Reduced Nitrofuran Sensitivity Conferred by R Factors

Takashi Aoki, Syuzo Egusa, and Toshihiko Arai

Department of Fisheries, Faculty of Agriculture, University of Tokyo, Tokyo, and Department of Microbiology, Keio University School of Medicine, Tokyo

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Nitrofuran derivatives are among the commonly used chemotherapeutics for human urinary tract infections. These drugs are also generally used for the treatment and prevention of fish infectious diseases and the preservation of fresh fish meat in Japan. Because of the continuous use of nitrofurans in Japan, the incidence of nitrofuran resistance has been reported as high [7, 8]. Most of the nitrofuran-resistant characters were found to be coded by the bacterial chromosome with none of the R factors detected in these bacteria found to carry nitrofuran-resistant genes [8].

We have been studying the R factors which conferred nitrofuran resistance in bacteria isolated from areas where nitrofurans are commonly used. Naturally occurring bacteria and bacteria which received R factors from these natural isolates were examined concerning their minimal inhibitory concentrations (MIC) to the nitrofurans: furazolidone (Takeda), nifuriprinol (Dainippon Seiyaku) and nifurprazine-HCl (Yamano-uchi). Heart infusion agar (Eiken) plates containing various concentrations of nitrofuran derivatives were used as solid media for MIC measurements. A 0.025-ml sample of a liquid culture containing $10^3$ cells was spotted on the above plates and incubated at the proper temperature (37°C for enteric bacteria and 25°C for the other bacteria). The nitrofuran concentrations which gave less than ten colonies per plate were taken as the MIC [2].

Naturally occurring nitrofuran-resistant strains which were found to carry R factors were cultivated in Bacto-penassay broth (Difco) at 25°C or 37°C. In the case of Vibrio strains, 2.0% NaCl was added as a supplement to this broth. Most of the naturally occurring strains were classified into nitrofuran-sensitive (MIC: 0.04–0.1 µg/ml or lower) and nitrofuran-resistant (MIC: 5–20 µg/ml) strains by their MIC of furazolidone, but a few strains fell into an intermediate resistant group (MIC: 0.2–1.0 µg/ml) [1, 2].

To avoid the fluctuations in MIC caused by differences in host strains, R factors were transferred to the standard strains of each species, and also Escherichia coli K-12: RC85 nal (methionine-requiring, nalidixic acid-resistant, F derivative) by selecting for tetracycline, or aminobenzyl penicillin resistance. Further examinations of resistance levels were carried out not only by MIC on the agar plates but also by their growth curves in broth in the presence of a certain concentration of furazolidone. For the growth curve experiments, logarithmically growing cultures were diluted one hundredfold by prewarmed penassay broth in cuvettes of a biophotometer (Bio-Log II, Jouan-Quetin), and the growth of the cells was measured not only by their transmittances but also by their viable cell counts/ml.

All strains with intermediate resistance to nitrofuran transferred their nitrofuran-resistant character to other strains by conjugation, but none of the highly nitrofuran-resistant strains transferred their resistant character. Of course, none of the R factors detected in nitrofuran-sensitive strains conferred nitrofuran resistance to any strains.

Correlation between the reduced nitrofuran-sensitive character and other genetic characters of these R factors was also examined by transduction using phage P1 in
Table 1. MIC of E. coli RC85 nal carrying various R factors against furazolidone and the origins and groups of these R factors

<table>
<thead>
<tr>
<th>R factor</th>
<th>MIC (µg/ml)</th>
<th>Other resistance markers</th>
<th>Incompatibility group</th>
<th>Origin of R factor [References]</th>
</tr>
</thead>
<tbody>
<tr>
<td>R&lt;sup&gt;-&lt;/sup&gt; (wild type)</td>
<td>0.1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>R6K</td>
<td>0.2</td>
<td>str, amp</td>
<td>X</td>
<td>E. coli [5,6,9]</td>
</tr>
<tr>
<td>pJA5017</td>
<td>0.2</td>
<td>sul, tet</td>
<td>A</td>
<td>A. liquefaciens [3,4]</td>
</tr>
<tr>
<td>pJA5082</td>
<td>0.2</td>
<td>sul, tet</td>
<td>A</td>
<td>A. liquefaciens [3,4]</td>
</tr>
<tr>
<td>pJA4733</td>
<td>0.3</td>
<td>str, cml, amp</td>
<td>N (?)</td>
<td>Enterobacter</td>
</tr>
<tr>
<td>pJA4320</td>
<td>0.3</td>
<td>tet</td>
<td>E&lt;sup&gt;a&lt;/sup&gt;</td>
<td>V. anguillarum [2]</td>
</tr>
<tr>
<td>R&lt;sup&gt;-&lt;/sup&gt; (nitrofuranresistant)</td>
<td>3.2</td>
<td>—</td>
<td>—</td>
<td>(mutation from wild type)</td>
</tr>
</tbody>
</table>

Abbreviation: str; streptomycin, amp; aminobenzyl penicillin, sul; sulfonamide, tet; tetracycline, cml; chloramphenicol.

Logarithmically growing cultures of E. coli K-12: RC85 nal carrying each R factor were diluted and 0.025 ml samples of diluted culture containing 10<sup>3</sup> cells were spotted on the heart infusion agar plates containing various concentrations of furazolidone. Concentrations which gave less than ten colonies were given as MIC after 18-hr incubation at 37°C.


Fig. 1. The growth curves of standard strains carrying each R factor. Logarithmically growing cultures of standard strains carrying each R factor were diluted to one hundredth into prewarmed Bacto-penassay broth containing various concentrations of furazolidone, and per cent transmittances were plotted by biophotometer. R factors: see Table 1. a, Growth curves of E. coli K-12: RC85 nal in penassay broth containing 2 µg/ml of furazolidone at 37°C. b, Growth curves of A. liquefaciens 67-P-24 in penassay broth containing 5 µg/ml of furazolidone at 30°C.

E. coli: RC85 nal [9]. Transductants selected by other resistance characters were then examined for their MIC to nitrofurans and their growth in the presence of nitrofurans. All the transductants examined were found to have reduced nitrofuransensitivity. The MIC to furazolidone for strain RC85 nal carrying various R factors, and the origins and incompatibility groups of these R factors are shown in Table 1. Figure la shows the growth curves at 37°C of strain RC85 nal carrying each R factor in the presence of 2 µg/ml of furazolidone and Fig. 1b shows growth curves at 30°C of Aeromonas liquefaciens (the standard strain 67-P-24 carrying A. liquefaciens originating R factors pJA5017 or pJA5082) in the presence of 5 µg/ml of furazolidone. Viable cell counts of the cultures had the same patterns as those of cell density from turbidity measurements.

The use of nitrofurans has always been followed by high incidences of nitrofuran-resistant bacteria, but because of their chromosomal localization, bacteria with nitrofuran resistance were thought not only to be unable to transfer their nitrofuran resistance to other bacteria by cell to cell contact but also to be unable to select other drug-resistant bacteria. In other words, the existence of nitrofuran-resistant normal flora was thought neither to effect the nitrofuran resistance of new pathogens nor to select other resistance characters. Possibly for this reason, nitrofuran derivatives have been used carelessly.

The finding that R factors exist which confer to their host bacteria increased resistance to nitrofuran derivatives warns against the careless use of nitrofurans because of their selection of such R factors, even though the resistance level conferred is lower than that of the more prevalent nitrofuran-resistant bacteria.
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