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Commentary

Inhibition of Random Amplified Polymorphic DNAs (RAPDs) by Plant Polysaccharides

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Abstract: A survey of the inhibition of the amplification of spinach DNA by various plant polysaccharides revealed that neutral polysaccharides (arabinogalactan, dextran, gum guar, gum locust bean, inulin, mannan, and starch) were not inhibitory. In contrast, the acidic polysaccharides (carrageenan, dextran sulfate, gum ghatti, gum karaya, pectin, and xylan) *were* inhibitory. In the process of preparing random amplified polymorphic DNAs (RAPDs), the loss of large DNA bands appears to be an indicator that the fingerprint pattern has been affected by polysaccharides. The addition of various concentrations of Tween 20, DMSO, or PEG 400 to the PCR reaction mixture resulted in partial restoration of amplification of RAPDs for the acidic polysaccharides. The most effective way to eliminate the effects of polysaccharide inhibition was by diluting the DNA extracts, and thereby diluting the polysaccharide inhibitors.

RAPDs (random amplified polymorphic DNAs) are now widely used in plant breeding, gene linkage analyses, and evolutionary and taxonomic studies (Caetano-Anolles et al., 1994). Analysis using a single short (5- to 10-bp, or 20- to 39-bp) primer of random sequence results in a fingerprint. Caetano-Anolles et al. (1994) classify these kinds of analyses as random amplified polymorphic DNA (RAPD), amplification fragment length polymorphisms (AFLPs), DNA amplification fingerprinting (DAF), or arbitrary primed PCR (AP-PCR). The DNA fingerprints are very dependent on exacting PCR conditions as well as the quantity and quality of DNA used.

Abbreviations: RAPD, random amplified polymorphic DNA.

Polysaccharides are common contaminants in DNA extracted from plant tissues (Demeke and Adams, 1992; Do and Adams, 1991). Although methods for the purification of plant DNAs are effective in removing some kinds of polysaccharides (Demeke and Adams, 1992; Do and Adams, 1991), we have recently experienced polysaccharide contamination that cannot be removed by current methods.

Do and Adams (1991) had shown acidic plant polysaccharides were inhibitory for *Hind* III enzyme restrictions of λ DNA, whereas neutral plant polysaccharides were not inhibitory. In addition, acidic polysaccharides have been shown to inhibit classical, two-primer PCR (Demeke and Adams, 1992).

We initiated the present study because polysaccharides present such a large problem in PCR and there has been no report on the effects of polysaccharides on RAPDs amplification of genomic plant DNA. Our objectives were to examine the effects of neutral and acidic plant polysaccharides on RAPD amplification and to investigate the effects of various PCR buffer enhancers on RAPD profiles.

Materials and Methods

Polysaccharides were obtained from Sigma Chemical Company and mixtures prepared as previously outlined (Do and Adams, 1991), except spinach DNA was used instead of λ DNA. DNA was extracted from locally obtained, fresh spinach (*Spinacia oleracea* L.) leaves using the hot CTAB protocol (Doyle and Doyle, 1987). PCR was performed in a volume of 12.5 μL containing 50 mM KCl, 10 mM Tris-HCl (pH 9), 2 mM $MgCl_2$, 0.01% gelatin and 0.1% Triton x-100, 0.2 mM of each dNTPs, 0.36 μM primer, 1.5 ng of spinach DNA, and 0.5 unit of Promega TAQ DNA polymerase. Initially, a ratio of polysaccharide to DNA of 33.3:1 (w/w) was used. Lower ratios of some polysaccharide:DNA were investigated for polysaccharides that showed inhibition at the 33.3:1 ratio. The highest ratio of polysaccharide/DNA tested was 1333:1. The primer used for RAPDs was 5'-3' (ATA CGG CGT C, #327, University of British Columbia). Amplification was performed in an MJ Research Programmable Thermal Cycler. The thermal cycle used was: 94 °C (1.5 min) for initial strand separation, then 40 cycles at 38 °C (2 min) for annealing, 72 °C (2 min) for extension, 91 °C (1 min). Two additional steps were used: 38 °C (2 min) and 72 °C (5 min) for final extension. Amplification products were analyzed by electrophoresis in 1.5% agarose (Sigma) gels and were

detected by staining with ethidium bromide. DMSO (2.5, 5, 7, 9, 12, 14, 16, 18, 20, 22, 24 and 50%), PEG 400 (2.5, 5, 7, 9, 12, 14, 16, 18, 20, 22, 24 and 50%) and Tween 20 (0.1, 0.25, 0.5, 1.0, 2, 5, 7, 9, 12, and 14% w/w) were added to the PCR buffer to attempt reversal of the inhibition of PCR (Demeke and Adams, 1992; Gelfand, 1989; Pomp and Medrano, 1991).

