HAL
open science

Stimulation of Genetic Instability and Associated Large Genomic Rearrangements in Streptomyces ambofaciens by Three Fluoroquinolones

Jean-Nicolas Volff, Dominique Vandewiele, Bernard Decaris

To cite this version:

Jean-Nicolas Volff, Dominique Vandewiele, Bernard Decaris. Stimulation of Genetic Instability and Associated Large Genomic Rearrangements in Streptomyces ambofaciens by Three Fluoroquinolones. Antimicrobial Agents and Chemotherapy, 1994, 38 (9), pp.1984-1990. 10.1128/AAC.38.9.1984 . hal-01659606

HAL Id: hal-01659606

https://hal.univ-lorraine.fr/hal-01659606v1

Submitted on 8 Dec 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1994, p. 1984–1990
0066-4804/94/$04.00+0
Copyright © 1994, American Society for Microbiology
Vol. 38, No. 9

Stimulation of Genetic Instability and Associated Large Genomic Rearrangements in *Streptomyces ambofaciens* by Three Fluoroquinolones

JEAN-NICOLAS VOLFF, DOMINIQUE VANDEWIELE, AND BERNARD DECARIS*

Laboratoire de Génétique et Microbiologie, Unité associée INRA, Faculté des Sciences, Université de Nancy I, 54506 Vandoeuvre-lès-Nancy, France

Received 16 February 1994/Returned for modification 5 May 1994/Accepted 29 June 1994

In *Streptomyces ambofaciens* NSA2002, pigmented wild-type colonies spontaneously give rise to pigment-negative (Pig$^-$) mutants at a frequency of about 0.5%. This genetic instability is related to large deletions which can be associated with amplifications of DNA sequences. The influence of three fluoroquinolones (ciprofloxacin, enoxacin, and norfloxacin) on this property was investigated. At a survival rate higher than 60%, most colonies showed a patchwork phenotype consisting of phenotypically heterogeneous colonies harboring numerous mutant sectors. Moreover, the frequency of Pig$^-$ mutants rose to more than 90% at survival rates equal to or higher than 10%. Induced Pig$^-$ mutants showed the same phenotypical features as did spontaneous mutants. Most of them also harbored deletions, associated in some cases with DNA amplifications, in two loci of the large unstable region, *AUD6* and *AUD90* (derived from amplifiable unit of DNA). The size of deletions in induced mutants could rise to 1.5 Mb. These results show that ciprofloxacin, enoxacin, and norfloxacin greatly stimulate genetic instability and the occurrence of DNA rearrangements in *S. ambofaciens*. Moreover, these three fluoroquinolones had the same rank order for both toxic (i.e., antibacterial) and genotoxic activities. If the antibacterial effect of fluoroquinolones in *S. ambofaciens* is due to their interference with DNA gyrase, as shown for some other organisms, the genotoxic effect observed could be due to their interaction with this type II topoisomerase. This suggests that DNA gyrase is involved in the process of genetic instability in *S. ambofaciens*.

The ability of a genome to undergo genomic rearrangements is thought to be an important factor in the evolution and survival of living organisms in changing environments. Large deletions and high-copy-number amplifications can inactivate genes, change their expression level, or create novel coding sequences. Such rearrangements have been detected at varying frequencies in bacteria and eucaryotic cells and are also related to numerous types of cancer (27). The frequency of genome rearrangements can be modified by exogenous factors. Studying the potential of these factors to induce DNA rearrangements is very important, since these agents can be classified as genotoxic or mutagenic in some cases and are therefore hazardous. Moreover, such studies can contribute to elucidating the mechanisms responsible for spontaneous genome plasticity in living organisms.

Previous studies have shown that members of the eubacterial genus *Streptomyces* show a very high degree of genetic variability called genetic instability (3, 15). This phenomenon involves the occurrence of spontaneous mutants at frequencies higher than 10^{-3} (3, 15). In most mutants arising because of genetic instability, the ability to differentiate is often affected. For example, mutants produced at a high frequency in *Streptomyces ambofaciens* have altered abilities to produce pigment, and in some cases, antibiotics (physiological differentiation), and some mutants are unable to produce aerial mycelia, which prevents sporulation from occurring (morphological differentiation) (32). In addition, some characters related to primary metabolism can also be affected, such as prototrophy of some amino acids (15). Two major types of DNA rearrangements have been associated with genetic instability in *Streptomyces* spp. The most frequent is the deletion of DNA sequences. These deletions can be very large, i.e., up to 2,000 kb, and can remove about one quarter of the total bacterial genome (16). High-copy-number DNA amplifications are sometimes associated with these deletions. In *Streptomyces lividans*, the region that can sustain both kinds of rearrangements has been located opposite the replication origin of the chromosome (18). This chromosome is linear in the wild-type (WT) strain (19) but circularized in some mutants harboring deletions (19, 24).

Some agents are known to stimulate genetic instability. In *S. ambofaciens*, UV, mitomycin C, and nitrous acid increase the frequency of pigment-negative (Pig$^-$) mutants from 0.5% (spontaneous level) to more than 30% (32). Some DNA intercalating dyes such as ethidium bromide also possess this property (4). Finally, two inhibitors of DNA gyrase (the bacterial type II topoisomerase), novobiocin (coumarin) and oxolinic acid (quinolone), were found to greatly stimulate genetic instability and genomic plasticity (31).

