Morphological Changes in *Pseudomonas aeruginosa* Treated with Rod-Shaped Pyocin 28

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An attempt to classify bacteriocins into 2 groups was made by Bradley [3]. One group of bacteriocins has a low molecular weight form which is thermostable, trypsin-sensitive, and cannot be distinctly resolved by the electron microscope. The other group contains high molecular weight bacteriocins which were thermolabile, trypsin-resistant, and visible under the electron microscope as phage-like objects.

Those bacteriocins ("pyocins") of *Pseudomonas aeruginosa* that belong to the "phage-like" group exhibit a rod-shaped contractile structure with core and sheath: for example, pyocin C10 [3, 9], pyocin R [8, 11], pyocin Alme [7], and other unnamed pyocins [2, 4].

Pyocin 28, produced by strain P28 of *P. aeruginosa*, has been studied in some detail here. It is morphologically different from other pyocins [16, 17], and resembles the tail of λ phage of *Escherichia coli*. This pyocin is 90 Å wide and 200–4000 Å long, with a sharp tip. By means of the tip, the pyocin 28 rod attached perpendicularly to the sensitive cell [17], but the time course of the attachment was not clear in the earlier work. To study it further, a preparation of pyocin 28, partially purified by ammonium sulfate fractionation and 3 cycles of differential centrifugation, was added to a log-phase culture (5.8×10^8 cells/ml) and treated with various concentrations of pyocin 28 (closed circles), diluted at the indicated times to stop adsorption, and plated for survivors. To controls were added dilution fluid containing 10 mM Tris-HCl buffer, pH 7.5 (open circles).

![Fig. 1. Kinetics of adsorption of pyocin 28 to *Pseudomonas aeruginosa* P29 at 37°C. Cells grown in nutrient broth to a concentration of 5.8×10^8 cells/ml were treated with various concentrations of pyocin 28 (closed circles), diluted at the indicated times to stop adsorption, and plated for survivors. To controls were added dilution fluid containing 10 mM Tris-HCl buffer, pH 7.5 (open circles).]
Fig. 2. Electron micrographs of control cells of *P. aeruginosa* strain P29 in exponential growth phase. a. Longitudinal section. ×38 000. b. Cross section. ×54 000.

Fig. 3. Cells of strain P29 treated with pyocin 28 for 15 min. ×38 000.
10^8 cells/ml) of the sensitive strain P29 in nutrient broth at 37°C in varying amounts (1/10, 1/50, 1/100, 1/500 arbitrary units). At intervals, samples were withdrawn, diluted with dilution fluid (0.145 M NaCl–0.005 M MgCl_2–0.005 M MgSO_4), and plated on nutrient broth agar to determine the fraction of surviving bacteria. Fig. 1 shows that it takes 15–30 min for effective adsorption of pyocin 28.

In the electron microscope, morphological changes of the sensitive bacteria treated with pyocin 28 can be discerned. Samples of the treated cells were fixed for examination by the procedure of Kellenberger, et al. [13]. Fig. 2 shows thin sections of the untreated cells, with low electron density in the fibrous nucleoplasm, surrounded by a clear margin of cytoplasm. This indicates that the nucleoplasm was well preserved by the R-K conditions (0.1% CaCl_2, 0.1% tryptone or casamino acid, pH 6) [12].

At 15 min, 0.1% of the cells treated with pyocin 28 survive. Some cells contain dispersed nucleoplasm (Fig. 3). The left cell in Fig. 3, which was cut diagonally, contains dispersed nucleoplasm, while the cell at the right shows apparently normal nucleoplasm.

After 30 min, cell survival was down to 0.01 to 0.03%, and many pyocin-treated cells show dispersed nucleoplasm markedly different from that of control cells (Fig. 4). No clear boundary could be seen between the nucleoplasm and the cytoplasm. Cell walls and cytoplasmic membranes still appeared to be intact.

Figures 5 a and b show longitudinal and cross sections after treatment with pyocin 28 for 60 min. Nuclear material now appears to extend throughout the cells. Numerous fine fibers found on the cell surface (Figs. 4 and 5) may represent adsorbed rods of pyocin 28.

The frequency of morphologically changed cells was determined among longitudinal cells cut perpendicularly, and found to be 20% at 15 min, 60% at 30 min and 80% at 60 min.

The mode of action of bacteriocins has been studied in many laboratories [14], but there are few reports about morphological effects. Šmarda described morphological...
changes of *E. coli* cells exposed to certain colicins, but these were observed only after several hours of incubation [15]. Instead, the morphological changes in pyocin 28-treated cells are common even 15 min after incubation starts.

The detailed mechanism of the morphological change of the nucleoplasm in the pyocin 28-treated cell is not clear, and the receptor site of pyocin 28 may be on the cytoplasmic membrane or on the cell wall. However, it is suggestive that the receptor sites for colicin E1 may be on the cytoplasmic membrane [1]; and it has also been suggested that bacterial DNA has a membrane attachment site [10]. Possibly pyocin 28 at a receptor site may affect bacterial DNA bound to the membrane, resulting in dispersion of the DNA. Alternatively, permeability changes in the membrane, caused by pyocin adsorption, might themselves induce the dispersion of nucleoplasm: Hirota showed that an *E. coli* mutant, temperature sensitive in DNA synthesis, CRT 257, showed dispersed nucleoplasm along with increased permeability [5, 6].
REFERENCES


