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Interaction of Cobalt and Iron in the Riboflavin Production of *Candida guilliermondii*

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The effect of cobalt on the production of riboflavin by *Candida guilliermondii* has been studied. The yeast was grown in a continuous culture with iron as growth limiting factor. When the iron concentration of the nutrient medium was small enough (0.5 μg/l) cobalt had no effect. At higher iron concentrations cobalt stimulated the riboflavin production and the highest riboflavin production was obtained at an iron concentration of 5 μg/l and a cobalt concentration of 15–25 mg/l. It was concluded that cobalt specifically replaces iron at the site were iron is necessary for the binding of riboflavin in the cell. Thus cobalt competes with iron and produces the same effect as iron deficiency.

Iron is known to inhibit the production of riboflavin by the yeast *Candida guilliermondii*. The optimal iron concentration in the medium is, according to Tanner *et al.*[1], 5–10 μg/l. The effect of some other divalent cations on the riboflavin production of *C. guilliermondii* has also been studied. Thus Levine *et al.*[2] found that manganese, copper, zinc, tin, nickel, and aluminium in concentrations of 1 mg/l do not affect it. Enari [3,4] showed that cobalt and, to a lesser degree, zinc stimulate riboflavin production. He found that the addition of 10^{-4} M of cobalt to the medium shifts the optimum iron concentration from about 10^{-7} M to about 10^{-5} M. On the basis of a study of the uptake of iron and cobalt and their interaction it was suggested that cobalt acted by preventing the uptake of iron into the yeast cell. The cobalt would thus cause an iron deficiency in the cell. Iron deficiency in the cell, whether caused by a low iron concentration in the medium or by the addition of cobalt, would lead to the excretion of riboflavin into the medium.

If this hypothesis is correct, cobalt should have no effect in an iron deficient medium. Attempts to test this in a closed culture were not successful. The failure was obviously due to the manner in which the iron was taken up by the yeast [4]. When grown in a medium with low iron concentration the yeast cell rapidly binds most of the iron. The iron concentration in the cell is thus

normal in the early stages of the experiment. As the yeast multiplies, the iron concentration in the cells gradually decreases until it finally becomes low enough to prevent any further growth. If cobalt is added to such a culture it inhibits the iron uptake in the beginning and an iron deficient yeast is produced much earlier than when no cobalt is added. The only way to produce a yeast culture with a constant low iron concentration is to use continuous cultivation in a chemostat with iron as growth limiting factor [5,6].

The effect of cobalt on the riboflavin production of *C. guilliermondii* when grown in a chemostat with iron as growth limiting factor was studied in order to test the hypothesis mentioned above.

EXPERIMENTAL

The yeast *C. guilliermondii* was grown with vigorous aeration in an all-glass chemostat[5] which was easy to keep free of iron. The capacity of the chemostat was 250 ml and a flow rate of 5 ml/h was used in all experiments. The nutrient medium used contained 30.0 g glucose, 3.8 g diammonium citrate, 4.1 g citric acid (H_2O), 15.2 g trisodium citrate (5 1/2 H_2O), 0.20 g KH_2PO_4, 1.0 g $(NH_4)_2HPO_4$, 0.25 g $MgSO_4 \cdot 7H_2O$, and 5 μg biotin per litre. The citrate was added to buffer the medium to pH 6.0. This buffering was necessary to keep the pH constant. Iron was removed from the medium by shaking with a chloroform solution of ferron and tributylamine [7]. Ferrous sulphate and cobaltous sulphate were then added to give the concentrations desired. In the experiments in which glucose was used as growth limiting factor the amount of glucose was reduced to 5 g/l and 200 μg/l of iron was added. The concentrations actually used in each experiment are given in connection with the results.

The riboflavin produced was determined by direct spectrophotometry of the centrifuged medium [4].

