ferentiation at rDNA loci, may rarely also involve homogenization of repeat unit lengths at the two rDNA loci.

In wheat, transfer of rDNA nucleotide sequences was suggested to be responsible for interlocus homogenization of rDNA between NOR loci on different chromosomes[10,11]. Sequence comparison of spacer region and thermal dissociation technique have proved high homology between the spacer sequences which led to interlocus homogenization in wheat. Although such a transfer would lead to sequence homogenization, if whole repeat unit is thus transferred, this may also lead to homogenization of spacer length. In barley, though no direct evidence on the mechanism of interlocus homogenization is available, the mechanism of transfer of rDNA nucleotides sequences (including transfer of whole repeat units) between the loci, as evidenced in the case of wheat, is the most plausible explanation. High frequency of single slv phenotype must have resulted due to an adaptive value of this phenotype.

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Comparative RAPD analysis of causative agent of rhinosporidiosis and *Microcystis* isolated from pond waters

Rhinosporidiosis is endemic in several states of South India. The disease does not respond to any form of treatment, and after surgical excision of polyps, recurrences are common. Polypoidal masses of rhinosporidiosis contain microscopic round bodies which are diagnostic for this disease, and are believed to be the causative agent. The identity of a round body filled with numerous daughter cells, has been controversial for many decades[1,2]. Investigations on samples of polyps have led earlier authors to suggest that the round body could be a protozoan[1,3], a fungus named *Rhinosporidium seeberi*[4], alga with precursors of chlorophyll[5], an organism similar to that found in plant crown gall tumours[6], and an agent showing phylogenetic relationship with protoctistan parasites of fish referred to as DRIP clade[7]. Most authors however, consider the round body as the sporangium of a fungus *R. seeberi* filled with spores, but fungal etiology could not be proved. Since patients have a history of bathing in ponds, water samples were collected from exact locations in ponds where patients had been dipping, from four endemic states of India, and analysed in the laboratory. A cyanobacterium *Microcystis* could be isolated from all water samples and the same cyanobacterium was demonstrated microscopically in round bodies *in vivo*[8]. Daughter cells in round bodies, which had not been cultured successfully in the laboratory so far[9], could now be grown on media used for cyanobacteria[10]. These studies suggested that daughter cells in round bodies could be cells of prokaryotic *Microcystis* instead of fungal spores, as pointed out by some authors[9]. This study was undertaken to find out whether or not a pathogenic strain of *Microcystis* exists in ponds in which patients had been bathing, by comparing the RAPD profile of DNA extracted from round bodies in clinical samples with that of *Microcystis* in pond-water samples.

RAPD analysis has been considered valuable for determining genetic diversity and relatedness among diverse isolates of pathogenic organisms, and is widely accepted as a taxonomic tool, including strain identification. A modified low-stringency PCR technique using short arbitrary primers which anneal with mismatches in the template DNA to produce polymorphism profiles can be analysed and compared to distinguish organisms at subspecies and strain levels[12]. Since previous genomic sequence information is not required, a universal set of primers of arbitrary nucleotide sequence and simple amplification protocol using small amount of DNA allows quick detection of several polymorphic DNA markers in a few

amplification reactions[13,14]. In this study, single primers were used to amplify genomic DNA extracted from round bodies in clinical samples as well as DNA from pond-water *Microcystis*, and banding patterns were compared. Primers which give identical banding pattern in *Microcystis* and round bodies in clinical sample have identified the pathogenic strain of *Microcystis* in natural pond waters. Therefore the same primers were used to amplify DNA from other clinical samples and pond-water *Microcystis* from other ponds in India.

