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Short Communication

Polymorphism of Microbody Malate Dehydrogenase in *Opuntia basilaris*[1]

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ABSTRACT

Electrophoretic survey of malate dehydrogenase (EC 1.1.1.37) in *Opuntia basilaris* showed intraspecific polymorphism. Further experiments with microbody malate dehydrogenase-specific antiserum suggest that the polymorphism occurs in microbody malate dehydrogenase independent of the soluble and mitochondrial forms. The pattern of polymorphism is one expected from a two-allele Mendelian system.

Multiple forms of malate dehydrogenase (L-malate: NAD oxidoreductase, EC 1.1.1.37) are known to exist in eucaryotic plant cells. Different isozymic forms are localized in the cytoplasm, mitochondria, and microbodies (4, 6, 8, 10). An electrophoretic survey of MDH[2] isozymes in various plant species showed that the microbody enzymes are the most cathodal (8) and lack intraspecific polymorphism (unpublished data). This communication reports the existence and intraspecific polymorphism of microbody-MDH in *Opuntia basilaris*.

Stem tissues of *O. basilaris* were collected in the Deep Canyon Desert Research Center (Riverside County, Calif.). Tissue was immersed in ice and taken to the laboratory for isozyme analysis. Three g of green outer cortex from phyloclades were ground with a Teflon-glass homogenizer in 12 ml of K-phosphate (0.1 M, pH 7.4) for a period of 20 sec. The ground material was pressed through four layers of cheesecloth and centrifuged for 20 min at 10,000*g*. The resulting supernatant fluid was used for separation and detection of isozymes. Electrophoresis was according to Fine and Costello (2) at 150 v for 19 hr. A modification of the Ouchterlony double diffusion analysis (5) was used to confirm the presence of the microbody form of MDH in *O. basilaris* (9). The antiserum used in the Ouchterlony tests was prepared from purified microbody-MDH of cucumber cotyledons (9). Wainwright (9) observed that this antiserum not only precipitated microbody-MDH from various cucumber tissues but also the microbody-MDH from many other species. The antiserum does not precipitate the soluble or mitochondrial forms of MDH (9). In order to determine which isozymes are located in the microbodies, a fresh homogenate was incubated with antiserum for 3 hr and centrifuged at 12,000*g* for 30 min. The supernatant fluid was used for electrophoresis. The antiserum should selectively precipitate microbody-MDH and thus zymograms of incubated and centrifuged homogenate should have the soluble and mitochondrial forms but lack the microbody isozymes.

Of all *O. basilaris* assayed electrophoretically (160 individuals), only three genotypes were found (Fig. 1). Polymorphism with respect to MDH is due to the slower moving bands (α_1 and α_2). Genotype A (58 individuals) had only the slightly more anodal isozyme α_1, genotype B (77 individuals) had both α_1 isozyme and the slower moving isozyme α_2, and genotype C (25 individuals) had only the α_2 isozyme. Ouchterlony tests show precipitates for all three genotypes (Fig. 2). Precipitation bands

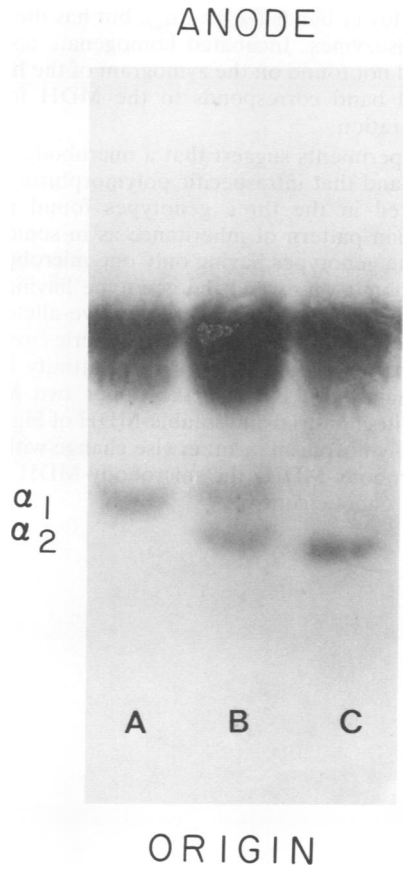

FIG. 1. Starch gel zymogram of three MDH genotypes found in *O. basilaris*. The two slower moving bands (α_1 and α_2) are microbody MDH. Based on previous analyses of MDH isozymes (8), we conclude that the two anodal bands are soluble (most anodal) and mitochondrial (second most anodal).

