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Effects of Six Different Agars on Tracheary Element Differentiation in Explants of *Lactuca*

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Agar gel has been the traditional supportive agent for semisolid plant tissue culture media. Agar is not physiologically inert, and this natural product contains varying amounts of growth-stimulating and growth-inhibiting contaminants.[1-3] The gross chemical analysis of Difco agars has been published.[4] Few investigators have examined the possible role of agar as a nutritive source in plant tissue culture media. Romberger and Tabor[5] reported that an "agar inhibitory effect" was considerably decreased by autoclaving the agar medium with sucrose. Activated charcoal has been used to adsorb organic and inorganic molecules from culture media, and this agent may remove inhibitory contaminants from the agar gel.[6]

Because of the variety of plant tissue culture agars that are now commercially available, a series of experiments was conducted in order to test the relative effectiveness of several agars in acting as a supportive matrix for explants exhibiting xylogenesis. Since this was a preliminary investigation, no efforts were made to isolate any physiologically-active contaminants from the agars. Because of numerous studies, the induction of xylogenesis in lettuce pith explants was chosen as a test system.[7,8]

Explants were prepared from the core of pith excised from heads of commercially-grown Romaine lettuce (*Lactuca sativa* L. Romana) as described by Dodds and Roberts.[3] After surface sterilization in 10% (v/v) Clorox for 10 min followed by three successive rinses in sterile double distilled water, borings of the pith parenchyma were made with a 5 mm I.D. cork borer. The pith cylinders were then cut into explants approximately 2 mm in thickness. The explants were rinsed twice with sterile double distilled water, blotted on sterile Whatman No. 1 filter paper, and transferred individually to glass vials (15 ml capacity) containing 10 ml of a xylem induction medium. The medium consisted of a Murashige and Skoog (1962)[9] basal salt mixture, supplemented with *myo*-inositol (100 mg/*l*), thiamine·HCl (0.1 mg/*l*), glycine (2.0 mg/*l*), nicotinic acid (0.5 mg/*l*), pyridoxine·HCl (0.5 mg/*l*), IAA (10 mg/*l*), kinetin (0.1 mg/*l*), D-glucose (2% w/v), and one of the agars (1% w/v) listed below. The agars employed in the present study included Difco Bacto-Agar, Gibco Phytagar, KC-TC agar, Flow agar, FMC SeaPlaque, and FMC SeaPrep.* The pH of the medium was adjusted to 5.7, and the complete medium was sterilized by autoclave. The vials, capped with KimKap

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Table 1. The effects of six different agars on the induction of tracheary element cytodifferentiation in explants of *Lactuca*.[a]

Agar preparation (1% w/v)	Tracheary Elements ($\times 10^3$) Experiment Number			
	1	2	3	4
Difco Bacto-Agar	10.5a	13.3a	19.3a	13.2a
Gibco Phytagar	10.6a		17.4ab	13.6a
Flow agar		15.9b	16.3a	12.4a
KC-TC agar	13.2a		23.3c	14.0a
FMC SeaPlaque agarose		8.9b	9.4d	6.2b
FMC SeaPrep agarose		0.5c	1.5e	0.01c

[a] Each agar was tested at least three times with a minimum of 10 explants for each treatment. Thus, tracheary element counts represent a mean of 10 samples.
Treatments not followed by the same letter are significantly different at the 5% level, as determined by Duncan's Multiple Range Test.

Closures, were dark incubated at 25°C for 7 days. Each agar was tested at least three times with a minimum of 10 samples for each treatment. Data were analyzed using Duncan's Multiple Range Test on the Statistical Analysis System at the University of Idaho.

Tracheary element cell counts were performed on the explants after 7 days of culture by a modification of the Brown and Rickless[10] maceration technique.[3] Explants were placed individually in vials containing 1 ml of a maceration fluid consisting of equal parts of chromium trioxide (5% w/v) and HCl (5% v/v) for 24 hr at room temperature. The maceration fluid was then removed and replaced with 1 ml of distilled water. The sample was drawn repeatedly into a syringe (2 ml) equipped with a 22-guage needle. The sample was brought to a total volume of 2 ml, thoroughly mixed by syringe, and 1 ml of the sample was transferred to a Sedgwick-Rafter plankton counting chamber. The number of tracheary elements was counted in each of 7 optical fields with the aid of a Whipple eyepiece micrometer at 100× magnification. The total number of tracheary elements in each explant was calculated.[3]

No significant differences in tracheary element count per explant was found between Difco Bacto-Agar, Gibco Phytagar, and Flow agar (**Table 1**). The KC-TC agar produced a considerably greater number of tracheary elements in one of the three experiments in which it was tested. This anomalous result could be due to the lack of an inhibitor substance, the presence of some xylogenic promoter, or to some unknown variable in the technique. The inconsistency in the data suggests that additional experiments should be performed with KC-TC agar. Significantly smaller numbers of tracheary elements were differentiated in the presence of the two agaroses in comparison to the other agars tested. SeaPrep has a low gelling temperature (approximately 15°C), and the medium containing this agarose had a viscous liquid consistency during incubation of the explants. This property may have been inhibitory to the initiation of xylogenesis. SeaPlaque, on the other hand, gels completely in less than 10 min at temperatures below 25°C. Since both FMC agaroses were highly inhibitory to xylogenesis, some additional factor influencing cytodifferentiation must be involved. Similar inhibitory effects on xylogenesis in lettuce pith explants were found using an agarose (Type A) obtained from Calbiochem-Behring** prior to the present study (unpublished, Roberts). Agarose is a purified linear galactan hydrocolloid isolated from agar, and it was

** Calbiochem-Behring, P.O. Box 12087, San Diego, CA 92112.

devised for the diffusion and electrokinetic movement of biopolymers (Guiseley and Renn, 1977).[11] A hypothesis can be advanced that the typical tissue culture agar does play a nutritive role in plant tissue culture media, and that this role(s) is largely eliminated during the removal of anionic polysaccharides and/or other constituents in the preparation of agarose. A review of the purification procedures employed in the manufacture of agarose is available from FMC Corporation.[11]

The xylem differentiation effect by hardness of 1% medium agars among six different agars used was little. The explants used were thin, and there was little effect by hardness of the medium agar.

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《和文要約》

レタスの茎の細胞の木部分化に際して培地に用いた6種類の寒天の効果

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レタスの茎の柔細胞を用いて木部 (tracheary elements, T. E.) 分化を誘導させる実験において，培地に用いる寒天 (1% w/v) 6種類 (市販品) について比較検討した。その結果 Difco Bacto-Agar, Gibco Phytagar および Flow agar では，木部分化にほとんど影響を与えなかった。KC-TC agar は非常に多くの T. E. が形成されたが FMC SeaPlaque, FMC SeaPrep では T. E. の形成が少なかった。