

culture was inoculated by spore suspension as a broad streak about the edge of the petri plate on potato dextrose agar medium. After three days of incubation at $28 \pm 2^\circ\text{C}$, the different microorganisms were streaked at right angles to the J_4 streak. The inhibition zone, if formed, was measured as a clear distance between the J_4 streak and the growth of the test organism.

J_4 was found to be active against *Alternaria brassicae*, *A. brassicicola*, *A. solani*, *A. raphani*, *Aspergillus niger*, *A. sulfureus*, *Fusarium solani*, *Curvularia lunata*, *Phoma palmarum*, *Penicillium janthenellum*, *Cladosporium sphaerospermum*, *Humicola fusco-atra*, *Botrydiplodia theobromae*, *Gleosporium candidum*, *Salmonella typhi*, *Pseudomonas pyocanea*, *Escherichia coli*, *Bacillus subtilis*, *Candida albicans*, *Staphylococcus aureus*, *Shigella* and *Proteus*.

Properties of antibiotic substance

The antibiotic produced by J_4 is thermolabile. With rise in temperature from 40 to 80°C the activity decreases gradually, and at temperatures above 80°C the antibiotic substance gets completely inactivated after 30 min.

On storage the activity is lost rapidly at $35\text{--}40^\circ\text{C}$. The antibiotic can be best stored without any appreciable loss in activity for up to 40 days at pH 7.0 at 7°C . The antibiotic is soluble in *n*-butanol, methanol, isopropyl alcohol, ethanol and ethyl acetate and moderately so in benzene, petroleum ether, toluene and chloroform. It was found to give a single spot in two-dimensional paper chromatography as well as by bio-autographic technique. The antibiotic contained only one component with R_f value 0.733 when the chromatogram was run in butanol:oxalic acid: H_2O (50:2.5 g:50), and with R_f value 0.802 when the chromatogram was run in methanol:ethyl acetate (95:5).

The isolate J_4 was identified as a species of *Streptomyces* as its mycelium does not break up in bacilli or coccoid forms and instead forms a tough, textured mycelium.

The *Streptomyces* isolate described above was found to resemble *Streptomyces bobili*^{4,5}. It produces active substance very similar to cinerubin produced by a known strain of *S. bobili*. However, it differs from the latter in the production of pigments of various shades in most natural media (table 1) and in the ability to utilize xylose. It has therefore been designated as a pigmented and xylose-utilizing strain of *S. bobili*.

The authors thank CSIR, New Delhi, for financial help.

10 October 1988; Revised 17 February 1989

1. Sinha, S. K. and Basuchaudhary, K. C., *Curr. Sci.*, 1977, 46, 784.
2. Shirling, E. B. and Gottlieb, D., *Int. J. Syst. Bacteriol.*, 1966, 16, 313.
3. Maerz, A. and Paul, M. R., *A Dictionary of Colour*, McGraw-Hill Company Inc., New York, 1950.
4. Pridham, T. G. and Tresner, H. D., *Bergey's Manual of Determinative Bacteriology*, 8th ed, The Williams and Wilkins Co., Baltimore, 1974, p. 748.
5. Waksman, S. A., *The Actinomycetes*, The Williams and Wilkins Co., Baltimore, 1961, Vol. 2.
6. Lyons, A. J. and Pridham, T. G., *Dev. Ind. Microbiol.*, 1973, 14, 205.
7. Shirling, E. B. and Gottlieb, D., *Int. J. Syst. Bacteriol.*, 1972, 22, 265.

SCREENING FOR OLEAGINOUS YEASTS USING REPLICA PRINTING TECHNIQUE COUPLED WITH DENSITOMETRIC SCANNING

SNEHA GOGTE, KALPAGAM POLASA and C. RUKMINI

National Institute of Nutrition, Indian Council of Medical Research, Hyderabad 500 007, India

MICROORGANISMS have been used as a source of protein and vitamins¹. Yeast is commercially produced for animal feed². Oleaginous yeasts are capable of accumulating oil within the cells³. Microbes can be exploited to produce fat. The advantages of using microbes are their rapid rates of growth and ability to accumulate fat using naturally occurring substrates. Thus single cell oil has recently acquired importance⁴. With this in view, we have screened selected environments for the presence of oleaginous yeast⁵. For screening a large number of isolates, routine methods like pure culture isolation and extraction of fat by chemical methods from every isolated culture are laborious and time-consuming. There is need for a quick screening method for picking up promising oleaginous strains.

The replica printing method was first reported by Evans *et al.*⁶ to select high fat-accumulating mutants of *Candida curvata*, *Lipomyces starkeyi* and *Trichosporon cutaneum* produced by artificial mutagenesis.

