DNA Probes with Different Specificities from a Cloned 23S rRNA Gene of *Micrococcus luteus*

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A 7500 bp *Pst*I restriction fragment of chromosomal DNA from *Micrococcus luteus* containing a 23S rRNA gene was cloned in vector pHE3 in *E. coli* RR 28 (the recombinant plasmid was designated pAR1). A recombinant phage (pAR5) hybridizing to all eubacteria tested was constructed by shotgun subcloning of the *Pst*I fragment in phage M13mp8. Further subcloning of the fragments of the 23S rRNA gene in the vectors pTZ18R and pTZ19R using selected restriction sites of the gene enabled us to select cloned fragments of the 23S rRNA gene representing different specificities. Probes specific for *Micrococcus luteus*–*Micrococcus lylae* (pAR28), for the *Arthrobacter*–*Micrococcus* group (pAR27), for eubacteria (pAR5), and for the detection of eu- and archaebacteria (the so-called universal probe pAR17) were constructed. The specificity of each probe was analysed by dot hybridization to the chromosomal DNAs of representatives of most of the main phyla of eu- and archaebacteria.

INTRODUCTION

During the last few years specific DNA probes have gained importance in different fields, for example for the detection of bacterial and viral infections, the diagnosis of inherited diseases and genetic DNA fingerprinting for forensic applications. Specific probe hybridization techniques allow rapid analysis of pure and mixed cultures. It should also be possible to identify organisms that are difficult or impossible to cultivate. Ribosomal RNA genes are especially useful for the construction of specific DNA probes for various groups such as the *Pseudomonas fluorescens* group (Festl *et al.*, 1986), legionellas (Edelstein, 1986), members of the genus *Proteus* (Haun & Göbel, 1987) and mycoplasmas (Göbel *et al.*, 1987).

To date the sequence comparison of 16S rRNA is the best way to reveal close and distant phylogenetic relationships. Cataloguing and full sequence data and some 23S rRNA total sequences have revealed that rRNA genes are composed of regions exhibiting different degrees of sequence conservation. Since these regions are longer and more distinct in the 23S rRNA than in the smaller 16S rRNA we chose a 23S rRNA gene for the construction of probes specific for close and distant relationships. Probes specific for *Micrococcus luteus*–*Micrococcus lylae*, for the *Arthrobacter*–*Micrococcus* group, for eubacteria and a universal probe have been constructed from a cloned 23S rRNA gene of *M. luteus*.

METHODS

Organisms and growth conditions. The strains used are listed in Table 2. Micrococci, arthrobacters, *Bacillus subtilis*, *brevibacteria*, *cellulomonads*, *corynebacteria*, *Microbacterium lacticum* and *Oerskovia turbata* were cultivated aerobically at 30 °C in a medium containing (g l⁻¹): peptone (10); NaCl (8); yeast extract (5); glucose (5) (adjusted to pH 7.5). For cultivation on solid medium, 13 g agar l⁻¹ was added.

Propionibacteria were grown anaerobically at 30 °C in a medium containing (g l⁻¹): peptone (10); yeast extract (5); glucose (10) (adjusted to pH 7.1). Bifidobacteria were cultivated anaerobically at 37 °C in a medium

containing (g l⁻¹): peptone from meat (5); peptone from casein (5); yeast extract (1); meat extract (5); NaH₂PO₄ (2); Tween 80 (1); cysteine (5); glucose (5) (pH 7.0).

Cells of *Chondromyces apiculatus*, *Cytophaga lytica*, '*Deinobacter*' species, flavobacteria, *Nannocystis exedens* and *Leucothrix mucor* were gifts from H. Reichenbach (Braunschweig, FRG). *Methanococcus vannielii* cells were a gift from H. Märkl (München, FRG). Cells of *Pyrococcus furiosus* and *Thermotoga maritima* were gifts from K.-O. Stetter (Regensburg, FRG). Cells, chromosomal DNAs and filter-bound chromosomal DNAs of the remaining archaeobacteria were gifts from W. Zillig and H. Klenk (Martinsried, FRG). Chromosomal DNAs of *Nocardiopsis dassonvillei*, *Stomatococcus mucilaginosus*, streptomycetes, and *Streptosporangium roseum* were gifts from E. Stackebrandt (Kiel, FRG).