Fluoroquinolones are a recent class of antibiotics whose target is DNA gyrase (the type II topoisomerase) in numerous bacteria (25). As it is of great importance to appraise the genotoxic effects of compounds used in human therapy, we have tested the ability of three fluoroquinolones (ciprofloxacin, enoxacin, and norfloxacin) to stimulate the formation of large DNA rearrangements such as deletions and amplifications in the *S. ambofaciens* model.

MATERIALS AND METHODS

Bacterial strains, antibiotics, and culture conditions. *S. ambofaciens* NSA2002, used as the WT strain (32), was derived

* Corresponding author. Mailing address: Laboratoire de Génétique et Microbiologie, Unité associée INRA, Faculté des Sciences, Université de Nancy I, B.P. 239, 54506 Vandoeuvre-lès-Nancy, France. Phone: (33) 83 91 20 96. Fax: (33) 83 91 25 00.

Downloaded from http://aac.asm.org/ on December 8, 2017 by INRA - old

from strain ATCC 23877 (22). NSA2002 colonies are gray pigmented (Pig^+) and have aerial mycelia present on the whole of their surface (Amy^+).

Ciprofloxacin was a gift from Bayer-Pharma Co. Enoxacin and norfloxacin were from Sigma. Antibiotic solutions were prepared in sterile water at a concentration of 100 mg ml^{-1} and were adjusted to pH 7.0 with 5 M NaOH. Colonies of *Streptomyces* spp. were grown on Hickey-Tresner (HT) (23) complete solid medium at 30°C. Fluoroquinolone treatments were performed by adding the appropriate antibiotic to this medium. At the highest antibiotic concentration used, the pH of the medium was not modified.

Surviving fractions, colony diameters, and phenotype frequencies were estimated on the 14th day of growth. Two replicate experiments were performed on separate occasions for each antibiotic concentration used. The values indicated in this text and its figures correspond to the mean of the values obtained in each separate experiment. Twenty colony diameters were measured for each concentration. To determine both surviving fractions and phenotype frequencies, more than 150 colonies were scored for each antibiotic concentration. For analysis of mutants, two successive restreakings of mutant colonies were done immediately after the phenotype had been scored.

DNA manipulations. Total DNA was extracted as described previously (11) after growth in HT liquid medium. Pulsed-field gel electrophoresis (PFGE) DNA preparations were performed as indicated previously (18), and *S. ambofaciens* DNA was digested with the rare-cutting restriction endonuclease *Ase*I for 12 h at 37°C. Electrophoreses were performed in a contour-clamped homogeneous electric field apparatus (Bio-Rad Laboratories, Richmond, Calif.). Lambda ladders and *Saccharomyces cerevisiae* YNN295 chromosomes (Bio-Rad) were used as size markers. Several migration conditions were used to establish the different strain patterns.

Southern blotting was done by the capillary transfer method with the Vacugene system (LKB) onto Hybond-N membranes (Amersham). Plasmids pOS15 (32) and pNSA6 (29) were used as probes to study the structure of *AUD90* and *AUD6*, respectively (derived from amplifiable unit of DNA). DNA labelling was performed with digoxigenin-labelled dUTP, and specific hybrids were detected by using a digoxigenin detection kit (Boehringer Mannheim). Probes were also labelled with [α-^{32}P]dCTP to a specific activity of about 10^9 dpm/μg of DNA by using the Multiprime labelling system kit (Amersham).

Estimation of toxic and genotoxic potencies of fluoroquinolones against *S. ambofaciens* NSA2002. The toxic (i.e., antibacterial) potency was defined as the ability of the fluoroquinolones to reduce both the colony diameter and the percentage of mutants surviving (surviving fraction, number of colonies on medium containing antibiotics divided by the number of colonies in the absence of antibiotic $\times$ 100). The genotoxic potency was estimated by the ability of the antibiotics to stimulate (i) Pig^- mutant sectors on Pig^+ colonies, resulting in the patchwork phenotype, (ii) Pig^- mutant colonies, and (iii) DNA rearrangements, such as deletions and amplifications of DNA sequences.

RESULTS

Toxic effect of fluoroquinolones against *S. ambofaciens* NSA2002. Spores of strain NSA2002 were plated on HT medium free of antibiotic (as a control) or containing ciprofloxacin, enoxacin, or norfloxacin at various concentrations. After 14 days' growth, the surviving fractions were estimated (Fig. 1). On HT medium containing ciprofloxacin, no effect or a slight effect was observed at 1.3 μM, at which point 93.7% survived. At ciprofloxacin concentrations of 1.9 μM, 2.6 μM, and 3.9 μM, the frequencies of survivors were 63.6, 53, and 13.2%, respectively. On medium containing enoxacin, a small effect on survival was detected at concentrations of 7.8 μM (100% survival) and 15.6 μM (83.1% survival), but the surviving fraction was significantly reduced at 23.4 μM (9.9% survival). Finally, norfloxacin had no effect on survival at a concentration of 15.7 μM, and the frequencies of survivors were 86, 51.5, and 5.5% at concentrations of 23.5 μM, 31.3 μM, and 47 μM, respectively. Therefore, ciprofloxacin showed the greatest antibacterial effect against *S. ambofaciens*, and norfloxacin showed the smallest. Colony diameters were also measured. On medium containing the antibiotics, the colony diameters were greatly reduced compared with those on the controls (Fig. 2). Ciprofloxacin had the largest effect on colony growth (causing the greatest reduction in size), and norfloxacin had the smallest. These results show that the rank order for

