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## Studies on DNA modification in *Oscillatoria* sp. MKU 178

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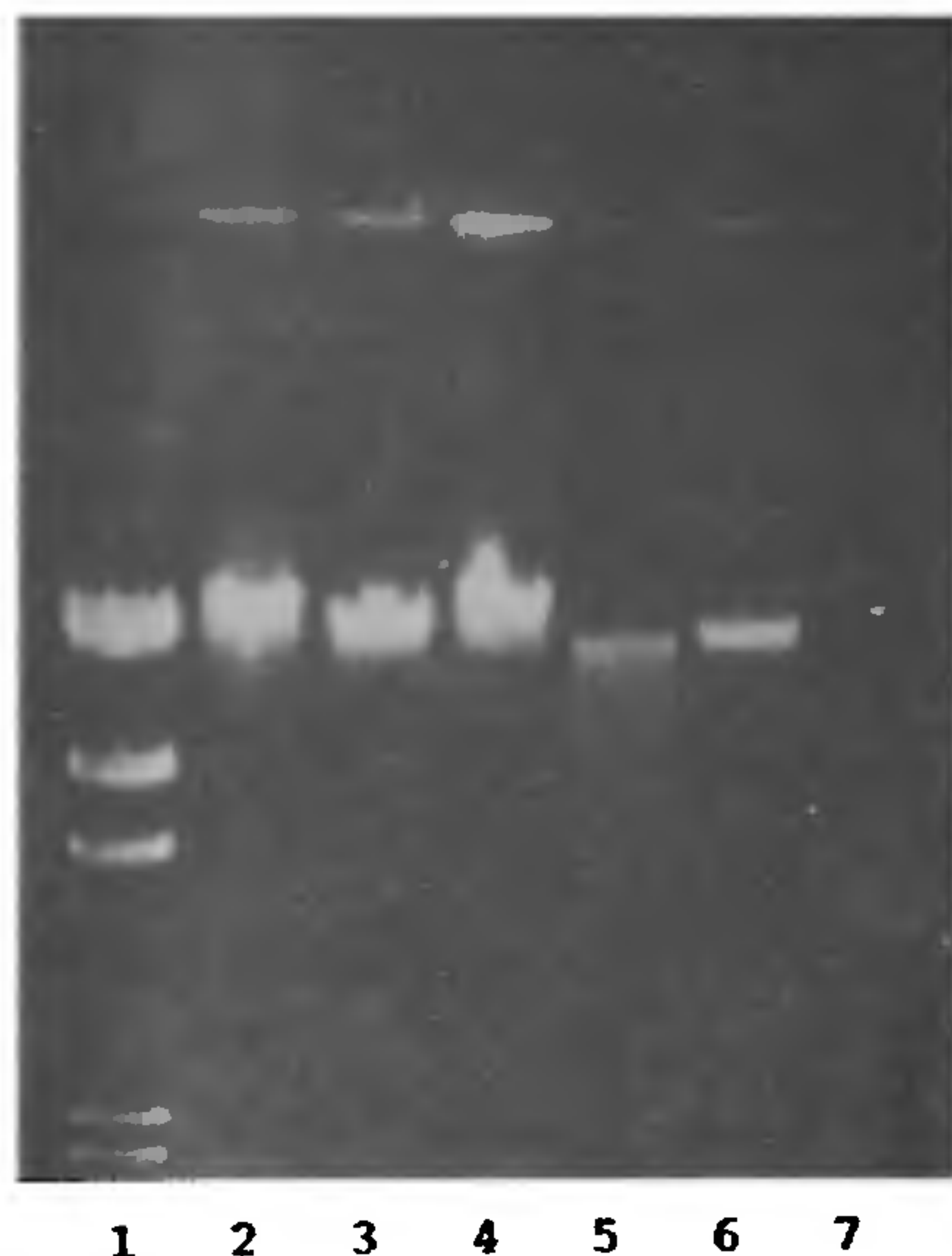
**Cyanobacterial DNA are generally believed to be resistant to restriction by endonucleases due to extensive modification of the DNA. We report here the absence of a *dam*-mediated modification in a cyanobacterium, *Oscillatoria* sp. MKU 178.**

RESTRICTION and modification systems of DNA have been well documented in several organisms<sup>1</sup> and the roles which have been postulated for the same include, protection of cells against invasion by foreign DNA<sup>2</sup>, replication and repair of DNA and control of gene expression<sup>3,4</sup>. However, as the type of restriction/modification is distinct for each organism, the worker interested in using an organism for recombinant DNA work, needs a thorough understanding of its restriction/modification system to be successful in his venture. Our laboratory has been working on the genetic and physiological aspects of cyanobacteria and our present study is aimed at studying the DNA modification in cyanobacteria. We took for our study, *Oscillatoria* sp., a non-heterocystous cyanobacterium, which exists in a wide range of habitats like streams, ponds, lakes, irrigation canals, rice fields and even sewage and industrial wastewaters<sup>5</sup>; besides, it is useful to agricultural lands

because of its soil-binding property and polysaccharide production. These properties make it an ideal choice for the introduction and expression of other agronomically important traits such as production of plant growth regulators and insecticides for its better use in agriculture.

