

BACTERIOLOGY¹

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ABSTRACTS

Lysozyme and gram positive bacteria. LOIS MYERHOLTZ and S. E. HARTSELL, Abbett Laboratories, North Chicago, and Purdue University.—The sensitivity of certain Gram positive bacteria to lysozyme and the relation of the Nakamura reaction to bacteriolysis indicates that this enzyme may act on the cell in three ways: (1) cause a change in the gram reaction and complete lysis; i.e. *M. lysodeikticus*, *B. subtilis* var. *globigii*, *S. lutea*, (2) cause a change in the gram reaction and partial lysis only in a certain range of the pH scale, i.e. *S. aurantiaca*, *M. roseus*, (3) cause no change in the gram reaction and no lysis, i.e. *M. pyogenes* var. *albus*.

The Nakamura reaction was essential for the observation of the effects of lysozyme on *S. aurantiaca* and *M. roseus*. No conditions have yet been found for observing a change in the gram reaction or lysis of *M. pyogenes* var. *albus*.

Varietal differences were noted with *B. subtilis* (insensitive) and *B. subtilis* var. *globigii* (sensitive).

The age of the culture was also found to influence the response to lysozyme—18 hour cultures have a greater lytic response than 24 hour cultures.

The sensitivity of *M. roseus* and *S. aurantiaca* was observed for the first time.

Effect of antibiotics on bacterial reduction of triphenyltetrazolium chloride (TTC). E. D. WEINBERG, Indiana University.—Several papers have recently appeared describing the ability of various antibacterial agents to interfere with the reduction of TTC by certain bacterial species. This phenomenon is of interest because it offers the possibility of developing a simple rapid assay method for the substances that inhibit TTC reduction, as well as the possibility of providing information concerning the selective mechanism of action of these agents.

In this laboratory, seven antibiotics were tested with resting cell suspensions of antibiotic sensitive strains of *Bacillus subtilis*, *Micrococcus pyogenes* var. *aureus*, and *Proteus vulgaris*. Aureomycin and terramycin consistently interfered with the ability of the cells to reduce

¹Joint session of the Indiana Academy, Bacteriology Division, and the Indiana Branch, Society of American Bacteriologists.

TTC. Chloramphenicol was active only in high concentrations; polymyxin, bacitracin, and streptomycin were inactive. In sharp contrast, penicillin stimulated TTC reduction.

A rapid method of assay for aureomycin and terramycin was devised. Advantages of the method include, in addition to speed, avoidance of three characteristics of overnight growth tests: need for sterility, possible deterioration of the antibiotic, and possible overgrowth of resistant variants. Disadvantages of the method include the facts that: a) the extent of TTC reduction is influenced by the kind and amount of nitrogenous and carbohydrate material present, and b) the quantity of aureomycin or terramycin required to inhibit reduction of TTC is considerably greater than that required to inhibit growth.

Evidence for the coexistence of alternate routes of carbohydrate metabolism in *Penicillium chrysogenum* Q-176. EDWARD C. HEATH, HENRY KOFFLER and E. P. GOLDSCHMIDT, Purdue University.—Preliminary studies on the breakdown of glucose by *Penicillium chrysogenum* Q-176 have led to the tentative conclusion that this organism is potentially capable of utilizing both the hexosemonophosphate shunt and a mechanism (or mechanisms) by which glucose is broken into two three-carbon-fragments. Evidence in support of this contention was obtained partly with cell-free extracts prepared from alumina-ground cells, which had been grown in a synthetic medium containing glucose. Such enzyme preparations oxidized glucose-6-phosphate and 6-phosphogluconate in the presence of triphosphopyridine nucleotide (TPN), as indicated by increased absorption at 340 m μ . Apparently ribose-5-phosphate also was metabolized; pentose disappeared rapidly when ribose-5-phosphate was the substrate. Further support for the hypothesis that the hexosemonophosphate shunt exists in this organism was obtained by experiments in which the dissimilation of glucose-1-C¹⁴ was studied with whole cells in the presence or absence of 6×10^{-3} M arsenite. The pyruvate that accumulated in the presence of arsenite and the respiratory carbon dioxide were collected and their specific activities (cpm/ μ MC) determined. In the presence of arsenite the specific activities of the CO₂ and the pyruvate were six times and two-thirds the initial activity of the glucose, respectively, while in the absence of arsenite the specific activity of the CO₂ was only slightly greater than that of the initial glucose. The high radioactivity in the carbon dioxide indicates the existence of a shunt mechanism involving the preferential removal of the first carbon of glucose, while the observation that pyruvate was labeled indicates another mechanism or mechanisms by which the glucose is split into two three-carbon fragments. It is suggested that these two systems function simultaneously, at least in the presence of arsenite, but nothing is yet known about the extent to which these reactions participate in uninhibited cells.

