BioFeedback

Dr. Robert P. Adams
Plant Biotechnology
Baylor University
P. O. Box 669
Gruver, TX 79040 USA

A Simple Technique for Removing Plant Polysaccharide Contaminants from DNA

ABSTRACT

*A survey of the inhibitory effects of various plant polysaccharides on DNA restrictions (HindIII and EcoRI) revealed that neutral polysaccharides (arabinogalactan, dextran, gum guar, gum locust bean, β-glucan, inulin, laminaran, mannan and starch) were not very inhibitory. In contrast, acidic polysaccharides (carrageenan, dextran sulfate, gum ghatti, gum karaya, pectin and xylan) were very inhibitory, even at low concentrations. The Elutip-d (RPC-5 type resin) was evaluated for removal of the inhibitory polysaccharides. Used alone or in combination with a phenol/chloroform wash, it proved effective in removing the polysaccharide so that HindIII digestion was possible, except in the cases of carrageenan and dextran sulfate. In addition, the genomic DNA extracts from live oak (*Quercus virginiana*) and magnolia (*Magnolia grandiflora*) were sufficiently purified so that the DNAs could be restricted with both EcoRI and HindIII.*

INTRODUCTION

Polysaccharides are common contaminants in DNA extracted from plant tissues (3,5). Most conventional plant DNA purification methods can remove proteins, but may not be effective for removing polysaccharides (3,5,6,9,10). However, because both the kinds and yields of plant polysaccharides vary among plant species (2,13), the DNA extraction method often does not produce DNA with sufficient purity for enzymatic reactions (1,7,9,11).

Several products are available commercially for purifying and concentrating DNA from agarose. These include Elutip-d from Schleicher and Schuell, Keene, NH; NACS column from GIBCO BRL/Life Technologies, Gaithersburg, MD; and Qiagen tips from Qiagen, Studio City, CA. It seemed reasonable that one of these products might be useful for removing plant polysaccharides from DNA. Due to the diversity of plant polysaccharides in nature, a number of kinds of plant polysaccharides were surveyed to determine if they had differential effects on *Hin*dIII activity. *Hin*dIII was chosen because it is sensitive to polysaccharide contaminants, and a protocol was available for determining relative digestions over time (11). The Elutip-d was evaluated for its effectiveness in removing plant polysaccharides that exhibited inhibitory effects on *Hin*dIII restrictions.

Table 1. Comparison of the Effects of Various Plant Polysaccharides on the Restriction of λ DNA by *Hin*dIII

	*Hin*dIII activities (%) on 1 μg λ DNA			
	with polysaccharide			after elution from
Polysaccharides	10 μg	100 μg	500 μg	Elutip-d
Neutral:(2,13)				
Dextran	++++	++++	++++	NT
Gum guar*	++++	+++	+++	NT
Gum locust bean*	++++	+++	+++	NT
Inulin	++++	+++	+++	NT
Laminaran[1]	++++	++++	++++	NT
Starch	++++	++++	++++	NT
Arabinogalactan	++++	+++	+	++++
β-glucan	+++	+++	+	+++
Mannan	++++	+++	+	++++
Acidic:(2,13)				
Carrageenan	-	-	-	-
Dextran sulfate	-	-	-	NR
Gum ghatti	++	-	-	-(++++[2])
Gum karaya	++++	-	-	+++
Pectin	+++	+	-	+++
Xylan	++++	+++	-	++++

In each test, 1 μg of λ DNA was mixed with 10, 100 or 500 μg of polysaccharide. The Elutip-d was evaluated using 1:500 (DNA/polysaccharide) mixtures. ++++ = 90%–100% activity; +++ = 65%–90%; ++ = 25%–65%; + = 5%–25%; - = no observable activity. NT = Not tested. NR = No recovery of DNA.

*Causes distortion of band migration on gel electrophoresis. Distortion eliminated after elution from an Elutip-d.

[1]Initially no digestion but after a single phenol/chloroform extraction and ethanol precipitation digestion was uninhibited, suggesting a protein contaminant.

[2]100% digestion after a single phenol/chloroform extraction and ethanol precipitation and a subsequent elution through the Elutip-d.

