Studies on the Nucleic Acids of Avirulent, Attenuated, and Virulent Isolates of Agrobacterium tumefaciens

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ABSTRACT

Deoxyribonucleic acid preparations from virulent and attenuated strains of *Agrobacterium tumefaciens* were compared using buoyant density and melting point determinations. No significant differences were found using either method of comparison. Ribonucleic acid (RNA) from virulent and avirulent *A. tumefaciens* strains were compared by fractionation on sucrose gradients. Both RNA preparations exhibited similar absorbance profiles. The magnesium concentration of the extraction buffer was found to be critical for the isolation of undegraded ribosomal RNA. A buffer containing 0.02 M magnesium was suitable for the isolation of native 16 and 23s ribosomal RNA components. Phytopathology 61:337-338.

There is evidence that bacterial nucleic acids are directly involved in plant tumor induction by *Agrobacterium tumefaciens* (1, 8, 9). This paper reports attempts to find differences between the DNA and RNA of virulent, avirulent, and attenuated strains of *A. tumefaciens* which might explain differences in pathogenicity among the isolates.

Four isolates of *A. tumefaciens* were used in this investigation: (i) virulent strain ATCC 15955; (ii) a virulent strain isolated from a tomato tumor (TI); (iii) a strain which had been attenuated by the method of Van Lanen et al. (10); and (iv) an avirulent isolate.

Deoxyribonucleic acid was isolated from bacteria according to the methods of Saito & Miura (6). Ribonucleic acid was isolated as outlined by Oishi & Sueoka (5), with the exception that the magnesium concn of the Tris-$MgCl_2$ buffer [tris(hydroxymethyl)amino methane], 0.01 M, $MgCl_2$, 0.02 M, pH 7.4) used during extraction was kept at 0.02 M unless otherwise stated.

Deoxyribonucleic acid preparations from virulent (TI) and attenuated cultures of *A. tumefaciens* were subjected to isopycnic equilibrium centrifugation in CsCl (60%, w/w, Matheson, Coleman, & Bell) to determine if the DNA's differed in buoyant density and whether more than one type of DNA can be detected in either virulent or attenuated cells. The buoyant densities were found as described by Schildkraut et al. (7) by centrifuging the DNA (3-5 μg) at 44,000 rpm and 20 C for 18 hr in a Beckman Model E analytical ultracentrifuge. Photographs of the gradients were taken with ultraviolet optics, and densitometer tracings made of the photographs to determine the positions of DNA in the gradients. The mole fractions of guanine plus cytosine (GC) for both types of DNA were found from the equation denoting a linear relationship between GC and buoyant density (7). The buoyant density values were 1.718 g/ml and 1.717 g/ml for DNA from virulent and attenuated cells, respectively, which corresponded to 58 and 59% GC. When a sample containing both types of DNA was centrifuged, only 1 DNA band (buoyant density = 1.718 g/ml) was detected, indicating that both types of DNA were identical with respect to buoyant density. Furthermore, if any unique type of DNA is present in virulent bacterial cells, it is not detectable with CsCl centrifugation as used here.

Melting point determinations made by the method of Doty et al. (2) did not reveal any differences between virulent and attenuated DNA (94.4 and 94.0 C, respectively).

