

Homology of Ribosomal Ribonucleic Acid of Diverse Bacterial Species with *Escherichia coli* and *Bacillus stearothermophilus*

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Hybridization competition experiments were used to examine the ribosomal ribonucleic acid (rRNA) homologies of 22 bacteria and 3 higher organisms with *Escherichia coli* and *Bacillus stearothermophilus*. Although little or no homology was observed with the higher organisms, the bacteria showed a wide range of homologies. Organisms whose rRNA showed closer homology to *E. coli* rRNA showed less rRNA homology to *B. stearothermophilus* rRNA and vice versa.

The observed differences in the thermal stability of ribosomes of *Escherichia coli*, *Bacillus stearothermophilus*, and other *Bacillus* species (4, 6) led Pace and Campbell (12) to examine the thermal stability of ribosomes from a variety of bacteria possessing widely different maximal growth temperatures. A positive correlation was demonstrated between the maximal growth temperature and the thermal stability of the ribosomes of the organisms studied. Although the thermal stability of the ribosomes tended to increase with increasing guanine plus cytosine (GC) content of the ribosomal ribonucleic acid (rRNA), no positive correlation could be demonstrated. Further, the GC content of the deoxyribonucleic acid (DNA) exhibited no apparent correlation to that of the rRNA of the organisms examined (12).

The differences in base composition and thermal stability of the ribosomes and rRNA have prompted us to examine the rRNA primary structural homology within the group of organisms previously studied to determine if the observed functional relationship of ribosome thermal stability was reflected in the genetic relationship of the ribosomal DNA. The refinement of the DNA-RNA hybridization technique by Gillespie and Spiegelman (8) permits RNA homologies from a variety of organisms to be quantitatively examined. Several studies on the rRNA primary structure homology of a number of microorganisms with *E. coli*, various *Bacillus* species, and *Desulfovibrio vulgaris* have been reported (2, 3, 10, 11, 13, 17).

This paper reports the homologies of rRNA (as judged by hybridization competition experiments) from a group of organisms to the homologous DNA of *E. coli* and *B. stearothermophilus*. The rRNA homologies did not correlate with the thermal stability of the ribosomes and rRNA of the organisms studied. Nevertheless, the results are interesting from a phylogenetic viewpoint in that the rRNA of *E. coli* and *B. stearothermophilus* showed a continuum of homologies with the rRNA of the other organisms examined.

MATERIALS AND METHODS

Organisms and growth media. *B. stearothermophilus* strains 10 and 1503R and unclassified thermophiles T-107, 194, and B (Teccé), *B. megaterium* strain Paris, *B. subtilis* SB-19, *Aerobacter aerogenes*, *Alcaligenes faecalis*, *Proteus vulgaris*, and *Serratia marcescens* were grown at their optimal temperatures (65 C for the thermophiles; 37 C for *A. faecalis*, *P. vulgaris*, *B. megaterium*, and *B. subtilis*; and 30 C for *A. aerogenes* and *S. marcescens*) in TYC medium containing per liter of distilled water: 20 g of Trypticase (BBL), 3 g of yeast extract, 8.5 g of NaCl, 1.47 g of CaCl₂·2H₂O, 7 mg of FeCl₃·6H₂O, 15 mg of MgSO₄·7H₂O, and 1 mg of MnCl₂·4H₂O. The pH was adjusted to 7.3. Twenty grams of agar per liter were added for plates and slants. *B. coagulans* strain 43P was grown at 45 C in TYC medium minus yeast extract.

E. coli strain K38 was grown in a modified 3XD (5) medium as described by Pace and Campbell (13).

Organisms 2-1 and 7E-3 (unidentified psychrophiles obtained from J. L. Ingraham) were grown at 30 and 18 C, respectively, in Trypticase Soy Broth (16).