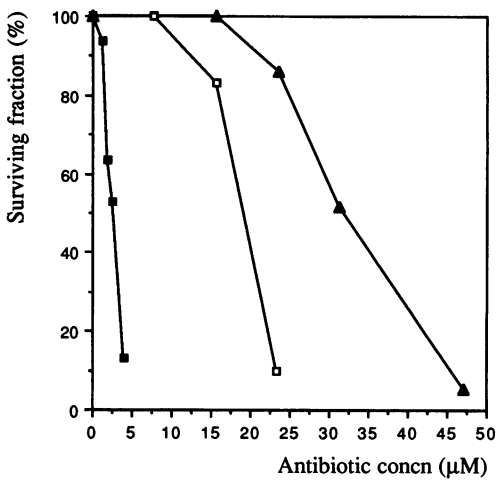

FIG. 1. Surviving fraction of *S. ambofaciens* NSA2002 on HT medium containing fluoroquinolones (■, ciprofloxacin; □, enoxacin; ▲, norfloxacin).

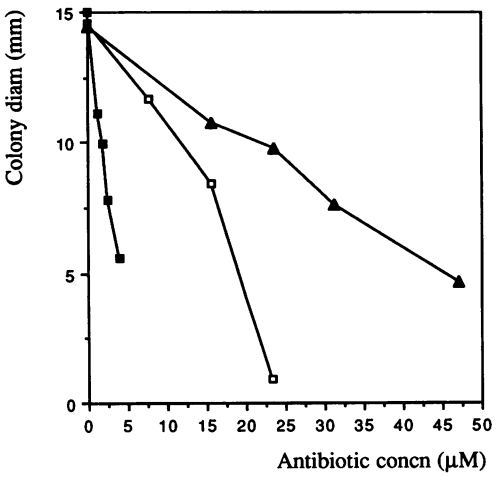

FIG. 2. Diameter of *S. ambofaciens* NSA2002 colonies on HT medium containing fluoroquinolones (■, ciprofloxacin; □, enoxacin; ▲, norfloxacin).

Downloaded from http://aac.asm.org/ on December 8, 2017 by INRA - old

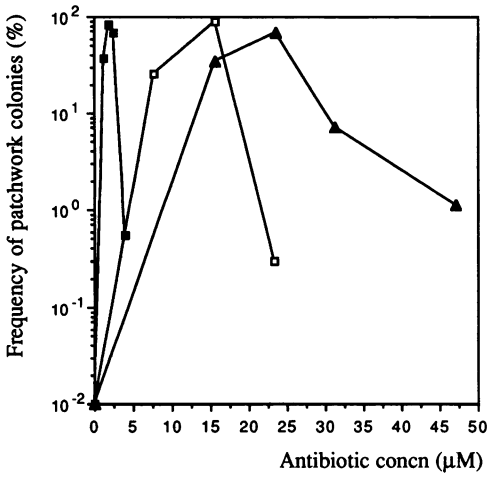

FIG. 3. Frequency of patchwork colonies on HT medium containing fluoroquinolones (■, ciprofloxacin; □, enoxacin; ▲, norfloxacin).

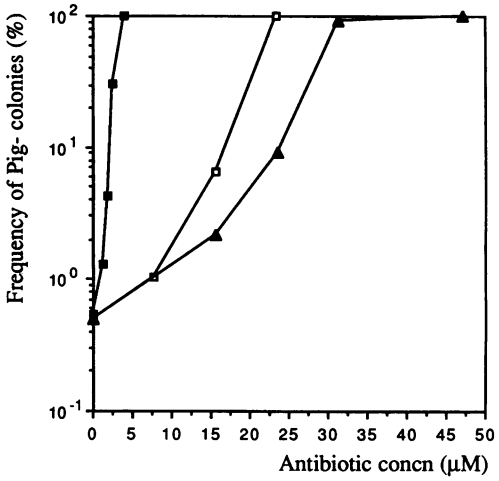

FIG. 4. Frequency of Pig$^-$ mutants on HT medium containing fluoroquinolones (■, ciprofloxacin; □, enoxacin; ▲, norfloxacin).

toxic effect against *S. ambofaciens* NSA2002 is ciprofloxacin > enoxacin > norfloxacin.

Effect of fluoroquinolones on the occurrence of patchwork colonies. After 14 days' growth, colony phenotypes were scored. At the lowest antibiotic concentrations used, some colonies containing numerous Pig$^-$ sectors were observed (Fig. 3). These colonies showing a heterogeneous phenotype were called patchwork and were detected at a frequency of about 0.01% in the absence of antibiotics.

(i) Ciprofloxacin. On media containing ciprofloxacin at concentrations of 1.3 μM, 1.9 μM, and 2.6 μM, patchwork colonies were detected at frequencies of 37.5, 82.5, and 67.5%, respectively. Seventeen sectors from 17 different patchwork colonies were restreaked. Sectors gave rise to Pig$^-$ mutant progeny, but two categories were observed. The progeny of 15 sectors were phenotypically homogeneous (15 of 17 = 88.2%), while the offspring of the remaining two sectors showed no preponderant phenotype (2 of 17). Colonies found for the latter type of progeny were of varying sizes, and in some cases they harbored aerial mycelium deficiencies, which differed between colonies. This phenomenon, named hypervariability, has been described in the spontaneous genetic instability of *S. ambofaciens* NSA2002 (32) and also in the strain DSM40697 (14). Seventeen Pig$^-$ mutants derived from the 17 sectors were restreaked on antibiotic-free medium. Three mutants possessed aerial mycelia covering the surface of the colony (Amy$^+$), 10 showed a partial aerial mycelium deficiency (Amy^d), and 4 had no aerial mycelium at all (Amy$^-$).

