Effects of the Recipient Strain and Ultraviolet Irradiation on Transduction Kinetics of the Penicillinase Plasmid of *Staphylococcus aureus*

SALLY J. RUBIN AND E. D. ROSENBLUM

Department of Microbiology, The University of Texas Southwestern Medical School, Dallas, Texas 75235

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When the penicillinase plasmid of *Staphylococcus aureus* PS 81(Ps1)(Ts1) was transferred to its cured derivative of PS 81(Np)(Ts1), there was a fivefold increase in the transduction frequency of penicillinase plasmid markers after ultraviolet (UV) irradiation of the phage instead of the expected decrease typical for plasmid-borne markers. These results were independent of the transducing phage, the donor, and the method of curing the recipient and were also obtained with a cured derivative of PS 80(P180). With PS 52, a naturally occurring penicillin-sensitive strain, and a cured transductant of PS 52 as the recipients, typical plasmid kinetics were observed. The plasmid location of penicillinase plasmid markers in transductants was confirmed by their instability in ethidium bromide (EB). In a cross between isogenic plasmids (P1234penZ cad × P1234pen1 asa ero), transductants were doubly selected for cadmium and erythromycin resistances. There was a twofold increase in transduction frequency after UV irradiation of the transducing phage and an increase in the proportion of recombinant type transductants. CsCl-EB density centrifugation revealed that plasmid deoxyribonucleic acid (DNA) was present in PS 81(Ps1)(Np) and its cured derivative [PS 81(Np)(Ps1)], but not in PS 52. Sucrose gradient analysis of plasmid DNA showed that the penicillinase plasmid of PS 81(Ps1)(Np) was larger than the plasmid in its cured derivative. Thus, the cured derivative contains plasmid DNA which appears to recombine with the incoming plasmid, causing the rise in transduction frequency noted after UV irradiation of transducing phage.

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Arber (1) found that low doses of ultraviolet (UV) irradiation to transducing phage stimulate the transduction frequency of chromosomal determinants while the rate of transfer of plasmid-borne markers is decreased. The increase in transduction frequency of chromosomal markers has been attributed to a stimulation of recombination. Similar results have been observed with chromosomal (10) and extrachromosomal (2, 11, 16) determinants of *Staphylococcus aureus*.

The characteristics of the kinetics of UV irradiation in transducing experiments have been used by a number of investigators to support the plasmid location of unstable staphylococcal markers (6, 8, 9, 18). The most studied plasmids of *S. aureus* have been the penicillinase plasmids which have been shown to carry the determinants conferring resistance to penicillin and to a number of metal ions (14). Their plasmid location has been substantiated by both curing and a decreased transduction frequency after irradiation of transducing phage (17).

The present study shows that this UV-induced decrease in transduction rate, typical of plasmid-borne markers, does not always occur and that the rate of transfer of some penicillinase plasmids may be increased rather than decreased after UV irradiation of transducing phage. Our data indicate that the increase may be due to the presence of homologous plasmid deoxyribonucleic acid (DNA) in the recipient strain.

**MATERIALS AND METHODS**

**Bacteria and bacteriophages.** Strain nomenclature is based on the convention of Peyru, Wexler, and Novick (15) when possible. The strains used, their derivations, and their relevant genotypes are listed in Table 1. Because the compatibility group of the penicillinase plasmid of PS 81 has not been confirmed, its plasmid is designated P81. The symbols penZ and pen1 refer to the penicillinase structural gene and inducibility locus, respectively. Genes conferring resistance to arsenate, cadmium, and mercury are represented by *asa*, *cad*, and *mer*. Loci controlling resistance to the antibiotics tetracycline and erythromycin are denoted by *tet* and...
Table I. Nomenclature, derivation, and genotype of bacterial strains

