Survey of Multicopy Single-Stranded DNAs and Reverse Transcriptase Genes among Natural Isolates of *Myxococcus xanthus*

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Twenty different isolates of the soil bacterium *Myxococcus xanthus* were examined for the presence of multicopy single-stranded DNA (msDNA)-producing retroelements, or retrons. Each strain was analyzed by ethidium bromide staining for msDNA, ^{32}P labeling of the msDNA molecule by the reverse transcriptase (RT) extension method, and DNA hybridization experiments with probes derived from two retrons, Mx162 and Mx65, previously cloned from *M. xanthus* DZF1. These analyses revealed that all *M. xanthus* strains contain an msDNA very similar to Mx162 msDNA, and 13 strains also contain a second smaller msDNA very similar to Mx65 msDNA. In addition, the strains contained retron-encoded genes *msr* and *msd*, which code for msDNA, and a gene for RT responsible for the synthesis of msDNA. These genes show greater than 80% nucleotide sequence similarity to retrons Mx162 or Mx65. The near-ubiquitous occurrence of msDNA retrons among *M. xanthus* strains and their homogeneous nature are in marked contrast to the highly diverse but rarely occurring msDNA-producing elements of *Escherichia coli*. The possible origin and evolution of RT and retron elements is discussed in view of these findings.

An extrachromosomal RNA-DNA molecule known as multicopy single-stranded DNA (msDNA) was first discovered in the soil bacterium *Myxococcus xanthus* (33). This molecule proved to be the key piece of evidence which led to the novel discovery of reverse transcriptase (RT) in bacteria (13, 17, 20). The msDNA molecule originally discovered in *M. xanthus* (Mx162 msDNA) consists of a 162-base single-stranded DNA joined to an internal residue of a 77-base single-stranded RNA. Branching out from the 2' position of a specific internal guanosine residue is a unique 2',5' phosphodiester bond joining the RNA strand to the 5' end of the DNA strand (7, 9) (for a review of the structure and synthesis of msDNA, see reference 16). Similar msDNA molecules have been found in other myxobacteria (4) as well as a number of *Escherichia coli* strains (10, 19, 28).

The ability of the host cell to produce msDNA, which is made in large quantities of over 500 copies, requires a single locus found on the chromosome (5). This locus contains *msr* and *msd* genes, which code for the RNA and DNA strands, respectively, of msDNA and are situated in opposite orientation. Located a short distance upstream of *msd* is an open reading frame (ORF) encoding the bacterial RT, which is related to retroviral RTs. The RT is required to synthesize msDNA by reverse transcription of a folded mRNA transcript from this locus, which serves as both a primer and a template (15). Thus, the locus responsible for the production of msDNA appears to represent a primitive, prokaryotic form of retroelement which has been given the term retron (30).

Coresiding with the Mx162 retron of *M. xanthus* (strain DZF1) is a second species of msDNA designated Mx65 msDNA (6). It is encoded by a different retron element containing a different RT and located at a different position

on the host chromosome from retron Mx162 (12). In addition to *M. xanthus*, retron elements and their msDNAs have been found in *E. coli*. A recent survey of *E. coli* strains from the ECOR collection (a standard reference collection [24]) indicates that only a small number of the 72 strains (13%) produce msDNA (10). However, hybridization studies (28) and the DNA sequence determination of three elements from *E. coli*, retrons Ec67, Ec86, and Ec73 (17, 20, 29), indicate little similarity between them, with msDNAs that differ in nucleotide sequence and RTs that differ considerably in amino acid sequence. In contrast to the diversity among *E. coli* retrons and their msDNAs, an early study by Dhundale et al. (4) indicated that all *M. xanthus* strains examined appear to harbor an msDNA essentially the same as that of the DZF1 prototype, Mx162, as determined from size and DNA hybridization.

This report describes a follow-up study of the earlier work by Dhundale et al. (4) which examines a larger collection of independent, natural isolates of *M. xanthus* for the prevalence and diversity of msDNAs and their retron-associated genes. In addition, the origin and evolution of RT and retroelements will be discussed in view of the contrary features of the msDNA-producing elements found in *M. xanthus* and those observed in *E. coli*.

MATERIALS AND METHODS

Bacterial strains, plasmids, and probes. The *M. xanthus* laboratory strain DZF1 (8) was obtained from David Zusman. The other 20 strains were kindly provided by Hans Reichenbach and Dale Kaiser and are listed in Table 1. Bacteria were cultured in CYE (3) broth or agar plates.

DNA hybridization probes were derived as follows. Probe Mx162MS was obtained by digesting the pUC9 plasmid clone pmsAlu (33) with *Eco*RI and *Hin*dIII, followed by purification of the 0.7-kb restriction fragment from electro-

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TABLE 1. *M. xanthus* strains surveyed