(ii) Enoxacin. On media containing enoxacin at concentrations of 7.8 μM and 15.6 μM, the patchwork colony frequencies were 25.8 and 88.7%, respectively. Eighteen different Pig$^-$ sectors from 18 different patchwork colonies were replated. The offspring of 16 sectors (88.9%) were phenotypically homogeneous, but the progeny of the remaining 2 were hypervariable. Eighteen Pig$^-$ mutants were retained for further analysis. Eleven of the 18 (61.1%) showed the Amy^d phenotype, while all of the others were Amy$^+$.

(iii) Norfloxacin. At concentrations of 15.7 μM and 23.5 μM norfloxacin, patchwork colonies were detected at frequencies of 34.3 and 68%, respectively. Twenty different Pig$^-$ sectors were restreaked from 20 different patchwork colonies. The ratio of mutant to phenotypically homogeneous progeny from these sectors was 17 of 20 (85%) or hypervariable. The 20 Pig$^-$ mutants which were retained showed different aerial mycelium forms: 3 were Amy$^+$, 13 were Amy^d, and 4 were Amy$^-$.

Therefore, the three fluoroquinolones greatly stimulated the occurrence of Pig$^-$ mutant sectors on Pig$^+$ colonies, with survival rates higher than 60%.

Effect of fluoroquinolones on the occurrence of Pig$^-$ colonies. The frequency of Pig$^-$ colonies was investigated (Fig. 4). On medium without antibiotic, the frequency of Pig$^-$ mutants was estimated to be about 0.5%, and this reflected the spontaneous level of genetic instability in strain NSA2002.

(i) Ciprofloxacin. On medium containing ciprofloxacin (2.6 μM, 53% survival), the Pig$^-$ colony frequency was greatly enhanced (about 60-fold, to 30.3%) and was higher than 99% at 3.9 μM. Thirteen colonies were restreaked. Four gave rise to homogeneous offspring and nine (69.2%) gave rise to hypervariable progeny. The 13 mutants harbored different phenotypes: 6 were Amy$^+$, 2 were Amy^d, and 5 were Amy$^-$. Among the last five, one mutant had colonies with severely reduced diameters. This phenotype has been called Doc$^-$ (derived from diameter of colony) (30).

(ii) Enoxacin. On medium containing 15.6 μM enoxacin, with 83.1% surviving, the frequency of Pig$^-$ colonies was increased 10-fold (to 6.5%). At 23.4 μM enoxacin (about 10% survival), the Pig$^-$ frequency was found to be close to 100%. Eleven Pig$^-$ colonies were restreaked, and their progeny were either phenotypically homogeneous (3 of 11) or hypervariable (8 of 11). The phenotypes of the 11 retained mutants differed: one was Amy$^+$, 4 were Amy^d, 5 were Amy$^-$, and one was Amy$^-$Doc$^-$.

(iii) Norfloxacin. At a concentration of 31.3 μM norfloxacin, which reduced the survival by only about twofold, Pig$^-$ colonies were observed at a frequency of 92.7%. This Pig$^-$ colony frequency was estimated to be 98.9% at 47 μM norfloxacin. The progeny of nine Pig$^-$ colonies were analyzed and were found to be mutant. Four of the nine progeny were phenotypically homogeneous, while the remaining five (55.5%) were hypervariable. Pig$^-$ mutants obtained were Amy$^+$ (2 of 9), Amy^d (2 of 9), Amy$^-$ (4 of 9), and Amy$^-$Doc$^-$ (1 of 9).

Therefore, at survival rates equal to or higher than 10%, the three fluoroquinolones tested stimulate the occurrence of Pig$^-$ mutants, ciprofloxacin being the most efficient antibiotic and norfloxacin being the least efficient antibiotic. Some of these

Downloaded from http://aac.asm.org/ on December 8, 2017 by INRA - old

TABLE 1. Number of fluoroquinolone-induced Pig⁻ mutants harboring the different combinations of *AUD6* and *AUD90* structures

AUD90-AUD6 deletion	No. of Pig⁻ mutants[a] induced by:		
	Ciprofloxacin	Enoxacin	Norfloxacin
None-none	7 (0)	6 (0)	8 (0)
Partial-none	0 (0)	0 (0)	1 (0)
Partial-partial	0 (0)	1 (0)	0 (0)
Total[b]-none	0 (0)	0 (0)	1[c] (0)
Total[b]-partial	10 (9 + 1[d])	9 + 2[d] (8 + 1[d])	9 + 1[d] (5)
Total[b]-total[b]	0 (3)	0 (2)	0 (3 + 1[e])

[a] Number of mutants derived from Pig⁻ sectors of patchwork colonies (number of mutants derived from Pig⁻ colonies).
[b] Total deletion of the region homologous to the probe.
[c] Mutant harboring an amplification of the whole region of *AUD6* homologous to pNSA6.
[d] Mutant(s) harboring amplifications in *AUD6*.
[e] Mutant harboring an amplification outside the *AUD90* region or the *AUD6* region.

mutants are pleiotropic, as was observed for some spontaneous Pig⁻ mutants (32).

