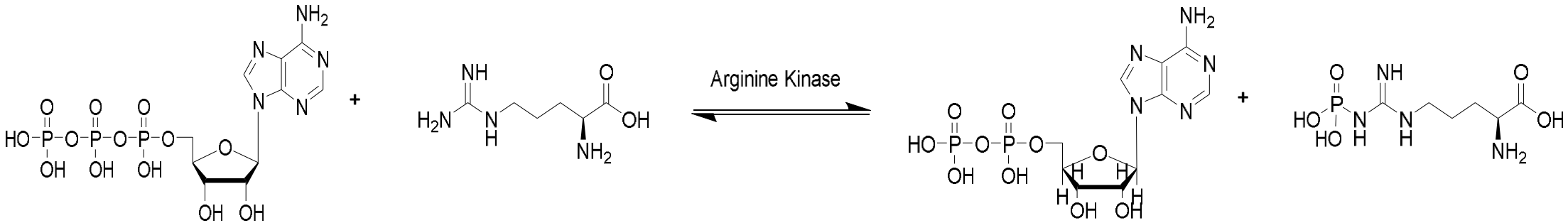
- Wooster Local Chapter of the A.C.S.

Energy Buffering Enzymes of *Myxococcus xanthus*



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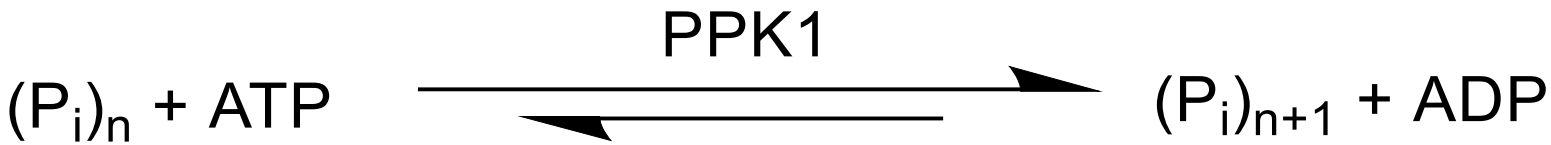
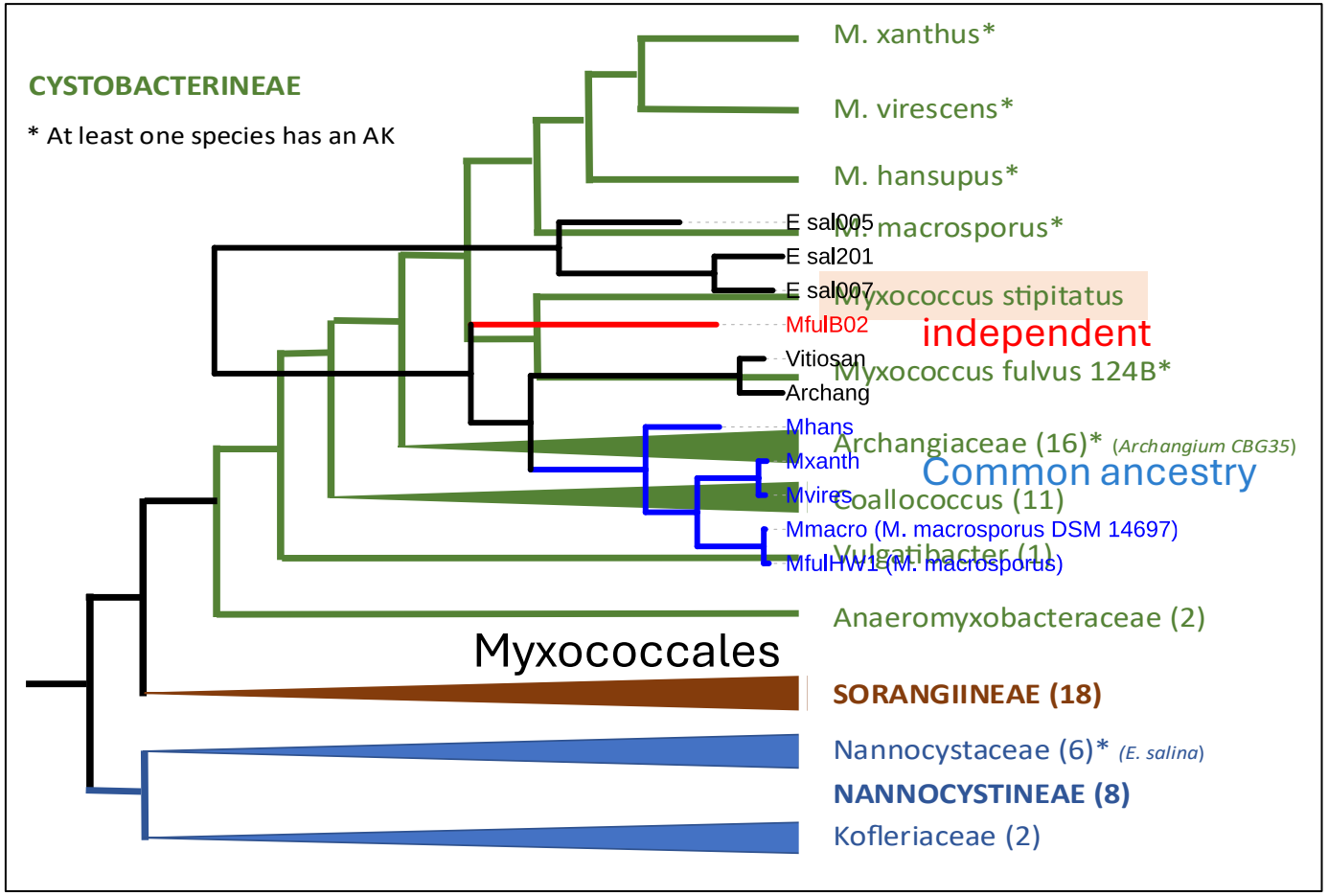


