Reports

Use of Fat-Soluble Fluorescent Brighteners on Microorganisms

Abstract. We have applied a fat-soluble fluorescent aid to certain microorganisms. The aid, a stilbyl triazole compound, fluoresced more in the presence of certain strains of flocculating brewing yeasts than in the presence of nonflocculating types. The work suggested the possibility that flocculating yeasts may possess more surface lipids than the nonflocculating organisms. The performance of the compound on rapidly growing cell centers and bacterial spores is described, and the possible application of the technique to other areas of microbiology is outlined.

Very few references are available on the application of fluorescent compounds in visual studies of lipids on microorganisms. Bekker and Tasman in 1942 (*1*) and Mankiewicz in 1952 (*2*) studied the use of auramine O dye in the visualization of lipids or waxes of *Mycobacterium*. The extraction of such organisms with fat solvents caused loss of auramine O fluorescence and, at the same time, loss of acid-fastness. Unlike O, the fat-soluble stilbyl triazole substances are extremely hydrophobic. The compound used in the present work was 2-(stilbyl-4″)-(naphtho-1′,2′:4,5)-1,2,3-triazole-2″-sulfonic acid *n*-octylamide (STC) (*3*).

Darken (*4*) reported on the successful use of more polar type substances of the diaminostilbene class. As applied to yeasts and other microorganisms, her compound fluoresced more strongly in active growing centers and was transferred by growing cells to their offspring. However, the brighteners used by Darken were substantive to protein or cellulose, whereas the substance used in the present work was found to be specific for lipids rather than for proteins, carbohydrates, or yeast nucleic acid.

A typical experiment in which STC was applied to flocculating (class III) and nonflocculating (class I) Guilliland brewing yeasts (*5*) is described below. In this procedure the cells were grown in Wickerham's YM broth, made by rehydrating 3 g of Difco yeast extract, 3 g of Difco malt extract, 5 g of Difco peptone, and 10 g of glucose per liter of medium. Yeasts were washed free of medium with 0.001*M* phthalate buffer at *p*H 3.8 and resuspended in 10-percent buffered glucose at 26°C, glucose being added as a dispersant for the flocculated class III cells. The suspension was adjusted to an optical density of 1.30 at 610 mμ in a Coleman universal spectrophotometer. To 9.0 ml of suspension was added exactly 1.0 ml of STC solution (10 mg of STC per 100 ml of fat-free acetone diluted with three parts of distilled water). The mixture was shaken for 10 minutes at 90 to 100 cy/min; the cells were centrifuged and washed three times with buffer. Fluorescence readings were taken on a model 12B Coleman photofluorometer with primary filter No. 12,221, which does not pass radiation above 365 mμ, and secondary filter 12,222, which passes radiation above 400 mμ. The primary filter was five-sevenths masked to reduce the amount of radiation applied to the suspensions, and the instrument was operated at maximum sensitivity. The fluorescent standard was aqueous quinine sulphate at 1.0 μg/ml adjusted to *p*H 1.0 with concentrated H_2SO_4, and this gave a reading of 100 with the above setting. Class I cells gave readings of about 10 percent fluorescence, while class III cells gave readings of about 100 percent fluorescence. Without STC the readings for both classes were 0. The flocculent cells consistently gave greater fluorescence, which suggested larger amounts of lipids on their surfaces.

The fluorescence of yeast cells after treatment with and centrifugation from 2.50 μg of STC per milliliter of suspension was observed through an ultraviolet microscope. The ultraviolet source was a 200-watt mercury arc lamp (*6*). The exciter filter was Corning No. 5840, and this was protected by heat-absorbing glass. A Wratten 2B barrier filter was placed in the microscope eyepiece. Mature cells exhibited fluorescence on the surface. In contrast with these older cells, the buds and particularly their bases demonstrated much greater fluorescence. In a similar manner, the spores of *Bacillus coagulans* 6125 (*7*) gave greater fluorescence than did vegetative cells.

At present it is not possible to say whether STC is actually absorbed by rapidly growing cell centers or whether it is simply adsorbed on the surface of these centers and becomes more fluorescent owing to higher concentrations of fat-like compounds present there. Experiments are in progress to determine whether the uptake of STC follows any of the classical adsorption laws. It is possible the fat-soluble stilbyl-type brighteners could answer questions in growth and reproduction that the more polar types could not. The fat-soluble optical brighteners should be tried in the study of different classes of cell membranes, as well as in the study of fat metabolism. Experience indicates that a general technique could be developed with STC for detecting traces of adsorbed fatty acids on yeasts and other organisms which might have value in following assimilation or excretion of lipids by a wide range of cell types.

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References and Notes

1. J. H. Bekker and A. Tasman, *Arch. Hyg. u. Bakteriol.* **127**, 7 (1942).
2. C. Mankiewicz, *Can. J. Biochem. and Physiol.* **30**, 106 (1952).
3. Made by Geigy Chemical Co., Ardsley, N.J.
4. M. A. Darken, *Science* **133**, 1704 (1961).
5. R. B. Guilliland, *Brewers' Guild J.* **41**, 246 (1955). Yeasts obtained from Dr. R. B. Guilliland, Arthur Guinness, Son & Co., Ltd., Dublin, Ireland.
6. American Optical Co., Chicago, Ill.
7. This organism was supplied by Dr. C. F. Schmidt, Continental Can Co., Chicago, Ill.

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].