

tion; heat resistance, stainability, and optical density were unchanged. On the other hand, at 75 C in caramelized glucose, spores of this organism and several other *Clostridium* strains have shown progressive loss of heat resistance.

Since DPA release at 75 C from spores in water and in caramelized glucose was entirely comparable, the results do not elucidate the question of possible germination in carmelized glucose at 75 C.

INDUCTION AND CULTIVATION OF STAPHYLOCOCCAL L FORMS IN THE PRESENCE OF METHICILLIN

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Clinical staphylococcal infections often recur in patients who have been thoroughly treated with penicillin or methicillin until they are clinically well and cultures are negative for staphylococci. This "persister" state is well known but not understood. The possibility that

1956; Marston, J. *Infectious Diseases* **108**:75, 1961; Prozorovskii, Zhur. *Mikrobiol. Epidemiol. Immunobiol.* **30**:116, 1959). However, induction of L forms from penicillin G-resistant and methicillin-resistant staphylococci with methicillin has not been previously reported.

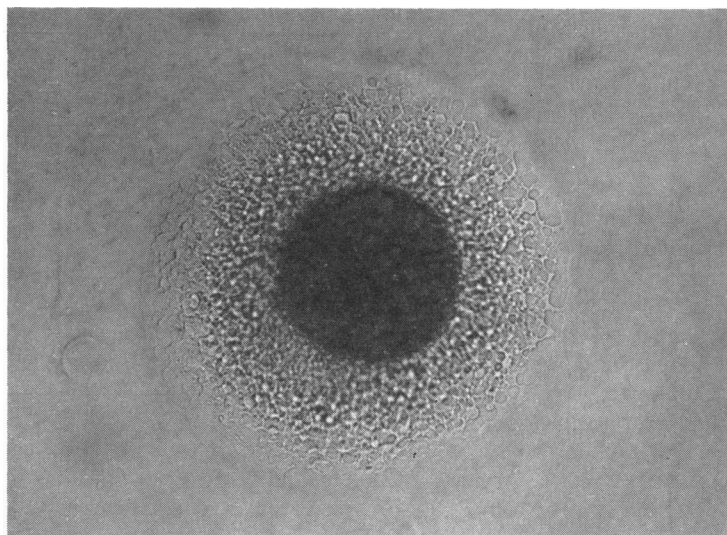


FIG. 1. Unstained L colony induced from a penicillin G-resistant *Staphylococcus aureus* in a medium containing 500 µg/ml of methicillin (115X).

this phenomenon might be explained by the capacity of staphylococci to convert to and revert from L forms led to the preliminary studies reported here.

The induction of L forms from penicillin G-sensitive strains of staphylococci in a medium containing penicillin G and a high concentration of NaCl has been reported by several investigators (Dienes and Sharp, *J. Bacteriol.* **71**:208,

Three penicillin G-sensitive, three penicillin G-resistant, and two methicillin-resistant staphylococcal strains were studied. Seven of these eight strains were isolated from human subjects. One penicillin-sensitive strain (6538P) was obtained from the American Type Culture Collection. All strains were coagulase positive. All were inoculated on solid agar medium essentially similar to that of Marston, but containing either inacti-

vated, filtered, human plasma or distilled water in place of horse serum, and from 0 to 5,000 $\mu\text{g/ml}$ of methicillin. After incubation under aerobic conditions at 37 C for 2 to 5 days, typical L colonies developed. These were identified microscopically by Dienes' methylene blue staining procedure (Dienes and Weinberger, *Bacteriol. Rev.* **15**:245, 1951).

Figure 1 shows a typical L colony derived from a penicillin G-resistant strain of *Staphylococcus aureus* containing numerous large bodies and granular elements. Although all strains produced L-type colonies, induction varied with the concentration of methicillin, antibiotic sensitivity of the strains, and presence of serum. In general, as the concentration of methicillin increased in serum-containing medium, the confluent bacterial growth was replaced by large and small discrete bacterial colonies mixed with, in some instances, a few L colonies and finally only L colonies. Induction of penicillin G-sensitive strains was observed at methicillin concentrations of 1 to

5,000 $\mu\text{g/ml}$; of penicillin G-resistant strains from 10 to 5,000 $\mu\text{g/ml}$; and of methicillin-resistant strains from 50 to 5,000 $\mu\text{g/ml}$. When serum was omitted from the medium, very sparse or no growth of L colonies was observed at any concentration of methicillin, with the exception of those from the penicillin G-sensitive strains. At some inducing concentrations of methicillin, L colonies from these strains appeared to grow even better in the absence of serum.

Induction of L-type colonies from methicillin-resistant and penicillin G-resistant strains was not observed on medium containing 600 to 6,000 $\mu\text{g/ml}$ of penicillin G.

L-type colonies from all but one methicillin-resistant strain have been maintained successfully for ten or more transfers on inducing medium containing 500 $\mu\text{g/ml}$ of methicillin. Gram-positive cocci were observed again in two L cultures (one penicillin G-sensitive and one penicillin G-resistant strain) after two or three passages on methicillin-free maintenance medium.

A FERRICHROME-REQUIRING ARTHROBACTER WHICH DECOMPOSES PUROMYCIN AMINONUCLEOSIDE

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An organism, presumably *Arthrobacter flavescens* Lochhead (Lochhead, *Arch. Mikrobiol.* **31**:163, 1958), which decomposes puromycin aminonucleoside [6-dimethylamino-9-(3'-amino-3'-deoxy- β -D-ribofuranosyl) purine] and requires ferrichrome (Neilands, *J. Biol. Chem.* **205**:647, 1953), or its equivalent, for growth has been isolated. [This organism, used by Burnham and Neilands (*J. Biol. Chem.*, **236**:554, 1961) for studies on the metabolism of ferrichrome compounds, has previously been designated *Arthrobacter* JG-9.]

The isolation of this organism is being reported for three reasons. (i) The otherwise conventional

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enrichment techniques were complicated by the uncommonly observed requirement for ferrichrome. (ii) This organism provides the only biological system known to decompose puromycin aminonucleoside (PAN); a nonspecific riboside hydrolase from *Lactobacillus delbrückii* failed to decompose this compound (Takagi and Horecker, *J. Biol. Chem.* **225**:77, 1957). (iii) Finally, the tentative identification of the organism as *A. flavescens* is complicated by the fact that authentic *A. flavescens* does not decompose the nucleoside.

The enrichment medium was tap water buffered with 0.05 M potassium phosphate (pH 7.0), containing 0.1% PAN and 0.1% yeast extract. The initial culture (100 ml) was inoculated with about 1 g of a mixed soil sample and incubated in a shallow layer with agitation at 30 C. The decomposition of PAN was followed by observing the decrease in absorbancy at 270 $\mu\mu$, the absorption maximum of PAN, in samples suitably