A Study of the Effect of Fuchsin Red on the Metabolism of Yeasts

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Introduction

In recent years a good deal of work has been done upon the influence of substances which, although not essential as nutrients, still affect the physiological properties of yeast cells[1]. In this paper I have studied the growth and metabolic activity of yeasts in cultures which have the property of absorbing visible light. I selected such a water-soluble dye, which in small doses, does not harm the yeast to a great extent but gives the culture a distinct color. Fuchsin red was chosen as one of such dyes for the study.

These cultures have deep red color. They naturally absorb all the radiations of the visible light except the red light. This absorption of light is followed by simultaneous change in the cells of the cultures. *Chaetomium*[2] and *Serratia* have been found to be mutated by high doses of wave length above 350mμ. The latter bacterium indicates 6 per cent color sector changes by 10^7 erg/cm^2. without noticeable killing. When cells are vitally stained by photodynamic dyes, e. g., erythrosine, the sensitivity to visible light becomes high 3—6).

The influence of dyeing time, concentration, *p*H, and temperature on photodynamic effects can be interpreted in terms of permeability and absorption of the dye. In *Penicillium*[6] and *E. coli* 15 h [7,8] photodynamic and ultraviolet induced mutation processes are similar regarding close curve and correlation to killing. Phage resistance[9] and Sd 4 mutations[10] are also induced by photodynamic action.

1) W. A. Krehl, and S. J. Llao, *Ann. Rev. Microbiol.*, **5**, 121 38 (1951).
2) A. L. Mc Aulay, J. M. Ford, and D. L. Dobie, *Heredity*, **3**, 109 (1949).
3) R. W. Kaplan, *Naturwissenschaften*. **35**, 127 (1948).
4) R. W. Kaplan, *Nature*, **163**, 573 (1949).
5) R. W. Kaplan, *Arch. Microbiol.*, **15**, 152 (1949).
6) R. W. Kaplan, *Planta*, **33**, (1949).
7) R. W. Kaplan, *Naturwissenschaften*, **31**, 547 (1950).
8) R. W. Kaplan, *Microbiol, Genet. Bull.*, **4**, 12, (1951).
9) R. W. Kaplan, *Naturwissenschaften*, **37**, 308, (1950).
10) M. Demerec, G. Bertani, and J. Flint, *Am. Naturalist*, **85**, 119 (1951).

When fuchsin red is present in yeast cultures it stains the cells and effects a change in the physiological properties of yeasts. To study this change varying amounts of fuchsin red were taken in yeast cultures containing sucrose as source of carbon and ammonium sulfate as source of nitrogen. The metabolic substances formed during the yeast growth were estimated to investigate the effect of the dye in the cultures. These studies were done with *Saccharomyces carlsbergensis* and Dhar Yeast[11]

Experimental

Five cultures, each containing 0.2 g. of calcium carbonate, 0.2 g. of magnesium carbonate, 0.2 g. of sodium chloride, 0.2 g. of potassium sulfate, 0.25 g. of disodium hydrogen phosphate and 2.5 g. of ammonium sulfate were prepared. Above substances were digested in dilute hydrochloric acid. The amount of fuchsin red, as mentioned against each culture in the table of *Saccharomyces carlsbergensis*, was added in them. This was done by making a standard solution of fuchsin red and then taking out a certain volume of this solution containing the requisite amount of fuchsin and introducing it in the culture solution. Total volume of each culture was made to 400 cc. with distilled water and *p*H adjusted to 4.5. These cultures were kept in 750 cc. flatbottom pyrex flasks and 20 g. of sucrose were added in each of them. The flasks were cotton plugged and sterilised by heating in an autoclave for 20 minutes at 10 lbs. pressure. Cultures were cooled and seeded with activated sample of *Saccharomyces carlsbergensis*.

Four cultures, each containing the requisite minerals in the above mentioned amounts, were prepared and the amount of fuchsin red mentioned against each culture in the table of Dhar Yeast was added in each of them by the procedure mentioned above. These cultures were kept in 500 cc. flatbottom pyrex flasks and the total volume of each culture was made 200 cc. To each of these cultures 20 g. of sucrose were added as the source of carbon. These cultures were sterilised as mentioned above and after cooling they were seeded with a trace of an activated sample of Dhar Yeast.

Observation

Following tables show the result of the effect of fuchsin red on the growth of yeast, alcohol formation, acid production and sugar consumption in 5 per cent (w/v) sucrose solution using *Saccharomyces carlsbergensis* variety of yeast under non-aerated conditions. The *p*H of the cultures was 4.5 in the beginning and the temperature variations during the period of fermentation was between 29.4 to 32.4°C.

These cultures were analysed after 18 days. The figures mentioned in the following tables are for 100 cc. of the cultures.