Molecular analysis of fluoroquinolone-induced mutants. The presence of DNA rearrangements in induced mutants was investigated in two loci of the large unstable region, *AUD90* and *AUD6*. This was done by Southern blot experiments using plasmids pOS15 and pNSA6, which specifically reveal the *AUD90* locus and the *AUD6* locus, respectively, as probes. Neither locus has been rearranged in the WT clones so far tested, but both can be deleted or amplified in spontaneous Pig⁻ mutants (32). Both probes were hybridized with *Bam*HI-digested total DNA of Pig⁻ mutants induced by fluoroquinolones. pOS15 used as a probe revealed 8 fragments, whose sizes ranged from 1.1 to more than 23 kb, while pNSA6 revealed 11 fragments, whose sizes ranged from 0.5 to 15.5 kb (32). Several categories of rearrangements were detected. In some cases, some bands were missing compared with those of the WT, indicating that a partial deletion event had occurred. Furthermore, the fact that all bands were lacking in some patterns was interpreted as a total deletion of the region homologous to the probe. This was confirmed by the PFGE experiments (see below). Finally, amplifications of DNA sequences (ADSs) were seen on agarose gel directly after electrophoresis or/and were detected as signals heavier than those of the WT pattern after hybridization experiments. Results for fluoroquinolone-induced mutants are given in Table 1.

No rearrangement was detected in 41.2% (7 of 17) of mutants isolated from ciprofloxacin-induced sectors, while deletions have been found in 58.8% of cases. All 13 mutants derived from Pig⁻ colonies showed deletions, associated in one case with an ADS in *AUD6*.

Mutants induced by enoxacin were studied further. Among the 18 mutants derived from sectors of patchwork colonies, 12 (66.6%) contained DNA rearrangements in the loci studied. Two of them harbored ADSs. In contrast, no rearrangement in the remaining six mutants was found. The 11 mutants derived from the Pig⁻ colonies contained deletions, 1 of which was associated with an ADS.

Sixty percent (12 of 20) of norfloxacin-induced mutants isolated after restreaking the sectors harbored rearrangements, including two examples of ADS, but the remaining 40% possessed the WT pattern for both *AUD6* and *AUD90*. All nine mutants obtained from norfloxacin-induced Pig⁻ colonies had deletions, one of which had an unknown ADS.

It is interesting to note that all of the nonrearranged mutants were isolated from sectors of patchwork colonies. In contrast, all mutants deleted for both *AUD6* and *AUD90* were obtained from the progeny of fluoroquinolone-induced Pig⁻ colonies. Nevertheless, the combination "total deletion of *AUD90*-partial deletion of *AUD6*" is the main rearrangement observed in both categories of clones. In addition, ADSs have been found in both sector- and colony-derived Pig⁻ mutants.

The genomes of several mutants induced by ciprofloxacin, enoxacin, or norfloxacin were analyzed by PFGE to investigate the presence of any large DNA rearrangements. The restriction enzyme used was *Ase*I, which has previously been used to analyze the *Streptomyces* genome (16, 18). With the use of this enzyme, the genome size of strain NSA2002 was estimated at about 6,500 kb (17). Some mutants induced by fluoroquinolones showed no rearrangement detectable by PFGE (Fig. 5), as observed in 40% of spontaneous mutants isolated from the WT strain NSA2002 (unpublished results). In contrast, some induced mutants had large deletions whose sizes were estimated at between 800 and 1,600 kb (Fig. 5C). The sizes of deletions were estimated as in reference 17. This has also been observed for 60% of spontaneous Pig⁻ mutants of the NSA2002 strain (unpublished results) and for all the spontaneous Pig⁻ mutants from the *S. ambofaciens* DSM40697 strain (16).

Therefore, fluoroquinolones are capable of stimulating the occurrence of very large rearrangements in *S. ambofaciens*, as well as the occurrence of other kinds of mutations that have not been detected by the methods used.

Origin of strains. Strain origins were as follows: NSA2002, WT strain; NSA2039, NSA2040, and NSA2041, Pig⁻ mutants derived from Pig⁻ sectors from patchwork colonies on medium plus enoxacin; NSA2043, NSA2044 and NSA2045, Pig⁻ mutants derived from Pig⁻ sectors from patchwork colonies on medium plus norfloxacin; NSA2046, Pig⁻ mutant derived from Pig⁻ colony on medium plus norfloxacin; NSA2047, NSA2048, and NSA2049, Pig⁻ mutants derived from Pig⁻ sectors from patchwork colonies on medium plus ciprofloxacin.

To conclude, the three fluoroquinolones tested are able to stimulate Pig⁻ mutants, all of which show the same kind of rearrangement in similar proportions. Therefore, the rank order for genotoxic potency (i.e., the ability to increase the occurrence of Pig⁻ mutants and DNA rearrangements) is ciprofloxacin > enoxacin > norfloxacin.

DISCUSSION

Three fluoroquinolones (ciprofloxacin, enoxacin, and norfloxacin) greatly stimulate the occurrence of Pig⁻ mutants, which show the same phenotypical and molecular features as do the spontaneous Pig⁻ mutants. Therefore, fluoroquinolones are capable of stimulating genetic instability in *S. ambofaciens*, as has already been observed with oxolinic acid and novobiocin (31). Furthermore, the three fluoroquinolones tested showed the same rank order for both toxic (i.e., antibacterial) potency and genotoxic potency, that is ciprofloxacin > enoxacin > norfloxacin. It therefore seems possible that the two potencies could be related. In numerous bacteria, the toxic effect of quinolones and fluoroquinolones is due to their interaction with DNA gyrase (25). If the target of these antibiotics is the same in *S. ambofaciens*, the stimulation of both genetic instability and genomic plasticity could result from the interaction between fluoroquinolones and the type II topoisomerase (DNA gyrase).

