Investigation of malathion sensitivity in *Saccharomyces cerevisiae* lacking the mitophagy receptor Atg32p

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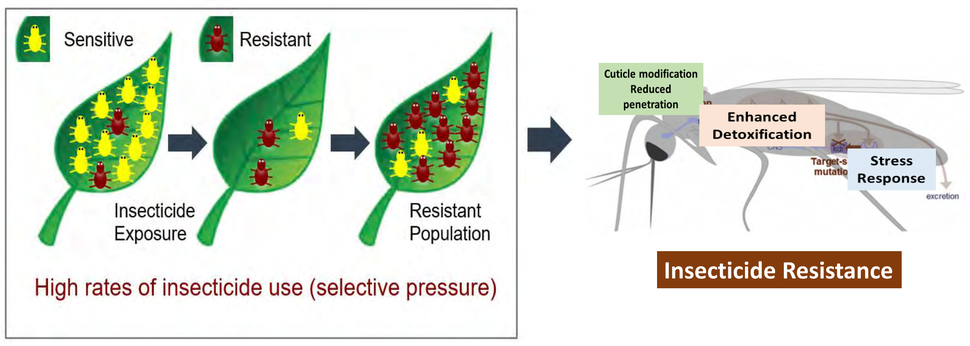
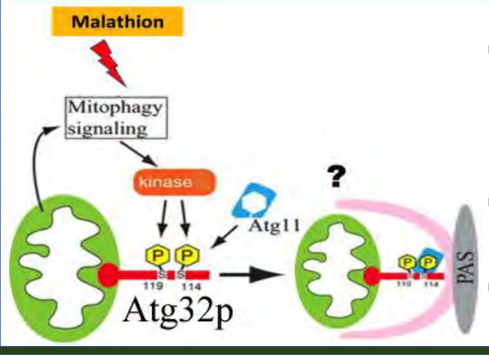
ABSTRACT : Organophosphate (OP) insecticides are widely used in agriculture for controlling insects that can damage crops. Malathion is among the most commonly used of this class. Misapplication and extensive use of insecticides has contributed to the development of OP resistance in insect populations as well as decreasing efficacy of insecticides resultinin crop loss and the spread of insect-borne diseases. In addition, increased insecticide use can lead to environmental contamination and human exposure. However, the limited genetic tools available for insect systems prevents comprehensive screening for mutations or deletions of genes that promote insecticide sensitivity directly. Simple model systems such as the baker's yeast *Saccharomyces cerevisiae* can be utilized to aid in the identification of pathways that lead to insecticide sensitivity. A collection of yeast deletion strains was screened for malathion sensitivity under conditions requiring respiratory growth. This screen identified *ATG32* as being required for malathion tolerance. However, the role of this protein in autophagic processes under other stress conditions has not been previously examined. Using the dual fluorescence Rosella system increased levels of autophagy were observed WT and yeast lacking Atg32p following malathion exposure. In contrast, increased levels of mitophagy were not apparent due to malathion treatment. It appears that malathion sensitivity in yeast lacking Atg32p is not due to disruption in autophagy. Overall, these findings suggest that loss of Atg32p can promote malathion sensitivity. Further characterization of pathways affected in yeast lacking Atg32p may identify molecular targets with utility in the development of agents to sensitize cells to malathion.

Extensive use of insecticides leads to resistance



- A major resistance mechanism is point mutations in genes that encodes AChE enzyme in insects.
- Thus, targeting independent molecular pathways may increase the effectiveness of insecticides.
- However, the limited genetic tools in insect systems hinders the investigation of molecular pathways that promote resistance to insecticides.

