

## The presence of glycolate oxidase in the prasinophycean alga *Mesostigma viride* as an ancestor of land plants.

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The prasinophycean alga *Mesostigma viride* is a freshwater microalgae. Recently, it is strongly suggested that the alga is in the evolutionary line to higher plants by ultrastructural studies and the small subunit rDNA phylogeny of the green algae. The author firstly showed that this alga possesses glycolate oxidase as glycolate oxidizing enzyme, which is essential for the landing and oxygen tolerant for plants. The result also suggests the relativity of this alga with higher plants other than the green algae, physiologically, and *Mesostigma* is the most primitive ancestor of higher plants.

New type of glycolate oxidase was found in the crude extract from the prasinophycean alga *Mesostigma viride* as glycolate oxidizing enzyme, which is essential for the landing and oxygen tolerant for plants. It was assayed by the detection of formed glyoxylate, formed hydrogen peroxide, reduced dichlorophenolindophenol (DCPIP), and consumed oxygen. Unlike the ordinary glycolate oxidase, the enzyme showed slight glycolate oxidizing activity without flavin mononucleotide (FMN), though it showed high activity in the presence of FMN in all assay methods. Flavin adenine dinucleotide (FAD) also made a few contributed to the activity. The affinity for FMN glycolate was 6 micromolar. The enzyme preferentially catalyzed the oxidation of glycolate at pH 8.0 &#8211; 9.5, and its affinity was and 0.27 mM. Glycolate oxidase also catalyzes the oxidation of L-lactase with the affinity 9.2 m M. Other than glycolate and lactate, this enzyme also had broad specificity for other straight chain alpha-hydroxy acids but not for beta-hydroxy acids. Cyanide, azide, N-ethylmaleimide and p-chloromercuribenzoic did not affect the enzyme, whereas 2-pyridylhydroxymethansulfonic acid, rotenone, bipyridine, o-phenanthroline and 8-hydroxyquinoline strongly inhibited it. Except the requirement of FMN, these properties of this enzyme from the prasinophycean alga *Mesostigma* are similar to the properties of glycolate oxidase from higher plants rather than glycolate dehydrogenase from other green algae.

By the way, two apparently different enzymes in green plants and algae act as glycolate oxidizing enzymes, key enzymes of photorespiratory carbon metabolism. The distribution of glycolate oxidase or glycolate dehydrogenase among different taxa is generally in accordance with evolutionary lines in green algae. One line is in the evolutionary line to higher plants and possessed glycolate oxidase in peroxisome. The charophycean algae are belonging to this line. The chlorophycean and the ulvophycean algae are belonging to other line and possess glycolate dehydrogenase in mitochondria. The fact that the algae in the evolutionary line to higher plant possess glycolate oxidase implies some relation between glycolate oxidase and landing.

So, the results in this study suggest the relativity of this alga with higher plants, physiologically adding to the former ultrastructural studies and the small subunit rDNA phylogeny of the green alga, and *Mesostigma* is the most primitive ancestor of higher plants.