No.[a]	Strain	Source/year	Geographic location
	DZF1	David Zusman/NA[b]	NA
1	Mx-x2	Hans Reichenbach/1969	Black Forest, Germany
2	Mx-x3	Hans Reichenbach/1970	Kaiserstuhl Mountains, Germany
3	Mx-x10	Hans Reichenbach/1977	Teneriffa, Spain
4	Mx-x11	Hans Reichenbach/1978	Sicily, Italy
5	Mx-x18	Hans Reichenbach/1974–77	Near Bonn, Germany
6	Mx-x49	Hans Reichenbach/1980	Djerba Island, Tunisia
7	Mx-x56	Hans Reichenbach/1980	Crete, Greece
8	Mx-x63	Hans Reichenbach/1980	Aix en Provence, France
9	Mx-x67	Hans Reichenbach/1981	Poland
10	Mx-x73	Hans Reichenbach/1982	Alanya, Turkey
11	Mx-x77	Hans Reichenbach/1982	Crete, Greece
12	Mx-x78	Hans Reichenbach/1982	Mallorca, Spain
13	Mx-x82	Hans Reichenbach/1983	Braunschweig, Germany
14	DK823	Dale Kaiser/NA	Solvang, Calif.
15	DK829	Dale Kaiser/NA	Morgan Hill, Calif.
16	DK843	Dale Kaiser/NA	Cold Spring Harbor, N.Y.
17	DK851	Dale Kaiser/NA	Salinas, Calif.
18	DK853	Dale Kaiser/NA	St. Louis, Mo.
19	DK862	Dale Kaiser/NA	Point Reyes, Calif.
20	DK870	Dale Kaiser/NA	Stanford, Calif.

[a] Each strain is assigned a number corresponding to sample lanes in Fig. 3 to 6.
[b] NA, information not available.

phoresis gels (5% acrylamide). Likewise, probe Mx162RT was obtained by *EcoRI-HindIII* digestion of the plasmid clone pmsSS (13), probe Mx65MS was obtained by *EcoRI-HindIII* digestion of plasmid pmrAlu (6), and probe Mx65RT was obtained by *EcoRI-HindIII* digestion of plasmid pBPv-1 (12). The resulting fragments were labeled by the nick translation method (25) using $[\alpha\text{-}^{32}P]dCTP$ and DNA polymerase (Kornberg fragment). The 16S rRNA probe corresponds to a 3.8-kb *PvuII* restriction fragment from plasmid pT711 (a gift from L. Lindahl and J. Zengel), which contains a 5.5-kb region (*BclI* fragment) of the *rrnB* operon of *E. coli* (2, 22). The 3.8-kb probe encodes the 16S rRNA gene, the $tRNA_2^{Glu}$ gene, and part of the 23S rRNA gene of *E. coli*.

Preparation of msDNA and chromosomal DNA. msDNA was prepared by the alkaline lysis method used for plasmid DNA (1). Samples were treated with RNase A prior to electrophoresis.

Chromosomal DNA was prepared as follows. *M. xanthus* cells were harvested either from CYE plates or from 10-ml broth cultures (100 to 150 Klett units). Cells were washed in 50 mM Tris (pH 8.0) and then resuspended in 0.5 ml of lysis solution (10 mM Tris [pH 8.0], 1 mM EDTA, 350 mM NaCl, 2% sodium dodecyl sulfate [SDS]) in a 1.5-ml microtube. The solution was incubated at 37°C for 5 to 15 min and then extracted twice with 0.5 ml of phenol-chloroform (1:1 mix) and twice with 0.5 ml of chloroform alone (any flocculent interface was saved until the last chloroform extraction). The aqueous phase was retrieved (~0.45 ml), and to this was added slowly 1 ml of cold (100%) ethanol. The tube was gently inverted 10 to 20 times until a small white clot of precipitated chromosome was present. The chromosome was then spooled onto the end of a pipet tip and washed several times in 70% ethanol. The chromosome pellet was then left to air dry or dried under a vacuum for only a few seconds (the pellet must not be overdried). Chromosomal DNA was then gently dissolved in 100 μl of 0.1× TE (1 mM Tris [pH 8.0], 0.1 mM EDTA).

Southern blot hybridization. Chromosomal DNA preparations were digested with *PstI* in a 100-μl volume with the manufacturer-supplied buffer. About 1 μg of digested DNA from each *M. xanthus* strain was electrophoresed in a 0.7% agarose gel. DNA was then transferred from the agarose gels to nitrocellulose (26), and hybridization was carried out in 50% (vol/vol) formamide–5× SSPE–5× Denhardt's solution–0.3% SDS at 42°C with probe (10^6 cpm/ml) (1× SSPE is 180 mM NaCl, 10 mM sodium phosphate [pH 7.4], and 10 mM EDTA; Denhardt's solution is 0.2% Ficoll, 0.2% polyvinylpyrrolidone, and 0.02% bovine serum albumin).

RT extension. The RT extension reaction to ^{32}P label msDNA was carried out with total RNA prepared from each strain plus RT from avian myeloblastosis virus (Boehringer Mannheim). For a detailed description of this reaction, see references 17 and 18.

RESULTS

Collections of *M. xanthus* strains. Environmental isolates from two collections of *M. xanthus* were used in this study to investigate the distribution of msDNA in this species (Table 1). A group of 13 *M. xanthus* strains isolated from various locations throughout Europe was obtained from the collection of Hans Reichenbach. A second group of seven strains was from the collection of Dale Kaiser and has been described previously (4). In addition, a common laboratory strain of *M. xanthus*, DZF1 (8), was examined. Three methods were used to detect msDNAs or their genes among these strains. Ethidium bromide was used as a UV fluorescent stain to detect the extrachromosomal msDNA molecules. Various retron probes were used in Southern hybridizations of chromosomal DNA to detect the genes encoding msDNA and RT. Finally, the RT extension method was used to specifically radiolabel the msDNA molecule.