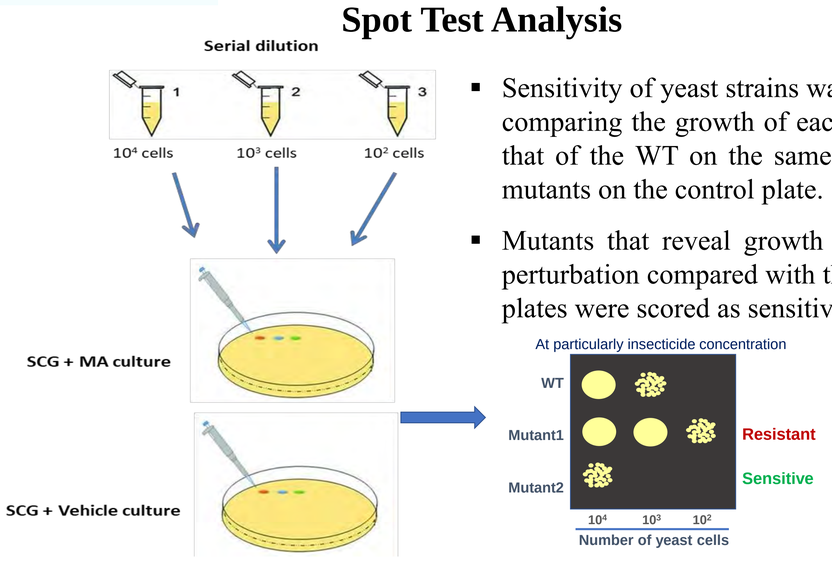
A pathway involving Atg32p may be a target to enhance malathion sensitivity in yeast



- Atg32p is an outer mitochondrial membrane protein involved in mitophagy in yeast cells following nitrogen starvation and perhaps other stresses.
- Altered autophagy/mitophagy pathways may be associated with malathion sensitivity.
- The analysis of *atg32* deletion strain should allow to enhance malathion sensitivity.

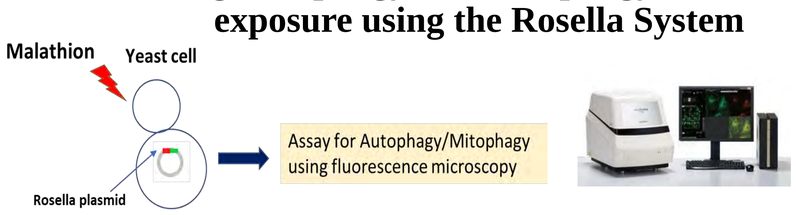
METHODS

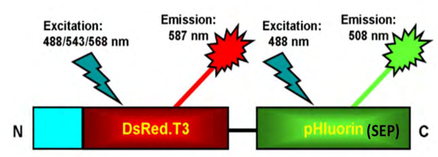
Spot Test Analysis



- Sensitivity of yeast strains was observed by comparing the growth of each mutant with that of the WT on the same plate and the mutants on the control plate.
- Mutants that reveal growth retardation or perturbation compared with the WT on MA plates were scored as sensitive phenotypes.

Monitoring Autophagy and Mitophagy from malathion exposure using the Rosella System





Rosella Biosensor

Rosella : A dual color emission biosensor

Reporter for

Autophagy (Cytoplasmic Rosella)

Mitophagy (Mitochondrial Rosella)

RESULTS AND DISCUSSION

Deletion of ATG32 sensitized yeast to malathion

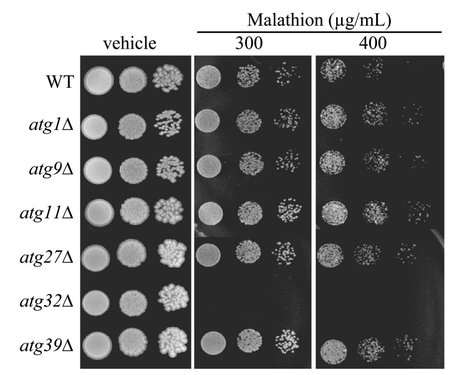


Figure 1. The strains were spotted onto SCG plates supplemented with the indicated concentration of malathion. Plates were incubated at 30˚C for 3 days.

❖ *ATG32* is required for malathion resistance.