Surgically excised polypoidal masses of rhinosporidiosis were collected in sterile 1 M Tris (pH 8.0). Samples of pond water containing *Microcystis* were obtained from ponds in Mathura (Uttar Pradesh), Raipur, Bhopal, Gwalior (Madhya Pradesh) and Delhi. Presence of *Microcystis* in pond-water samples was confirmed by microscopy. All chemicals used in DNA extraction were from Sigma, USA (Analytical grade). DNA molecular-weight markers, dNTPs and Taq DNA polymerase were from MBI Fermentas, Lithuania. Lysozyme, RNAase and Proteinase K were from Promega. Oligonucleotides were synthesized by Biobasic (Canada). DNA was extracted using a modification of the method of Porter[15], directly from the rhinosporidial tissue and from pond-water samples containing *Microcystis* colonies.

For RAPD–PCR, the PCR reaction was performed in a 50 µl reaction volume containing 10–25 pM RAPD primer, 0.25 U Taq DNA polymerase, 0.2 mM dNTP, 1.5 mM $MgCl_2$ and 80–100 ng DNA template. Thirty-five cycles at a denaturation temperature of 94°C for 20 s, primer annealing step at 45°C for 30 s, and extension step at 72°C for 1 min; an initial denaturation step at 94°C for 5 min and a final extension step at 72°C for 7 min were carried out. Except one reaction where equimolar concentration of a forward and reverse primer was used, a single primer was used in all other reactions. RAPD primers used in the study are shown in Table 1.

Table 1. Primers used in this study

Primer	Sequence (5′–3′)
1	GACAGACAGACAGACA
2	AACGCGCAAC
3	CAATCGCCGT
4	CCGCAGCCAA
5	GCGATCCCCA
6	TGTGTGTGTGTGTGTGCC
7	CAATATGGTGCGCGACAAT
8	AGGTGACCGT
9	GCCGTGCTGCCCCTGGTA

Primers at serial nos 4 and 5 were added in equimolar concentration.

PCR conditions were optimized to facilitate binding of an arbitrary primer. Out of nine primers used, three gave distinct bands in DNA extracted from clinical samples as well as DNA from one or more water samples, with bands in corresponding positions. Figure 1 shows the position of bands obtained with DNA from the clinical sample and DNA extracted from *Microcystis* cells isolated from three sources, namely Mathura (Gangaji), a pond near Delhi Zoo, and a pond called Buddha Talaab in Raipur. Bands with DNA from two clinical samples are seen to correspond in position with band from *Microcystis* DNA found in the pond near Delhi Zoo and Buddha Talaab. The primer used had the sequence 5′GCCGTGCTGCCCCTGGTA3′. In Figure 2, one band each in DNA from *Microcystis* found in Mathura and Delhi Zoo corresponds in position with a band in the clinical sample DNA. The primer 5′AACGCGCAAC3′ was used. Figure 3 shows the result obtained with a longer primer 5′GCCGTGCTGCCCCTGGTA3′. Distinct bands in the clinical sample correspond in position with the band from *Microcystis* DNA isolated from Mathura and the pond near Delhi zoo.

Results of this study showing bands in corresponding positions in clinical sample DNA and pond-water *Microcystis* indicate existence of a pathogenic strain of *Microcystis* for humans. This implies that individuals dipping in a pond having the pathogenic strain are at risk of getting the disease. However, it is well known that all individuals coming in contact with a particular strain of *Microcystis* do not develop the disease, and possible

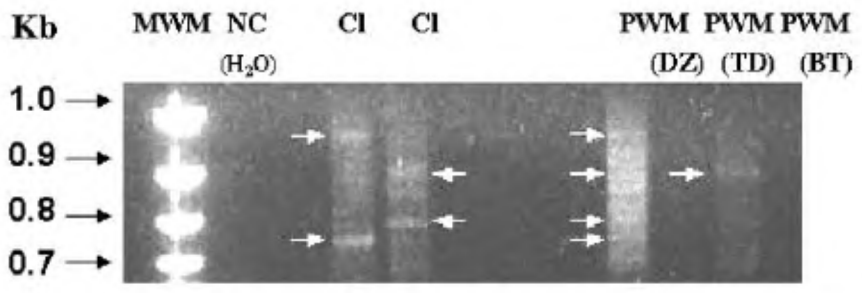

Figure 1. RAPD–PCR bands from clinical sample (Cl) in two lanes have the same positions as those from *Microcystis* found in a pond near Delhi Zoo (DZ–PWM) and Buddha Talab (BT–PWM). No bands are seen with *Microcystis* DNA from Tiger Dam in Gwalior (TD–PWM). MWM dentoes molecular weight markers. DNA fragment sizes (in Kilobase) are shown on left side of figure. PWM and NC denote the Pond Water *Microcystis* and Negative Control respectively.