Ethidium bromide staining of msDNAs. DNA extracted from the 13 Reichenbach strains was electrophoresed through a 5% polyacrylamide gel and then ethidium bromide stained (33). As shown in Fig. 1, ethidium bromide staining revealed that each strain contains a satellite DNA band about 160 to 150 bases in size. This size is nearly identical to
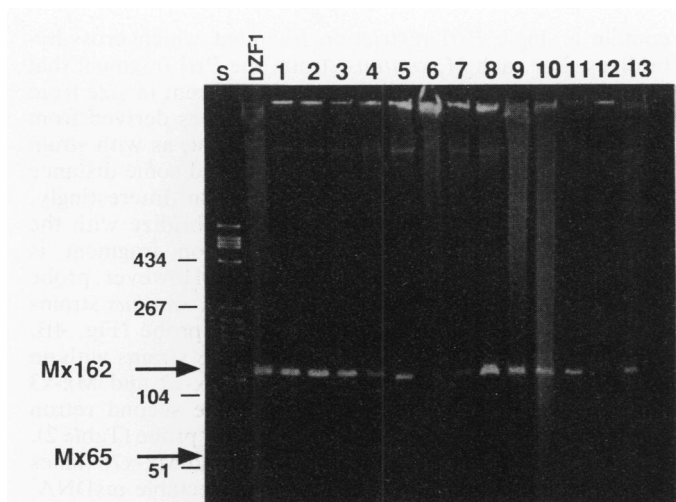
FIG. 1. Ethidium bromide staining of msDNAs. DNA prepared by the alkaline lysis method (1) and treated with RNase A was separated by electrophoresis in a 5% acrylamide gel and then ethidium bromide stained. Numbered lanes correspond to the *M. xanthus* strain numbers in Table 1. Lane S contains pBR322 plasmid DNA digested with *Hae*III as size standards; lane DZF1 contains the two prototype msDNAs, Mx162 (upper arrow) and Mx65 (lower arrow), from the *M. xanthus* laboratory strain DZF1.

that of Mx162 msDNA, the large species originally isolated from *M. xanthus* DZF1 (Fig. 1). This finding is similar to that described by Dhundale et al. (4) in which each of the Kaiser strains (numbers 14 to 20 in Table 1) also contained a DNA band identical in size to Mx162 msDNA. The slight variability in size observed in some strains in Fig. 1 may be due to

differences in the amount of msDNA loaded onto the gel. Coresiding with Mx162 msDNA in strain DZF1 is a smaller, unrelated msDNA (Mx65 msDNA) which is just discernible in Fig. 1 (lane DZF1). A smaller DNA band, similar in size to Mx65 msDNA, is clearly detectable in some but not all of the strains surveyed in Fig. 1. A summary of the findings from ethidium bromide staining (Table 2) suggests that all strains of *M. xanthus* examined contain an msDNA very similar to the large speci s, Mx162 msDNA of DZF1. Some strains, however, may lack the smaller Mx65 msDNA.

Southern hybridization. DNA hybridization experiments using four different probes were conducted to examine the kinship of msDNAs detected with ethidium bromide. The hybridization probe designated Mx162MS corresponds to the 0.7-kb *Alu*I restriction fragment from the Mx162 retron previously cloned from the DZF1 chromosome (33). This region of the retron encodes genes *msr* and *msd* (Fig. 2A), for the RNA and DNA portions, respectively, of the msDNA molecule. The Mx162MS probe was ^{32}P labeled by nick translation and hybridized to *Pst*I-digested chromosomal DNA prepared from each strain (Fig. 3A). Except for strain DK851 (lane 17), a single *Pst*I restriction fragment from each *M. xanthus* strain cross-hybridized with this probe. For DK851 (lane 17) a second fragment also hybridized, possibly because of incomplete digestion of the chromosomal preparation. Some of the *M. xanthus* strains showed a weak hybridization signal with the Mx162MS probe (lanes 4, 6, and 7), probably because these lanes contained smaller amounts of DNA. An identical hybridization pattern is also obtained with the probe designated Mx162RT (Fig. 3B). This probe corresponds to the 2.7-kb *Sma*I-*Sal*I restriction fragment from the cloned DZF1 retron (Mx162) and encompasses the C-terminal two-thirds of the RT ORF (Fig. 2A). A summary of the hybridization results (Table 2) strongly

TABLE 2. Summary of data to detect msDNAs in *M. xanthus*

No.	Strain	Mx162 msDNA				Mx65 msDNA			
		EtBr[a]	Probe[b]		^{32}P[c]	EtBr	Probe		^{32}P
			Mx162MS	Mx162RT			Mx65MS	Mx65RT	
	DZF1	+	+	+	+	+	+	+	+
1	Mx-x2	+	+	+	+	−	−	−	−
2	Mx-x3	+	+	+	+	−	−	−	−
3	Mx-x10	+	+	+	+	−	−	+	−
4	Mx-x11	+	+	+	+	−	+	+	+
5	Mx-x18	+	+	+	+	−	+	+	+
6	Mx-x49	+	+	+	+	−	+	+	−
7	Mx-x56	+	+	+	+	−	−	+	−
8	Mx-x63	+	+	+	+	+	+	+	+
9	Mx-x67	+	+	+	+	+	+	+	+
10	Mx-x73	+	+	+	+	+	+	+	+
11	Mx-x77	+	+	+	+	−	−	+	−
12	Mx-x78	+	+	+	+	−	+	+	−
13	Mx-x82	+	+	+	+	−	+	+	+
14	DK823	+[d]	+	+	+	ND[e]	+	+	+
15	DK829	+[d]	+	+	+	ND	+	+	+
16	DK843	+[d]	+	+	+	ND	+	+	+
17	DK851	+[d]	+	+	+	ND	+	+	+
18	DK853	+[d]	+	+	+	ND	+	+	+
19	DK862	+[d]	+	+	+	ND	+	+	+
20	DK870	+[d]	+	+	+	ND	+	+	+