Vibrio marinus strains 15381 and 15382, obtained from the American Type Culture Collection, were grown at 18 and 30 C, respectively, in a medium (1) containing per liter of distilled water: 10 g of Proteose

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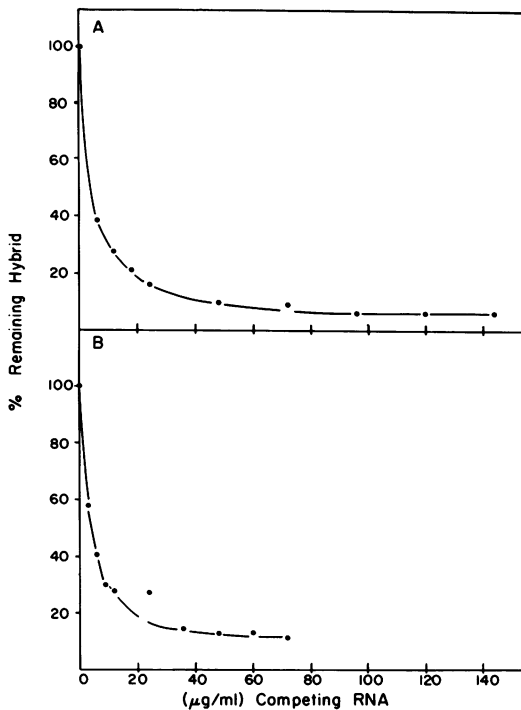


FIG. 1. Hybridization competition of *Escherichia coli* rRNA by homologous RNA. Membrane filters with 8 µg of ^3H -labeled *E. coli* DNA were immersed in 0.5 ml of $2 \times \text{SSC}$ containing 3 µg of ^{32}P -labeled methylated-albumin-kieselguhr (MAK)-purified *E. coli* rRNA and the indicated amounts of unlabeled MAK-purified *E. coli* rRNA or *E. coli* bulk RNA and held at 65 C for 8 hr. After the annealing period, filters were washed, treated with ribonuclease, again washed, and monitored for ^3H and ^{32}P radioactivity as detailed in Methods and Materials. Data are expressed as per cent of ^{32}P hybridized relative to samples containing no competing RNA. All points are the average of two identical filters. (A) ^{32}P -labeled *E. coli* rRNA competed by unlabeled, MAK-purified *E. coli* rRNA. (B) ^{32}P -labeled *E. coli* rRNA competed by unlabeled *E. coli* bulk RNA.

Peptone, 3 g of yeast extract, and 25.2 g of artificial sea salt. The pH was adjusted to 7.2.

Spirillum itersonii (SI-1), *Desulfotomaculum nigrificans* strain 8351, *D. africanus* strain Benghazi, *D. desulfuricans* strain VC, *D. vulgaris* strain 8303, and *D. salexigens* strain British Guiana were grown as described by Pace and Campbell (13).

Saccharomyces chevalieri Y32 was grown at 37 C in 5% glucose plus 0.5% yeast extract.

Tetrahymena pyriformis variety 1 strain C16643 was grown at 30 C in 1% Proteose Peptone plus 0.1% yeast extract.

Isotopic labeling of bacterial nucleic acids. Organisms from which ^{32}P -labeled rRNA was isolated were transferred to their respective low-phosphate media containing ^{32}P (orthophosphate). After the first one-third to one-half of the exponential phase of growth, a

200-fold excess of Na_2HPO_4 , pH 7.3, was added and the cells were grown for a further one to two generations. *E. coli* K38 was grown in a modified SC (14) medium containing: 2 g of NH_4Cl , 5 g of NaCl , 0.37 g of KCl , 1 ml of a 1% solution of MgCl_2 , 2.6 ml of a 1% solution of Na_2SO_4 , 300 ml of tap water, and 694 ml of distilled water. After autoclaving, the following sterile components were added: 0.175 ml of a 0.0668% solution of FeCl_3 , 5 ml of 1 M tris(hydroxymethyl)amino-methane (Tris), pH 7.3, 0.1 ml of 2 M CaCl_2 , 4 ml of 5% glucose, and 1 ml of 0.05 M Na_2HPO_4 . *B. stearothermophilus* strain 10 was grown in 1% peptone plus 0.1% fructose.

