A non-radioactive method for detection of Tn5 Kanamycin phosphotransferase activity in cyanobacteria

The wide use of Kanamycin phosphotransferase encoding gene, also known as NPT II gene, as one of the reporter genes in transformation studies has prompted the development of a number of procedures to detect and assay Kanamycin phosphotransferase in the transformed cells[1–3]. All procedures reported so far need the use of radioactive ATP and also adequate care to account for other phosphotransferase activities in crude cell extracts. The present report deals with a simple, non-radioactive assay for Kanamycin phosphotransferase in two genetically transformed cyanobacteria.

The cyanobacteria, *Oscillatoria* sp. MKU277 and *Westiellopsis* sp. MKU154 isolated from rice field soils were electrotransformed using pRL1063a. The plasmid contained psbA promoter from *Amaranthus hybridus* and downstream to that were five genes namely, Neomycin/Kanamycin phosphotransferase gene (Tn5), Bleomycin resistance gene, Streptomycin resistance gene and *lux*A and *lux*B of *Vibrio fischeri*. Transformants growing in BG-11 medium containing 50 µg/ml of Kanamycin were harvested at the exponential stage, washed several times with Tris (100 mM pH 8.0) containing 10 mM $MgCl_2$ and suspended in the same buffer. PMSF was added to a final concentration of 5 mM. Cell suspension was sonicated and the lysate was clarified by centrifuging at 10,000 *g* for 10 min at 4°C. The clear lysate was transferred to a fresh sterile Eppendorf tube and stored at 0°C until use. Protein content in the lysate was estimated following Bradford's procedure[4].

Reaction mixture contained 50 µg to 100 µg crude protein, 1.0 mM ATP and 4 µg Kanamycin. Reaction was carried out at 16°C for 12 h. *E. coli* HB101 from exponential growth phase was mixed with LB soft agar (0.7%) and spread on plates. Soon after agar solidification, sterile Whatman No. 1 filter paper discs were carefully placed on the agar surface. Equal volume of samples from various treatments were spotted on the discs. Kanamycin with ATP alone was used as control. The plates were incubated at 37°C for 24 h and the inhibition zones were qualitatively assayed.

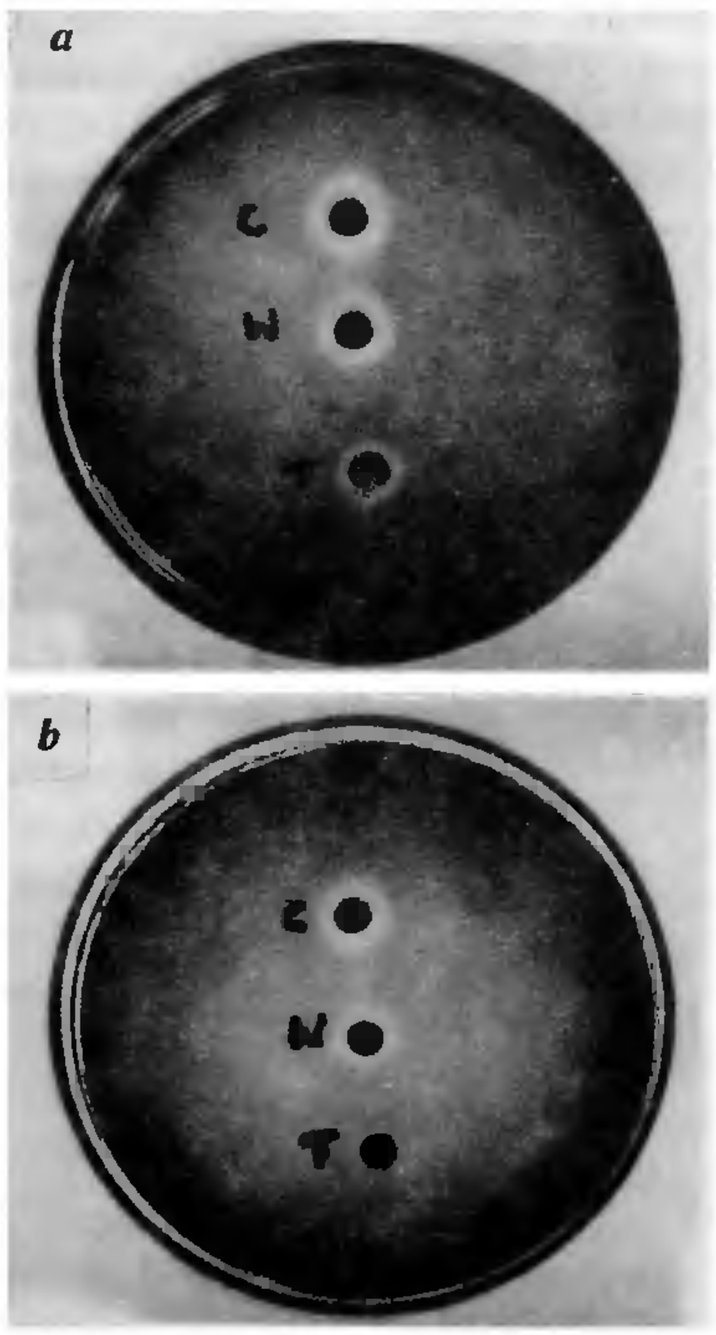

Figure 1 *a, b*. *E. coli* sensitivity to phosphorylated and non-phosphorylated Kanamycin. *a*, *Westiellopsis* sp. MKU154; *b*, *Oscillatoria* sp. MKU277. C – disc with untreated Kanamycin (control), W – disc with Kanamycin treated with wild type cell lysate; and T – disc with Kanamycin treated with lysates of transformed cells. pRL1063a was used for transforming the cyanobacterial strains.

When the sizes of inhibition zone formed around the discs (Figure 1 *a, b*) were compared, there was a significant reduction in the inhibition zone around the discs containing Kanamycin treated with lysate prepared from transformed cyanobacteria, indicating inactivation of Kanamycin due to phosphorylation. Inactivation of Kanamycin depended on the amount of protein added and the time of incubation. 50 µg crude protein was required to inactivate 4 µg of Kanamycin.

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