MATERIALS AND METHODS

The Elutip-d, a RPC-5 type anion-exchange resin, was evaluated by use of a model system of λ DNA and various plant polysaccharides, without further purification (Sigma Chemical, St. Louis, MO): larch wood arabinogalactan, Irish moss carrageenan, dextran, dextran sulfate, barley seed β-glucan, gum ghatti, gum guar, gum karaya, gum locust bean, chicory root inulin, laminaran (laminarin in Sigma Chemical catalog), mannan, apple pectin, starch and oat xylan. Polysaccharide solutions were made in a 1% concentration and kept at 70°C overnight to inactivate nucleases that might be present.

The λ DNA/polysaccharide mixtures were prepared in three different ratios (μg DNA:μg polysaccharide): 1:10, 1:100 and 1:500. The mixtures were incubated with *Hin*dIII according to the manufacturer's protocols and examined by field inversion gel electrophoresis (FIGE) (1.0% agarose, 7

V/cm, 3 s forward, 1 s reverse, ramped to 12 s forward and 4 s reverse over 4 h [2]). *Hin*dIII restriction activities were determined by the protocol of Perbal (11) that compares the presence/absence of bands with standardized, partial *Hin*dIII digestions of λ DNA. The following 5 classes were scored according to the presence/absence of λ/*Hin*dIII bands (11): - = no activity; + = 5%–25%; ++ = 25%–65%; +++ = 65%–90%; ++++ = 90%–100% activity.

The following protocol was used for elution from the Elutip-d:

1) Prime with 2 ml of high salt buffer (2.5 M NaCl, 20 mM Tris, pH 7.4, 1 mM EDTA).

2) Wash the column with 5 ml of low salt buffer (0.5 M NaCl, 20 mM Tris, pH 7.4, 1 mM EDTA).

3) Dilute the DNA/polysaccharide mixture to 3 ml with low salt buffer to obtain a low flow through the column.

4) Wash the column with 5 ml of low salt buffer.

5) Elute DNA with 0.4 ml of high salt buffer.

6) Precipitate DNA with 2× volume of ethanol.

The eluted DNA was digested with *Hin*dIII and the restriction pattern scored as above.

Genomic DNAs from live oak (*Quercus virginiana* Miller) and magnolia (*Magnolia grandiflora* L.) were extracted by the hot CTAB method (5). The final DNA pellet was resuspended in 500 µl sterile water and left overnight at 4°C (to aid solution). The salts were adjusted to 0.5 M NaCl, 2 mM EDTA and 20 mM Tris (final volume 1 ml of low salt buffer) prior to loading onto the column.