Reversible reaction catalyzed by Polyphosphate kinase 1 converting ATP into a linked chain of inorganic phosphates (polyphosphate) and ADP.

Bragg et al. (2012). Journal of Bacteriology, 194(10). https://doi.org/10.1128/JB.06435-11

Arginine Kinase Distribution in Bacteria



- **Distribution of AK homologs in cystobacterineae is not continuous, suggesting multiple independent HGT events.**
- **In myxococcales specifically, independent HGT events have been documented.**

M. xanthus Stress Response upon AK Loss

TABLE 2 Effects of various stressors on generation times of wild-type and Δ*ark* mutant strains[a]

Strain	Generation time (h)[b]			
	No stressors	0.2 M sucrose	0.2 M KCl	0.2 M NaCl
Wild type	5.1 ± 0.26	6.6 ± 1.38	6.5 ± 0.95	7.8 ± 0.57
Δ*ark* mutant	4.9 ± 1.2	7.5 ± 1.2	9.1 ± 1.6	11.6 ± 1.2

TABLE 3 Generation times after pH shift[a]

Strain, pH	Generation time after pH shift (h)[b]
WT, 7.6	5.22 ± 0.47
WT, 5.0	5.28 ± 0.40
Δ*ark* mutant, 7.6	4.53 ± 0.14
Δ*ark* mutant, 5	7.36 ± 0.53

Bragg et al. (2012). Journal of Bacteriology, 194(10). https://doi.org/10.1128/JB.06435-11

Troubleshooting Stress Response Assays

Table 1. Generation doubling times (in hours) of *M. xanthus* strains under 0.2M NaCl stress using methodologies from Praul (2025) and Bragg et al. (2012).