TABLE I

Serial Number	Fuchsin dye added in the culture (mg.)	Reducing sugar left in the culture after fermentation (g.)	Total sugar left in the culture (g.)	Sugar consumed during fermentation (g.)	Percentage of sugar consumed calc. on the basis of sugar originally present.
1	0.25	2.13	2.25	2.75	55.00
2	0.50	1.85	1.99	3.01	60.20
3	1.00	1.58	1.66	3.34	66.80
4	2.00	1.31	1.33	3.67	73.40
5	4.00	0.00	0.00	5.00	100.00

TABLE II

Serial no. according to Table I.	Total acid formed during fermentation (g. equiv.)	Percentage of acid formation calc. on the basis of sugar consumed	Ethyl alcohol formed during fermentation (g.)	Percentage of alcohol formation calc. on the basis of sugar consumed	Dry yeast grown in the culture (g.)	Percentage of yeast yield calc. on the basis of sugar consumed
1	0.0238	0.86	0.64	23.27	0.4284	15.57
2	0.0524	1.74	0.71	23.65	0.4148	13.76
3	0.0619	1.82	0.96	28.74	0.3938	11.79
4	0.0762	2.07	1.39	37.87	0.3888	10.59
5	0.2142	4.28	2.01	40.20	0.3288	6.57

11) N. R. Dhar, and Krishna Bahadur., *The Proceedings of the National Acad. of Sci.*, Vol. 19, Part II, 55–9 (1950).

Following tables indicate the results of the effect of fuchsin red on Dhar Yeast cultures containing 10 percent (w/v) of sucrose as source of carbon and ammonium sulphate as nitrogen source, under non-aerated conditions. These cultures were analysed after 10 days and the temperature variation during the period of fermentation was between 26.4° to 29.8°C. The *p*H of the cultures in the beginning was 4.5. The results mentioned below are for 100 cc. of the cultures.

TABLE III

Serial No.	Fuchsin added in the culture (mg.)	Total sugar left in the culture after fermentation (g.)	Reducing sugar left in the culture (g.)	Nonreducing sugar left in the culture (g.)	Sugar consumed during fermentation (g.)	Percentage of sugar consumed calc. on the basis of sugar originally present
1	0.125	8.36	6.86	1.50	1.64	16.4
2	0.250	7.76	6.86	0.90	2.24	22.4
3	0.500	6.86	6.85	0.01	3.14	31.4
4	1.000	7.66	7.18	0.48	2.82	28.2

TABLE IV

Serial No. according to Table III	Total acid formed during fermentation (g. equiv.)	Percentage of acid formation calc. on the basis of sugar consumed during fermentation	Ethyl alcohol formed (g.)	Percentage of alcohol formation calc. on the basis of sugar consumed	Dry yeast grown during fermentation (g.)	Percentage of yeast yield calc. on the basis of sugar consumed
1	0.4324	26.21	0.69	44.07	0.7356	44.85
2	0.4232	18.75	0.58	25.89	0.7440	33.21
3	0.4232	13.37	0.53	16.87	0.7694	24.50
4	0.4232	15.24	0.44	15.67	0.7862	24.36

Inference

Dhar Yeast shows a marked decrease in alcohol forming property up to the concentration of 5×10^{-4} per cent of fuchsin red in the culture after which this decrease becomes constant. *Saccharomyces carlsbergensis* indicates a rapid increase in alcohol forming property up to the concentration of 2×10^{-3} per cent of fuchsin in the culture after which this rise becomes gradual and slow.

The acid producing property of Dhar Yeast rapidly decreases up to the concentration of 5×10^{-4} per cent of fuchsin in the culture after which there is an increase in the acid production; where as *Saccharomyces carlsbergensis* shows a gradual increase in the acid forming property from the very beginning. This increase is gradual up to the concentration of 2×10^{-3} per cent of fuchsin in the culture after which the production of acid increases very rapidly.

The sugar consuming property of Dhar Yeast increases gradually up to the concentration of 5×10^{-4} per cent of fuchsin in the culture after which this shows an upward trend. In the case of *Saccharomyces carlsbergensis* the sugar consuming property gradually decreases with the increase of fuchsin concentration in the culture.

The yield of dry yeast decreases in both cases. Dhar Yeast indicates a more sudden fall but *Sacchoromyces carlsbergensis* shows a very gradual decrease in the yeast yield.

Thus we see that there is a correlation between the radiation induced and spontaneous change in the physiological properties of the microorganism. The different effects of the same influencing factor on different organisms is in accordance with the observation made in the case of *E. coli* whose one strain, particularly streptomycin dependent strain frequently changes spontaneously and after ultra violet radiation, whereas another strain was stable in both respects[12].

Summary

When fuchsin dye is added in the yeast cultures it causes a change in the physiological properties of the yeasts which is characterstic of the variety of yeast used. For

12) M. Demerce, B. Wallace, E. M. Witkin, and G. Bertani, *Carnegie Inst. Wash. Yearbook*, **48**. 154 (1949).

many properties this change becomes more gradual as the concentration of dye increases in the culture. Although there is a vast difference in the nature of change of alcohol forming property of the yeasts studied above, changes of the other properties show a similar trend in both the yeasts under examination, when higher concentrations of fuchsin red is present in the cultures.

The presence of fuchsin in the culture is poisonous to the yeasts. This is indicated by the fact that it hinders their growth. This hindrance is directly proportional to the amount of dye in the culture.

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