[a] Ethidium bromide staining to detect msDNA (Fig. 1).
[b] Southern blot hybridization with indicated retron probe (Fig. 3 or 4).
[c] Labeling of msDNA with ^{32}P by the RT extension method (Fig. 5).
[d] Data determined by Dhundale et al. (4).
[e] ND, not determined.
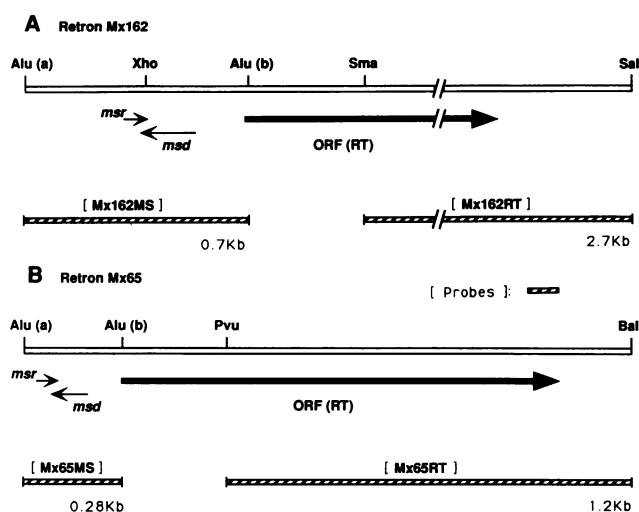
A Retron Mx162

B Retron Mx65

FIG. 2. Locations of hybridization probes to detect retron genes for msDNA and RT. (A) Partial restriction map of the chromosomal region of *M. xanthus* encoding retron Mx162. The region encoding the RNA portion of msDNA is shown by an arrow labeled *msr*, and the DNA portion of the molecule is indicated by an arrow labeled *msd*. The neighboring ORF coding for RT is shown with a large arrow. The restriction fragments used for hybridization probes are denoted by bars below the restriction map. Probe Mx162MS corresponds to the *AluI* (a)-*AluI* (b) fragment containing the *msr-msd* genes from the plasmid clone pmsAlu. Probe Mx162RT corresponds to the *SmaI-SalI* fragment (from plasmid pmSSS) encompassing the RT ORF and a region downstream. (B) Partial restriction map of the chromosome region encoding the Mx65 retron of *M. xanthus* DZF1. Gene designations are as for panel A. Probe Mx65MS is the *AluI* (a)-*AluI* (b) fragment from plasmid clone pmrAlu (horizontal bar below). Probe Mx65RT is derived from the *PvuI-BalI* fragment from plasmid pBPv-1. Abbreviations for restriction sites: Alu, *AluI*; Xho, *XhoI*; Sma, *SmaI*; Sal, *SalI*; Pvu, *PvuI*; Bal, *BalI*.

suggests that, like strain DZF1, each *M. xanthus* isolate contains a chromosomal restriction fragment encoding a single retron element. In addition, the retron element encodes an msDNA and RT closely homologous to those of the DZF1 retron, Mx162 (13). The degree of similarity is high since under the conditions for Southern hybridization any nucleotide sequences less than 80% homologous with Mx162 will not be detected (28). For example, in the Southern blot presented in Fig. 3A, chromosomal DNA prepared from the related myxobacterium *Stigmatella aurantiaca* was also included (lane not shown). *S. aurantiaca* is known to encode a retron, Sa163, related to Mx162, with DNA sequence homology of about 81% for the *msr* and *msd* genes of these two retrons (7, 9). However, under the hybridization conditions used, only a very weak signal is observed with the Mx162MS probe. Given that roughly the same amount of DNA is present in the Southern blots for both *S. aurantiaca* and DZF1 (which gives a strong signal), it is estimated that only sequences greater than 80% homologous are detected.

Hybridization experiments were also conducted using probes derived from the second retron element of DZF1, Mx65 (6). The probe designated Mx65MS matches the 280-bp *AluI* fragment of the retron previously cloned from a different location on the host chromosome (6). This *AluI* fragment contains the genes (*msr* and *msd*) which encode the smaller (Mx65) msDNA (Fig. 2B). Hybridization with the Mx65MS probe to *PstI*-digested chromosomes (Fig. 4A) reveals that most but not all of the *M. xanthus* strains