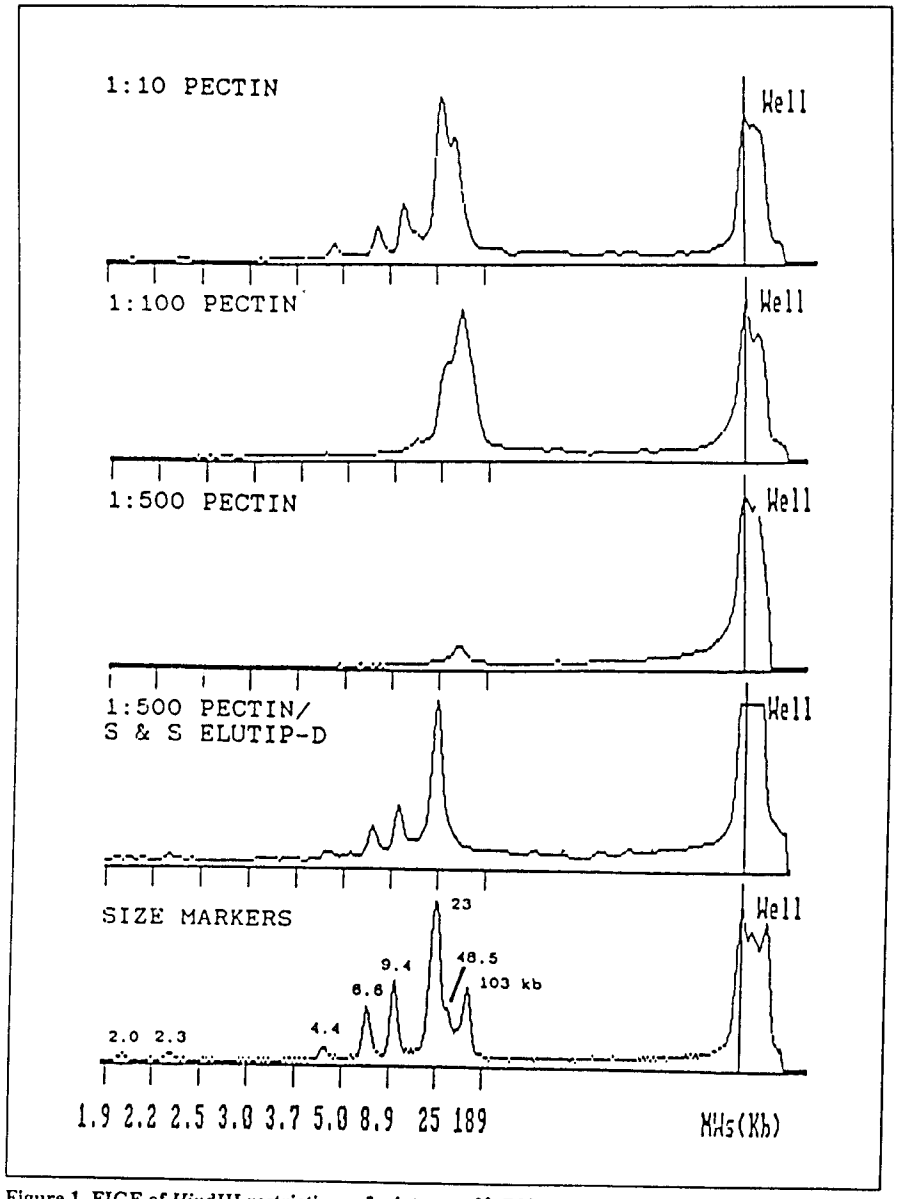

Figure 1. FIGE of *Hin*dIII restrictions of mixtures of λ DNA and pectin, and the effect of a single elution from an Elutip-D and *Hin*dIII restriction of a 1:500 DNA:pectin mixture.

RESULTS AND DISCUSSION

It is apparent from Table 1 that the neutral polysaccharides were much less inhibitory than the acidic polysaccharides. However, even among the acidic polysaccharides, there were several differences. Xylan was the least interfering, whereas carrageenan and dextran sulfate caused very serious problems, even at the lowest concentration tested. The highly inhibitory nature of polysaccharides with free acidic groups is further demonstrated by comparing dextran and dextran sulfate. Dextran (neutral) had no interfering effects at any concentration tested, whereas dextran sulfate (anionic) was highly inhibitory (Table 1).

The Elutip-d columns were effective in removing polysaccharides from DNA except for carrageenan, dextran sulfate and gum ghatti. The addition of a single phenol/chloroform (1:1) extraction on the DNA/gum ghatti mixture did not remove the inhibition. However, subsequent elution from the column resulted in full *Hin*dIII activity (Table 1). This suggests that inhibition was due to both contaminating protein and gum ghatti.

No DNA from the DNA/dextran sulfate mixture was recovered from the column (Table 1). It may be that the dextran sulfate binds tightly even in the low salt buffer and occupies almost all the sites. Note that the ratio of 1:500 DNA:dextran sulfate was used. The basis for the separation of DNA and acidic polysaccharides is the tighter

binding of DNA during the 0.5 M NaCl wash and then freeing the DNA with the high salt buffer (2.5 M). Additional studies using different salt concentrations are being conducted as well as evaluations of alternative products.

In general, the Elutip-d gave moderate yields of DNA (ca. 60%–80%) with sufficient purity for digestion with *Hin*dIII. Similar results were obtained using *Eco*RI (data not shown).

Figure 1 shows densitometer scans of FIGE for the various mixtures of λ DNA and pectin. Notice (Figure 1, top) that even with a 1:10 mixture (DNA/pectin), there is incomplete digestion. At a concentration of 1:500, much of the λ DNA stayed in the well during FIGE and essentially none was cut by *Hin*dIII (Figure 1). Yet, despite the extremely high concentration (1:500), the λ DNA appears to have been effectively restricted by *Hin*dIII after elution.

Genomic DNA from live oak (*Quercus virginiana* Mill.) and magnolia (*Magnolia grandiflora* L.), as extracted using only the hot CTAB method (5), could not be restricted by either *Eco*RI or *Hin*dIII. However, after elution from this column, the DNAs could be restricted with both *Eco*RI and *Hin*dIII.

If possible, it is important to reduce the bulk of polysaccharides in standard extraction procedures such as the hot CTAB protocol. When the DNA extract is very slimy, a phenol/chloroform (1:1) extraction should be done before loading the extract onto this column for optimum binding and DNA recovery. Another useful procedure for viscous extracts is to dilute the DNA/polysaccharide mixture with low salt buffer (see above) before loading.

