Regulation of symbiotic interactions and primitive lichen differentiation by *UMP1* MAP kinase in *Umbilicaria muhlenbergii*

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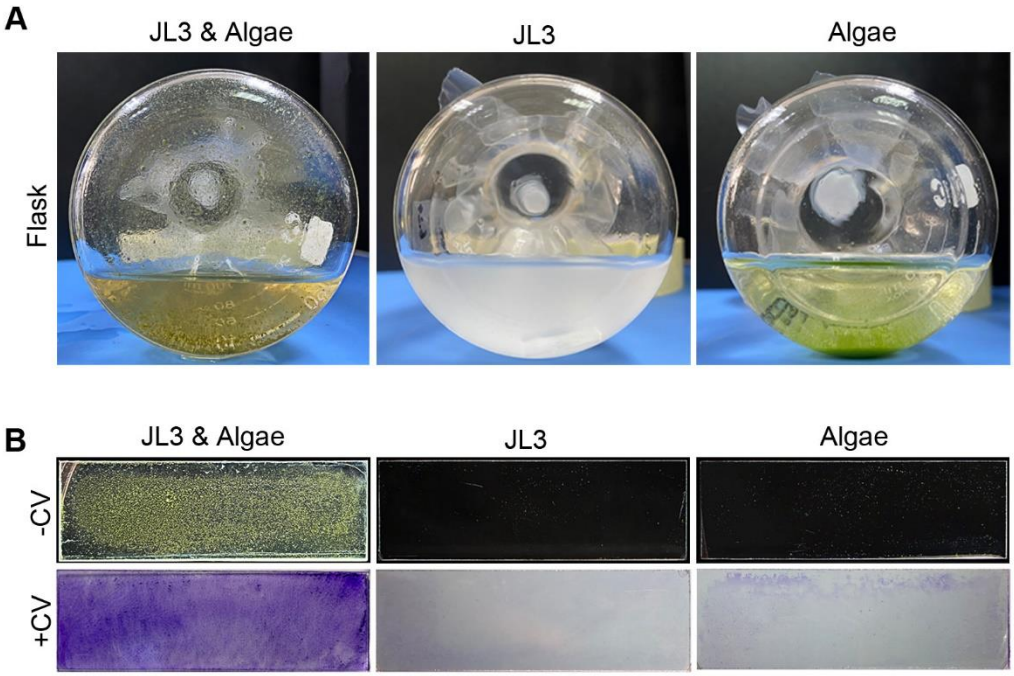
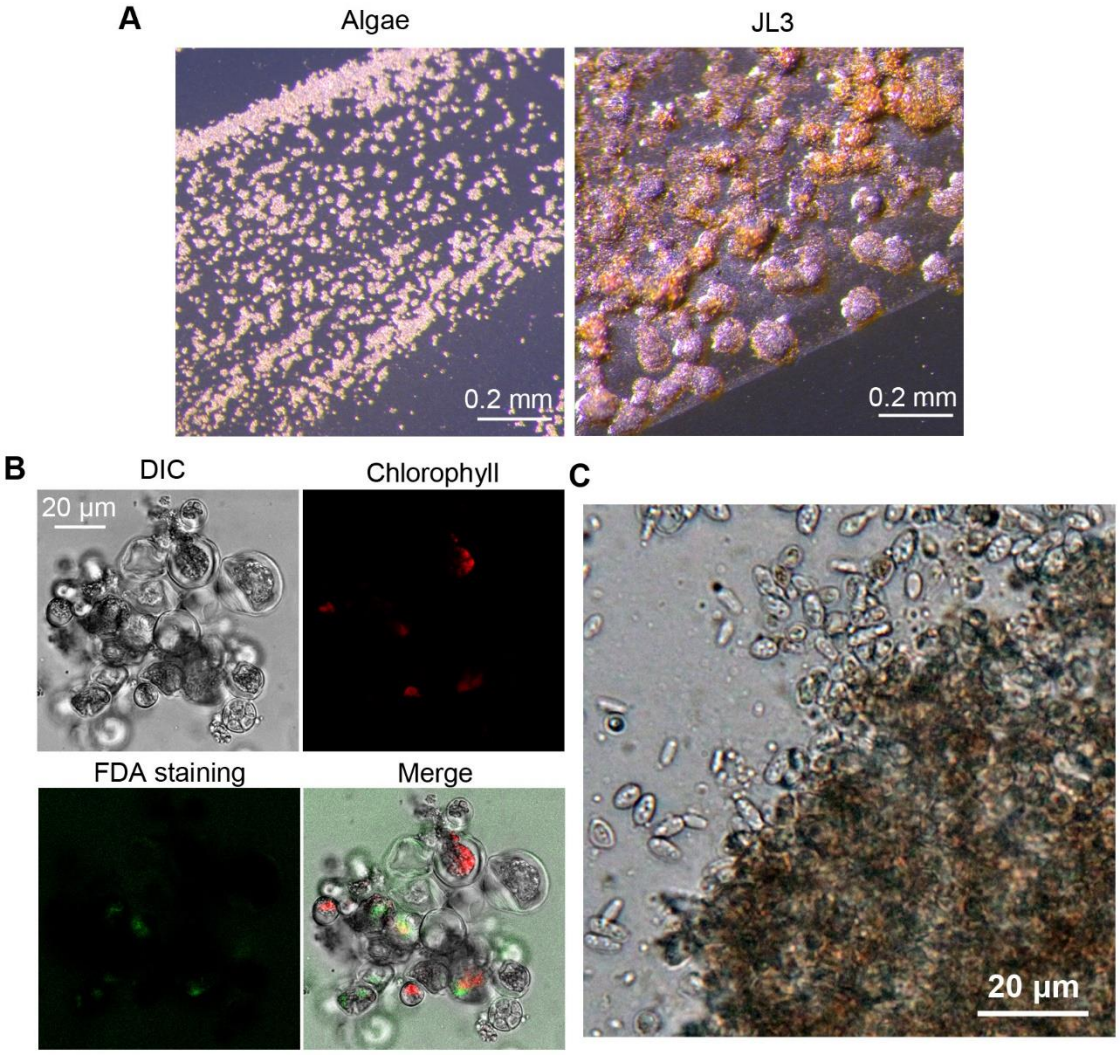
Supplemental Figures 1-5