Taken together, these results led us to propose a mechanism for genomic plasticity involving gyrase-mediated recombination (30). In bacteria, DNA gyrase shows an A2B2 structure

Downloaded from http://aac.asm.org/ on December 8, 2017 by INRA - old

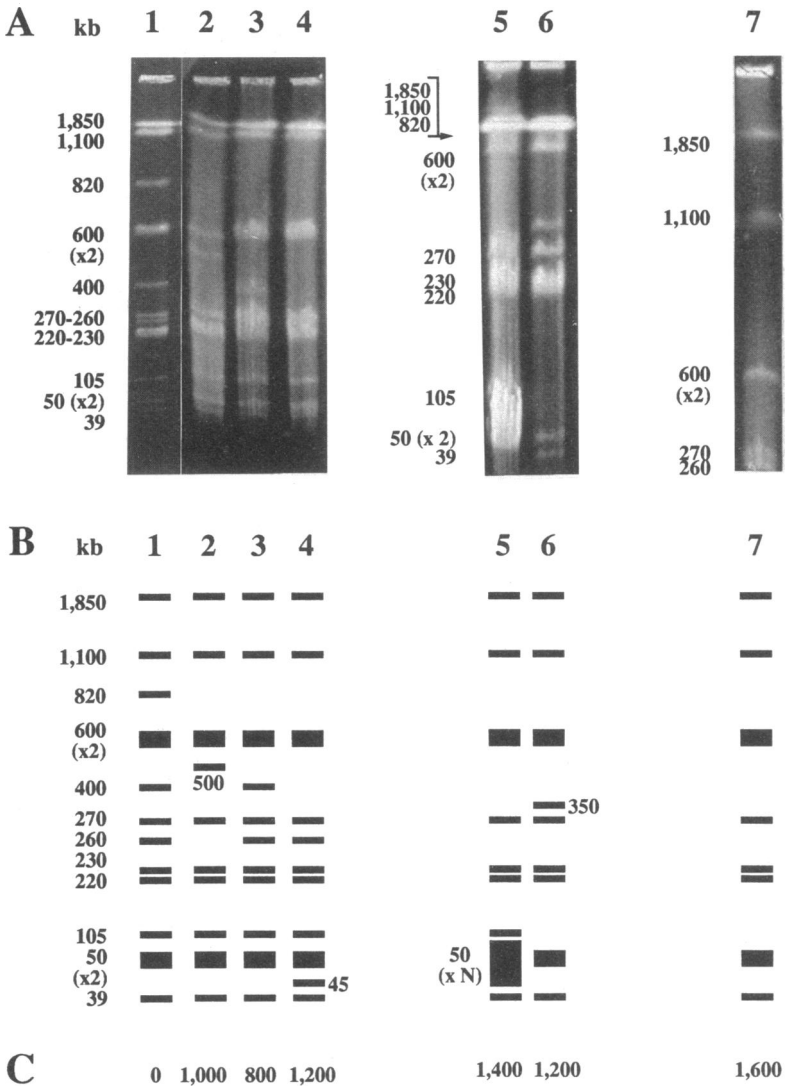

FIG. 5. PFGE analysis of genomic DNA of the WT strain NSA2002 and fluoroquinolone-induced Pig$^-$ mutants using the rare-cutting restriction endonuclease *Ase*I. (A) Examples of PFGE patterns. Pulse times and voltages were as follows: lanes 1 to 4, 40 to 100 s, 200 V for 28 h; lanes 5 and 6, 20 to 60 s, 200 V for 25 h; lane 7, 80 to 120 s, 200 V for 40 h. (B) Schematic interpretation of PFGE patterns resulting from several electrophoresis experiments performed under different migration conditions. The sizes of fragments additional to those shown in panel A are indicated. The relative fragment stoichiometry is represented by line thickness. (C) Approximate sizes of deletions, estimated as the total of missing fragment size minus the size of additional fragments, when they have been detected. Lanes: 1, NSA2002 (example shown in panel A), NSA2041, NSA2043, and NSA2049; 2, NSA2040; 3, NSA2039 (example shown in panel A) and NSA2048; 4, NSA2044; 5, NSA2045 (harbors an amplified 50-kb sequence in the *AUD6* locus which contains an *Ase*I site); 6, NSA2047; 7, NSA2046. Fragment and deletion sizes are indicated in kilobases.

(25) and is capable of binding to DNA at specific sites. Ikeda et al. (12) postulated that in *Escherichia coli*, an AB subunit exchange between two molecules of gyrase bound on DNA could generate the deletion of a sequence between the two binding sites. Furthermore, we suggest that the amplification could occur by a mechanism involving the passage of the replication fork and a subunit exchange between two molecules of gyrase, one on the newly replicated chromosome region and the other on the nonreplicated part of the chromosome. This could lead to the formation of a loop, as postulated in the model of Young and Cullum (34). This loop could undergo rolling-circle replication, generating an amplification. Rearrangement hotspots observed in some cases of genetic instability (5) could correspond to regions rich in high-affinity binding sites for gyrase, which would explain why some regions are very frequently prone to deletions and amplifications.

Fluoroquinolones may exert their effect by trapping the gyrase on the chromosome and thus enhancing the contact time between the enzyme and DNA. This could increase the number of molecules bound on the chromosome and therefore the frequency of rearrangements.

