A Fourth Malate Dehydrogenase (MDH) Locus in Cucumber

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Abstract. A new malate dehydrogenase (MDH) locus (*Mdh*) was identified in cucumber (*Cucumis sativus* L.) by using an extraction buffer containing sucrose and 2-mercaptoethanol. This fourth locus localizes on starch gels between the previously described *Mdh-2* and *Mdh-3* loci, necessitating the renaming of existing loci. Given previous MDH nomenclature in cucumber, this locus should be *Mdh-3*, and the former *Mdh-3* is more correctly labeled *Mdh-4*. *Mdh-x* designates the interlocus heteromer formed between *Mdh-2* and *Mdh-4*. Interpreting our data in terms of previous work, we hypothesize that *Mdh-1* codes for a microbody isozyme; *Mdh-2*, *Mdh-4*, and *Mdh-x* code for cytosolic isozymes; and *Mdh-3* codes for a mitochondrial isozyme.

Three polymorphic malate dehydrogenase (MDH) isozyme loci (*Mdh-1*, *Mdh-2*, *Mdh-3*) have been detected in cucumber (Knerr and Staub, 1992; Wehner, 1993). Knerr and Staub (1992) suggested that an interlocus heterodimer, formed between two MDH isozyme loci, may overlap with a protein product representing a fourth MDH locus in cucumber (Fig. 1a). Lanes 1, 2, 4, 6, 7, and 8 show individual plants possessing two bands in the *Mdh-x* region but only one band each in the *Mdh-2* region. The band in the cathodal region of *Mdh-x* stained more intensely than the anodal bands, suggesting that this in fact may represent two enzymes whose protein products migrate to slightly different but overlapping locations on the gel. The fact that the *Mdh-x* region stained differentially and variation was observed in this region (anodal band) indicated that another MDH locus might be present. If this fourth locus could be characterized and a polymorphism identified, the value of MDH for studying cucumber would increase. Therefore, our study was designed to test the hypothesis that a fourth MDH locus exists in cucumber, using starch gel electrophoresis.

Progeny seeds, segregating for allozymes at all three MDH loci, were germinated in vermiculite in a greenhouse (16-h photoperiod

plus cool-white fluorescent lights providing $\approx 100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; 17/35C day/night cycles). Progeny were obtained by self-pollinating line 6424E, which has the pedigree [(PI 285606 x PI 412188) x (PI 432858)] x (PI 412188 x PI 412118). One cotyledon was removed from each germinated seedling 3 days after emergence for electrophoretic analysis.

We followed Knerr et al.'s (1989) procedures when performing electrophoresis and subsequent staining for MDH with the following exception: an additional extraction buffer was used that added 5% sucrose (w/v) and 14 mM mercaptoethanol to the original 50 mM Tris-HCl buffer (pH 7.5) (Wendel and Weeden, 1989). These components can improve the extract quality because sucrose acts as an enzyme-stabilizing osmoticum and mercaptoethanol functions as an antioxidant. Electrophoresis was performed on two gels using each extraction buffer (original and modified) on a separate gel with duplicate tissue samples. Horizontal starch gel electrophoresis was conducted using 200-V electric potential (maximum of 75 mA). Gels were cut horizontally into four slices, and the top slice was discarded. The remaining slices were stained for MDH using standard methods (Shaw and Prasad, 1970).

Adding 5% sucrose (w/v) and 14 mM mercaptoethanol resulted in further band separation on zymograms stained for MDH (Fig. 1b). This enhanced separation confirms the hypothesis that another MDH locus overlapped the interlocus heteromer formed between the *Mdh-2* and *Mdh-3* (Fig. 1a) loci of Knerr and Staub (1992).

The consistent band identified in Fig. 1a as *Mdh-x* has been separated from the interlocus heteromer formed between *Mdh-2* and *Mdh-3*. Figure 1b has been labeled to account for this additional locus. Relative mobility and reference to previous research (Knerr and Staub, 1992) allow for clarification of this new locus. In lane 1 of Fig. 1a, the band intensity of the

cathodal-most band of *Mdh-x* is less than that for other individuals (lanes 2 through 10). When the extract of an individual, phenotypically like that observed in lane 1 Fig. 1a, is treated with mercaptoethanol (e.g., lane 1; Fig. 1), a cathodal-most band in the *Mdh-x* region is absent, lending support to the hypothesis of interlocus dimerization.

The previously described *Mdh-3* (Knerr et al., 1989) is now more correctly *Mdh-4*. The cathodal-most region of *mdh-x* (Fig. 1a) is labeled *mdh-3*, and the hypothesized interlocus heteromer is designated *Mdh-x*. Variation has been observed at *Mdh-2*, which is not reflected

Fig. 1. (a) A diagrammatic representation of malate dehydrogenase (MDH; dimeric) variation in 10 cucumber plants at three loci. Interlocus dimerization can be seen between *Mdh-2* and *Mdh-3* when using 50 mM Tris-HCl extraction buffer (pH 7.5). *Mdh-x* is hypothesized to overlap with a fourth nonvariable MDH locus as one *Mdh-x* band is consistent, regardless of variation at *Mdh-2* or *Mdh-3*. (b) Variation in the same individuals (a) showing interlocus dimerization between *Mdh-2* and *Mdh-4* [labeled *Mdh-3* in (a)] when using 50 mM Tris-HCl extraction buffer (pH 7.5) containing 5% sucrose (w/v) and 14 mM mercaptoethanol. *Mdh-x* is separated from a fourth MDH locus (now labeled *Mdh-3*).

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at the *Mdh-4* (data not presented); thus, this putative locus (*mdh-x*) is not a conformational isomer of *Mdh-2*. Segregation at *Mdh-3* has been observed, and linkage analyses (isozyme and morphological markers) are needed to characterize its breeding and genetic potential. Although the genetic basis of *mdh-x* is not known, it may be that it is an interlocus dimerization product of *Mdh-2* and *Mdh-4*.

Including the interlocus heteromer, we have identified five activity zones for MDH in cucumber cotyledons (unpublished data), which is consistent with the work of Liu and Huang (1976), who discovered activity for five MDH isozymes in cucumber cotyledons using starch gel electrophoresis. By interpreting our data in terms of their work, we hypothesize that *Mdh-1* codes for a microbody isozyme; *Mdh-2*, *Mdh-4*, and *Mdh-x* code for cytosolic isozymes; and *Mdh-3* codes for a mitochondrial isozyme.

A fourth MDH locus has been identified in cucumber by using an extraction buffer containing sucrose and 2-mercaptoethanol. This locus appears on starch gels between the previously described *Mdh-2* and *Mdh-3* loci, necessitating the renaming of existing loci. Given previous MDH nomenclature in cucumber, this locus should be named *Mdh-3*, and the former *Mdh-3* is more correctly labeled *Mdh-4*. *Mdh-x* designates the interlocus heteromer formed between *Mdh-2* and *Mdh-4*. The more complete characterization of this complex enzyme system provides information useful for the eventual subcellular localization of MDH isozymes and the analysis of potential linkage relations.

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