Supplemental Table 1



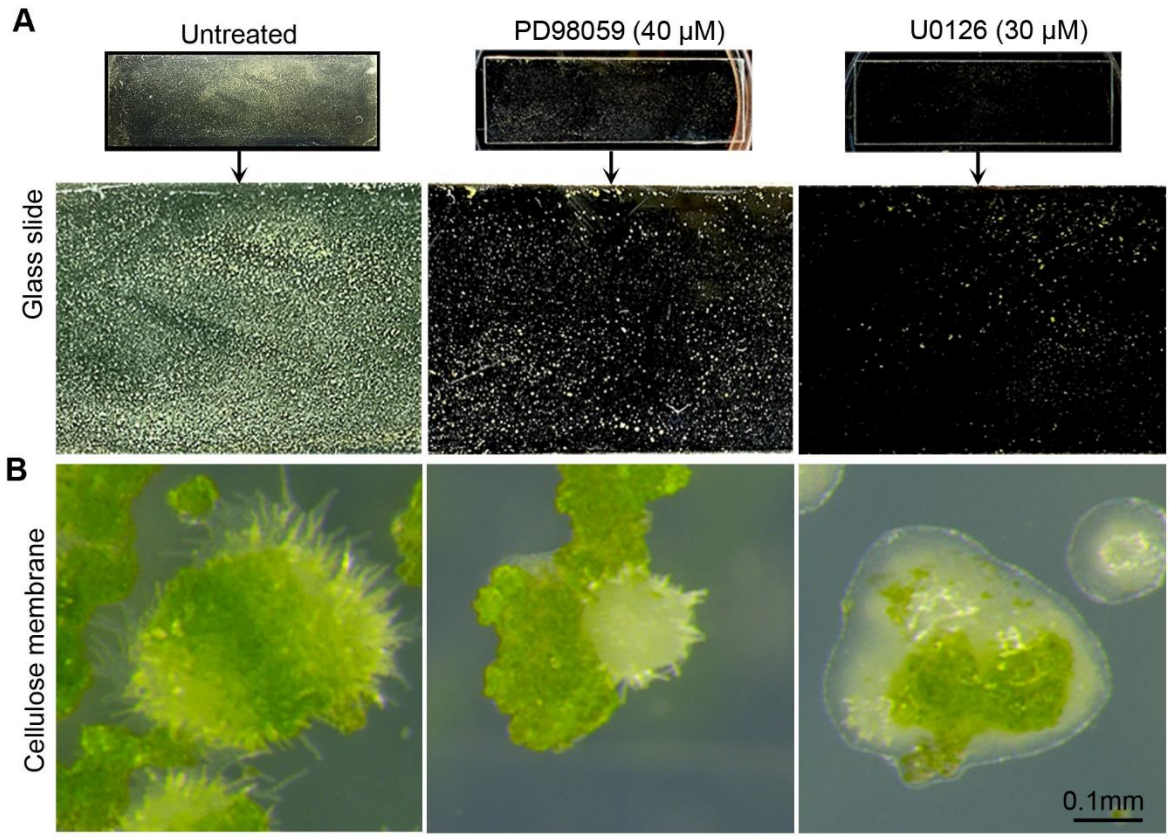
Supplemental Figure 1. Formation of the fungal-algal complex on glass.