contain a single *PstI* restriction fragment which cross-hybridizes. For each *M. xanthus* strain, the *PstI* fragment that hybridizes with the Mx65MS probe is different in size from the fragment that hybridizes with the probes derived from the Mx162 retron. This finding indicates that, as with strain DZF1, the two retrons are probably situated some distance apart on the chromosome of each host strain. Interestingly, some of the *M. xanthus* strains do not hybridize with the Mx65MS probe. No detectable restriction fragment is present in lanes 1, 2, 3, 7, and 11 of Fig. 4A. However, probe Mx65RT gives a signal with some of the *M. xanthus* strains that did not hybridize with the Mx65MS probe (Fig. 4B, lanes 3, 7, and 11). Of the seven *M. xanthus* strains with no detectable Mx65 msDNA, two strains, Mx-x2 and Mx-x3 (lanes 1 and 2), probably lack entirely the second retron element since they do not hybridize to either probe (Table 2). Chromosomal DNA from strains Mx-x49 and Mx-x78 (lanes 6 and 12), although not producing any detectable msDNA, hybridize to both probes (Mx65MS and Mx65RT) derived from the Mx65 retron of DZF1. Perhaps these strains produce too little of the smaller msDNA to be detected. It has been reported that Mx65 msDNA is produced in considerably fewer copies (100) than the larger Mx162 msDNA (500) in strain DZF1 (6). *M. xanthus* Mx-x10, Mx-x56, and Mx-x77 (lanes 3, 7, and 11) are peculiar in that they do not produce any detectable Mx65 msDNA, nor do their chromosomal DNAs hybridize to the Mx65MS probe (Fig. 4A) derived from the region of the retron which encodes the genes (*msr* and *msd*) for the msDNA molecule. Surprisingly, these three strains did hybridize to the Mx65RT probe (Fig. 4B, lanes 3, 7, and 11), which corresponds to the gene encoding RT (Table 2). One possible explanation is that these three strains contain an Mx65 retron which does not contain the genes (*msr* and *msd*) coding for msDNA. Alternatively, perhaps these strains contain a new species of retron element which encodes an RT gene similar enough to cross-hybridize with the Mx65RT probe but codes for msDNA entirely different from Mx65 msDNA. The Mx65RT probe did hybridize to the same *PstI* fragments as did the Mx65MS probe in lanes 4, 5, 6, 8, 9, 10, 12 to 20, and DZF1 of Fig. 4B. Table 2 presents a summary of all the DNA hybridization experiments.

Labeling of msDNAs with ^{32}P. The experiments described above demonstrate that *M. xanthus* Mx-x2 and Mx-x3 do not produce the smaller Mx65 msDNA and do not appear to encode the retron element that is responsible for its synthesis. It is not clear whether strains Mx-x10, Mx-x56, and Mx-x77 (Fig. 4A, lanes 3, 7, and 11) produce the small species of msDNA since they do not hybridize with the Mx65MS retron probe (Table 2). The RT extension method was used to detect the smaller msDNA molecule in the *M. xanthus* collection, especially for the strains mentioned above. This method specifically labels msDNA with ^{32}P because of its unique secondary structure and is much more sensitive in detecting msDNA than is ethidium bromide staining (17, 20). Briefly, avian myeloblastosis virus RT added exogenously to purified msDNA will incorporate label into the molecule by extending the DNA strand, using the RNA portion as a template and the hybrid structure (where the 3' ends of the DNA and RNA chains remain base paired) as a primer (18). With this method, the larger (Mx162) msDNA is readily labeled and detected by autoradiography in each *M. xanthus* strain (Fig. 5). The smaller (Mx65) msDNA was easily detected in most strains, just detectable in others (strains Mx-x18 and Mx-x82; lanes 6 and 13), and not detectable at all in strains Mx-x2, Mx-x3, Mx-x10, Mx-x56, Mx-x77, and Mx-x78 (lanes 1 to 3, 7, 11, and 12).
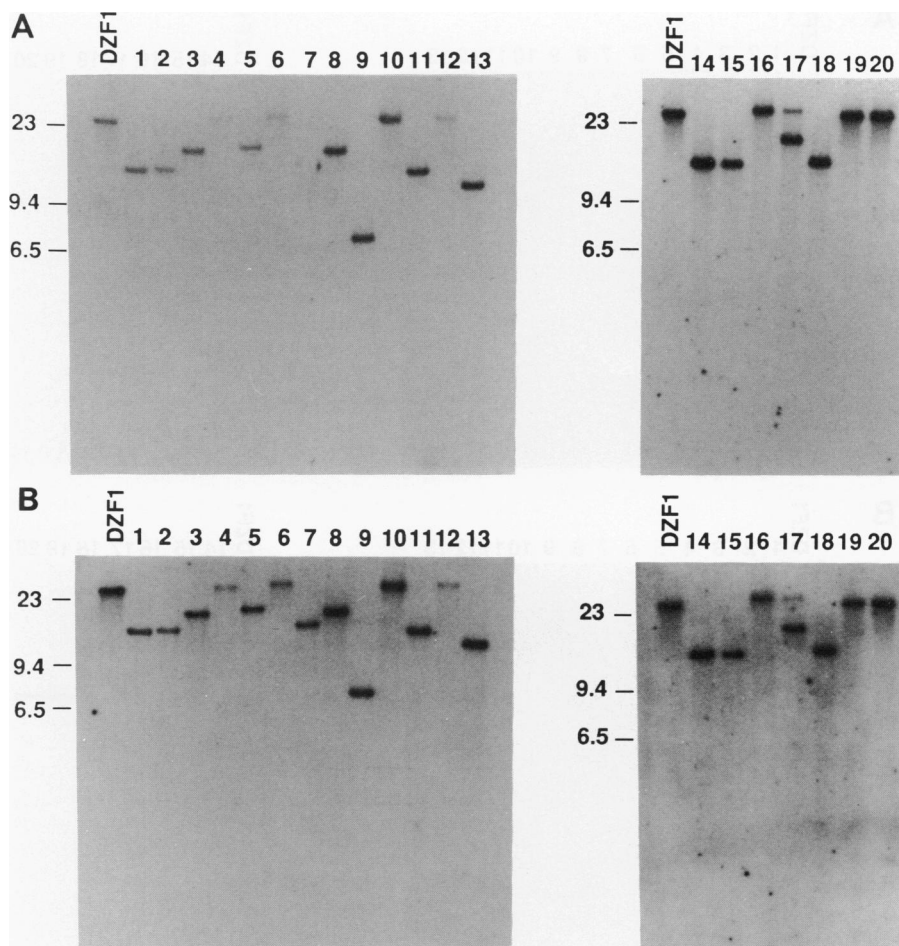
FIG. 3. Southern hybridization with probes Mx162MS and Mx162RT. (A) Chromosomal DNA from the Reichenbach strains (lanes 1 to 13) and the Kaiser strains (lanes 14 to 20) was digested with *Pst*I, and the restriction fragments were separated on a 0.7% agarose gel. The DNA was then transferred to a nitrocellulose filter (26) and hybridized with the Mx162MS probe (^{32}P labeled by nick translation). This probe hybridizes with genes encoding an msDNA homologous to Mx162 msDNA. (B) Southern blots of chromosomal DNA digested with *Pst*I were hybridized with probe Mx162RT. This probe detects genes coding for an RT similar to the Mx162 RT of DZF1. Lane numbers correspond to strain numbers in Table 1. Positions of *Hin*dIII-digested λ DNA size markers are indicated in kilobases. Lane DZF1 contains DNA from the laboratory strain DZF1.