<table>
<thead>
<tr>
<th>Strain nomenclature</th>
<th>Derivation</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chromosomal genes</strong></td>
<td><strong>Plasmid genes</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>penI</td>
<td>penZ</td>
</tr>
<tr>
<td>PS 81(P81)(T81)</td>
<td>Wild-type parent carrying penicillinase and tetracycline plasmids</td>
<td>-</td>
</tr>
<tr>
<td>PS 81(N8)(T81)</td>
<td>Parent cured of P81 by EBc</td>
<td>-</td>
</tr>
<tr>
<td>PS 81(T3)(N8)(T81)</td>
<td>Parent cured of P81 by 43 C</td>
<td>-</td>
</tr>
<tr>
<td>PS 81(P81)(N8)</td>
<td>Parent cured of T81 by EVd</td>
<td>-</td>
</tr>
<tr>
<td>PS 81(N8)(N8)</td>
<td>PS 81(N8)(T81) cured of T81 by EVd</td>
<td>-</td>
</tr>
<tr>
<td>PS 80(P182)b</td>
<td>Wild-type parent carrying metal-ion resistance on a penicillinase-like plasmid</td>
<td>+</td>
</tr>
<tr>
<td>PS 80(N8)</td>
<td>Parent cured of P180 by EBc</td>
<td>+</td>
</tr>
<tr>
<td>PS 80(P81)</td>
<td>PS 80 transductant from donor PS 81(P81)(T81)</td>
<td>+</td>
</tr>
<tr>
<td>PS 52</td>
<td>Wild-type parent, no known plasmids</td>
<td>-</td>
</tr>
<tr>
<td>PS 52(P81)</td>
<td>PS 52 transductant from donor PS 81(P81)(T81)</td>
<td>-</td>
</tr>
<tr>
<td>PS 52(N8)</td>
<td>PS 52(P81) cured of P81 by EBc</td>
<td>-</td>
</tr>
<tr>
<td>8325(T816)</td>
<td>8325 Carrying tetracycline plasmid from 169</td>
<td>-</td>
</tr>
<tr>
<td>8325(P182, penI asa ero)</td>
<td>8325 Carrying mutant penicillinase plasmid of 258</td>
<td>-</td>
</tr>
<tr>
<td>8325(P182, penI cad)</td>
<td>8325 Carrying mutant penicillinase plasmid of 258</td>
<td>-</td>
</tr>
</tbody>
</table>

*a PS 81 and PS 80 are NCTC 9716 and NCTC 9789, respectively; although less convenient, P81, T81, and P182 could also be designated P81; T81; and P182.

*b Obtained from R. P. Novick.

c Ethidium bromide.

d Ethyl violet.

The symbols (N8) and (N7) refer to the loss or curing of a penicillinase plasmid or tetracycline plasmid.

Strain PS 81(N8)(T81) is synonymous with 81-6-S described in the accompanying paper (19).

Bacteriophages 80, 52HJD, and 53 of the International Phage Typing Series were used. Phage stocks were prepared by the soft agar overlay method (4, 21).

Media. Stock cultures were maintained on Tryptone Soy Agar (TA; Oxoid). Broth cultures were grown in Trypticase Soy Broth (TSB; BBL) or CY medium (11).

Detection of penicillinase production and metal-ion resistance. Tests for penicillinase production and metal-ion resistance were described by Novick and Richmond (13), and Novick and Roth (14).

Transduction experiments. Recipient cells were harvested in 2 ml of TSB from an overnight culture on TA slants, diluted to about 5 × 1010 or 5 × 109 colony forming units (CFU)/ml. Equal volumes of cells and phage were mixed to give a multiplicity of infection (MOI) of about 0.15; CaCl2 at a final concentration of 4 mM was added, and the mixture was shaken at 37°C for 25 min. The mixture was then diluted with an equal volume of cold 1% sodium citrate (w/v) and centrifuged, and the cells were resuspended in cold 0.5% sodium citrate to a volume twice that of the original phage-cell mixture. As a control, TSB was substituted for the phage lysate. The transduction mixture was plated within 50 min after the addition of phage.

For UV irradiation of lysates, the phage lysate was gently rotated in a glass petri dish 53 cm from a 15-w General Electric germicidal lamp and exposed for 90 to 420 sec.

Transductants were selected on Brain Heart Infusion Agar (BHIA; Difco). Selective levels were 2.5 × 10−4 m Cd(NO3)2, 10 μg of erythromycin per ml (Ilotycin; Eli Lilly), and 0.25 units of penicillin G per ml (Squibb). Cadmium- and erythromycin-resistant transductants were selected by direct plating. To select for penicillin resistance, the transduction mixture was plated in 3 ml of soft agar, and, after 3 hr at 37°C, the plates were overlaid with 3 ml of soft agar containing 4 U of penicillin G. Ten transductants from each time period were overlaid with 3 ml of soft agar containing 4 units of markers. In most experiments, five of these were also tested for co-elimination of the plasmid markers by ethidium bromide (EB) treatment.

