

NOTES

Plasmid-Determined Resistance to Erythromycin: Comparison of Strains of *Streptococcus faecalis* and *Streptococcus pyogenes* with Regard to Plasmid Homology and Resistance Inducibility

YOSHIHIKO YAGI, ARTHUR E. FRANKE, AND DON B. CLEWELL*

Dental Research Institute, Departments of Oral Biology and Microbiology, Schools of Dentistry and Medicine, The University of Michigan, Ann Arbor, Michigan 48104

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Streptococcus faecalis strain DS-5 and *Streptococcus pyogenes* strain AC-1 both have a 17 million dalton plasmid that determines resistance to erythromycin, lincomycin, and vernamycin B_α. The results of deoxyribonucleic acid-deoxyribonucleic acid hybridization experiments indicate that the two plasmids are about 95% homologous. It was also shown that erythromycin resistance is inducible in AC-1 and constitutive in DS-5.

Recent reports from our laboratory have demonstrated the presence of plasmid deoxyribonucleic acid (DNA) molecules in such members of the genus *Streptococcus* as *S. faecalis* (4), *S. pyogenes* (2), and *S. mutans* (6). A multiple drug-resistant strain of *S. faecalis* (strain DS-5) isolated at Jackson Memorial Hospital in Miami approximately 13 years ago, was shown to harbor three plasmid molecules designated α , β , and γ with molecular weights of approximately 6, 17, and 35 million, respectively (4). It was demonstrated that the β -plasmid determines resistance to erythromycin (Ery) and lincomycin. More recently it has been shown that resistance to vernamycin B_α is also determined by this plasmid. Interestingly, a plasmid in a clinical isolate of *S. pyogenes* (strain AC-1) isolated 3 to 4 years ago in Alberta, Canada, was found (2) to possess properties similar to those displayed by the β -plasmid of *S. faecalis* strain DS-5 with regard to its molecular weight (about 17 million) and pattern of co-resistance. These similarities prompted us to make some further comparisons between these two organisms. Thus, experiments were performed (i) to measure the extent of homology between these plasmids and (ii) to determine the type (inducible or constitutive) of expression of Ery resistance in the two organisms.

The homology studies involved DNA-DNA hybridization experiments that employed an albumin-coated membrane method (3). Plasmid DNA from strains DS-5 and DS-5C1 (an

Ery-susceptible derivative devoid of the β -plasmid [4]) was utilized, and the α - and γ -plasmids were present in both strains as a common background. Since the β -plasmid constitutes approximately 10% of the total DS-5 plasmid DNA (4), the amount of DNA fixed on the membranes was always in a large excess, allowing for the availability of at least 0.1 to 0.2 μ g of β -plasmid DNA to hybridize with the AC-1 plasmid DNA which was present to the extent of about 0.01 μ g during the annealing process. The result of a typical DNA-DNA hybridization experiment is presented in Table 1. It is clearly demonstrated that there is a high degree of homology (about 95%) between the β -plasmid and the AC-1 plasmid. Less than 6% is seen between the AC-1 plasmid and the combined $\alpha + \gamma$ -plasmids (i.e., of DS-5C1).

There is convincing evidence that Ery resistance is a result of the methylation of a ribonucleotide in 23S ribosomal ribonucleic acid (9). Ery resistance may be expressed inducibly or constitutively depending on the particular isolate (7, 11-13). We, therefore, were interested in investigating the type of expression determined by the highly similar Ery plasmids but in the different hosts, DS-5 and AC-1. The response of these strains to challenge by different concentrations of Ery is depicted in Fig. 1. Typifying constitutive expression, the rate of growth of strain DS-5 was unaffected by the addition of as much as 1,000 μ g of drug per ml (the data were similar regardless of whether Todd-Hewitt

TABLE 1. Polynucleotide sequence relationship between *S. faecalis* (strains DS-5 and DS-5C1) plasmid DNA and *S. pyogenes* (strain AC-1) plasmid DNA^a