Thus, it is likely that these strains are not able to produce Mx65 msDNA or produce it at an extremely low level.

RFLPs. Examination of msDNAs and their retron elements by the various detection methods used (Table 2) reveals a striking lack of heterogeneity, particularly for the Mx162 msDNA-retron system, among the *M. xanthus* strains surveyed. An argument can be made, although it is unlikely, that the lack of heterogeneity observed among the msDNAs arises from the fact that the different isolates are closely related or even derived from the same clone. To demonstrate genetic diversity among the 20 *M. xanthus* strains, *Pst*I-digested chromosomal DNAs were hybridized with the *E. coli rrnB* (16S rRNA) operon probe (2, 22). rRNA sequences are highly conserved among bacteria (23, 27), and variation in the length of the restriction fragments or polymorphisms (RFLP) upon which they reside is an indication of genetic diversity between two isolates (27).

Hybridization with the rRNA probe revealed 17 distinct restriction fragment patterns among the 20 *M. xanthus* isolates, indicating extensive diversity (Fig. 6). Clearly, however, many of the isolates are, to various degrees,

related. For example, strains Mx-x11 and Mx-x78, DK823 and DK829, and DZF1, DK862, and DK870, respectively, have identical *Pst*I restriction fragment profiles (Fig. 6, lanes 4 and 12, 14 and 15, and DZF1, 19, and 20, respectively). Likewise, the only two strains, Mx-x2 and Mx-x3, in which the Mx65 retron appears to be absent share three identical *Pst*I fragments out of four which hybridize to the rRNA probe (lanes 1 and 2), and all but four of the 20 strains appear to share a common restriction fragment of about 9.1 kb.