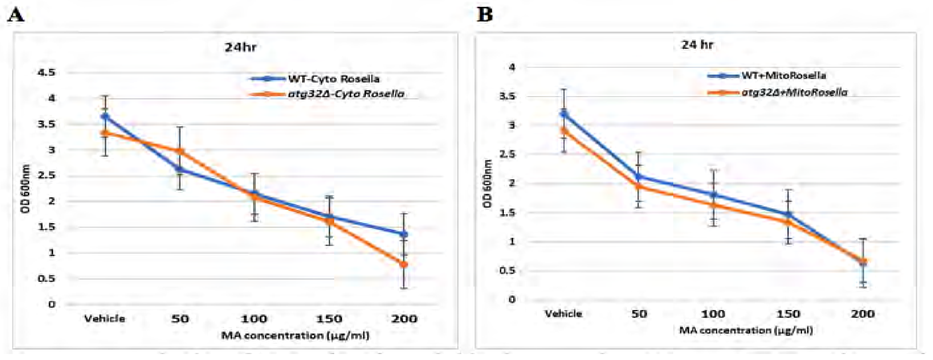


Figure 2. Determination of IC50 values for malathion in WT and *atg32*Δ yeast, (A) Growth curve for Cyto-Rosella transformants, and (B) Growth curve for Mito-Rosella transformant. Cells (starting at OD_{600} = 0.05) were inoculated into 10 mL of SC-Leu liquid medium containing a range of malathion concentrations and grown for 24 hr at 30˚ C in a rotary shaker. The OD_{600} was measured and IC_{50} was determined.

❖ *atg32*Δ Rosella transformants were sensitized to malathion in a concentration dependent manner compared to the wild type.

Autophagy is induced following malathion exposure

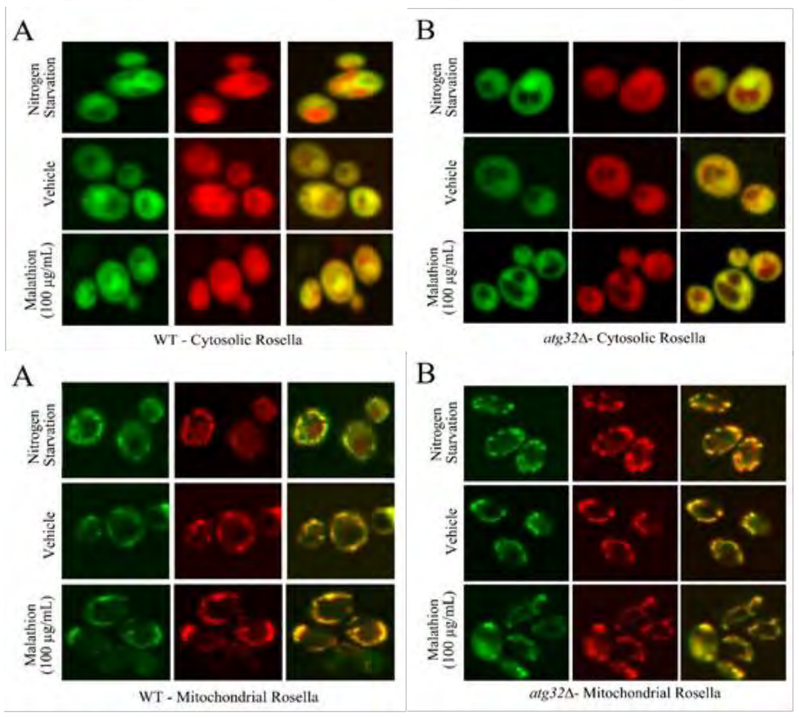


Figure 3. Induction of autophagy following malathion exposure. Wild-type (WT), *atg32*Δ strains expressing Cyto-Rosella were cultured in SC-Leu medium to mid-log phase, and then starved in SCG-N for 4hr as a positive control. In addition, samples treated with vehicle or 100 µg/mL malathion were also examined for induction of autophagy (A) Wild-type cells and (B) *atg32*Δ cells.

Figure 4. Delivery of Mitochondrial Rosella to the vacuole by mitophagy under nitrogen starvation. Wild-type (WT) and *atg32*Δ strains expressing Mito-Rosella were cultured in SC-Leu medium to mid-log phase, and then starved in SCG-N for 6hr as a positive control. In addition, samples treated with vehicle or 100 µg/mL malathion were also examined for induction of mitophagy (A) WT and (B) *atg32*Δ cells.

❖Malathion is capable of inducing the autophagy in cells lacking Atg32p.
❖*atg32*Δ cells exhibit lack of mitophagy activity under nitrogen starvation, and vehicle or malathion exposure.

CONCLUSION

- Bulk autophagy was elevated in *atg32*Δ cells following malathion exposure.
- Yeast *atg32*Δ cells showed impaired mitophagy compared to the wild type under mitophagy-inducing conditions.
- Atg32p function in mitophagy may be important for malathion tolerance.
- Better understanding of the molecular mechanism and regulatory pathways of Atg32p should assist in finding novel strategies to sensitize cells to malathion.

REFERENCES

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