Source of ³ H-labeled plasmid DNA fixed on membranes	Amount of ³² P-labeled AC-1 plasmid DNA bound to membranes	
	Counts/min (³² P) bound ^b ± SD	% of control
DS-5C1 (α + γ) + AC-1	1282 ± 33	100.0
DS-5 (α + β + γ)	1289 ± 40	100.5
DS-5C1 (α + γ)	71 ± 11	5.5

^a The procedures concerning the purification of plasmid DNA have been described in detail elsewhere (1, 3). The plasmid preparations here represent satellite DNA obtained after centrifugation of Sarkosyl lysates to equilibrium in CsCl-ethidium bromide buoyant density gradients. The ³H-labeling of DNA was described previously (2, 4). Labeling the DNA of *S. pyogenes* strain AC-1 with ³²P was accomplished by growing the cells in Todd-Hewitt broth (Difco; 50 ml) containing H₃³²PO₄ (0.07 mCi/ml; New England Nuclear Corp.). The DNA-DNA hybridization was carried out by the method described by Clewell and Helinski (3) except that the plasmid DNA was denatured by heating the DNA samples to 90 C for 20 min. This step nicks the covalently closed circular plasmid DNA, as well as denatures it. The ³²P-labeled AC-1 plasmid DNA was sheared to an average size of 2.5 × 10⁵ daltons by sonic treatment and then denatured. Plasmid DNA (2.0 μg) from either DS-5 (2.4 × 10⁴ counts/min per μg) or DS-5C1 (5.4 × 10⁴ counts/min per μg) was immobilized on filter membranes. (The ³H-labeling was to permit monitoring the amount of DNA that fixed to the membranes.) Serving as a control, a mixture of ³H-labeled DS-5C1 plasmid DNA (2.0 μg) and AC-1 plasmid DNA (0.2 μg; 2.2 × 10⁵ counts/min per μg) was fixed to membranes. Quantities of 0.01 μg of ³²P-labeled AC-1 plasmid DNA (2.3 × 10⁵ counts/min per μg) were allowed to anneal with the membrane-fixed ³H-labeled DNA. Nonspecific binding of ³²P-labeled AC-1 plasmid DNA (as measured by the amount of radioactivity bound to DNA-free membranes) during the hybridization process amounted to less than 1%.

^b The hybridizations were carried out in triplicate. SD, Standard deviation.

broth or Penassay broth was used); whereas in the case of strain AC-1 concentrations of 100 and 1,000 μg per ml resulted in an inhibition followed by an increase in the growth rate after about 2 h. Resistance in the case of AC-1 is inducible as seen in the experiment of Fig. 2 where a 1-h exposure to a subinhibitory concentration (0.05 μg/ml) of drug made the cells totally insensitive to subsequent challenge with 100 μg of drug per ml. Similar data were obtained when cultures were plated (after sonic

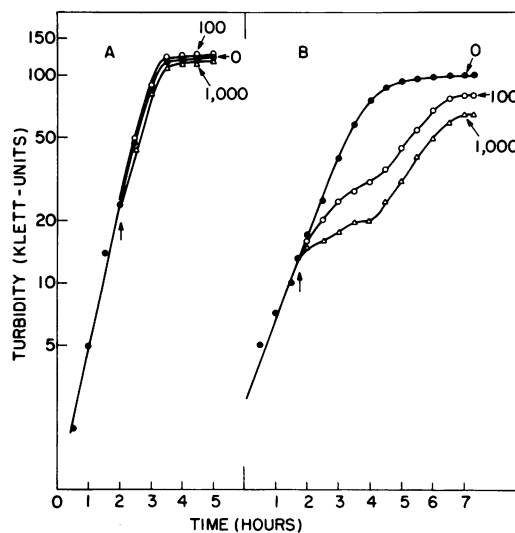


FIG. 1. Effects of the addition of various concentrations of Ery on strain DS-5 (*S. faecalis*) and strain AC-1 (*S. pyogenes*). A 60-ml quantity of broth was inoculated with either DS-5 (Penassay broth, Difco) or AC-1 (Todd-Hewitt broth, Difco). Cell growth at 37 C was monitored by measuring turbidity by using a Klett-Summerson colorimeter with a no. 54 filter. During log phase (indicated by vertical arrow), each culture was divided into equal portions which were then challenged with different concentrations of Ery as indicated (μg/ml). (A) represents *S. faecalis* strain DS-5; (B) represents *S. pyogenes* strain AC-1.