M. xanthus strains	Salt stress (i) method from Praul (2025)		Salt stress (ii) method from Bragg et al. (2012)	
	Control	0.2M NaCl	Control	0.2M NaCl
WT	7.8 ± 1.1	13.9 ± 2.3		
ΔPPK1	5.8 ± 3.2	10.2 ± 5.4	7.9 ± 3.0	17.8 ± 8.1
ΔAK-2b	6.4 ± 3.0	10.6 + 5.7	7.8 ± 1.9	24.0 ± 6.9
ΔAK-REV	6.0 ± 3.0	8.8 ± 7.5	8.4 ± 2.9	20.7 ± 7.9

Stress Response Results

Table 2. Generation doubling times (in hours) of *M. xanthus* strains under pH 7.6 and pH 5.5 ambient stress.

M. xanthus strains	pH 7.6	0.2M NaCl
WT	7.8 ± 1.1	137 ± 2.3
ΔPPK1	6.6 ± 1.4	167 ± 5.3
ΔAK-2b	8.2 ± 2.3	147 ± 3.5
ΔAK-REV	8.9 ± 1.8	138 ± 2.5

Limitations and Future Directions

- The lack of an AK-PPK1 double deletion mutant limited the extent to which functional compensation could be explained by this study
- The double mutant needs to be constructed in the future to repeat development, stress response, and gene expression analyses
- The phenotypic stability of the double mutant must be studied to see if there is a third energy buffering enzyme in the development program of *M. xanthus*
- Stress response assays should be repeated with salt, pH, osmotic, and oxidative stressors
- New heavy metal salts should be added as stressors to test the lethality of the double mutant
- RNA sequencing should be conducted on the double mutant and the change in expression of specific pathways, such as chaperone proteins and oxidative phosphorylation, must be studied for differential expression
- *Myxococcus stipitatus* PPK2 could be inserted into the *M. xanthus* ΔAK-2b genome to test if it can stably rescue fruiting body formation

Table A1. Description of strains, Plasmid, and Primers used for this study.

Strain, plasmid, or primer	Description or sequence	Reference
Strains		
DK1622	Wildtype and parental M. xanthus strain	(Kaiser, 1979)
dAK-2b	DK1622 with AK gene (MXAN_2252) deletion	(Bragg et al., 2012)
dAK-REV	DK1622 with AK gene (MXAN_2252) deletion	Fraga lab
dPPK1	DK1622 with PPK1 gene (MXAN_0056) deletion	(Harita et al., 2024)
Plasmids		
pBJ114	M. xanthus cloning vector containing galK and Kanamycin resistance gene	(Julien et al., 2000)
pBJ114_AK	pBJ114 with AK gene (MXAN_2252) deletion	Fraga lab
pTF1_Kam^{R}	Plasmid used for InFusion reaction with Kanamycin resistance gene	(Harita et al., 2024)
Primers		
Inner Myxo KO- A2	5’ CGG CAA CGC CCA GGA CTC CTT CGT GTT C 3’	(Bragg et al., 2012)
Inner Myxo KO- D2	5’ CGG ACC TGG ACA TCA CCA GCA TGC AGC AG 3’	(Bragg et al., 2012)
Outer MXAN2252 Forward	5’ TCT TCT ACG CGA GCG GCT ACT 3’	(Bragg et al., 2012)
Outer MXAN2252 Reverse	5’ CCC GGA TGC AAG CAG GAG AAC 3’	(Bragg et al., 2012)
Ppk1-N (Forward)	5’ CAC CCC GGG CTC GAT TTG GCG CGG ACG TGG CGG AC 3’	(Harita et al., 2024)
Ppk1-C (Reverse)	5’ ACC CGG GGT CAG CAC CCC TTG AGG GGC AGG AAG CG 3’	(Harita et al., 2024)

Statistical Analysis for Salt Stress Data

$$Salt\ effect = Doubling\ Time_{0.2M\ NaCl} - Doubling\ Time_{control}$$

$$DiD_{mutant} = Salt\ effect_{mutant} - Salt\ effect_{WT}$$

Thank you!