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[2] Abbreviations: MDH: malate dehydrogenase (L-malate:NAD oxidoreductase, EC 1.1.1.37).

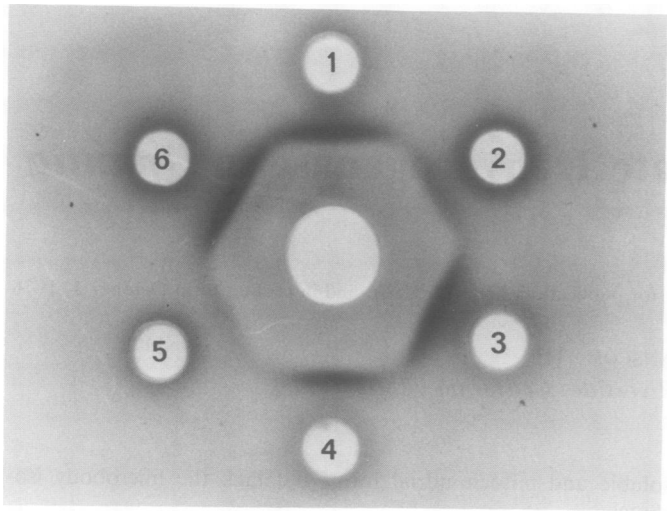

FIG. 2. Ouchterlony tests for the three genotypes of *O. basilaris*. Wells 1 and 4 are for genotype C; 3 and 6 for genotype A; and 2 and 5 for genotype B. Agar gel was stained for MDH after incubation.

from wells 1 and 4 are for genotype C, precipitation bands for wells 2 and 5 are from genotype B, and precipitation bands for wells 3 and 6 are for genotype A. Figure 3 shows the electrophoresis of a fresh homogenate with and without incubation with antibody. The zymogram of the antiserum-incubated homogenate lacks the slower bands (α_1 and α_2), but has the soluble and mitochondrial isozymes. Incubated homogenate also shows an additional band not found on the zymogram of the homogenate. This additional band corresponds to the MDH found in the antibody preparation.

Antibody experiments suggest that a microbody-MDH exists in *O. basilaris* and that intraspecific polymorphism occurs. The pattern observed in the three genotypes found resembles a classic Mendelian pattern of inheritance as in some peptidases (3, 7) where the genotypes having only one microbody isozyme represent the homozygous and the genotype having two bands represents the heterozygous genotype of a two-allele Mendelian system (7). Since MDH is known to be a dimeric enzyme (1), the heterozygous pattern could imply a strict affinity for identical polypeptide chains (7). Because the upper two MDH bands representing mitochondrial and soluble-MDH of Figures 1 and 3 do not show polymorphism or otherwise change with the α_1 and α_2 of the microbody-MDH, the microbody-MDH is evidently under separate genetic control.

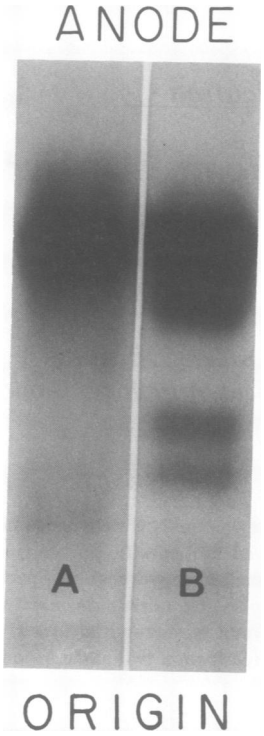

FIG. 3. Zymogram of homogenate with (A) and without (B) incubation with antiserum.

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