

cultivars CSC 2, Co 36, TKM 4, IR 20 and GR 3 in the leaves due to salinization (table 3). We had earlier reported that the salt-tolerant cultivars AU 1, Co 43 and CSC 1 maintain high levels of  $K^+$  in their shoots than the other salt-sensitive cultivars to saline treatment<sup>10</sup>. The capacity to increase ion uptake, particularly of  $K^+$  and to synthesize small molecules, like glycinebetaine and total QAC, might represent an important adaptation by rice to a shortage of water under saline conditions. Moreover these total QAC could indicate a sparing of  $K^+$  in the cytoplasm by some of these compounds like glycinebetaine and act as a cytoplasmic osmoticum<sup>11</sup>.

It is evident from the results that the salt-tolerant cultivars AU1, Co 43 and CSC 1 exhibited less reduction in the dry matter accumulation of shoot, higher values of SI and higher magnitude of increase in glycinebetaine and total QAC in their leaves than the salt-sensitive cultivars under saline conditions. The accumulation of glycinebetaine was consistently associated with salt tolerance of rice and may be used as a cumulative index for evaluation of salt-tolerant rice cultivars.

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## A METHOD FOR THE ISOLATION OF MEGAPLASMID FROM *PSEUDOMONAS SOLANACEARUM*

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A rapid and sensitive 'out gel' lysis technique is described to isolate mega plasmid from *Pseudomonas solanacearum*. Screening of *Azotobacter* sp., *Bacillus subtilis* and *Xanthomonas* spp. for plasmids by this method gave reproducible results. Our procedure can be employed routinely to rapidly screen the strains for plasmids.

Occurrence of plasmids in phytopathogenic bacteria is ubiquitous and they carry genes, implicated in pathogenesis<sup>1</sup>. *P. solanacearum* is an important plant pathogen infecting a variety of plants. Currier and Morgan<sup>2</sup> detected plasmids in 6 out of 20 strains. Rosenberg *et al*<sup>3</sup> observed a slowly migrating plasmid DNA band in 8 out of 9 strains of *P. solanacearum*. Morales and Sequeira<sup>4</sup> screened 39 strains of this bacterium, collected from different geographic regions and noted 1 or 2 plasmids with relative masses from 7.5 kb to about 750 kb in 22 strains. In *P. solanacearum* involvement of plasmid pAMB1 in catechin dissimilation was shown<sup>5</sup>. Both preparative and analytical methods have been used to visualize plasmid DNA. These are not only time-consuming but result in doubling of bands. Morales and Sequeira<sup>4</sup> observed the duplication of plasmid band of *P. solanacearum* strain pps13 when isolated by 'in gel' lysis technique. Often the results are not reproducible. In this communication, we report a simple and rapid method to detect mega-plasmid in *P. solanacearum*.

*P. solanacearum* isolated from infected pseudostems of banana was subcultured in BG medium<sup>6</sup> for 12 h. Cells, (5 ml, 0.6 to 0.8 OD) were harvested by centrifugation at 10,000 g for 10 min at 4°C. The pellet was washed with TES buffer (sucrose, 0.3 M; tris-HCl, pH 8, 25 mM; EDTA pH 8, 25 mM) and suspended in 400  $\mu$ l TES buffer to get  $8 \times 10^7$  cells/ml. The suspension was transferred to a microfuge tube (1.5 ml) and kept in ice. Lysozyme (100  $\mu$ l, 1 mg) was added to the suspension and incubated for 20 min, with mixing at 2 min interval. Lysis was completed by the addition of 30  $\mu$ l SDS (2%). To the lysate, 30  $\mu$ l loading dye (10 $\times$  bromophenol blue) were added and mixed. Aliquots (24  $\mu$ l) were loaded on to 0.7% agarose gel submerged in tris acetate EDTA buffer. Elec-

trophoresis was performed at 20 V for 1 h and 60 V for 3 h. The gel was stained with ethidium bromide (5  $\mu\text{g/ml}$ ) for 15 min, destained with double-distilled water for 15 min and visualized under UV.

The preparative method gave satisfactory result for the isolation of megaplasmid and routinely used. A plasmid with very low mobility was obtained (figure 1). Since the exact size of the megaplasmid cannot be calculated by a negative logarithmic correlation between mobility and molecular weight<sup>7,8</sup>, the size of the megaplasmid was  $450 \times 10^6$  as revealed from its low mobility<sup>3,5</sup>. The plasmid was highly stable, as it was lost at a frequency<sup>5</sup> of 0.25%.

The plasmid was purified by the following method. The lysate was extracted with phenol : chloroform (1 : 1 v/v), the aqueous phase was transferred to a microfuge tube containing 3 M sodium acetate and precipitated with ethanol. At each step, an aliquot was removed and used to assess the plasmid DNA. The megaplasmid was detected in the gel up to upper aqueous phase of phenol : chloroform and in further steps, intact plasmid was not detected. When the aqueous phase of phenol : chloroform was again extracted, no plasmid was detected. It is therefore believed that the large plasmid did not survive the extraction procedure even though shearing was kept to a minimum.