Plasmid elimination. Methods used for plasmid elim-
inination of plasmid DNA. Log phase cultures were grown for 40 min at 37°C with 5 μCi/ml 3H-thymidine. Plasmid DNA was isolated and separated from chromosomal DNA by CsCl-Et density centrifugation by the method of Novick and Bouanouch (12). Labeled cells were harvested and washed by filtration, resuspended to a final cell concentration of 10^9 CFU/ml, and centrifuged at 7,500 rev/min in a Sorvall SS34 rotor for 15 min. Protoplasts were formed by resuspending the cells to the original volume in 2.5 M NaCl-0.05 M ethylenediaminetetraacetic acid (EDTA), pH 7.0, and incubating for 15 min at 37°C with 15 μg of lysostaphin per ml (Schwarz/Mann). The protoplasts were lysed as described by Novick and Bouanouch (12). The cleared lysate was centrifuged for 30 min at 20,000 rev/min in a Sorvall SS34 rotor at 0°C. The supernatant fluid was mixed with CsCl to give a final density of 1.54, 10 mg of EB per ml was added, and the mixture was centrifuged at 20°C for 38 hr at 43,000 rev/min in a type 50 rotor of a Spinco preparative ultracentrifuge.

Sucrose-gradient centrifugation. Sucrose gradients (5 ml) were prepared with 5 and 20% sucrose in 0.05 M EDTA, pH 7.0. In some cases, 0.2 ml of 50% sucrose was placed in the bottom of the polycyliner (Beckman) tubes. EB was removed from CsCl fractions containing plasmid DNA by dialysis (3), and 0.2 ml of the pooled fractions was layered on the top of the gradients. The gradients were centrifuged for 3.5 hr at 39,000 rev/min in an SW39 rotor of a Spinco model L ultracentrifuge.

Collection and assay of fractions. Both CsCl and sucrose gradients were fractionated by puncturing the bottom of the tube. Fractions were prepared for assay of trichloroacetic acid-precipitable radioactivity by the procedure of Bollum (5). The samples were counted in a Nuclear Chicago Unilux II scintillation counter.

RESULTS

Transduction of the penicillinase plasmid to a cured derivative of PS 81. The known markers on the penicillinase plasmid of PS 81 are co-eliminated at rates of 20 to 30% after growth in 6 × 10^{-4} M EB. To substantiate the plasmid location of these markers, phage 80 was propagated on PS 81(P_{81})(T_{81}), and the resulting phage lysate was irradiated and used to transfer these markers to PS 81(N_{P})(T_{81}) (Table 2). After 90 sec of UV irradiation of the phage lysate, there was an approximately threefold increase in the transduction frequency of cadmium resistance instead of the expected decrease typical of plasmid-borne genes. Cadmium, arsenate, mercury, and penicillin resistance were cotransduced. These markers were co-eliminated from the transductants by growth in EB at rates of 22 to 29% which were comparable to those obtained with the parent strain PS 81.

Transduction of cadmium resistance to independently cured derivatives. Four independently isolated EB-cured derivatives and one temperature-cured derivative, PS 81-T-3(N_{P})(T_{81}), were used as recipients of cadmium resistance determinants from PS 81(P_{81})(T_{81}) (Table 3). There was about a fivefold increase in transduction frequency after 90 sec of irradiation for all five recipients. Penicillin, arsenate, mercury, and cadmium resistance were co-eliminated from all transductants tested at rates typical for parental PS 81. Therefore, the stimulation of transduction frequency is independent of the curing method and is not unique to the original recipient PS 81(N_{P})(T_{81}). A similar stimulation was observed when PS 81(N_{P})(N_{P}) was the recipient or when PS 81(P_{81})(N_{P}) was the donor.