DISCUSSION

***M. xanthus* msDNAs.** msDNA and the retron genes required for their production were examined in 20 different isolates of *M. xanthus*. These strains show considerable genetic diversity based on RFLPs and were isolated from many locations throughout the United States and Europe. Two features about the retron msDNAs of *M. xanthus* observed in this study are particularly important: the near-universal prevalence of msDNAs and the conspicuous absence of diversity in nucleotide sequence for these msDNA-
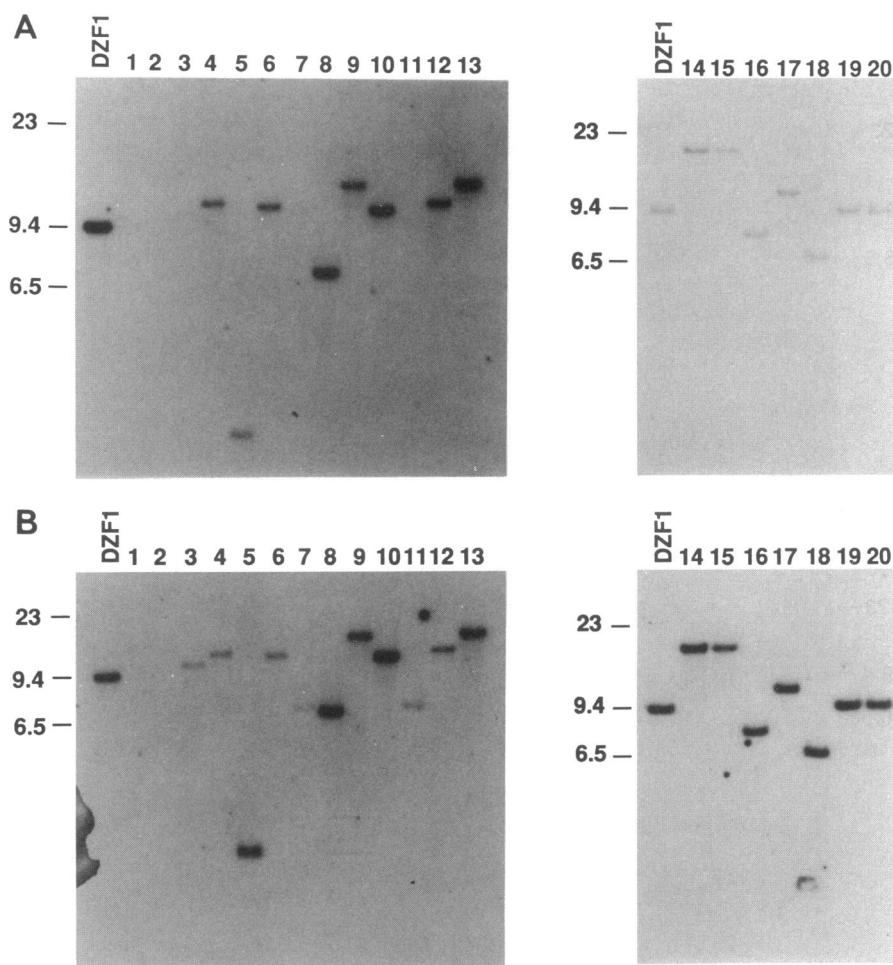
FIG. 4. Southern hybridization with probes Mx65MS and Mx65RT. (A) Chromosomal DNAs were digested with *Pst*I, and the resulting restriction fragments were separated on a 0.7% agarose gel. The DNA was transferred to nitrocellulose and hybridized with the ^{32}P-labeled Mx65MS probe. This probe hybridizes with DNA encoding msDNA homologous to Mx65 msDNA. Lane numbers correspond to the *M. xanthus* strain numbers in Table 1. Lane DZF1 contains DNA from the laboratory strain of *M. xanthus*. (B) Southern blots similar to those in panel A were hybridized with the ^{32}P-labeled probe Mx65RT. This probe hybridizes to DNA encoding an RT homologous to the RT from retron Mx65. Positions of size markers are indicated in kilobases.

producing elements. Ethidium bromide staining (Fig. 1) and the RT extension method (Fig. 5) make plain that all 20 *M. xanthus* strains produce an msDNA molecule indistinguishable from the first msDNA described, Mx162 msDNA, in *M. xanthus* DZF1 (7, 33). DNA hybridization experiments (Fig. 3) revealed that all *M. xanthus* strains contain a retron element, encoding the *msr*, *msd*, and RT genes, with greater than 80% DNA sequence similarity with the Mx162 retron. Although the other smaller species of msDNA (Mx65) among the *M. xanthus* strains do not appear to be distributed as universally as the larger Mx162 msDNA, clearly a second retron msDNA is present in most of the strains (13 of 20) and appears to be similar, if not identical, to the Mx65 msDNA of DZF1 (Fig. 4 and 5).

These characteristics of the *M. xanthus* retrons along with their simple functional organization, particularly the ORF encoding RT, which contains only a polymerase domain and lacks the protease, RNase, and integrase domains found in many eukaryotic *pol* genes (16), have led to speculations about the origin of RT and retroelements. One idea that has been proposed is that the RT gene of *M. xanthus* may have

existed before the divergence of prokaryotes and eukaryotes and thus represents the ancestor of all current retroelement RTs (14, 30, 31). This idea is supported by a comparison of the amino acid codons used by the *M. xanthus* RT genes. Codon usage of the Mx162 and the Mx65 RT genes is similar to the codon usage of other known host genes, implying a gene native to *M. xanthus* (13).

E. coli msDNAs. The msDNA-producing elements of *E. coli* share basic features with the *M. xanthus* msDNAs. These include the conserved secondary structures of their msDNAs (the 2',5' branch linkage, stem-loop folding, and short DNA-RNA hybrid) and the common gene organization of the retron element (*msr-msd*-RT) (14, 16). However, recent survey studies of *E. coli* msDNAs present a very different picture from that described here for *M. xanthus* msDNAs. Only a small number of *E. coli* strains (10 to 13%) produce msDNA, and the msDNAs vary considerably in size and nucleotide sequence. Also, the retron elements display little if any DNA sequence similarity (10, 19, 28). Recent mapping and DNA sequencing of two retrons, Ec67 and Ec73, from *E. coli* show that a 2.7-kb region which
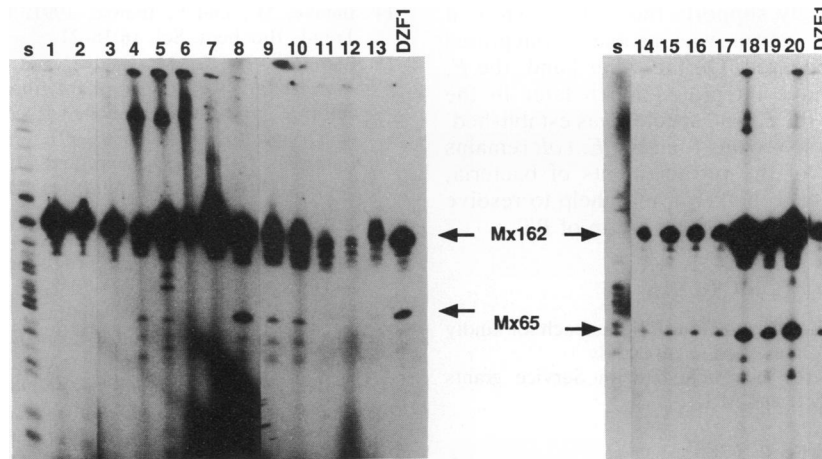
FIG. 5. Radiolabeled msDNA molecules from various *M. xanthus* strains. msDNAs were specifically labeled with ^{32}P by the RT extension method (17, 18, 20). Total RNA from each strain was incubated in a reaction buffer containing [α-^{32}P]dCTP and avian myelobastosis virus RT. Reactions were treated with RNase A, precipitated with ethanol, and electrophoresed on a 4% acrylamide–8 M urea slab gel. Labeled msDNA molecules were detected by exposing the gel to X-ray film. Arrows indicate the two species of msDNA specifically labeled by this method. The upper, large band in each lane corresponds to the Mx162 molecule, and the lower, smaller band, present in some lanes, corresponds to the Mx65 species. Lane numbers correspond to the *M. xanthus* strain numbers in Table 1. Lane DZF1 contains the *M. xanthus* laboratory strain; lane S contains pBR322 digested with *Msp*I for molecular size standards.