Isolation and labelling of rRNA. The rRNAs were isolated according to the method of Stackebrandt *et al.* (1981) and labelled with [γ -³²P]ATP (New England Nuclear) using T4 polynucleotide kinase (BRL) as described previously (Schleifer *et al.*, 1985).

Isolation of chromosomal DNA. This was done by the method of Marmur (1961) with the modifications described by Meyer & Schleifer (1975, 1978).

Molecular cloning of 23S rRNA genes. The identification, isolation and cloning of the 23S rDNA containing restriction fragments of chromosomal DNA from *M. luteus* in the vector pHE3 (Hennecke *et al.*, 1982) in *E. coli* RR28 as well as the identification of the rDNA clones by Southern hybridization (Southern, 1975) was done as described previously (Schleifer *et al.*, 1985). Subclones were constructed by using the phage vector M13mp8 (Messing & Vieira, 1982) and *E. coli* JM101 (Yannisch-Perron *et al.*, 1985) and the vectors pTZ18R and pTZ19R (Pharmacia) and *E. coli* JM83 (Yannisch-Perron *et al.*, 1985), respectively.

Restriction enzymes, calf intestinal alkaline phosphatase and DNA ligase were purchased from BRL.

DNA sequencing. This was done according to Sanger *et al.* (1977). The sequencing primers were purchased from Boehringer, the M13 Sequencing Reagent Kit from BRL and the [α -³²P]dATP from New England Nuclear.

The isolation of single strand M13 phage DNA and the following sequencing reaction were done as described by Messing (1983). For the extraction of pTZ plasmid DNA, the rapid alkaline lysis procedure (Maniatis *et al.*, 1982) and the double strand sequencing method as described by Chen & Seeburg (1985) were used.

Labelling of the probes. The probes cloned in the sequencing vectors M13mp8 or pTZ were labelled with [α -³²P]dATP (New England Nuclear), by primer elongation according to the sequencing method (see above) in the absence of dideoxy nucleotides. The labelled DNA was separated from the free dATP by Elutip-d column chromatography (Schleicher and Schuell).

Hybridizations. For phage dot hybridization the phage supernatants were spotted onto a nylon membrane (Zeta-Probe, BioRad) and baked for 2 h at 80 °C. Prehybridization was for 8 h at 50 °C in 3 × SSC (1 × SSC is 0.15 M-NaCl, 0.015 M-sodium citrate) containing 50 mM-sodium phosphate buffer (pH 6.5), 10 × Denhardt's solution [1 × Denhardt's solution is 0.02% (w/v) Ficoll, 0.02% (w/v) polyvinylpyrrolidone, 0.02% (w/v) bovine serum albumin], 0.1% (w/v) SDS, 100 µg sheared salmon sperm DNA ml⁻¹ and 26% (v/v) formamide. The DNA was denatured at 100 °C for 10 min.

The hybridization to ³²P-labelled 23S rRNA was done for 17 h under the same conditions with the exception that the concentration of the phosphate buffer (pH 6.5) was reduced to 25 mM and 2 × Denhardt's solution was used.

After hybridization, the filters were washed twice in 2 × SSC (pH 7.0) containing 0.1% (w/v) SDS for 15 min each and twice in 0.1 × SSC (pH 7.0) containing 0.1% (w/v) SDS for 30 min each at 50 °C. Before rehybridization the probe DNA was removed from the membrane as recommended by the manufacturer.

For dot hybridization of spotted chromosomal DNA (Kafatos *et al.*, 1979) 10 µg samples of chromosomal DNA [denatured by alkali treatment according to Chen & Seeburg (1985)] were spotted onto Zeta-Probe membrane using a Minifold (Schleicher and Schuell) filtration manifold and fixed by baking for 2 h at 80 °C. The prehybridizations and the hybridizations to the ³²P-labelled probes were done under optimal conditions (25 °C below the melting point of the probes) using the same solutions as for the dot hybridization (see above). For the calculation of the melting point the equation of Meinkoth & Wahl (1984) was used.