Results and Discussion

The neutral polysaccharides (arabinogalactan, dextran, gum guar, gum locust bean, inulin, mannan and starch) did not inhibit RAPD amplification of spinach DNA even at levels of 1500 ng polysaccharide (PS): 1.5 ng DNA (Table I). However, there was some inhibition (Table I) by mannan at 2000:1.5 (PS:DNA). In contrast, the acidic polysaccharides, carrageenan, dextran sulfate, gum ghatti, pectin and xylan completely inhibited RAPD amplification at 2000 ng, and all but gum karaya inhibited RAPD amplification at 1500 ng (Table I, Fig. 1). Additional studies (data not shown) revealed that dextran sulfate was inhibitory even at 50 ng and gum ghatti was inhibitory at 500 ng. Gum ghatti, which contains about 9.5% D-glucuronic acid (Smith and Montgomery, 1959),

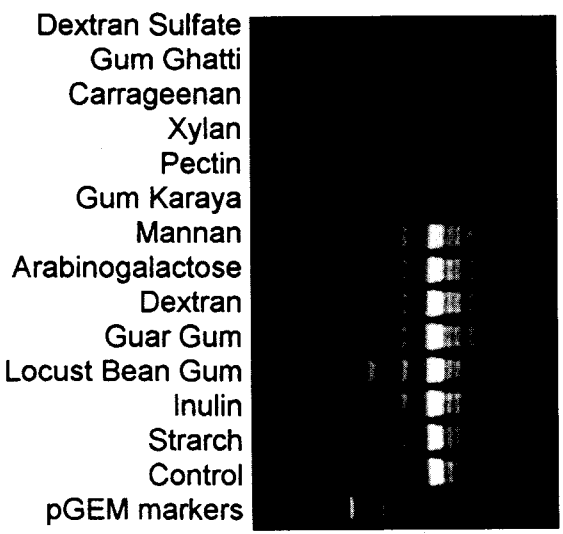

Fig. 1. Effects of neutral and acidic polysaccharides on RAPD analysis of spinach DNA. The indicated polysaccharides were analyzed in proportions of 2000 ng polysaccharide:1.5 ng DNA. The control was without polysaccharide.

Table I. Comparison of the effects of various plant polysaccharides on RAPD analyses of spinach DNA. Different levels of polysaccharides were employed (1500 and 2000 ng per reaction); ++, not inhibited; +, loss of larger bands; —, completely inhibited (no bands).

Neutral polysaccharides	RAPDs amplification 1500 ng	2000 ng	Acidic polysaccharides	RAPDs amplification 1500 ng	2000 ng
arabinogalactan	++	++	carrageenan	—	—
dextran	++	++	dextran sulfate	—	—
gum guar	++	++	gum ghatti	—	—
gum locust bean	++	++	gum karaya	+	—
inulin	++	++	pectin	—	—
mannan	++	+	xylan	—	—
starch	++	++			

is a natural product in the legume family. Carrageenan contains polymers with a strongly negative charge; κ and λ carrageenans are vegetable gelatins. Gum karaya, which has an acid number of from 13.4 to 22.7, is obtained from the *Sterculia urens* tree. Pectin, which contains 76% galacturonic acid, is obtained from various fruits, including apples. Xylan, which contains arabic and glucuronic acids, is an oat product. Although carrageenan, gum karaya, pectin and xylan did not appear to inhibit classical PCR (Demeke and Adams, 1992), these acidic polysaccharides had major effects on RAPDs, parallel to their effects on the activity of the *Hind* III restriction enzyme (Do and Adams, 1991). This suggests that polysaccharides may inhibit by complexing with the enzyme rather than complexing with the plant DNA.