encodes the Ec73 retron is incorporated into the right end of a P4-related prophage which has inserted into the host chromosome at 82.3 min (29). Similarly, the Ec67 retron also appears to be associated with a large 34-kb prophage genome inserted at 19 min on the *E. coli* chromosome (11, 21).

From these results, together with the atypical codon usage for *E. coli* RT genes, it was concluded that msDNA-producing retroelements were acquired independently, from an outside source, into several different clonal lineages of *E. coli* (10, 19). Indeed, a recent systematic comparison of the amino acid sequences of four bacterial RTs (two from *M. xanthus* and two from *E. coli*) with the sequences of 78 RTs encoded by various eukaryotic retroelements concluded that the bacterial RTs most resemble the RTs found in certain organelle genomes of mitochondria and plasmids. Moreover,

it was proposed that the present-day RTs of *M. xanthus* and *E. coli* were probably acquired by the bacterial chromosome (or phage) from an existing progenitor retrotransposon which also transmitted its *pol* gene to some mitochondrial plasmids and introns found in the fungi (32). However, this analysis did not account for the fact that retrons exist in the bacterial chromosome and are duplicated by bacterial DNA polymerase of extremely high fidelity. Importantly, *M. xanthus* and *S. aurantiaca*, two independent species of myxobacteria, contain highly homologous msDNAs; those of Mx162 and Sa163, respectively (9), indicating that the retron for this msDNA was integrated into the bacterial genome before these two myxobacterial strains were established during the course of evolution (14). The fact that the codon usage of RT of retron Mx162 is very similar to that of other
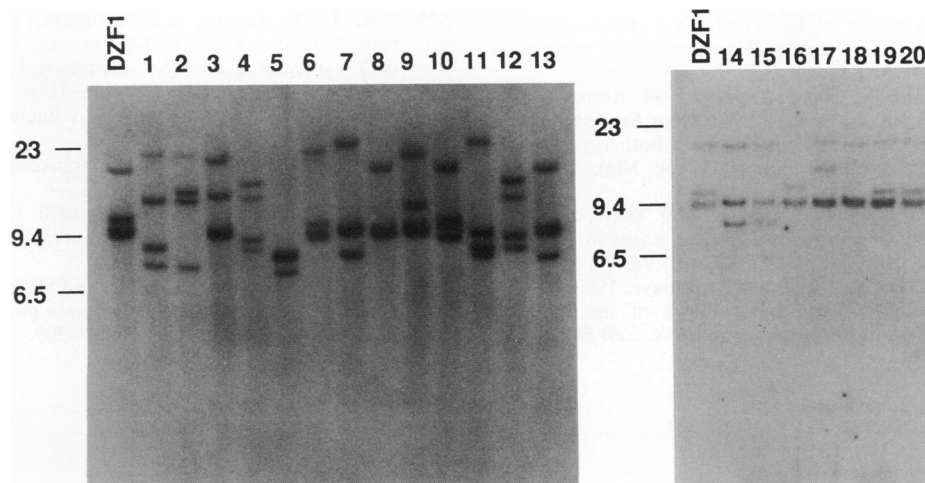

FIG. 6. Southern hybridization with the *E. coli* 16S rRNA gene. *Pst*I-digested chromosomal DNA from each *M. xanthus* strain was hybridized to a ^{32}P-labeled probe derived from the rRNA operon, *rrnB*, of *E. coli*. RFLPs of the restriction fragments that hybridize to the probe are an indication of the genetic diversity among the *M. xanthus* strains. Lane numbers correspond to the strain numbers in Table 1. Positions of size standards are indicated in kilobases on the left.

genomic genes also strongly supports this notion. Thus, it was speculated that the retron was probably integrated before the eukaryotes appeared. On the other hand, the *E. coli* retrons appear to have integrated much later in the course of evolution, after the *E. coli* species was established. The origin of highly diverse retrons found in *E. coli* remains unsolved. Further study of the retroelements of bacteria, particularly those of the myxobacteria, may help to resolve the conflicting evolutionary issue of the origin of RT.

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