A. Flasks containing marked 10-day-old 0.1×PDB cultures were examined for cell masses adhered to the bottom after vigorous shaking. A granular layer of cell masses adhered to the bottom of flasks was observed only in co-cultures of *U. muhlenbergii* JL3 and *T. jamesii* algal cells (1:10). **B**. Slide glasses submerged in the marked 0.1×PDB cultures for 10 days were gently rinsed and directly examined or examined after staining with crystal violet (CV). A greenish (no staining) or purplish (after CV staining) layer was observed in fungal-algal cocultures but not in JL3 and *T. jamesii* cells cultured alone.



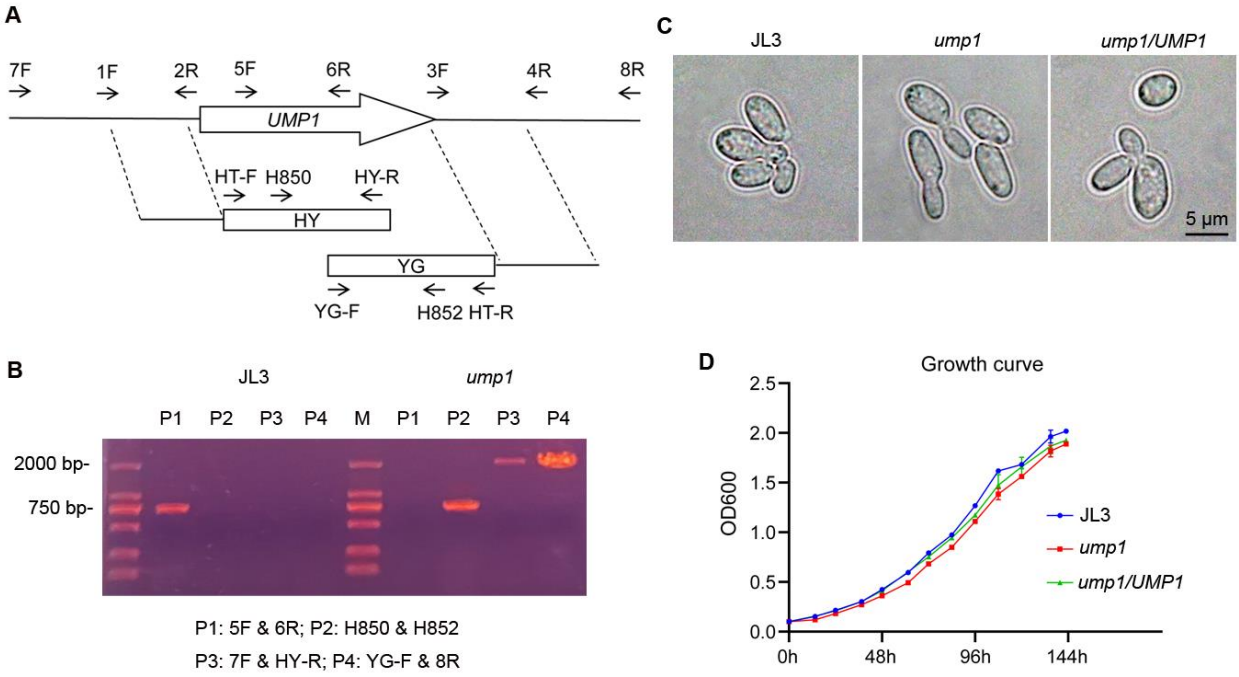
Supplemental Figure 2. Algal or fungal cells cultured separately on cellulose membranes for 3-month.

A) Algal or fungal cells (JL3) cultured on cellulose membranes for three months. **B)** Algal cells were stained with FDA and observed by DIC and epifluorescence microscopy. Most of the algal cells appeared to be dead or damaged and lack chlorophyll autofluorescence. **C**) Yeast cells of *U. muhlenbergii*. The aggregated cells tend to be brownish and some of yeast cells were empty or dead.