Furthermore, we report here that the molecular features of fluoroquinolone-induced Pig$^-$ mutants derived from sectors of patchwork colonies are not exactly the same as those of induced Pig$^-$ mutants isolated from Pig$^-$ colonies. One possible explanation is that the deletions occur earlier at the highest concentrations of fluoroquinolones used in this work, which induce Pig$^-$ colonies instead of Pig$^-$ sectors of patch-

Downloaded from http://aac.asm.org/ on December 8, 2017 by INRA - old

work colonies. This phenomenon may be due to the higher number of gyrase molecules bound on DNA as a consequence of the increased antibiotic concentration, which would enhance the probability of rearrangements occurring.

Understanding the genotoxic potencies of fluoroquinolones is important because this class of antibiotics is used in human therapy. Mutagenic activity of quinolones has been previously detected in one of the Ames tester strains (9). We report here that in *S. ambofaciens*, fluoroquinolones are capable of increasing the frequency of both very large deletions and amplifications of DNA sequences above the spontaneous level, which are characteristic of this species. These results do not allow us to conclude that these antibiotics could show such an effect in mammalian cells. In fact, no such observation exists. Nevertheless, a similar mechanism involving type II topoisomerase could be present in both procaryotic and eucaryotic cells. Topoisomerase II is able to promote illegitimate recombination in vitro (2, 12) and has been implicated in the generation of rearrangements in vivo (7, 21, 26, 33) in both categories of cells. Inhibition of both gyrase and eucaryotic topoisomerase II by drugs which interfere with the breakage-rejoining reaction by trapping the enzyme on DNA induces deletions and amplifications (1, 6, 21, 31). Furthermore, quinolones are capable both of producing a toxic effect on eucaryotic cells and of interfering with eucaryotic topoisomerase II (8, 13, 28), and some of these compounds could show a mutagenic effect in mammalian cells (10, 20). However, the affinity of fluoroquinolones for mammalian topoisomerase II is much lower than that for gyrase, thus allowing the use of these antibiotics in human therapy.

ACKNOWLEDGMENTS

This work was supported by grants from the French Ligue Nationale contre le Cancer. J.N.V. is the recipient of a fellowship from the French Ligue Nationale contre le Cancer and D.V. is the recipient of a fellowship from the French Ministère de la Recherche et de l'Enseignement Supérieur.

We thank Lois Silk for reading the manuscript. We are grateful to Bayer-Pharma Co. for the gift of ciprofloxacin.