A reduction in the number of lytic phage by UV killing could cause an apparent increase in the transduction frequency simply by reducing loss of transductants due to lysis. This possibility was excluded by the following experiment. Because 90 sec of UV irradiation produces a 10-fold drop in titer, the original unirradiated phage lysate was diluted 10-fold and used in a kinetics experiment. There was a sixfold increase in transduction frequency after 90 sec of UV irradiation of this diluted phage lysate, indicating that the stimulation of transduction frequency is

\[ \text{TABLE 2. Ultraviolet effect on transduction frequency of cadmium resistance from PS 81(P_{81})(T_{81})} \]

<table>
<thead>
<tr>
<th>UV irradiation (sec)</th>
<th>Transductants/10^{6} phage</th>
<th>PFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.3</td>
<td>4.8 × 10^6</td>
</tr>
<tr>
<td>90</td>
<td>14</td>
<td>5.0 × 10^6</td>
</tr>
<tr>
<td>120</td>
<td>12</td>
<td>2.9 × 10^6</td>
</tr>
</tbody>
</table>

a Phage 80 at a multiplicity of infection of 0.18 was used to transduce cadmium resistance to PS 81(N_{P})(T_{81}).

\[ \text{TABLE 3. Ultraviolet effect on transduction frequency of cadmium resistance to independently derived cadmium-sensitive recipients} \]

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Transductants/10^{6} phage</th>
<th>Unirradiated</th>
<th>90 sec of 90 sec of Elimination of plasmid markers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS 81-T3(N_{P})(T_{81})</td>
<td>1.5</td>
<td>8.6</td>
<td>22.0</td>
</tr>
<tr>
<td>PS 81(N_{P})(T_{81})</td>
<td>1.5</td>
<td>7.5</td>
<td>29.0</td>
</tr>
<tr>
<td>PS 81-54(N_{P})(T_{81})</td>
<td>2.6</td>
<td>12.0</td>
<td>30.5</td>
</tr>
<tr>
<td>PS 81-70(N_{P})(T_{81})</td>
<td>1.2</td>
<td>6.9</td>
<td>21.2</td>
</tr>
<tr>
<td>PS 81-90(N_{P})(T_{81})</td>
<td>3.2</td>
<td>13.0</td>
<td>34.8</td>
</tr>
<tr>
<td>PS 81-117(N_{P})(T_{81})</td>
<td>2.2</td>
<td>10.0</td>
<td>31.4</td>
</tr>
</tbody>
</table>

a Phage 80 propagated on PS 81(P_{81})(T_{81}) was used for transducing lysate.

b Selected transductants were treated with 6 × 10^{-4} M ethidium bromide as previously and were tested for co-elimination of penicillin, arsenate, cadmium, and mercury resistance.
related to the effects of UV irradiation and not to a decrease in the number of lytic phage.

Transduction of the penicillinase plasmid to PS 52. When a naturally occurring penicillin-sensitive strain, PS 52, was used as a recipient of cadmium resistance from PS 81, there was a decrease in the transduction after UV irradiation typical of plasmid markers (Fig. 1). These transductants were also resistant to mercury, arsenate, and penicillin, and all four markers were co-eliminated from the transductants after growth in EB at rates of 2 to 11%. When PS 52(Np) (a cured transductant) was used as a recipient, plasmid-type kinetics were again observed.

Effects of the donor strain and the transducing phage on transduction kinetics. The effect of using a heterologous donor, PS 80(P180), is shown in Fig. 2. The genes conferring penicillin resistance in this strain are chromosomal (2). Strain PS 81(Np)(T81) again exhibited chromosomal type kinetics as a recipient of cadmium resistance. These transductants were penicillin-sensitive, indicating no cotransfer of chromosomal penicillin resistance with the plasmid markers. Cadmium and mercury resistance were co-eliminated from the transductants tested at rates of 4.1 to 5.3%. Transduction of chromosomal penicillin resistance resulted in the expected increase in transduction frequency with irradiation. The initial rate of transfer of the plasmid-borne markers to the cured derivative was about 20 times greater than the initial rate of transfer of the chromosomal penicillin resistance markers. With the naturally occurring penicillin-sensitive strain, PS 52, transduction of cadmium resistance again resulted in typical plasmid-type kinetics at an initial rate 30 times greater than the rate of transfer of the same markers to the cured derivative. The transductants were penicillin-sensitive, and cadmium and mercury resistance were co-eliminated from them at rates of 0.5 to 7.3%. Attempts to transfer the chromosomal penicillinase genes to this strain were not successful. This was most likely due to a lack of genetic homology between the chromosomal penicillinase markers of PS 80 and the chromosome of PS 52. Similar results were obtained when phage 52HJD was used to transfer cadmium resistance from parental PS 80 or PS 81. In all cases the transduction frequency was stimulated by UV irradiation when a penicillinase plasmid-cured derivative of PS 81 was the recipient, and the transduction frequency decreased when PS 52 or its cured transductant
was the recipient.