RESULTS

Cloning of a 23S rRNA gene

A recombinant plasmid designated pAR1 (Fig. 1) was obtained by cloning a 23S rDNA-containing, *Pst*I restriction fragment from *M. luteus* DNA. It was characterized by restriction analysis and hybridization experiments with ³²P-labelled 23S rRNA, 16S rRNA and 5S rRNA from *M. luteus*. The cloned fragment was 7500 base-pairs (bp) in length and contained a 23S rRNA gene, a 5S rRNA gene and the major part of a 16S rRNA gene.

Fig. 1. Restriction map of the recombinant plasmid pAR1. The thick line represents vector pHE3 DNA; hatched lines represent cloned rRNA genes of *M. luteus*.

Subcloning of 23S rDNA fragments and screening of their specificity by phage dot hybridization

Plasmid pAR1 (Fig. 1) was digested with *Sma*I and the resulting fragments were separated by agarose gel electrophoresis. The 3.2 kb fragment containing most of the 23S rRNA gene was recovered from the gel and digested with various restriction enzymes (*Acc*I, *Cfo*I, *Hpa*II, *Taq*I and *Sau*3A). The resulting 40–400 bp fragments (length checked by agarose gel electrophoresis) were ligated with compatible phage vectors M13mp8 digested with *Acc*I or *Bam*HI or *Acc*I and *Bam*HI.

In dot hybridizations of the recombinant phage supernatants to 32 P-labelled 23S rRNAs of various prokaryotes, one of them hybridized to all 23S rRNAs tested, with the exception of the archaeobacterial rRNA. Sequence determination of the insertion of this recombinant phage (pAR5) revealed that it comprised two restriction fragments of the 23S rRNA gene, one *Taq*I fragment (115 bp) and one *Hpa*II–*Cfo*I fragment (67 bp) cloned in M13mp8 digested with *Acc*I.

Subcloning of 23S rDNA fragments and selection of specific probes by sequence comparisons

Subcloning of the cloned 23S rRNA gene of *M. luteus* in the vectors pTZ18R and pTZ19R and sequence determination of the cloned fragments enabled us to select cloned fragments with a definite degree of sequence conservation that might be appropriate for specific probes. This was elucidated by comparing the sequence of the 23S rRNA gene of *M. luteus* (Regensburger *et al.*, 1988) with published 23S rRNA sequences. Two cloned fragments representing variable regions of the gene designated pAR27 (127 bp) and pAR28 (99 bp) and one cloned fragment representing a highly conserved region of the gene (pAR17, 472 bp) were selected. Fig. 2 shows the sequences of the cloned fragments. The homology values between the probes and the corresponding regions of some published 23S rRNA gene sequences are shown in Table 1.

Screening of the hybridization probes

The specificity of the 32 P-labelled hybridization probes was determined by dot hybridization (Kafatos *et al.*, 1979) to the chromosomal DNAs of representatives of most of the main phyla of eubacteria and archaeobacteria. The results are listed in Table 2. Fig. 3 shows autoradiograms of the same membrane after hybridization to different probes.

The recombinant phage pAR5 hybridized to the 32 P-labelled 23S rRNAs of eubacteria but not to the archaeobacterial ones and showed the same specificity when used as a labelled probe itself. The universal probe pAR17 hybridized to all eubacterial and archaeobacterial chromosomal DNAs tested. pAR27 hybridized under optimal conditions to the chromosomal

pAR5 TTTGGTAGGGAGCGAAAGTCGGGACTAGTGATTTGGGGGCTTTTCTTCAACGGGCG
 TTTATTCACGGATAAAAGGTACCTGGGGAATACAGGCTGATCTTACCAAGAGT
 CATATTCGGGTCAGGGACAGTCGCTGATGTAAGTTTAACTGGGGGGGTTGGCTC
 GTAAATGTAAACGGAGGGC