REFERENCES

1. **Bae, Y. S., M. Chiba, M. Ohira, and H. Ikeda.** 1991. A shuttle vector for analysis of illegitimate recombination in mammalian cells: effects of DNA topoisomerase inhibitors on deletion frequency. Gene **101:**285–289.
2. **Bae, Y. S., I. Kawasaki, H. Ikeda, and L. F. Liu.** 1988. Illegitimate recombination mediated by calf thymus DNA topoisomerase II *in vitro*. Proc. Natl. Acad. Sci. USA **85:**2076–2080.
3. **Birch, A., A. Häusler, and R. Hütter.** 1990. Genome rearrangement and genetic instability in *Streptomyces* spp. J. Bacteriol. **172:**4138–4142.
4. **Dary, A., N. Bourget, N. Girard, J. M. Simonet, and B. Decaris.** 1992. Amplification of a particular DNA sequence in *Streptomyces ambofaciens* RP181110 reversibly prevents spiramycin production. Res. Microbiol. **143:**99–112.
5. **Demuyter, P., D. Schneider, P. Leblond, J. M. Simonet, and B. Decaris.** 1991. A chromosomal hotspot for multiple rearrangements associated with genetic instability of *Streptomyces ambofaciens* DSM40697. J. Gen. Microbiol. **137:**491–499.
6. **Di Leonardo, A., P. Cavolina, and A. Maddalena.** 1993. DNA topoisomerase II inhibition and gene amplification in V79/B7 cells. Mutat. Res. **301:**177–182.
7. **Di Leonardo, A., A. Maddalena, and P. Cavolina.** 1992. Nalidixic acid-resistant V79 cells with reduced DNA topoisomerase II activity and amplification-prone phenotype. Mutat. Res. **269:**319–327.
8. **Forsgren, A., S. F. Schlossman, and T. F. Tedder.** 1987. 4-Quinolone drugs affect cell cycle progression and function of human lymphocytes in vitro. Antimicrob. Agents Chemother. **31:**768–773.
9. **Gocke, E.** 1991. Mechanism of quinolone mutagenicity in bacteria. Mutat. Res. **248:**135–143.
10. **Holden, H. E., J. F. Barett, C. M. Huntington, P. A. Muehlbauer, and M. G. Wahrenburg.** 1989. Genetic profile of a nalidixic acid analog: a model for the mechanism of sister chromatid exchange induction. Environ. Mol. Mutagen. **13:**238–252.
11. **Hopwood, D. A., M. J. Bibb, K. F. Chater, T. Kieser, C. J. Bruton, H. M. Kieser, D. J. Lydiate, C. P. Smith, J. M. Ward, and H. Schrempf.** 1985. Genetic manipulation of *Streptomyces*: a laboratory manual. John Innes Foundation, Norwich, England.
12. **Ikeda, H., K. Moriya, and T. Matsumoto.** 1981. *In vitro* study of illegitimate recombination: involvement of DNA gyrase. Cold Spring Harbor Symp. Quant. Biol. **45:**399–408.
13. **Krajcovic, J., L. Ebringer, and J. Polonyl.** 1989. Quinolones and coumarins eliminate chloroplasts from *Euglena gracilis*. Antimicrob. Agents Chemother. **33:**1883–1889.
14. **Leblond, P., P. Demuyter, L. Moutier, M. Laakel, B. Decaris, and J. M. Simonet.** 1989. Hypervariability, a new phenomenon of genetic instability, related to DNA amplification in *Streptomyces ambofaciens*. J. Bacteriol. **171:**419–421.
15. **Leblond, P., P. Demuyter, J. M. Simonet, and B. Decaris.** 1990. Genetic instability and hypervariability in *Streptomyces ambofaciens*: towards an understanding of a mechanism of genome plasticity. Mol. Microbiol. **4:**707–714.
16. **Leblond, P., P. Demuyter, J. M. Simonet, and B. Decaris.** 1991. Genetic instability and associated genome plasticity in *Streptomyces ambofaciens*: pulsed-field gel electrophoresis evidence for large DNA alterations in a limited genomic region. J. Bacteriol. **173:**4229–4233.
17. **Leblond, P., F. X. Francou, J. M. Simonet, and B. Decaris.** 1990. Pulsed-field gel analysis of the genome of *Streptomyces ambofaciens* strains. FEMS Microbiol. Lett. **72:**79–88.
18. **Leblond, P., M. Redenbach, and J. Cullum.** 1993. Physical map of the *Streptomyces lividans* 66 genome and comparison with the related strain *Streptomyces coelicolor*. J. Bacteriol. **175:**3422–3429.
19. **Lin, Y. S., H. M. Kieser, D. A. Hopwood, and C. W. Chen.** 1993. The chromosomal DNA of *Streptomyces lividans* 66 is linear. Mol. Microbiol. **10:**923–934.
20. **McQueen, C. A., and G. M. Williams.** 1987. Effects of quinolone antibiotics in tests for genotoxicity. Am. J. Med. **82:**94–96.
21. **Miura-Masuda, A., and H. Ikeda.** 1990. The DNA gyrase of *Escherichia coli* participates in the formation of a spontaneous deletion by a *recA*-independent recombination *in vivo*. Mol. Gen. Genet. **220:**345–352.
22. **Pinnert-Sindico, S., L. Ninet, J. Preud'homme, and C. Cosar.** 1955. A new antibiotic: spiramycin. Antibiot. Annu. **1954–1955:**724–727.
23. **Pridham, T. G., P. Anderson, C. Foly, L. A. Linderfelser, C. W. Hesseltine, and R. C. Benedict.** 1957. A selection of media for maintenance and taxonomic study of Streptomyces. Antibiot. Annu. **1956–1957:**947–953.
24. **Redenbach, M., F. Flett, W. Piendl, I. Glocker, U. Rauland, O. Wafzig, R. Kleim, P. Leblond, and C. Cullum.** 1993. The *Streptomyces lividans* 66 chromosome contains a 1MB deletogenic region flanked by two amplifiable regions. Mol. Gen. Genet. **241:**255–562.
25. **Reece, R. J., and A. Maxwell.** 1991. DNA gyrase: structure and function. Crit. Rev. Biochem. Mol. Biol. **26:**335–375.
26. **Saing, K. M., H. Orii, Y. Tanaka, K. Yanagisama, A. Miura, and H. Ikeda.** 1988. Formation of deletion in *Escherichia coli* between direct repeats located in the long inverted repeats of a slime mold plasmid: participation of DNA gyrase. Mol. Gen. Genet. **214:**1–5.
27. **Salomon, E., J. Borrow, and A. D. Goddard.** 1991. Chromosome aberrations and cancer. Science **254:**1153–1160.
28. **Sato, K., K. Hoshino, T. Une, and Y. Osada.** 1989. Inhibitory effects of ofloxacin on DNA gyrase of *E. coli* and topoisomerase II of bovine calf thymus. Rev. Inf. Dis. **11:**915–916.
29. **Schneider, D., B. Aigle, P. Leblond, J. M. Simonet, and B. Decaris.**

Downloaded from http://aac.asm.org/ on December 8, 2017 by INRA - old

1993. Analysis of genome instability in *Streptomyces ambofaciens*. J. Gen. Microbiol. **139**:2559–2567.

30. **Volff, J. N.** 1994. Stimulation de l'instabilité génétique et des réarrangements génomiques chez *Streptomyces ambofaciens*. Thèse de l'Université de Nancy I, Vandoeuvre-lès-Nancy, France.
31. **Volff, J. N., D. Vandewiele, J. M. Simonet, and B. Decaris.** 1993. Stimulation of genetic instability by antibiotics that interact with DNA gyrase. J. Gen. Microbiol. **139**:2551–2258.
32. **Volff, J. N., D. Vandewiele, J. M. Simonet, and B. Decaris.** 1993. Ultraviolet light, mitomycin C and nitrous acid induce genetic instability in *Streptomyces ambofaciens* ATCC23877. Mutat. Res. **287**:141–156.
33. **Wang, J. C., P. R. Caron, and R. A. Kim.** 1990. The role of DNA topoisomerase in recombination and genome stability: a double-edged sword? Cell **62**:403–406.
34. **Young, M., and J. Cullum.** 1987. A plausible mechanism for large-scale chromosomal DNA amplification in streptomycetes. FEBS Lett. **212**:10–14.

Downloaded from http://aac.asm.org/ on December 8, 2017 by INRA - old