TABLE 1

Effectiveness of different complements for demonstrating the bactericidal action of normal mouse serum (NMS)

Complement		Titers* of NMS in Presence of Complement	Titer* of Complement	Titer* of NMS in Absence of Complement
Source	Dilution used			
Normal guinea pig (adsorbed)	1:5	16	<2	<2
Normal guinea pig (adsorbed-heated 56 C, 30 min)	1:5	<2	<2	
Irradiated guinea pig	1:10	64	<2	
Irradiated guinea pig (heated 56 C, 30 min)	1:10	<2	<2	
Irradiated rabbit	1:5	32	<2	
Irradiated rabbit (heated 56 C, 30 min)	1:5	<2	<2	

* Reciprocal of highest serum dilution showing bactericidal activity for *Escherichia coli*.

rious process of removing normal antibodies can be eliminated by the use of serum from irradiated animals. Normal bactericidins for *Bacillus subtilis* have been found to disappear from the sera of rabbits by the sixth day after total body X-irradiation with 650 r. Complement titers remained unchanged (Donaldson and Marcus, J. Immunol., **72**, 203–208, 1954).

Swift's snuffle-free white rabbits weighing 1 to 2 kg were subjected to 1000 r total body irradiation delivered in a single exposure at 250 kv, 30 ma, at a distance of approximately 79 cm with 0.5 mm copper and 1 mm aluminum filtration at a rate of 43 to 45 r per min. They were exsanguinated 48 hr later. Guinea pigs were given 200 or 300 r and bled 2 to 5 days later when normal bactericidins were absent. The optimum time for bleeding seemed to depend on the size of the animal. The sera were obtained in the usual manner and stored at −20 C. They retained their activity for at least 3 months.

Hemolytic titrations with sensitized sheep erythrocytes were performed on numerous paired serum specimens obtained before and after irradiation from both rabbits and guinea pigs. The titers were essentially identical. On the other hand, bactericidal tests with the same paired sera demonstrated that the normal bactericidal activity for a strain of *Escherichia coli* (isolated from a normal mouse) was lost following irradiation.

The optimum amount of complement required for activation of bactericidins in mouse serum was determined by titration. Briefly, the test method consisted of adding to each of several sets of tubes containing 0.3 ml of serially diluted mouse serum, 0.2 ml quantities of a complement dilution and 0.1 ml of bacterial suspension containing approximately 1000 *E. coli*/ml. After incubation in a waterbath at 37 C for 6 hr, 0.1 ml aliquots from each tube were spread on EMB agar plates and after 18 hr incubation, colony counts were made. The unusually long reaction period was necessitated by a peculiarity of the strain of *E. coli* chosen and was independent of the other test reagents. Reasons for this choice and details of the method will be published elsewhere.

For almost every lot of complement from irradiated animals tested, dilution of rabbit serum to 1:5 and guinea pig serum to 1:10 was indicated. The results of bactericidal tests with a normal mouse serum pool in the presence of complements from different sources are given in table 1. It is evident that a more sensitive reaction was obtained with serum from irradiated animals, particularly the guinea pig.

DROPLET METHOD FOR OBSERVATION OF LIVING UNSTAINED BACTERIA

A. S. MICHAEL

Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland

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Examinations of living unstained bacteria are usually made in "hanging-drop" slides or in a moist cover slip mount rimmed with Vaseline. A simplified method of making these examinations is described below.

Place a drop of immersion oil on a glass slide

and spread to a thin film covering an area slightly larger than a cover slip. With a wire loop place a drop of fluid containing the organisms on a cover slip. Invert this cover slip and place it directly on the film of oil on the slide and seal with gentle pressure. This procedure entraps the fluid in the oil in the form of droplets. Place the slide on the microscope, rack down the condenser slightly and partially close the iris diaphragm to obtain reduced illumination. Locate the droplets containing the organisms with the low-power objective. Turn the high-power dry or oil-immersion objective into position and focus on the organisms. Lower or raise the condenser to obtain the best illumination, and obtain sharp definition by reducing the aperture of the diaphragm.

The advantages of this method are immediately apparent. Preparation of the mount is simple, and the location of the organisms, which are concentrated in the droplets, is rapid. Focusing is easily accomplished because most of the organisms are in a single plane. No currents are possible in the fluid and streaming is eliminated. The organisms are confined to the droplets and therefore can be easily kept under continuous observation. Motility studies, as Lederberg (J. Bacteriol., **68**, 258, 1954), using a similar procedure, has already pointed out, are made more easily, because an entire droplet can often be observed in one field and the motile organism can move only from one wall of the droplet to another, therefore being in clear view at all times. The entrapment of a single organism in a small droplet is easily accomplished, thus making single cell observations a simple matter. Observation is not limited to bright field illumination, as dark field illumination and phase illumination can also be used to obtain any desirable increase in contrast and detail.

PRESENCE OF NITRITE-ASSIMILATING SPECIES OF *DEBARYOMYCES* IN LUNCH MEATS

LYNFERD J. WICKERHAM

Fermentation Section, Northern Regional Research Laboratory,[1] *Peoria, Illinois*

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Ability to assimilate nitrate is an important biochemical test used in classifying yeasts. The author is not aware of any publication which reports yeasts that assimilate nitrite but not nitrate. Such yeasts do exist, however; and one common source is lunch meat.

Several samples of cold meat cuts were obtained at a supermarket. Two slices of each were placed in sterile petri dishes. After several days at room temperature, colonies of yeasts stood rather prominently above the thin film of bacterial growth on the surface of the meat. Selected colonies were purified, and many of these strains produced sporulation characteristics of the genus *Debaryomyces;* i. e., the asci were formed predominantly by mother-daughter conjugation, and the single ascospores had the usual layer of granules which impart a warty appearance.

The purified cultures were inoculated into tubes of yeast carbon base (Difco[2]), a powdered medium containing pure B vitamins, trace elements, salts, and 1 per cent glucose as carbon source. In 100 ml of distilled water were dissolved 0.26 g sodium nitrite and 11.7 g of yeast carbon base. This 10× medium was sterilized by filtration, and 0.5 ml was added to 4.5 ml sterile distilled water in a test tube. The medium was inoculated from a fresh slant culture and incubated for a week at 25 C. If the resulting culture gave a 3+ density reading (Wickerham, U. S. Department of Agriculture Technical Bulletin No. 1029, 1951) and if the color test for nitrite was negative or nearly so, then the culture had assimilated nitrite. Confirmation was obtained by inoculating a loop of the week-old culture into a second tube of nitrite medium, and the same observations and tests were made a week later. The nitrate test was run concurrently.

[1] Of the Northern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.

[2] The name of the company is furnished for convenience and does not imply the Department's endorsement of its products over those of other manufacturers.