Isolation and study of an actinophage isolated from soil. M. M. HOEHN, L. SITLER, and J. M. MCGUIRE, Eli Lilly and Company, Indianapolis.—The actinophage designated as Lvl was isolated from soil by an enrichment procedure using *S. venezuelae* 8-44 as the host. Plaque counts were used to measure phage titers. Ca⁺⁺ was essential for phage multiplication in a glucose-peptone medium. Calcium concentration of 1M to 10⁻¹ M gave

the maximum phage count, but some multiplication occurred with only traces of Ca^{++} .

Lv1 actinophage is specific for its host *S. venezuelae* 8-44. It does not parasitize *S. rimosus*, *S. aureofaciens*, *S. griseus* No. 4, *S. griseus* No. 3463, or *S. erythreus*.

Plaque size is a distinguishing characteristic of this phage. Electron micrographs show a phage with a head and tail similar to those described in previous publications.

A statistical investigation into the factors of a starch-agar, filter-paper disc assay for amylases. RALPH WELLERSON, JR., EGON STARK, PHILIP A. TETRAULT and CARL F. KOSSACK, Purdue University.—The validity of a starch-agar, paper-disc, diffusion method for determining bacterial amylase potency has been investigated. The method consists of placing filter-discs on a starch-agar plate, applying an exact amount of enzyme solution and incubating the plates at 52°C . for six hours. The discs were removed, plates were successively flooded with an iodine solution and the zones read with a Fisher-Lilly zone reader. The optimum medium contained 1% agar and 0.2% soluble starch in M/15 Sorenson's buffer, pH 6.0. Storage of the medium at 12°C . for a week did not decrease the sensitivity of the method. A statistical analysis of the zones of hydrolysis revealed that slight differences in the concentration of starch, concentration of agar, amount of enzyme per disc, pH of the medium, temperature of incubation and the length of the incubation period introduced significantly different zone diameters. Differences in the depth of the medium and in the type and molarity of the buffer, within limits, did not affect the diameter of the zone. Reproducibility of results was very good; disc to disc, plate to plate and day to day variations having been eliminated through refinement of technique and development of a suitable testing medium. The method is simple, highly accurate and allows the simultaneous testing of a large number of samples. Since the potency of the amylase is related linearly to the diameter of the zone, an assay for bacterial, fungal, malt, pancreatic and salivary alpha amylase is being developed.

A nutritional and cytological study of a stalked bacterium from well water. E. A. GRULA and R. H. WEAVER, University of Kentucky.—Bowers (1951) was able to isolate on nutrient agar, a vibrio-shaped, gram-negative stalked bacterium from water obtained from a well in the vicinity of Sleepy Hollow, Kentucky. After rather extensive study of the organism, he suggested it to be a *Caulobacter* species. The purpose of this work has been to determine the specific vitamin and mineral requirements of the isolate of Bowers and to investigate further its cytology.

After completion of organic and mineral nutritional studies, the following chemically defined synthetic medium was found to be satisfactory for the cultivation of this organism at 30°C . FeSO_4 , $7\text{H}_2\text{O}$, 20 mg; Riboflavin, $5\mu\text{g}$; Dextrose, 500 mg; K_2HPO_4 and KH_2PO_4 , 250 mg each; and $(\text{NH}_4)_2\text{SO}_4$, 100 mg. pH can be between 6.0 and 8.0.

Two types of intracellular inclusions were observed in this organism and labeled Body "A" and Body "B" for ease of comparison.

Because bodies of the A Type were highly dense and opaque to electron and light transmission; because they could be melted under electron bombardment; because their direct relationship to phosphates could be shown and because they stained intensely with basic dyes, it was concluded that these bodies were aggregates of inorganic phosphate (possibly hexometaphosphate) in union with acidic component, possibly RNA. As such, they may be considered to be volutin.

Since bodies of the B Type are directly influenced by iron and riboflavin levels and, to a lesser extent, phosphate concentrations, and since these chemical entities are known components of respiratory systems; because they show a high transparency to electron beams, decrease with culture age and show a strong internal turgor pressure, it is suggested that the B Type bodies in this organism are cell sap vacuoles possessing a definite membrane and showing a marked response to enzyme precursor substances. It may be further suggested that, because of this growth response to these enzyme precursor substances, bodies of the B Type possess respiratory enzymatic activity and could be the actual sites or loci of oxidation and reduction in these cells.