pAR17 GTTGGGCGAAGCGGAGAACTTAAGCCCCAGTAAACGGGGTGGTAACTATAACCA
 TCGTAAGGTAGCGAAATTCCTTGTGGGTAAGTCCGACCTGCACGAATGGAGTA
 ACGACTTCCCGCTGTCTCAACCATGAACTCGGCGAAATTCGATTACGAGTAAAG
 ATGCTCGTTACGGCAGAAAGGCGGAAAGGCCCGTGACCTTACTATAGTTTGG
 TATTGGTCTTCGGGTGGGCTGTGTAGGATAGGTGGGAGACTGTGAAGCGGGCA
 GCGCAATGTTCTGGAGTCGTCTGAAATACCACCTCTGGTCACTCTGGATATCT
 AACTTCGGCCCGTGATCCGGGTCAGGGACAGTGCCTGATGGGTAGTTTAACTGGG
 GCGGTTGCCCTCCTAAATGTAACGGAGGCGCCAAAGGTTCCCTCAGCGGTGTG
 GTAATCAGGTGTCGAGTGCAAGTGCACAAGGGAG

pAR27 GGGCAGGGGTTGCATGTGCGGTGTGTGGGGCCCTCCTTGTATGTCTGCCGGCA
 TGGCGCAGTGAGGTCCGGCATATAGACGAACAGTGTGGGCTGGACCGTAGAG
 CTGAGAGTCCCGTAGT

pAR28 CCGGGCAATGGAAATGACCTGGTCTAAGGCTGTACGGCTGCCGGTAGGTAATCC
 CCGGGCTGTATGCTGAGACCTGATGGGCTCCACATGGTGGGTG

Fig. 2. Sequence of the insertions of the recombinant phage pAR5 and the recombinant plasmids pAR17, pAR27 and pAR28.

Fig. 3. Sequential hybridization of the same Zeta-Probe membrane containing DNAs from 48 organisms (numbers refer to Table 2) to ^{32}P -labelled probes pAR17 (a), pAR27 (b) and pAR28 (c). DNA of *E. coli* always gave a positive signal since the plasmid preparations contained trace-amounts of *E. coli* DNA.

Table 1. Sequence homology between the probes and the corresponding regions of some 23S rRNA genes

mc, *Micrococcus luteus*; bu, *Bacillus subtilis* (Green *et al.*, 1985); bt, *Bacillus stearothermophilus* (Kop *et al.*, 1984); an, *Anacystis nidulans* (Douglas & Doolittle, 1984); ec, *Escherichia coli* (Brosius *et al.*, 1980); mv, *Methanococcus vannielii* (Jarsch & Böck, 1985).

Probe	Homology values (%) to the rDNA of:					
	mc	bu	bt	an	ec	mv
pAR17	100	82*	82*	78*	79*	76*
pAR5	100	83	85	80	82	63
pAR27	100	33	36	36	24	30
pAR28	100	53	55	39	56	39

* A stretch of 93 bp showed homology values of 90–95%.

DNAs of all micrococci and arthrobacters studied and also to DNAs of some other Gram-positive bacteria. However, under stringent hybridization conditions (not shown in Tables 2 and Fig. 3) only DNAs of representatives of the genera *Arthrobacter* and *Micrococcus* gave a positive hybridization reaction with pAR27 and none of the other strains studied. pAR28 represents a very specific DNA probe that only hybridized to the DNAs of *M. luteus* and the closely related *M. lylae*.

DISCUSSION

Specific probes are required for definitive results in dot blot experiments. This could be expected of those regions of a 23S rRNA gene of which the sequence is not only homologous to the corresponding regions of the chromosomal DNAs of those bacteria that are to be detected, but also only moderately homologous to the corresponding regions of the chromosomal DNAs of other bacteria that are to be excluded. The hybridization conditions that we applied detected only organisms sharing at least 80% sequence homology. DNAs with lower homologies did not hybridize.