A lower ratio of 33.3:1 (= 50 ng PS:1.5 ng DNA) was further investigated for the acidic polysaccharides. Only dextran sulfate completely inhibited RAPDs at this ratio. At a ratio of 333.3:1 (= 500 ng PS:1.5 ng DNA) carrageenan and gum ghatti inhibited PCR completely. This suggests that the acidic polysaccharides are either changing the pH of PCR buffer or inhibiting the Taq enzyme, rather than interfering by binding to the DNA.

To investigate the effects of various buffer enhancers, we tried several concentrations of Tween 20, DMSO, or PEG 400 on the natural acidic polysaccharides. Different concentrations of Tween 20 produced variable effects among the polysaccharides, but no concentration was very effective (Table II). DMSO yielded more uniform effects and was optimal at 5 to 7% concentrations. PEG 400 was by far the best enhancer exam-

Table II. Comparisons of the effects of buffer enhancers on RAPD amplifications of spinach and acidic polysaccharides. ng of polysaccharide is the threshold for inhibition. ++ = not inhibited; + = loss of larger bands; - = loss of most bands; — completely inhibited (no bands).

Acidic polysaccharides	Tween 20 (%)					DMSO (%)			PEG 400 (%)		
	0.5	2	5	7	9	5	7	9	5	7	9
gum ghatti, 500 ng	NT	—	-	—	—	-	-	+	+	+	-
carrageenan, 1500 ng	—	—	+	-	—	+	-	-	+	-	-
gum karaya, 2000 ng	+	-	—	—	—	+	-	—	+	-	—
pectin, 1500 ng	-	—	—	—	—	+	-	—	—	+	—
xylan, 1500 ng	+	-	-	—	—	+	-	—	+	-	—

ined, often almost restoring the RAPD pattern (Fig. 2) at concentrations from 5 to 7% (Table II).

Of the natural acidic polysaccharides, gum ghatti was the most inhibitory. The inhibitory effects were not reversed with any concentrations of buffer additives when gum ghatti was present in amounts of 700 ng (data not shown). The inhibitory effects of dextran sulfate (50:1) could not be reversed by the use of buffer enhancers, in contrast to the previous report of reversal using restriction enzymes (Demeke and Adams, 1992).

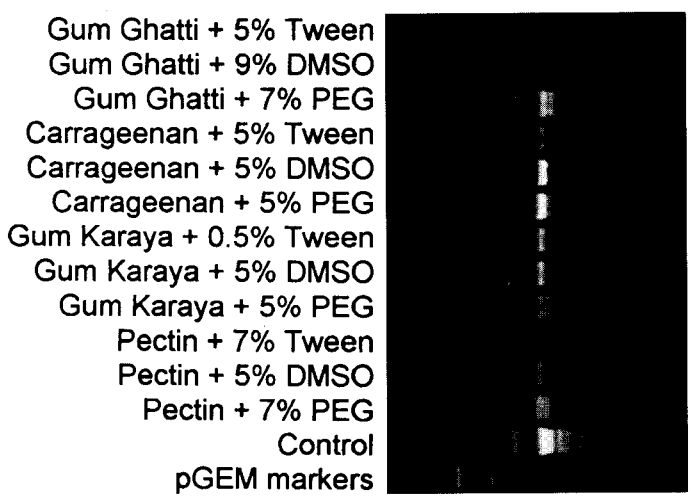

Fig. 2. Effects of buffer enhancers on RAPDs with various acidic polysaccharides. Reactions were carried out with selected polysaccharides plus the indicated additions; the control was without polysaccharide.

It appears that plant polysaccharides present a significant problem for RAPDs, as was previously found for restriction enzymes (Do and Adams, 1991). Although the addition of Tween 20, DMSO, or PEG might be useful to aid in the RAPD amplifications from acidic polysaccharide containing species, these additives will not completely reverse the inhibition. RAPD users should be aware that the varying amounts of acidic polysaccharides present in different species in a study may affect the RAPD profile. The loss of larger RAPD bands in a profile appears to be a good indicator that inhibition is occurring. When natural inhibitory polysaccharides are present, an interim solution is to dilute the DNA extracts. This also dilutes the polysaccharides, and generally one can amplify the DNA in the diluted state. Occasionally, we have found it necessary to dilute the DNA extracts to as low as 0.1 ng / reaction tube in order to get good RAPD bands.

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