Tritiated DNA was extracted from *E. coli* grown in the presence of ^3H -thymidine in 3XD medium (5). *B. stearothermophilus* strain 10 DNA was labeled with ^3H -thymidine in TC medium.

RNA extraction. Cells harvested during the late exponential phase of growth were washed twice with TM3 buffer (0.01 M Tris, pH 7.3, 0.001 M MgCl_2) and suspended in 2 volumes TM3 buffer. Lysozyme (400 µg/ml) and deoxyribonuclease (10 µg/ml) were added and the suspension was disrupted by three freeze-thaw cycles. In addition to this procedure, it was necessary to incubate yeast cells for 5 hr with 1% Glusulase (snail gut enzyme). RNA was extracted from these ly-

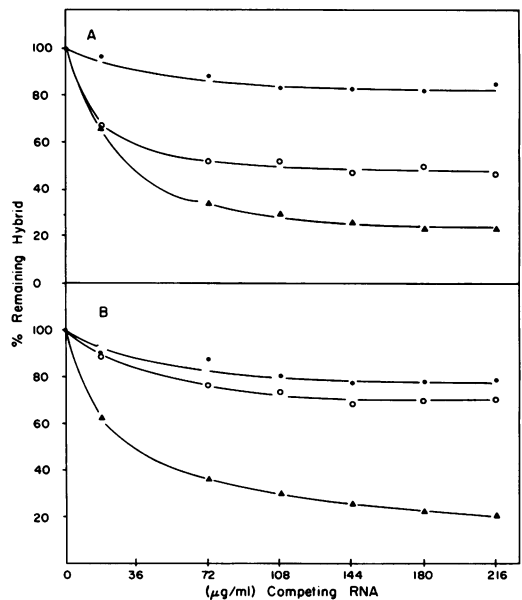


FIG. 2. Hybridization competition of *Escherichia coli* rRNA by heterologous bulk RNA. Membrane filters with 8 µg of ^3H -labeled *E. coli* DNA were immersed in 0.5 ml of $2 \times \text{SSC}$ containing 3 µg of ^{32}P -labeled, methylated-albumin-kieselguhr-purified *E. coli* rRNA and the indicated quantities of bulk RNA from the following organisms. Hybridizations were carried out as detailed in Methods and Materials and the legend to Fig. 1. All data are from duplicate filters (A) ●, *Desulfovibrio desulfuricans* strain VC; ○, *Vibrio marinus*; ▲, *Serratia marcescens*. B. ○, *Bacillus subtilis* strain SB-19; ○, *Alcaligenes faecalis*; ▲, *Aerobacter aerogenes*.

TABLE 1. Homology of heterologous ribonucleic acid (RNA) species to ribosomal RNA (rRNA) of *Escherichia coli*^a

Organism	% Homology of rRNA	% Homology of bulk RNA
<i>Aerobacter aerogenes</i>	88	77
<i>Proteus vulgaris</i>	83	79
<i>Serratia marcescens</i>		76
<i>Vibrio marinus</i> 15381	55	52
<i>Vibrio marinus</i> 15382		53
Psychrophile 2-1		36
<i>Alcaligenes faecalis</i>	31	30
<i>Spirillum itersonii</i> SI-1	31	23
<i>Desulfotomaculum nigrificans</i> 8351 ..		24
<i>Desulfovibrio vulgaris</i> 8303		24
<i>Desulfovibrio salexigens</i> British Guiana		22
<i>Desulfovibrio africanus</i> Benghazi ..		22
<i>Bacillus stearothermophilus</i> 10	21	17
<i>Bacillus megaterium</i> Paris		20
Psychrophile 7E-3		19
<i>Bacillus coagulans</i> 43P		18
<i>Desulfovibrio desulfuricans</i> VC		17
<i>Bacillus subtilis</i> SB-19		16
Thermophile 194		12
<i>Bacillus stearothermophilus</i> 1503R ..		11
Thermophile B (Tece)		9
<i>Tetrahymena pyriformis</i>		9
Thermophile T-107		7
<i>Saccharomyces chevalieri</i>		6
<i>Drosophila melanogaster</i> ^b	0	

^a Filters with 8 μg of ³H-labeled *Escherichia coli* deoxyribonucleic acid were immersed in 0.5 ml of 2 \times SSC containing 3 μg ³²P-labeled, methylated-albumin-kieselguhr-purified *E. coli* rRNA and up to 108 μg of unlabeled RNA. Hybridizations were carried out as detailed in Methods and Materials and the legend to Fig. 1.