treatment to break up chains) on broth agar containing 50 μg of drug per ml. By this method, log-phase cultures (20 to 60 Klett units) of strain AC-1, after 18 h of incubation on plates at 37 C, reproducibly gave rise to a number of colony-forming units corresponding to 25 to 40% of the colony-forming units observed on drug-free plates. Thus, under normal conditions (i.e., when drug is absent) full drug resistance is expressed in about a third of the cells. The observed resumption of growth (measuring turbidity) after 2 h in the presence of drug in the case of the experiment of Fig. 1B could be interpreted as simply reflecting the continuation of the unaltered growth of that fraction of the cells that were already expressing a high level of resistance. Exposure to a subinhibitory concentration of drug (0.05 μg/ml for 1 h) before plating resulted in a number of colony-forming units on drug-containing plates essentially identical to that on drug-free plates. This behavior of strain AC-1 closely resembles that reported by Hyder and Streitfeld (7) for inducible strains of resistant *S. pyogenes*.

Whereas the type of expression of Ery resistance in the two strains differs somewhat (i.e., one is inducible whereas the other is constitu-

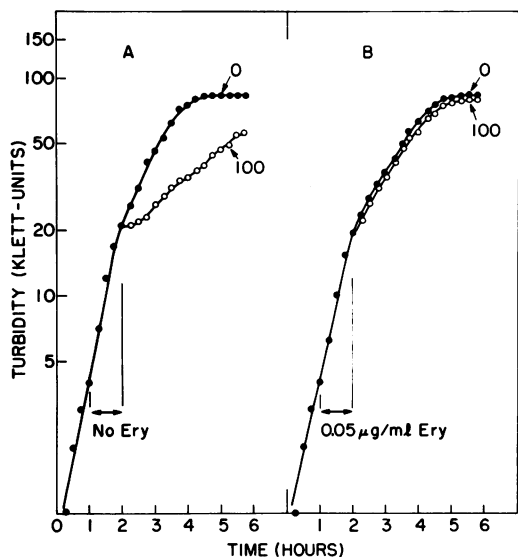


FIG. 2. Inducible nature of Ery resistance in strain AC-1. An 80-ml culture of strain AC-1 (in Todd-Hewitt broth) that had been growing logarithmically was divided into two equal portions (at Klett unit 4), one of which received 0.05 μ g of Ery per ml. After 1 h of further incubation, each portion was divided further into two equal portions, one of which was then challenged with 100 μ g of Ery per ml. (A) represents the growth of the cells that did not receive 0.05 μ g of Ery per ml before the challenge, and (B) represents that which received 0.05 μ g of Ery per ml.

tive), the similar molecular weights and the high degree of homology demonstrated here implies a common evolutionary origin. Courvalin et al. (5) have recently isolated from a strain of *S. faecalis* (isolated in Paris) an Ery resistance plasmid that determines a similar co-resistance pattern and has a molecular weight (17.6 million) very similar to that of the AC-1 and DS-5 plasmids. Conceivably, the plasmids in these strains could have appeared as a result of being transferred from one organism to another by transduction, transformation, or by the recently reported phenomenon of streptococcal conjugation (8, 10). Alternatively, Ery resistance could have originally arisen by independent mutations in different streptococcal species (or strains) but on very similar chromosomal segments; and in each case similar looping-out processes involving this segment of DNA (perhaps due to the interaction of similar spaced redundant sequences) could give rise to plasmids (assuming there is a capability of existing autonomously) of similar size.

To our knowledge the only other reported case of plasmid-determined erythromycin resistance

in streptococci (where plasmid size has been indicated) is that described by Jacob and Hobbs (8) for a strain of *S. faecalis* (isolated in London) where Ery resistance (along with tetracycline, streptomycin, and kanamycin resistance) was found to be determined by a much higher molecular weight plasmid (50×10^6). It was not reported whether their strain exhibited a pattern of co-resistance similar to the above cases or whether resistance was constitutive or inducible.

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