The first approach to obtain probes of various specificities was achieved by shotgun cloning of a cloned 23S rRNA gene and by selecting subcloned fragments with a certain specificity by phage dot hybridization. By this method a eubacterial specific DNA probe (pAR5) was obtained. Sequence determination and comparison with published 23S rRNA sequences revealed that the insertion in pAR5 represented a conservative region of the gene with a homology of more than 80% for eubacteria and only 63% for archaebacteria (Table 1). It only hybridized to DNA of eubacteria. To separate archaebacteria from eubacteria by a positive hybridization signal, a corresponding DNA probe specific for archaebacteria is still needed.

A direct subcloning of regions of the gene with a known degree of sequence conservation seemed to be more convenient for the construction of specific DNA probes than the random shotgun cloning technique used for the construction of pAR5. However a prerequisite for this approach is the knowledge of the sequence of the 23S rRNA gene of the organism from which these fragments are to be isolated and also the existence of usable restriction sites for cloning. We also stipulated that the region of the 23S rRNA gene should have a high degree of sequence homology (at least 80%) to that group of organisms for which it should be specific.

The largest, coherent, universal stretch of the 23S rRNA gene is present in the insertion of the recombinant plasmid pAR17 that produced positive hybridization signals with the chromosomal DNAs of all eubacteria and archaebacteria tested. Using this universal probe it was possible to estimate the amount of filter-bound chromosomal DNA that is accessible to a hybridization reaction. Therefore a correction of the hybridization results with the more specific probes could be done. False negative results, based upon failure of binding of the chromosomal DNA to the

Table 2. *Strains studied, their phylogenetic positions and their reactions with the ³²P-labelled probes under optimal hybridization conditions*