REFERENCES

1. Aoki, Y. and H. Koshihara. 1972. Inhibitory effects of acid polysaccharides from sea urchin embryos on RNA polymerase activity. Biochim. Biophys. Acta *272*:33-43.
2. Aspinall, G.O. 1982. Molecular biology: an international series of monographs and textbooks. Vol. 2. The Polysaccharides. Academic Press, New York.
3. Bendich, A.J., R.S. Anderson and L.B. Ward. 1979. Plant DNA: Long, pure and simple, p. 31-33. *In* C.J. Leaver (Ed.), Genome Organization and Expression in Plants. Plenum Press, New York.
4. Bostock, C.J. 1988. Parameters of field inversion gel electrophoresis for the analysis of pox virus genomes. Nucleic Acids Res. *16*:4239-4252.
5. Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities for fresh quantities of fresh leaf tissue. Phytochem. Bull. *19*:11-15.
6. Edelman, M. 1975. Purification of DNA by affinity chromatography: Removal of polysaccharide contaminants. Anal. Biochem. *65*:293-297.
7. Furukawa, K. and V.P. Bhavadna. 1983. Influences of anionic polysaccharides on DNA synthesis in isolated nuclei and by DNA polymerase α: Correlation of observed effects with properties of the polysaccharides. Biochim. Biophys. Acta *740*:466-475.
8. Heyn, R.F., A.K. Hermans and R.A. Schilperoort. 1974. Rapid and efficient isolation of highly polymerized plant DNA. Plant Science Letters *2*:73-78.
9. Ishi, K., S. Futaki, H. Uchiyama, K. Nagasawa and T. Andoh. 1987. Mechanism of inhibition of mammalian DNA topoisomerase I by heparin. Biochem. J. *241*:111-119.
10. Murray, M.G. and W.F. Thompson. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. *8*:4321-4325.
11. Perbal, B. 1988. A Practical Guide to Molecular Cloning. John Wiley and Sons, New York.
12. Shioda, M. and K. Marakami-Muofushi. 1987. Selective inhibition of DNA polymerase α by a polysaccharide purified from slime of Physarum polycephalum. Biochem. Biophys. Res. Commun. *146*:61-66.
13. Whistler, R.L. and J.N. BeMiller. 1973. Industrial Gums. Academic Press, New York.

This research was supported by the Helen Jones Foundation. Address correspondence to R.P. Adams.

Nhan Do and Robert P. Adams
Plant Biotechnology Center
B.U. Box 7372
Baylor University
Waco, TX 76798

ERRATUM

The following reference should be added to the article entitled "A rapid and inexpensive procedure for desalting synthetic oligonucleotides," which was published in the September 1990 issue (p. 300-301) of *BioTechniques*:

Jayaraman, K. 1987. A rapid isolation procedure for oligonucleotide purification. BioTechniques *5*:626.

Standardized Isolation of Protoplasts

ABSTRACT

Isolation of leaf mesophyll protoplasts from tobacco (Nicotiana tabacum) *is facilitated using a specially designed digestion chamber. The chamber's airtight seals and various ports reduce the complexity, time and contamination risks which may be associated with standard isolations. The polished glass viewing window allows continuous monitoring of protoplast release, thus facilitating more precise determinations of the optimal digestion time. In concert with a simplified centrifugation step, the protoplast isolation procedure is greatly standardized.*

INTRODUCTION

Isolated plant protoplasts are single cells stripped (enzymatically or mechanically) of their walls (Figure 1a). They provide a fairly uniform population of true single cells as well as allowing comparatively easy access to cellular components such as organelles (5). Protoplasts are required for many studies dealing with developmental, physiological and biochemical issues (4). They are also used in genetic engineering experiments (e.g., microinjection, electroporation and somatic hybridization (1–3).

Isolation of protoplasts from mesophyll cells and other tissues is generally accomplished in three major steps: 1) donor tissue sterilization, 2) enzymatic digestion of the cell wall and 3) purification—generally, large leaf debris is removed using a 30–60-μ syringe filter and is followed by centrifugation and washing cycles to remove the smaller debris and digestive enzymes. Many procedures utilize high density sucrose/ficoll (10) or density gradient centrifugation (7), which yields relatively pure cultures but is time-consuming and may reduce yield. If a gentle procedure is not employed, damage to the protoplast may occur during these steps.

The complexity of existing methods of protoplast isolation is such that apparently minor alterations in procedure