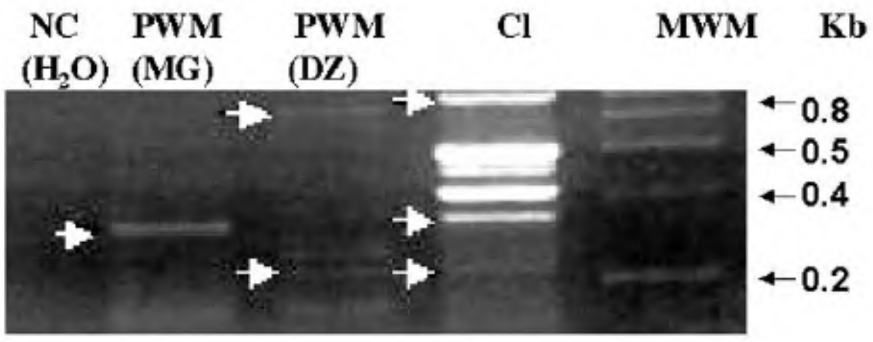

Figure 2. PCR bands from clincial sample (Cl) DNA correspond in position with those from *Microcystis* in Mathura–Manasi Ganga Pond Water *Microcystis* (MG–PWM) and Delhi Zoo Pond Water *Microcystis* (DZ–PWM). MWM denotes molecular weight markers. DNA fragment sizes (in Kilobase) are shown on right side of figure. PWM and NC denote the Pond Water *Microcystis* and Negative Control respectively.

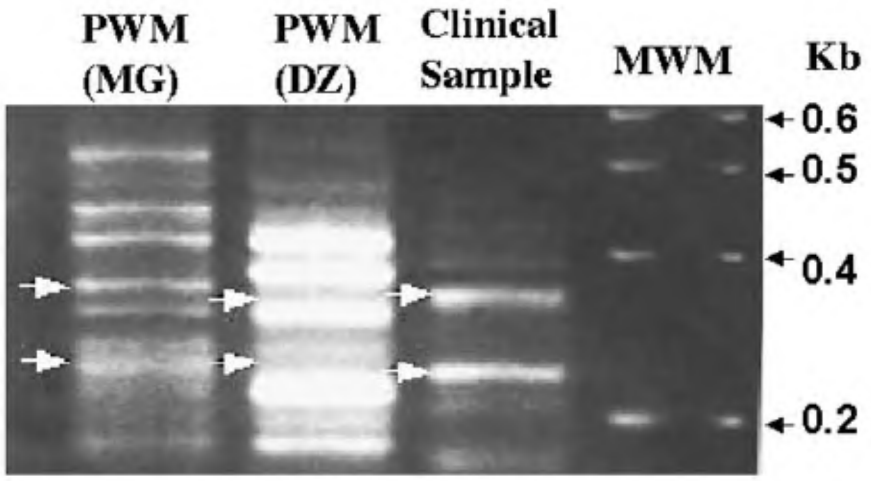

Figure 3. Clinical sample (Cl) DNA bands in the same position as those from *Microcystis* in Mathura–Manasi Ganga (MG–PWM) and Delhi Zoo (DZ–PWM). MWM denotes molecular weight markers. DNA fragment sizes (in Kilobase) are shown on right side of figure. PWM denotes the Pond Water *Microcystis*.

involvement of other host factors is suspected. Preventive control of the disease can be done by spreading awareness about the pathogenic strain of *Microcystis* in particular ponds.

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