Environments with high probability of isolating oleaginous yeasts were selected. Representative samples of different environments were obtained. About 120 samples were collected for screening from (i) industrial sewage from starch industries and breweries; (ii) dairy waste; (iii) bakery waste; (iv) soil from petrol filling stations and oil mills; (v) fruit waste from fruit juice and pulp industries; and (vi) oilseeds.

Yeast extract malt agar medium (YEMA) was used to plate the samples to isolate yeasts. Nitrogen-limited medium (NLM) with 2% agar as described earlier⁷ was used to cultivate oleaginous yeasts.

Samples obtained were immediately processed and homogenized and ten-fold serial dilutions were prepared. They were plated on YEMA plates and incubated at 26°C for 72–96 h.

From the YEMA plates, colonies were replica-plated onto NLM agar plates in duplicate in the following manner. Nitrogen-limited agar plates were divided into squares and were numbered. Individual colonies were picked from the YEMA plates with sterilized wooden sticks and spotted onto the NLM agar plate, one in each square. These were then incubated for 72–96 h at 26°C.

An impression of the colonies on the NLM agar plates was made on filter paper (Whatman No. 1) by pressing it against the plate. The filter paper was dried under vacuum for 30 min at 50°C and stained for 20 min with Sudan Black B (0.08% w/v in 95% ethanol). Excess stain was removed by washing in 95% ethanol and destaining again in 95% ethanol for 5–10 min. The filter paper was finally air-dried⁸.

A photovolt densitometer, model 52.CN.Y., was used. The densitometer is provided with a scanner that measures percentage absorption of the incident light (525 nm). The intensity of colour of colonies stained specifically for fat was recorded as peaks on a recording chart. Thus percentage absorption is a direct measure of the quantity of fat accumulated by the strain.

Lipid extraction was done chemically as described previously by Sobus and Holmlund⁹.

Yeast strains

i) Different isolates of yeasts as samples; ii) *Trichosporon cutaneum* NCIM 3151 as positive control,

high lipid-accumulating strain (lipid content was 40%); iii) *Saccharomyces cerevisiae* No. 3076 (NCIM, NCL, India) as negative control (lipid content was 10%).

Figure 1 shows the result of the staining of yeast strains isolated from different environments, along with a positive control and a negative control. Figure 2 shows a densitometric scan of stained colony impressions corresponding to figure 1. Per cent absorption is in direct proportion to the