Earlier workers have reported that DNA from several cyanobacterial species is resistant to cleavage by endonucleases<sup>6,7</sup>. In the course of our investigation on the resistance of cyanobacterial DNA to hydrolysis by restriction enzymes we identified one isolate, *Oscillatoria* sp. MKU 178, which was susceptible to cleavage by *Eco*R1. We took up a more detailed study in this isolate.

A fifteen-day-old culture of the strain, grown under a light/dark cycle of 16 h/8 h, at 25°C, was harvested for DNA extraction. DNA was purified following Sambrook *et al.*<sup>8</sup> The DNA was treated with *Dpn*1, *Mbo*1 and *Sau*3A enzymes (New England Biolabs, USA). The tubes were incubated at 37°C for 1 h and then electrophoresed on a 0.7% agarose gel. The DNA was visualized on a UV transilluminator. As can be seen from Figure 1, *Dpn*1 failed to cut the DNA (lane 3), while *Mbo*1 produced a streak showing partial digestion (lane 5) and *Sau*3A totally digested the DNA (lane 7). The corresponding controls showed no digestion. The above three enzymes are isoschizomers recognizing the sequence GATC. However the ability to cut depends on the nature of DNA methylation. The action of *Dpn*1 and *Mbo*1 is mutually exclusive because *Dpn*1 cuts the DNA if the adenine residues are



**Figure 1.** Restriction profile of DNA of *Oscillatoria* sp. MKU 178 digested with *Dpn*I (lane 3), *Mbo*I (lane 5) and *Sau*3A (lane 7). Lanes 2, 4 and 6 are the corresponding untreated controls, lane 1  $\lambda$  DNA  $\times$  HindIII molecular weight marker. (Equal volumes of the DNA was distributed into three eppendorf tubes. The DNA in the three tubes was suspended in *Dpn*I, *Mbo*I and *Sau*3A buffers respectively. A 5  $\mu$ l aliquot from each suspension was treated with one microtitre of the corresponding enzyme. A 5  $\mu$ l aliquot of each suspension without the enzyme was kept as control.)

methylated in both the strands, whereas *Mbo*I cuts only if the adenine is totally unmodified. *Sau*3A cuts irrespective of adenine modification, but in the absence of cytidine modification. From our results it is clear that DNA of this organism lacks modification of both adenine and cytidine residues in the GATC sequences. Reports regarding the unusual resistance of cyanobacterial DNA to restriction endonucleases have proposed the involvement of a deoxy-adenosine methylase (*Dam*) enzyme (*Dam* methylates the adenine in a GATC sequence) in DNA modification<sup>6,7</sup>. The results obtained in the present study prompts us to postulate that a *dam*-like system of modification is absent in this cyanobacterium, *Oscillatoria* sp. MKU 178.

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## *Apiospora camptospora*—A new fungus causing stalk rot on maize in India

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We observed stalk rot of maize (*Zea mays* L.) during regular survey of diseases. One of the pathogens responsible for disease was identified as *Apiospora camptospora* Penz and Sacc., a new fungus on maize in India.

DURING the regular survey of diseases, the maize culture Manjri composite was found to show symptoms which resembled stalk rot at the All India Co-ordinated Maize

Improvement Project of the Mahatma Phule Agricultural University, Rahuri.

The symptoms are similar to stalk rot caused by *Fusarium*. The lower leaves became flaccid, wilted and rolled inwardly, while upper leaves of such plants became pale green and subsequently the whole leaf sheath becomes chlorotic. The lower internodes developed purple to brownish discoloration. In completely wilted plants, the pith became hollow and developed a pinkish to dirty brown colour. In culture, the fungus is whitish in colour with irregular, patchy growth. Sporodochia appeared ellipsoidal or elongate with black conidiophores. Mother cells measured in the range of 8-12  $\times$  4-7  $\mu$ m, conidiophores often pale, brown, up to 140  $\mu$ m long, 3-4  $\mu$ m thick, conidia round or polygonal in the face view, 20-32  $\mu$ m diameter, 14-15  $\mu$ m thick, mid to dark brown with a distinct hyaline, rim or germ