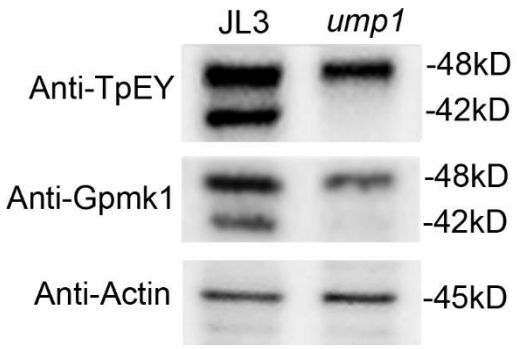


Supplemental Figure 3. Effects of MEK inhibitors on fungal-algal interactions.

A) Slide glasses submerged in the 0.1×PDB cultures of fungal and algal cells (1:10) with or without labelled MEK inhibitors for 10 days were gently rinsed before examination. U0126 was more effective than PD98059 in inhibiting the formation of greenish fungal-algal symbiotic complex. **B**) The mixture of fungal-algal cells (1:10) was cultured on cellulose membranes laid over 0.1×PDA with or without labelled MEK inhibitors for 10 days. Treatments with both inhibitors, particularly U0126 significantly reduced the formation of fungal-algal symbiotic complexes, resulting the growth of whitish yeast cells and greenish algal cells separately. U0126 inhibited pseudohyphal growth and eliminated hairy appearance of the fungal-cell masses.



Supplemental Figure 4. The *UMP1* gene replacement mutant and complementation. **A)** Diagram of the *UMP1* gene, fragments of the *hph* cassette (HY and YG), and PCR primers used to generate and verify the *ump1* deletion mutant. **B)** Verification of the *UMP1* gene replacement event by PCR with marked primers in the *ump1* mutant ud1. JL3 is the wild-type strain. **C)** Yeast cells of JL3, *ump1* mutant, and *ump1/UMP1* complementation transformant. **D)** Growth rate of the same set of strain was measured with OD_{600}. Yeast cells of each strain were inoculated into 200 ml PDB, adjusted to OD_{600} to 0.1, incubated at 25ºC with gentle shaking (100 rpm), and measured every 12 h for 144 h. Means and standard deviations were calculated from data from three independent replicates.



Supplemental Figure 5. Assays for the expression and phosphorylation of Ump1.

Western blots of proteins isolated from the wild-type strain JL3 and *ump1* mutant were detected with marked antibodies. The 42-kD Ump1 band was detected in JL3 but not in the *ump1* mutant. In contrast, the 48-kD UmMps1 band was detected in both strains. Detection with an anti-actin antibody was used as the loading control.

62 **Supplemental Table 1. Primers used in this study.**

Primer	Sequence (5'-3')	Application
1F	TGCGAGGGGATGTTAAGGGA	*UMP1* knockout
2R	AATGCTCCTTCAATATCATCTTCTGTACTTACTAACTCATGGCTGC	*UMP1* knockout
3F	CGTCCGCAATGTGTTATTAAGTCGACGTTGTAAGATTAGATGGTCG	*UMP1* knockout
4R	AGTATACTTTCCTGCGTCGG	*UMP1* knockout
5F	TACGGCTCCAGGTACGGGT	*UMP1* knockout
6R	ATCCAGGTATGCTAAACCTCA	*UMP1* knockout
7F	AGGATTTCCGGGTGGAGTT	*UMP1* knockout
8R	CCACGGTTGTGACAGTCGCT	*UMP1* knockout
HT-F	ACAGAAGATGATATTGAAGGAGC	*hph* cassette
HT-R	GTCGACTTAATAACACATTGCGGACGT	*hph* cassette
HY-R	GTATTGACCG ATTCCTTGCG GTCCGAA	*hph* cassette
YG-F	GATGTAGGAGGGCGTGGATATGTCCT	*hph* cassette
H850	TTCCTCCCTTTATTTCAGATTCAA	*hph* cassette
H852	ATGTTGGCGACCTCGTATTGG	*hph* cassette
C1F	AGGGAACAAAAGCTGGGTACCCCGACCTCCAAGCCCCTCACGT	*UMP1* complementation
C2R	TCGCCCTTGCTCACCATAAGCTTATAAAGATCGAAGCAAGAGGGA	*UMP1* complementation
C3F	ATGGACGAGCTGTACAAGTAAACTTACTAACTCATGGCTGCA	*UMP1* complementation
C4R	GATGATTTCAGTAACGTTAAGTATTTATCGAATGAAGACTTTC	*UMP1* complementation

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