^b Kindly provided by C. Radcliffe.

sates, which were diluted in TM3 buffer to 10 to 20 ml per g of cells, by a modification of the procedure of Gierer and Schramm (7), in the presence of 0.5% sodium dodecyl sulfate. Phenol was removed from the final aqueous layer by three extractions with 3 volumes of anhydrous ether, or by ethanol precipitation three times. The RNA pellet was dried and suspended in TE buffer (0.01 M Tris, pH 7.3; 0.001 M ethylenediaminetetraacetic acid).

DNA extraction. DNA was prepared by a slight modification of the procedure of Saito and Miura (15) as described by Pace and Campbell (13).

Methylated-albumin-kieselguhr (MAK) chromatography. MAK columns were prepared by the procedure of Mandell and Hershey (9) as detailed by Pace and Campbell (13). The RNA was eluted with a 0.7 to 1.1 M NaCl linear gradient. The isolated 16S and 23S peak fractions (free of 5S RNA) were pooled, ethanol precipitated, and suspended in 2 \times SSC (0.15 M NaCl plus 0.015 M sodium citrate, pH 7).

DNA-RNA hybridizations. The DNA-RNA hybridization technique of Gillespie and Spiegelman (8) was employed exactly as described by Pace and Campbell (13) except that 6 \times SSC was occasionally used in place of 2 \times SSC in some of the experiments. Hybridizations were carried out by immersing the filters in 0.5 or 1.3 ml of a 2 \times SSC solution of RNA of the desired concentration and incubating for 8 to 10 hr at 65 C. After chilling the tubes in an ice bath, the DNA and the blank control filters were washed with 50 ml of 2 \times SSC on each side by suction filtration and immersed in a solution of 2 \times SSC containing 25 μg of pancreatic ribonuclease per ml (EC 2.7.7.16) and 5 μg of T₁ ribonuclease per ml. After incubation at 37 C for 60 min the tubes were chilled and the filters were washed, dried, and counted in a Tri-Carb liquid scintillation counter (model 3002; Packard Instrument Co., Inc., Downers Grove, Ill.). DNA remaining on the filter was determined from the tritium counts and the amount of RNA hybridized was calculated from the ³²P counts on the DNA filter minus those on the blank filter.