No.*	Strain	Origin†	S_{AB} of 16S rRNA‡	Phylogeny§	Hybridization with pAR			
					17	5	27	28
1	<i>Micrococcus luteus</i>	DSM 20030 ^T	1.00	Gm + H	+	+	+	+
2	<i>Micrococcus lylae</i>	ATCC 17566	0.83	Gm + H	+	+	+	+
3	<i>Micrococcus varians</i>	DSM 20033 ^T	0.75	Gm + H	+	+	+	—
4	<i>Micrococcus roseus</i>	Back H15	0.71	Gm + H	+	+	+	—
5	<i>Micrococcus agilis</i>	CCM 1744	ND	Gm + H	+	+	(+)	—
6	<i>Arthrobacter globiformis</i>	DSM 20124 ^T	0.77	Gm + H	+	+	+	—
7	<i>Arthrobacter ureafaciens</i>	DSM 20126 ^T	ND	Gm + H	+	+	+	—
8	<i>Arthrobacter pascens</i>	DSM 20545 ^T	ND	Gm + H	+	+	+	—
9	<i>Arthrobacter citreus</i>	DSM 20133 ^T	0.71	Gm + H	+	+	+	—
10	<i>Arthrobacter citreus</i>	ATCC 21348	0.71	Gm + H	+	+	+	—
11	<i>Arthrobacter atrocyaneus</i>	DSM 20127 ^T	0.73	Gm + H	+	+	+	—
12	<i>Micrococcus kristinae</i>	ATCC 27570	ND	Gm + H	+	+	+	—
13	<i>Micrococcus nishinomiyaensis</i>	DSM 20448 ^T	0.68	Gm + H	+	+	+	—
14	<i>Cellulomonas flavigena</i>	DSM 20109 ^T	0.62	Gm + H	+	+	+	—
15	<i>Cellulomonas flavigena</i>	DSM 20109 ^T	0.62	Gm + H	+	+	—	—
16	<i>Cellulomonas biazotea</i>	DSM 10112 ^T	ND	Gm + H	+	+	—	—
17	<i>Oerskovia turbata</i>	NCIB 10585	0.68	Gm + H	+	+	+	—
18	<i>Cellulomonas cartae</i>	DSM 20106 ^T	ND	Gm + H	+	+	+	—
19	<i>Brevibacterium linens</i>	DSM 20158	0.62	Gm + H	+	+	+	—
20	<i>Microbacterium lacticum</i>	DSM 20427 ^T	0.58	Gm + H	+	+	—	—
21	<i>Brevibacterium imperiale</i>	DSM 20530 ^T	ND	Gm + H	+	+	+	—
22	<i>Corynebacterium betae</i>	DSM 20141	0.64	Gm + H	+	+	—	—
23	<i>Corynebacterium michiganense</i>	DSM 20134	ND	Gm + H	+	+	+	—
24	<i>Arthrobacter simplex</i>	DSM 20130 ^T	0.55	Gm + H	+	+	+	—
25	<i>Corynebacterium glutamicum</i>	DSM 20300 ^T	0.52	Gm + H	+	+	—	—
26	<i>Brevibacterium ketoglutamicum</i>	ATCC 15587	0.55	Gm + H	+	+	—	—
27	<i>Corynebacterium fascians</i>	DSM 20131	0.60	Gm + H	+	+	—	—
28	<i>Kitasatoa nagasakiensis</i>	DSM 43361 ^T	ND	Gm + H	+	+	+	—
29	<i>Nocardioptis dassonvillei</i>	DSM 43111 ^T	ND	Gm + H	+	+	—	—
30	<i>Stomatococcus mucilaginosus</i>	DSM 20445	0.67	Gm + H	+	+	+	—
31	<i>Streptomyces 'alborubidus'</i>	DSM 40465	ND	Gm + H	+	+	—	—
32	<i>'Streptomyces' salmonicida</i>	DSM 40472	ND	Gm + H	+	+	+	—
33	<i>Streptomyces capreolus</i>	DSM 40225	ND	Gm + H	+	+	+	—
34	<i>Streptosporangium roseum</i>	DSM 43021 ^T	0.55	Gm + H	+	+	+	—
35	<i>Propionibacterium freudenreichii</i>	DSM 20271 ^T	0.47	Gm + H	+	+	—	—
36	<i>Propionibacterium acidi-propionici</i>	DSM 20272	ND	Gm + H	+	+	—	—
37	<i>Bifidobacterium bifidum</i>	DSM 20456 ^T	0.41	Gm + H	+	+	—	—
38	<i>Bifidobacterium infantis</i>	DSM 20088 ^T	ND	Gm + H	+	+	—	—
39	<i>Bifidobacterium adolescentis</i>	DSM 20083 ^T	ND	Gm + H	+	+	—	—
40	<i>Bacillus subtilis</i>	DSM 10 ^T	0.36	Gm + L	+	+	—	—
41	<i>Staphylococcus aureus</i>	ATCC 12600 ^T	0.33	Gm + L	+	+	—	—
42	<i>Streptococcus pyogenes</i>	NCTC 8198 ^T	0.30	Gm + L	+	+	(+)	—
43	<i>Escherichia coli</i>	DSM 30083 ^T	0.23	PURC	+	+	+	+
44	<i>Ruminobacter amylophilus</i>	DSM 1361	0.21	PURC	+	+	—	—
45	<i>Enterobacter aerogenes</i>	DSM 30053 ^T	0.21	PURC	+	+	—	—
46	<i>Leucothrix mucor</i>	DSM 2157 ^T	0.22		+	+	—	—
47	<i>Rhodobacter capsulatus</i>	DSM 1710 ^T	0.22	PURA	+	+	—	—
48	<i>Alteromonas putrefaciens</i>	DSM 50426	0.21	PURC	+	+	—	—
49	<i>Proteus mirabilis</i>	DSM 788	0.21	PURC	+	+	—	—
50	<i>Chondromyces apiculatus</i>	DSM 436	ND	PURD	+	+	—	—