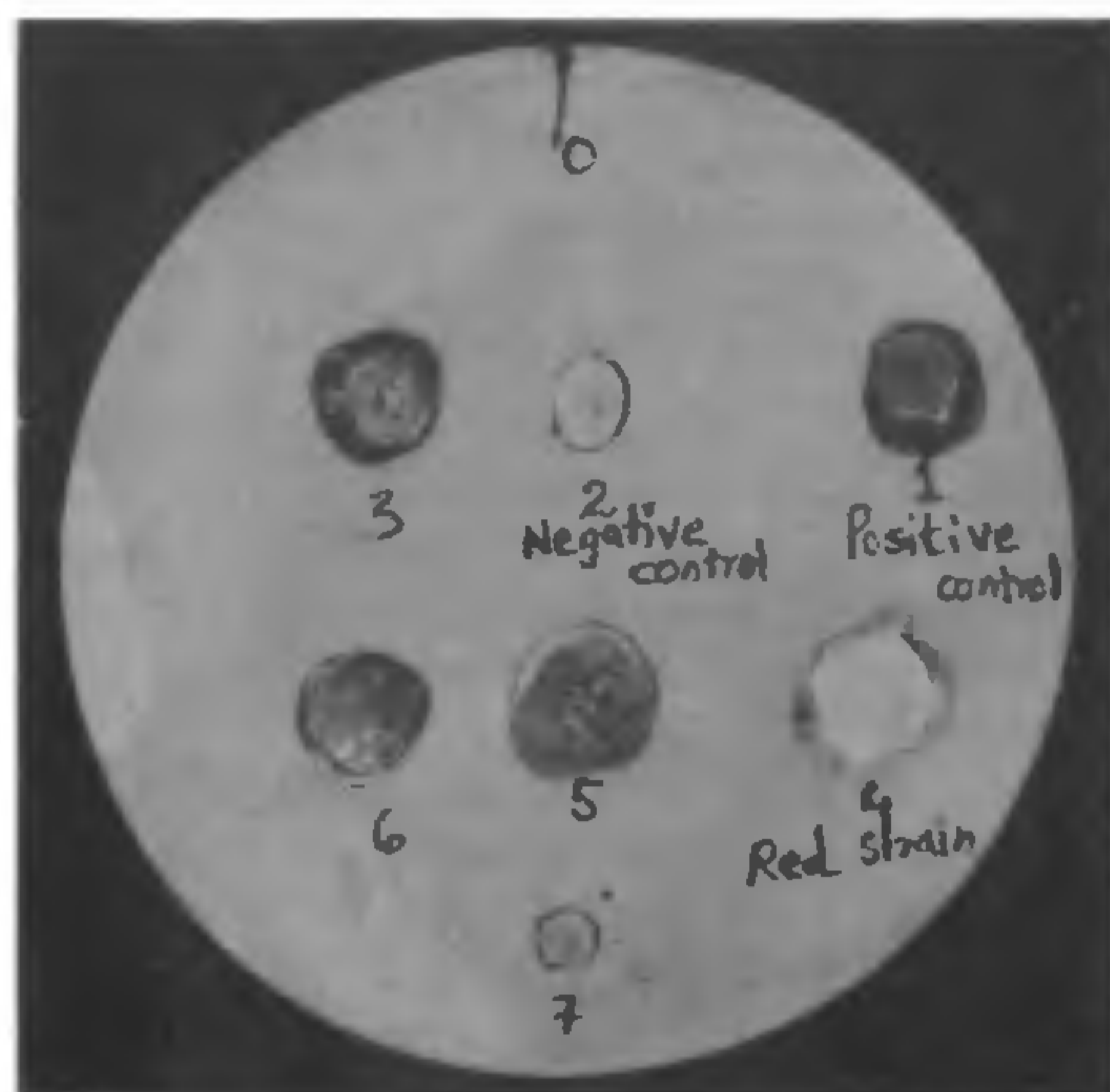


Figure 1. Yeast colony impressions after replica printing stained with Sudan Black B. 1, *Trichosporon cutaneum*—positive control; 2, *Saccharomyces cerevisiae*—negative control; 3, *Endomyces*; 4, *Rhodotorula rubra*; 5, *Debaromyces hansenii*; 6, *Hansenula jadinii*; 7, *Candida krusei*.