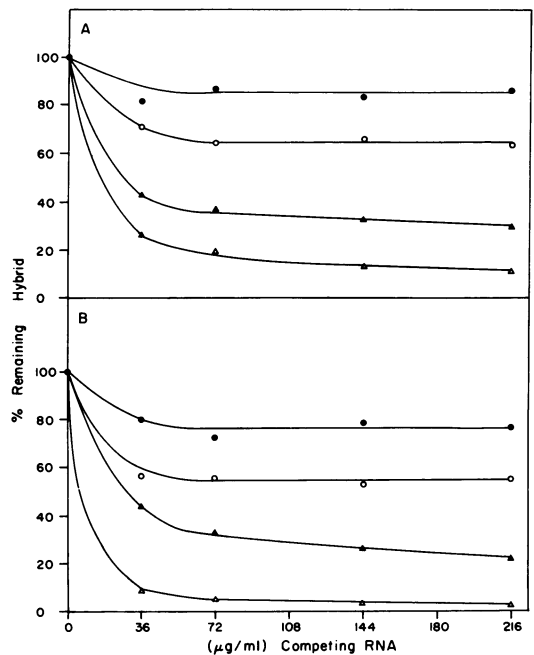


FIG. 3. Hybridization competition of *Bacillus stearothermophilus* strain 10 rRNA by heterologous bulk RNA. Membrane filters containing 3 μg of ³H-labeled *B. stearothermophilus* DNA were immersed in 0.5 ml of 2 \times SSC containing 1.5 μg of ³²P-labeled, methylated-albumin-kieselguhr-purified *B. stearothermophilus* rRNA and the indicated quantities of bulk RNA from the following organisms. Hybridizations were carried out as detailed in Methods and Materials and the legend to Fig. 1. All data are from duplicate filters. (A) ●, *Saccharomyces chevalieri*; ○, *Vibrio marinus* strain 15382; ▲, 7E-3. (B) ●, *Serratia marcescens*; ○, *Desulfovibrio africanus* strain Benghazi; ▲, *Bacillus stearothermophilus* strain 10.

TABLE 2. Homology of heterologous ribonucleic acid (RNA) species to ribosomal RNA (rRNA) of *Bacillus stearothermophilus* strain 10

Organism	% Homology of bulk RNA
<i>Bacillus stearothermophilus</i> 1503R	95
<i>Bacillus subtilis</i> SB-19	90
Thermophile T-107	89
<i>Bacillus coagulans</i> 43P	82
Thermophile 194	81
<i>Bacillus megaterium</i> Paris	78
Psychrophile 7E-3	71
<i>Desulfotomaculum nigrificans</i> 8351	65
<i>Desulfovibrio salexigens</i> British Guiana	51
<i>Spirillum itersonii</i> SI-1	51
<i>Desulfovibrio africanus</i> Benghazi	45
Thermophile B (Tecca)	45
<i>Alcaligenes faecalis</i>	44
<i>Desulfovibrio desulfuricans</i> VC	43
<i>Desulfovibrio vulgaris</i> 8303	43
Psychrophile 2-1	41
<i>Proteus vulgaris</i>	39
<i>Vibrio marinus</i> 15382	37
<i>Vibrio marinus</i> 15381	37
<i>Aerobacter aerogenes</i>	36
<i>Escherichia coli</i> K38	33
<i>Serratia marcescens</i>	25
<i>Saccharomyces chevalieri</i>	15
<i>Tetrahymena pyriformis</i>	0

^a Membrane filters containing 3 μ g of ³H-labeled *Bacillus stearothermophilus* deoxyribonucleic acid were immersed in 0.5 ml of 2 \times SSC containing 1.5 μ g of ³²P-labeled, methylated-albumin-kieselguhr-purified *B. stearothermophilus* rRNA and up to 108 μ g of unlabeled RNA. Hybridizations were carried out as detailed in Methods and Materials and the legend to Fig. 1.

RESULTS AND DISCUSSION

By using the competition hybridization technique, the homologies of homologous and heterologous bulk and rRNA species to *E. coli* rRNA were determined. Figure 1 illustrates the hybridization competition profiles of *E. coli* rRNA with its homologous rRNA and bulk RNA. The rRNA competition approximates the theoretical dilution curve, and it is apparent that the bulk RNA approaches the dilution asymptote.

Representative bulk RNA competitions of *E. coli* rRNA are shown in Fig. 2. *D. desulfuricans*, *V. marinus* 15381, *S. marcescens*, *B. subtilis*, *A. faecalis*, and *A. aerogenes* have homologies of 17, 52, 76, 16, 30, and 77%, respectively. Table 1 lists the rRNA homologies of 22 bacteria and 3 higher organisms to *E. coli* rRNA.

The rRNA homologies of the same group of organisms for *B. stearothermophilus* rRNA were also determined. Representative hybridization

competition experiments are illustrated in Fig. 3. In Fig. 3A, *Saccharomyces*, *V. marinus* 15382, 7E-3, and *B. subtilis* are seen to have homologies of 15, 37, 71, and 90%, respectively. In Fig. 3B the respective homologies of *S. marcescens*, *D. africanus*, *B. megaterium*, and homologous *B. stearothermophilus* are 25, 45, 78, and 97%. Table 2 lists the rRNA homologies of the organisms examined to *B. stearothermophilus* rRNA.