**Transduction of cadmium resistance to a cured derivative of PS 80.** PS 80 was cured by EB treatment of its plasmid PI80 which carries the markers conferring resistance to cadmium, mercury, and arsenate. The cured derivative, PS 80(Np), was used as a recipient of the PI80 and PS81 plasmids of PS 80 and PS 81 (Fig. 3). When PS81 was transferred to PS 80(Np), there was a sixfold increase in the transduction frequency after 90 sec of UV irradiation of the phage lysate. The transductants were resistant to all three metal ions, which were co-eliminated from the transductants at rates of 50 to 72% by growth in EB. When PI80 was transferred to PS 80(Np), there was a fourfold increase in the rate of transduction. These transductants were also resistant to all three metal ions and lost these markers jointly at rates of 43 to 81% after EB treatment. Therefore, UV irradiation appears to stimulate the transduction frequency when penicillinase plasmids are transferred to penicillinase plasmid-cured derivatives of the parent or a closely related strain.

**Effect of UV irradiation on an isogenic plasmid cross.** Because UV is thought to increase transduction frequency by stimulating recombination (1), the increase in transduction frequency observed after phage irradiation when a penicillinase plasmid is transduced to a cured derivative may be due to the presence of homologous extrachromosomal DNA. Thus, irradiation of transducing phage should stimulate the transduction frequency of an isogenic plasmid cross. The plasmid of 8325 (PI83 penZ cad) was transferred to 8325 (PI83 penI asa ero) and 8325 (T169) by phage 53 (Fig. 4). When the penicillinase plasmidless strain 8325 (T169) was the recipient and transductants were selected for erythromycin resistance, there was a decrease in transduction frequency with irradiation as expected for plasmid-borne markers. However, when the recipient contained an isogenic plasmid and transductants were doubly selected for erythromycin and cadmium resistance, irradiation caused a twofold increase in the transduction frequency.

Twenty doubly resistant transductants were picked at each time period, suspended in 1 ml of ice-cold TSB, and plated on .3CY medium. These plates were replica plated to .3CYM, BHIA containing erythromycin, and BHIA containing cadmium to determine whether the transductants were diploid or recombinant type. Diploids yield segregants, whereas recombinants do not. In two separate experiments, the proportion of recombinant-type transductants doubled from 30 to 60% after 90 sec of UV irradiation. Re-
combinant-type transductants yielded no segregants, and all plasmid markers were co-eliminated after growth in EB from three recombinants tested. Thus, UV clearly stimulated recombination between genetically homologous plasmids.

**Gradient analysis of plasmid DNA.** PS 81(P81)(NT) PS 81(Np)(Np), and PS 52 were labeled by growth in media containing H-thymidine. After lysis of the cells, a low speed prespin was employed to eliminate approximately 99% of the DNA presumed to be chromosomal. Plasmid and residual chromosomal DNA were then separated in CsCl-EB density gradients (Fig. 5). PS 81(P81)(NT) exhibited the expected satellite peak indicative of circular duplex DNA, whereas the naturally occurring penicillin-sensitive strain, PS 52, did not. PS 81(Np)(Np) also had a satellite peak indicating that it contained circular duplex DNA presumed to be a plasmid.

The plasmid DNA fractions from PS 81(P81)(NT) and PS 81(Np)(Np) were pooled separately and, after removal of EB by dialysis, were sedimented in 5 to 20% sucrose gradients (Fig. 6). The plasmid DNA from each strain formed a single peak; the plasmid of PS 81(P81)(NT) sedimented faster than that of PS 81(Np)(Np). When the two DNA fractions were mixed and centrifuged together, two distinct peaks were formed which sedimented at the same positions in the gradient as the DNA fractions centrifuged separately. These data suggest that each strain contains a single species of plasmid DNA and that the plasmid carried in PS 81(Np)(NT) is smaller than the plasmid of PS 81(P81)(NT).

