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AGAR, AN ALTERNATIVE TO AGAROSE IN ANALYTICAL GEL ELECTROPHORESIS

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SUMMARY

Agarose is purified from the polysaccharide complex, agar. This purified fraction forms a neutral gel matrix that is commonly used in gel electrophoresis. We have found that for many routine uses, agar which is considerably less expensive than agarose can be used with satisfactory results.

Agar is a complex polysaccharide extracted from the agarocytes of algae of the *Rhodophyceae*. It can be separated into agarose, a neutral gel and agaropectin which is highly sulphated and does not form a gel (Araki,1937). Purified agarose is considered to be a linear galactose polymer with little or no sulphate present (Maniatis et al, 1989). The structure of agarose as determined by Araki and Arai (1956), consists of alternating copolymers of 1,4 linked 3,6-anhydro-α-L-galactose and 1,3 linked ß-D-galactose. This structure forms a repeating unit referred to as agarobiose (Serwer, 1983). In contrast, agar, consists of a spectrum of polysaccharides (Morrice et al, 1983) including agarose and a sulphated galactan or porphyran (Santos and Doty, 1983) of which agarose has the lowest charge (Williams and Wilson, 1979).

Commercially available agarose is contaminated to a varying degree with other polysaccharides, salts and proteins. Agarose containing relatively high sulphate or other charged groups is thought to result in a high electro-endosmosis (EEO). Consequently a difference in charge is produced between the water molecules in the buffer and the agarose during electrophoresis. The outcome of this ionization is the production of positively charged oxonium ions

(H_3O^+). These migrate to the cathode carrying with them dissolved neutral substances. This movement accelerates the progress of the cations but retards the anions (Serwer, 1983, Sharp et al, 1973). Impurities in agarose can affect both the migration of substances as well as their biological activity if they are to be purified from the gel.

Electrophoresis through agarose gels is a standard method to separate, identify and purify DNA fragments (Hayward and Smith, 1972, Aaij, and Borst, 1972, McIndoe and Munro, 1967). Gel electrophoresis is often used only to check the size, amount and/or purity of a sample (Serwer, 1983, Sharp et al, 1973, McIndoe and Munro, 1967). The DNA in the gel is not isolated from the gel for further study. In these cases the effect of impurities in the agarose on the biological activity of the DNA is not relevant. The influence of these impurities, especially the charged groups, on the migration of the DNA is evident (Maniatis et al, 1989).

Electrophoresis of DNA is becoming an essential technique in all areas of biological research. It is often used purely to determine whether organisms contain plasmids, viruses or to visualize DNA restriction patterns (Sharp et al, 1973). The majority of agarose gels run in our laboratory serve an analytical purpose only. There is, therefore, little need to use highly purified agarose.

Due to the relative isolation of South Africa and its distance from manufactures of agarose, as well as a lower currency value, agarose is extremely costly. This is presumably also true in other third world countries.

We have recently attempted to find an inexpensive alternative to agarose for analytical purposes. We observed that agar is an adequate support medium for gel electrophoresis (Fig. 1). This is in contrast to the expected effect of the electro-endosmosis on the migration of the DNA. We speculate that the effects of the charged groups on the movement of the DNA are not significantly great enough to upset the electrophoresis of such a highly charged molecule.

In order to determine whether we had fortuitously used a particularly pure batch of agar, we tested a number of agar brands and lot numbers that were available to us. These included Difco Bacto-Agar (Detroit, U.S.A.), Merck Agar-Agar (Darmstadt, Germany), Oxoid agar (London, U.K.), Biolab Agar (Halfway House, R.S.A.), Bitek Agar (Detroit, U.S.A.) and bulk agar obtained from New Zealand. All these agar formulations gave good separation of our DNA

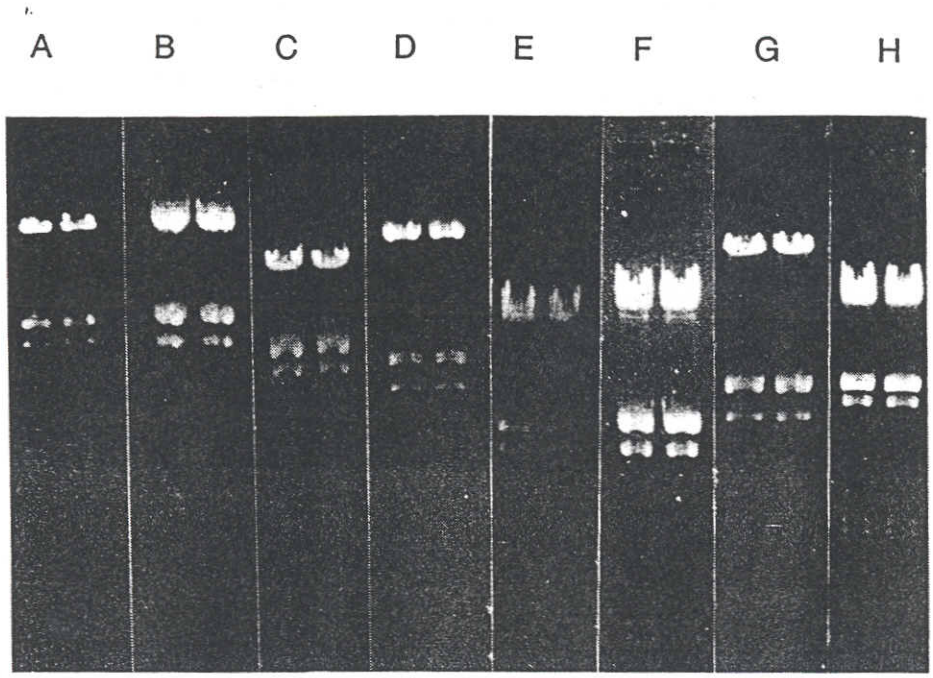

Figure 1. Gels (1%w/v) of agar or agarose run in 90 mM Tris-borate, 90 mM boric acid, 10 mM EDTA (pH 8.0) buffer. In all cases the first lane is lambda DNA cut with the restriction enzymes *Eco*R1 and *Hind*III. The fragments sizes are 21 226; 5148, 4973, 4268, 3530, 2027, 1904, 1584, 1375, 947, 831 and 564 base pairs (bp) respectively. The gels were made from the following brands of agar, Difco Bacto-Agar [A], Merck Agar-Agar [B], Oxoid agar [C], Biolab agar [D], Bitek agar [E], bulk agar [F]. Gels labeled G and H were made from Seravac and Promega agarose respectively.

fragments (Fig. 1). None of the agars tested were particularly cloudy or contained any obvious particulate matter. Most were an off white to light brown color but this did not affect the florescence of the DNA.

Although agar probably contains contaminants such as DNase, we did not observe appreciable degradation of DNA during electrophoresis. The migration of the DNA was similar in all cases to that of DNA in molecular biology grade agarose (Promega, [Madison, U.S.A.] and Seravac [Cape Town, R.S.A.]). In all cases the same buffer, gel percentage, voltage, type of gel apparatus and amount of DNA were used to test the different agar types.

The relative migration of the DNA was in linear proportion to the log molecular weight of the DNA fragments. This is in agreement with the commonly accepted migration of DNA through agarose gels (Helling et al, 1974).

In this study we have shown that agar can be used as a gel matrix in place of agarose in many instances. We do not recommend using agar if the DNA is to be purified from a gel. We have attempted experiments involving the southern blotting technique using agar as our gel matrix. Our results were, however, unsatisfactory. We therefore recommend that agar gels can be used as a cheap alternative to agarose to check the purity, size and amount of DNA in a sample.

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