Table 2. Continued

No.*	Strain	Origin†	S_{AB} of 16S rRNA‡	Phylogeny§	Hybridization with pAR			
					17	5	27	28
51	<i>Nannocystis exedens</i>	DSM 71	0.25		+	+	—	—
52	<i>Flavobacterium breve</i>	DSM 30096	0.27	CFB	+	+	—	—
53	<i>Flavobacterium ferrugineum</i>	DSM 30193 ^T	ND	CFB	+	+	—	—
54	<i>Cytophaga lytica</i>	DSM 2039 ^T	0.24	CFB	+	+	—	—
55	' <i>Deinobacter</i> ' sp.¶	R 5004	0.19	RD	+	+	—	—
56	<i>Herpetosiphon aurantiacus</i>	ATCC 23779 ^T	0.18	CX	+	+	—	—
57	<i>Thermotoga maritima</i>	DSM 3109 ^T	ND		+	+	—	—
58	<i>Methanococcus vannielii</i>	DSM 1224 ^T	0.07	ARC	+	—	—	—
59	<i>Methanococcus</i> ' <i>thermolithotrophicus</i> '	DSM 2095	ND	ARC	+	—	—	—
60	<i>Thermoproteus tenax</i>	DSM 2978 ^T	0.10	ARC	(+)	—	—	—
61	<i>Desulfurococcus mobilis</i>	DSM 2475	ND	ARC	(+)	—	—	—
62	<i>Thermococcus celer</i>	DSM 2161	ND	ARC	(+)	—	—	—
63	<i>Pyrococcus furiosus</i>	Stetter	ND	ARC	+	—	—	—
64	<i>Desulfurococcus mucosus</i>	DSM 2162	ND	ARC	(+)	ND	ND	ND

* Numbers refer to Fig. 3.

† ATCC, American Type Culture Collection, Rockville, Md, USA; Back, Weihenstephan, FRG; CCM, Czechoslovak Collection of Microorganisms, Brno, Czechoslovakia; DSM, Deutsche Sammlung von Mikroorganismen, Göttingen, FRG; NCIB, National Collection of Industrial Bacteria, Aberdeen, UK; NCTC, National Collection of Type Cultures, London, UK; R, H. Reichenbach, Braunschweig, FRG; Stetter, K. O. Stetter, Regensburg, FRG; T, type strain.

‡ S_{AB} values relate to *M. luteus*. References: Stackebrandt & Schleifer (1984); Ludwig *et al.* (1985); Ludwig (1981); E. Stackebrandt (personal communication); Woese *et al.* (1985); C. R. Woese (personal communication). ND, Not determined.

§ Abbreviations according to Woese *et al.* (1985): Gm+, Gram-positive eubacteria; L, low G + C subdivision; H, high G + C subdivision; PUR, purple bacteria and relatives, A, B, C and D, alpha, beta, gamma and delta (Fowler *et al.*, 1986) subdivisions, respectively; CFB, flavobacteria and cytophagas; CY, cyanobacteria; CX, green nonsulphur bacteria and relatives; RD, radioresistant micrococci; ARC, archaebacteria.

|| (+), Weakly positive; ND, not determined.

¶ Radioresistant rods related to *Deinococcus* species (H. Reichenbach, W. Ludwig and E. Stackebrandt, unpublished results).

filter or inaccessibility to a hybridization reaction, were excluded by control hybridizations using pAR17. This universal probe can also be used for screening for 23S rRNA genes, thus making the difficult isolation of the homologous 23S rRNAs unnecessary.

The plasmid pAR27 not only hybridized under optimal conditions to the DNAs of all representatives of the genera *Arthrobacter* and *Micrococcus* that were studied, but also to two brevibacteria, two cellulomonads, *Corynebacterium michiganense*, *Stomatococcus mucilaginosus*, a few streptomycetes and even *Streptococcus pyogenes*. However, the reactions clearly depended on the hybridization conditions applied. Stringent conditions (20 °C below the melting point of the probe) resulted in increased specificity. In this case only the chromosomal DNAs of closely related bacteria belonging to the genera *Arthrobacter* and *Micrococcus* ($S_{AB} > 0.7$) hybridized to the probe (data not shown).

The DNA probe pAR28 hybridized exclusively to the chromosomal DNAs of *M. luteus* and *M. lylae*.

23S RNA genes are, as the results of the present study show, a reservoir for the construction of DNA probes of various specificities. It has been demonstrated that it is possible to isolate not only DNA probes specific for closely related bacteria ($S_{AB} > 0.83$) but also for distantly related organisms (S_{AB} 0.1 and 0.2).

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