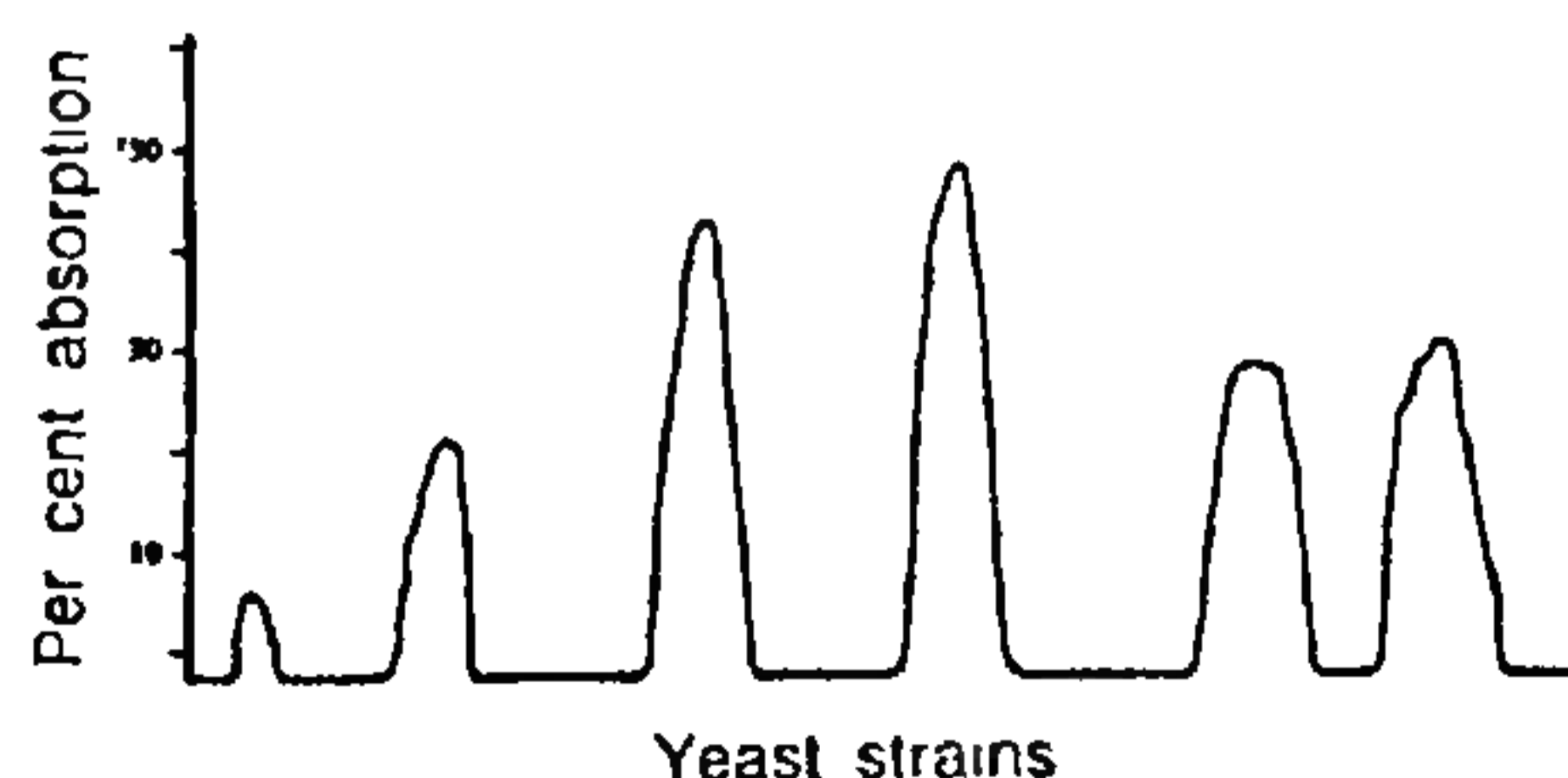


Figure 2. Densitometric scan of stained colony impressions from left to right: negative control *S. cerevisiae* NCIM 3076, positive control *T. cutaneum* NCIM 3151, *C. krusei*, *Endomyces*, *D. hansenii*, *H. jadinii*.

Table 1 Correlation between optical density of stained oleaginous yeasts after replica printing and fat yield

Yeast strain	Fat staining after replica printing	Optical density at 525 nm of stained colonies	Fat yield (mg of fat/100 mg of dried biomass, carbon source glucose)
<i>Trichosporon cutaneum</i> NCIM 3151	+	45	40
<i>Saccharomyces cerevisiae</i> NCIM 3076	—	5	10
<i>Endomyces</i>	+	52	33 (55)*
<i>Rhodotorula rubra</i>	**	**	35
<i>Debaromyces hansenii</i>	+	33	27
<i>Candida parapsilosis</i>	+	38	29
<i>Candida krusei</i>	+	22	23
<i>Hansenula jadinii</i>	+	33	27

Coefficient of correlation between optical density and fat yield +0.932.

*Carbon source xylose.

**Could not be detected.

intensity of colour development on the filter strip *Rhodotorula rubra* (red-pigmented) isolated from sewage was not stained after replica printing and was not scanned. It was seen that strains with less than 10% accumulated lipid were not stained and their per cent absorption was nil.

Intracellular lipid was extracted chemically from dried cell biomass. Fat was estimated gravimetrically as mg% fat. Table 1 shows the fat content of different yeast strains. It also shows that densitometric measurement of individual strains compares well with intracellular fat content (coefficient of correlation +0.932).

We have demonstrated that this method could be applied to selection of oleaginous microbes in samples containing a mixed group of microorganisms. Further we have quantitated the fat content of the strains by densitometric scan of stained colony impressions after replica printing. Thus, this method is as simple as the existing replica printing technique, and offers the advantage of simultaneous estimation of fat content. Colonies with high lipid content showed dark blue coloration and higher per cent absorption. Those with moderate lipid content showed light blue coloration after staining and lower absorption. Those with poor lipid accumulation could not be stained and gave no absorption. The oleaginous yeasts were cultivated on NLM as this medium enhances lipid accumulation. Even though the red-pigmented strain *R. rubra* accumulated lipid to as much as 35%, this technique could not be applied successfully to quantify the amount of fat accumulated by this strain as the red pigment produced by the strain interfered with the

fat staining. Thus the present procedure has limitations in screening pigmented yeast strains.

The authors are grateful to Dr B. S. Narasinga Rao for his suggestions and keen interest.

13 January 1989; Revised 1 March 1989

1. Khilberg, R., *Annu. Rev. Microbiol.*, 1972, **26**, 427.
2. Kharatyan, S. G., *Annu. Rev. Microbiol.*, 1978, **32**, 301.
3. Ratledge, C., *Progr. Ind. Microbiol.*, 1982, **16**, 119.
4. Nancy, J. M., Hammond, E. G. and Bonita, A. G., *J. Dairy Sci.*, 1978, **61**, 1537.
5. Hamid, Sh., Shakir, N. and Bhatti, N. K., *Fette, Seifen, Anstrichm.*, 1981, **83**, 30.
6. Evans, C. T., Ratledge, C. and Gilbert, S. C. G., *J. Microbiol. Methods*, 1985, **4**, 203.
7. Evans, C. T. and Ratledge, C., *Lipids*, 1983, **18**, 623.
8. John, A. Washington (ed), *Laboratory Procedures in Clinical Microbiology*, Springer Verlag, 1981, p. 87.
9. Sobus, M. T. and Holmlund, C. E., *Lipid*, 1976, **2**, 341.