Although the data obtained in this study do not reveal a correlation between the genetic relatedness of the rDNA and the thermal stability of the ribosomes and rRNA of the organisms examined (12), a wide range of rRNA homologies was observed for *E. coli* and *B. stearothermophilus* with diverse bacterial species. *E. coli* and *B. stearothermophilus* appears to represent the extremes of homology among the bacterial species examined; the other species are distributed between these two organisms with regard to homology. Of note is the observation that the rRNA species possessing near relationships to *E. coli* are correspondingly less related to *B. stearothermophilus* and vice versa. This approximately reciprocal relationship would seem to justify the use of rRNA homologies as one means of generating a numerical spectrum (in the sense of ordering) of genetic relatedness, based on primary sequence homologies of a particular class of functional molecules.

ACKNOWLEDGMENTS

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LITERATURE CITED

- Colwell, R. R., and R. Y. Morita. 1964. Reisolation and emendation of description of *Vibrio marinus* (Russell) Ford. *J. Bacteriol.* **88**:831-837.
- Doi, R. H., and R. T. Igarashi. 1965. Conservation of ribosomal and messenger ribonucleic acid cistrons in *Bacillus* species. *J. Bacteriol.* **90**:384-390.
- Dubnau, D., I. Smith, P. Morell, and J. Marmur. 1965. Gene conservation in *Bacillus* species. I. Conserved genetic and nucleic acid base sequence homologies. *Proc. Nat. Acad. Sci. U.S.A.* **54**:491-498.
- Farrell, J., and L. L. Campbell. 1969. Thermophilic bacteria and bacteriophages. *Adv. Microbial Physiol.* **3**:83-109.
- Fraser, D., and E. A. Jerrell. 1953. The amino acid composition of T₃ bacteriophage. *J. Biol. Chem.* **205**:291-295.
- Friedman, S. M. 1968. Protein-synthesizing machinery of thermophilic bacteria. *Bacteriol. Rev.* **32**:27-38.
- Gierer, A., and G. Schramm. 1956. Infectivity of ribonucleic acid from tobacco mosaic virus. *Nature (London)* **177**:702-703.
- Gillespie, D., and S. Spiegelman. 1965. A quantitative assay for DNA-RNA hybrids with DNA immobilized on a membrane. *J. Mol. Biol.* **12**:829-842.

9. Mandell, J. S., and A. D. Hershey. 1960. A fractionating column for analysis of nucleic acids. *Anal. Biochem.* **1**:66-77.
10. Moore, R. L., and B. J. McCarthy. 1967. Comparative study of ribosomal ribonucleic acid cistrons in enterobacteria and myxobacteria. *J. Bacteriol.* **94**:1066-1074.
11. Nomura, M., P. Traub, and H. Bechmann. 1968. Hybrid 30S ribosomal particles reconstituted from components of different bacterial origins. *Nature (London)* **219**:793-799.
12. Pace, B., and L. L. Campbell. 1967. Correlation of maximal growth temperature and ribosome heat stability. *Proc. Nat. Acad. Sci. U.S.A.* **57**:1110-1116.
13. Pace, B., and L. L. Campbell. 1971. Homology of ribosomal ribonucleic acid of *Desulfovibrio* species with *Desulfovibrio vulgaris*. *J. Bacteriol.* **106**:717-719.
14. Roberts, R. B., D. B. Cowie, P. H. Abelson, E. T. Bolton, and R. J. Britten. 1957. Studies of biosynthesis in *E. coli*, p. 5. Carnegie Inst. of Washington, Publication 607. Washington, D. C.
15. Saito, H., and K. Miura. 1963. Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochim. Biophys. Acta* **72**:619-629.
16. Straka, R. P., and J. L. Stokes. 1960. Psychrophilic bacteria from Antarctica. *J. Bacteriol.* **80**:622-625.
17. Takahashi, H., H. Saito, and Y. Ikeda. 1967. Species specificity of the ribosomal RNA cistrons in bacteria. *Biochim. Biophys. Acta* **134**:124-133.