**DISCUSSION**

The differential effects of UV irradiation on the transduction frequency of genetic markers has been widely accepted as an indication of a chromosomal or extrachromosomal location of the genes involved. However, our data show that when a penicillinase plasmid of *S. aureus* is transferred to a cured derivative of the parent or of a closely related strain, the transduction frequency may increase after irradiation in a manner typical of chromosomal markers. Similar observations were reported by Asheshov (2) who found there was a twofold increase in trans-
duction frequency after UV irradiation of the transducing lysate when unstable penicillin resistance was transferred from strain 2 to its cured derivative. She suggested that these results could be an indication of episomal behavior by the penicillinase plasmid.

This does not seem likely in our system, as we have shown that PS 81 cured of the penicillinase and tetracycline plasmids contains DNA presumed to be plasmid and, when PS 81(Np)(T81) or PS 81(Np)(Np) is used as a recipient of P81, the transductants are cured by EB as readily as the parent strain. UV irradiation stimulates recombination between genetically homologous plasmids as shown by the increase in the number of recombinant-type transductants after UV irradiation of transducing phage in an isogenic cross. This increase in recombination is accompanied by a stimulation of the transduction frequency.

Because the complete characterization of the circular duplex DNA species has not yet been performed, any conclusions as to the physicochemical nature must be tentative. However, the data strongly suggest the presence of a plasmid DNA species in PS 81(Np)(Np) which is smaller than that found in PS 81(P81)(Np). Assuming that the plasmid carried by PS 81(Np)(Np) has at least some regions genetically homologous to the plasmid P81, one explanation of our data is that the incoming penicillinase plasmid recombines with the plasmid in the cured derivative resulting in the observed increase in transduction frequency after irradiation of the transducing phage.

The plasmid in the cured derivative could be either a cryptic plasmid originally present or a remnant of the penicillinase plasmid now devoid of the known plasmid markers. The known plasmid markers map in a small region comprising only about one-fourth of the plasmid DNA (20). The latter hypothesis is more attractive because PS 81(P81)(Np) and PS 81(Np)(Np) appear to carry a single plasmid species which differs in size, with P81 being the larger.

Recent evidence indicates that certain R factors behave differently in different genera of Enterobacteriaceae. The same R factor that occurs as a single molecule in Escherichia coli can exist in Proteus mirabilis as two independent plasmids as well as a large composite plasmid (7). The penicillinase plasmid of PS 81(P81) may be behaving in a similar although not analogous manner. The penicillinase plasmid could exist under normal conditions as a composite molecule which, under certain conditions such as treatment with EB or growth at high temperature, dissociates into two molecules. The one carrying the resistance markers would be more sensitive to curing than the plasmid with no known markers. The stimulating effect of UV irradiation on transduction might be expected if each molecule carries a genetically homologous region.

Complete loss of the penicillinase plasmid does appear to occur in strains in which the plasmid has been artificially introduced by transduction. This can be demonstrated by transducing P81 to PS 52, curing the transductants, and then using a cured transductant as a recipient of P81. When these cured derivatives of PS 52 are used as recipients, there is still a decrease in the transduction frequency with irradiation. When PS 81 is treated in a similar manner, the transduction frequency is still stimulated (unpublished data). Thus, retention of a part of the penicillinase plasmid appears to be a property of naturally occurring penicillinase plasmid-bearing strains.

On the other hand, if transduction involves only that part of the plasmid which carries the resistance markers, then the absence of a change in the transduction kinetics when PS 52(Np) is used as a recipient would be due to the absence of the retained plasmid found in PS 81(Np)(Np).

It should be pointed out, however, that the previously cited work (20), which demonstrated that the known plasmid markers map in a region of about one-fourth of the plasmid DNA, was performed on a penicillin-sensitive strain (like PS 52) into which penicillinase plasmids were introduced. Analysis of the DNA from PS 81(P81) and PS 52(Np) is planned to resolve this possibility.

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LITERATURE CITED