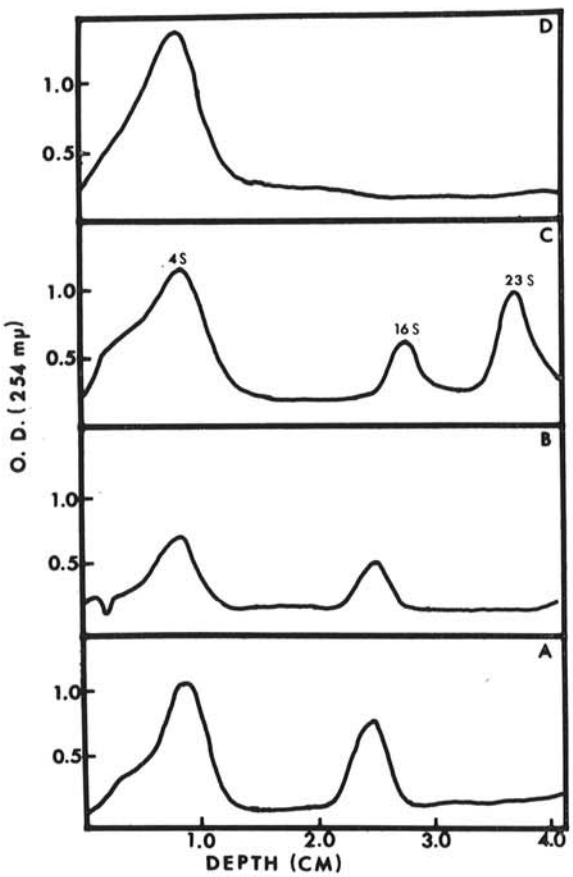

Fig. 1. Sucrose density-gradient separation of RNA isolated from virulent *Agrobacterium tumefaciens* cells using Tris-$MgCl_2$ buffer containing various concentrations of magnesium. **A)** 0.001 M; **B)** 0.005 M; **C)** 0.02 M; **D)** 0.1 M Mg++ (0.2 — 1.0 M sucrose, 250,000 g, 5 hr at 4 C).

Ribonucleic acid was isolated from virulent (ATCC 15955) and avirulent bacteria and layered on sucrose gradients (0.2-1.0 M) which were formed in cellulose nitrate tubes (0.5-inch × 2.0-inch) with a Buchler density gradient forming apparatus. The gradients were centrifuged at 250,000 *g* for 5 hr at 4 C with a SW65 Ti rotor in a Model L2 Beckman preparative ultracentrifuge, then monitored for absorbance at 254 mμ wavelength with an ISCO density gradient fractionator. Preliminary extractions and centrifugation in sucrose gradients of total RNA from the *A. tumefaciens* strains resulted in absorption profiles similar to that shown in Fig. 1-A in which the 4s transfer RNA and 16s ribosomal RNA (rRNA) components were present but the 23s rRNA fraction could not be detected. This result was obtained when the Tris-$MgCl_2$ buffer used during isolation and purification of RNA contained 0.001 M magnesium. The absence or degradation of 23s rRNA was further examined by isolating separate preparations of total RNA in the presence of buffers containing varying amounts of magnesium (0.001, 0.005, 0.02, and 0.1 M), then centrifuging on sucrose gradients. The use of buffer containing 0.005 M magnesium (Fig. 1-B) also did not allow the isolation of 23s rRNA. Increasing the magnesium concn to 0.02 M permitted the isolation of undegraded 23s rRNA (Fig. 1C). At 0.1 M magnesium (Fig. 1-D), both 16 and 23s rRNA components are degraded. When total RNA preparations from virulent and avirulent *A. tumefaciens* were compared on sucrose gradients, no differences were observed. Both absorbance patterns were identical to that shown in Fig. 1-C.

We could not detect any differences between the DNA's and RNA's of the *A. tumefaciens* strains examined by the methods employed. The inability to detect differences in the DNA's is not final proof of the absence of other DNA components in virulent cells. A small DNA fragment, such as that from a lysogenic phage, incorporated into the bacterial chromosome may not detectably change the melting point or buoyant density values of the DNA. If the fragment is not incorporated, it may be of the same density as the DNA from the bacterial chromosome and, therefore, not distinguishable. The possibility of a phage being the tumor inducing agent has been the subject of much speculation. Leff & Beardsley (3) have reported that tumors were induced on plants with DNA prepared from a phage first isolated from sterile crown gall tumor tissue.

The requirement for a specific magnesium concn during the isolation of *A. tumefaciens* RNA is of interest in view of the report by Le Goff (4) that *A. tumefaciens* cells possess only a trace amount of 24s (23s) rRNA and that this may have some bearing on its pathogenicity. Le Goff's extraction medium (4) contained no magnesium, which suggests that her findings could be explained by the absence of magnesium in the extraction medium rather than by the absence of 23s rRNA in the bacterium.

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