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Short Communication

Stability of Phenylacetic Acid in Liquid Media

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Abstract. The stability of phenylacetic acid (PAA) in H_2O and B5 and MS culture media was determined by HPLC. There was no loss of PAA when a nonsterile 10 mM stock solution was held at 5°C for 2 months. PAA was stable to autoclaving in full-strength MS and B5 media. After storage at 5°C or after agitation at 125 rpm at room temperature for 28 days, 100% of the PAA remained in B5 medium. Under comparable conditions, up to 15% of the PAA was lost in MS medium.

The role of phenylacetic acid (PAA) as a plant growth regulator has been investigated sporadically since it was first shown to have weak auxin activity (Zimmerman and Wilcoxon 1935). More recent investigations have established that PAA occurs in several plants (Abe et al. 1974, Fregeau and Wightman 1983, Wightman and Lighty 1982). Further, the amount of PAA in shoots is several times greater than the amount of indoleacetic acid (IAA), the compound considered to be the major auxin (Wightman and Lighty 1982). Recent research has demonstrated that PAA may be involved in the regulation of auxin transport in pea stems (Johnson and Morris 1987).

While tissue culture techniques can be used to study the effects and mode of action of plant growth regulators, there is only one report on the ability of PAA to serve as an auxin in such systems (Milborrow et al. 1975). When further studies are begun, it would be desirable to know the stability of PAA under conditions commonly employed in tissue culture. For example, IAA is unstable in light and is destroyed in the presence of MS basal salts (Dunlap et al. 1986). The objective of this study was to determine the stability of PAA in basal media to autoclaving, storage, and agitation over a period of 28 days.

Materials and Methods

The B5 and MS (with B5 vitamin mix) media were prepared with Millipore-filtered deionized H_2O and stock solutions (Dodds and Roberts 1985), adjusted to pH 5.5 (B5) and 5.7 (MS), and dispensed at 50 ml/250 ml flask. The flasks were stoppered with cotton, autoclaved at 1.05 kg/cm^2 for 15 min, and cooled to room temperature. The media were either stored at 5°C in the dark or placed on a rotary shaker operating in the dark at 125 rpm at room temperature (20–25°C) for 28 days. Volume losses due to evaporation were corrected with sterile distilled H_2O. The experiment was initially performed with 1 mM PAA and repeated at 500 μM PAA. To test the stability in H_2O, a 10 mM PAA (Sigma Chemical Co., St. Louis, MO) stock solution was prepared in Millipore-filtered deionized H_2O and stored at 5°C.

All analyses were made with a Beckman model 332 HPLC unit fitted with a Bio-Rad Aminex HPX-87 ion exclusion column (300 × 7.8 mm) for organic acids. The column was heated to 50°C and eluted isocratically with 30% acetonitrile, 70% 0.01 N H_2SO_4 at a flow rate of 0.5 ml/min. The detector was set at 254 nm, and the maximum injection volume was 20 μl. Under these conditions PAA had a retention time of 14 min.

A fresh 10 mM PAA standard was prepared daily, and dilutions were made in the range of 1 mM to 25 μM. In order to obviate changes in column efficiency with time, a standard curve was prepared daily from at least three PAA standards run in duplicate. All experimental samples were run in duplicate, with peak height as the measure of PAA concentration.

Results and Discussion

Analyses showed that autoclaving did not destroy PAA in liquid B5 or MS media. Results obtained with autoclaved media placed on a rotary shaker for 28 days are summarized in Table 1. Within the limits of detection, there was no loss of PAA in B5 medium. With the MS medium, a decrease in the amount of PAA was first noted at 8 days. As indicated in Table 1 and in related experiments, about 15% of the PAA was lost after 3–4 weeks on the shaker.

Similar results were obtained when autoclaved media were refrigerated at

Table 1. Percentage of phenylacetic acid (PAA) remaining in autoclaved B5 and MS media after shaking at 125 rpm at room temperature for various periods of time. Results reported are average values for experiments carried out with 500 μM and 1 mM PAA.

	% PAA remaining in	
Days	B5 medium	MS medium
0	104.1 ± 5.2%	101.1 ± 3.0%
3	99.8 ± 1.8%	98.7 ± 3.3%
8	99.5 ± 4.2%	96.5 ± 2.0%
20	102.7 ± 8.9%	86.7 ± 2.7%
28	94.8 ± 6.8%	89.0 ± 3.0%

5°C. This indicates that some component(s) of the MS medium react with the PAA. In studies of IAA stability, the rate of degradation was reduced when the concentration of the salts in the MS medium was decreased (Dunlap et al. 1986). We have not investigated the relationship of salts concentration and PAA stability.

There was no loss of PAA when the 10 mM aqueous solution (pH 3.4–3.5) was stored at 5°C for 28 days. In related experiments, aqueous, nonsterile PAA solutions in the range of 0.025 to 1 mM usually became contaminated within a week or so.

While some loss of PAA was noted in MS medium, it would appear that this naturally occurring auxin is sufficiently stable to allow its use in experiments with plant suspension cultures.

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