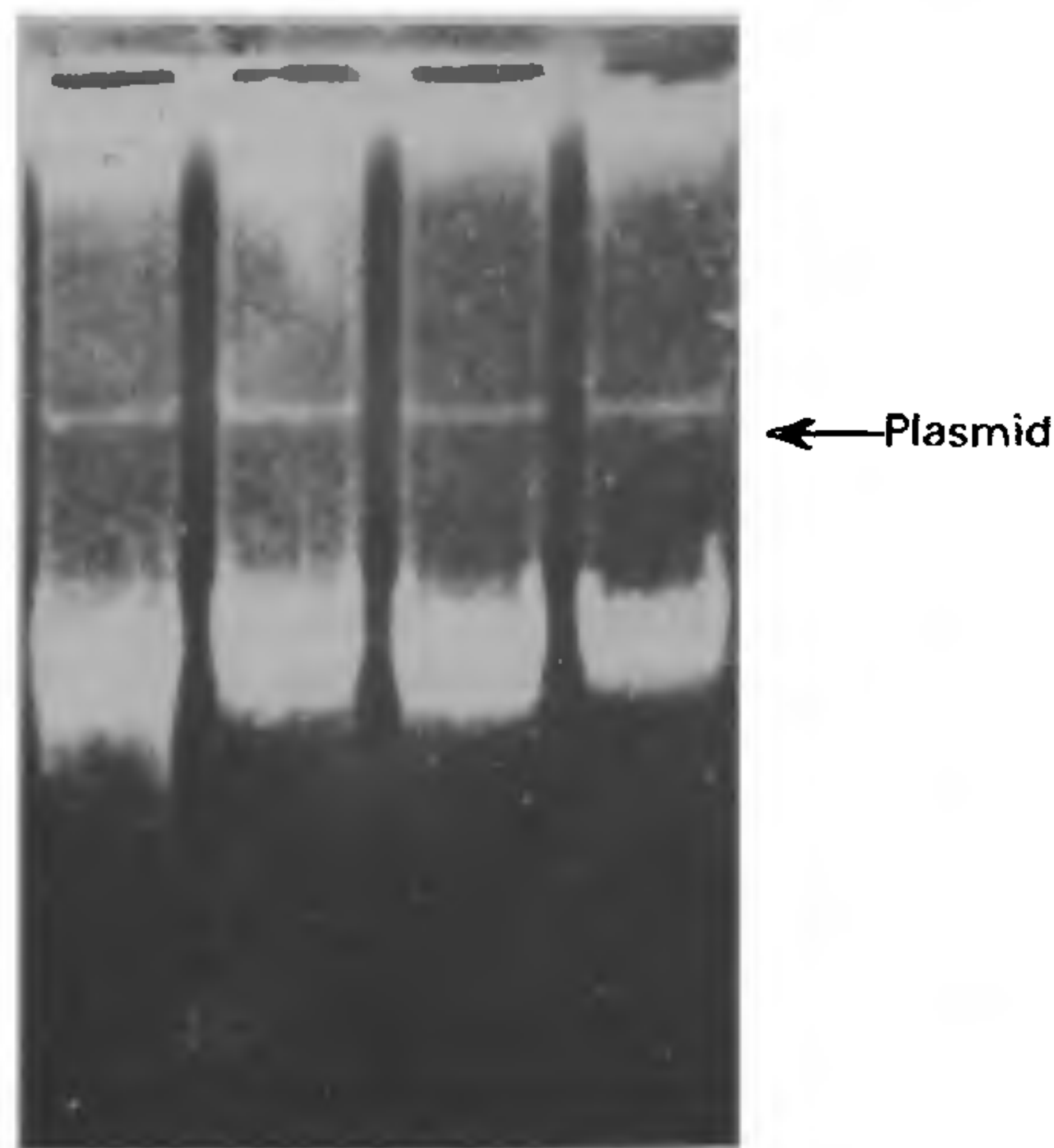


Figure 1. Agarose gel electrophoresis of plasmid preparation from *P. solanacearum* (all the lanes indicate the presence of plasmid of similar mobility).

Kado's procedure<sup>9</sup> was successfully employed to visualize plasmids with molecular weights ranging from 60 to 210 kb. The failure to detect plasmids in some strains of *P. solanacearum* is due to the harsh conditions needed to lyse this bacterium. Rosenberg *et al*<sup>3</sup> employed the 'in gel' lysis technique and observed a megaplasmid in *P. solanacearum*. Although the technique is effectively employed to detect megaplasmids, the procedure is time-consuming and cumbersome. Our simplified method of 'out gel' lysis gave reproducible results and can be routinely used for rapid screening. Cell concentration and SDS profoundly influence the result; high concentration of cells (above  $8 \times 10^7$  cells/ml) and SDS ( $< 30 \mu\text{l}$ ) gave extremely viscous lysate which interfered with loading of the wells.

Our technique is simple and sensitive. Screening of *Azotobacter* spp., *Bacillus subtilis* and *Xanthomonas campestris* pv. *oryzae* for plasmids gave reproducible results. We suggest that the procedure can routinely be used to rapidly screen strains for plasmids.

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