RESULTS AND DISCUSSION

The effect of iron on riboflavin production when iron was used as growth limiting substance was first studied (Fig. 1). The culture was started with an iron concentration of 5 μg/l. At this concentration the yeast produced about 2.4 μg of riboflavin per mg of yeast dry weight. When the iron concentration was gradually increased the riboflavin production decreased until it was

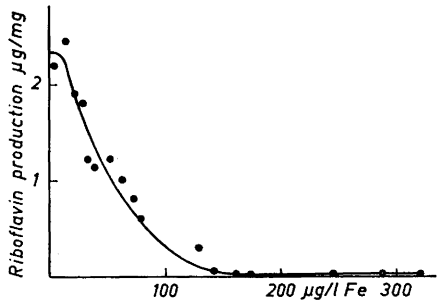

Fig. 1. Effect of iron on the riboflavin production of *C. guilliermondii*.

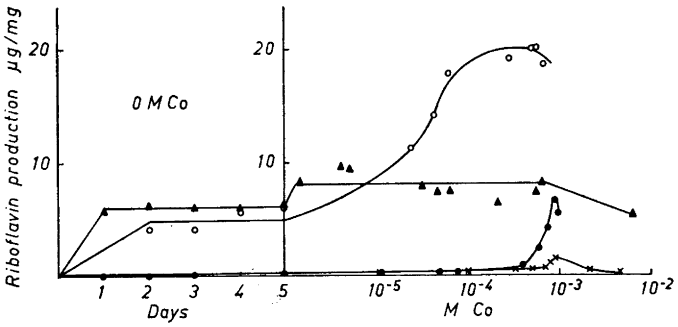

Fig. 2. Effect of cobalt on riboflavin production. × = 200 µg/l Fe in the medium, glucose growth limiting; ● = 200 µg/l Fe in the medium, Fe growth limiting; ○ = 5 µg/l Fe in the medium, Fe growth limiting; ▲ = 0.5 µg/l Fe in the medium, Fe growth limiting.

almost completely inhibited at an iron concentration of 140 µg/l. Thus also when iron is the growth limiting factor it can prevent the production of riboflavin by the yeast. This is in good agreement with our previous studies on the iron uptake of yeast [6]. In these it was found that at iron concentrations below 140 µg/l all the iron in the medium was taken up by the yeast. At iron concentrations higher than this value an excess of iron was left in the medium and the iron concentration of the yeast remained at a steady value of 15 µg/g dry yeast.

In the next experiment we studied the effect of cobalt on the riboflavin production at different iron concentrations (Fig. 2). In all the experiments the yeast was cultivated in a medium without any cobalt until a steady state was reached. After five days cobalt was added to the medium in increasing concentrations until finally a cobalt concentration of $1-8 \times 10^{-3}$ M was reached in 7—8 days. When glucose was used as growth limiting factor almost no riboflavin was produced. When iron used as growth limiting factor riboflavin was excreted into the medium. The lower the iron concentration in the medium, the lower was the cobalt concentration at which the highest riboflavin yield was obtained. Thus for an iron concentration of 200 µg/l the optimal

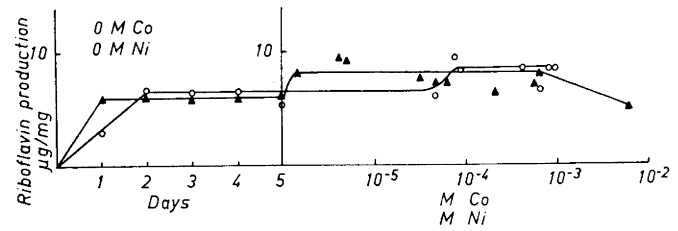

Fig. 3. Effect of nickel and cobalt on the riboflavin production of iron deficient yeast. ▲ = Co added, Fe growth limiting at 0.5 µg/l; ○ = Ni added, Fe growth limiting at 5 µg/l.

cobalt concentration was 10^{-3} M and for an iron concentration of 5 μg/l the optimal cobalt concentration was about 5×10^{-4} M. When the iron concentration was reduced to 0.5 μg/l cobalt had only a very slight effect.

The greatest amount of riboflavin was produced with an iron concentration of 5 μg/l and a cobalt concentration of about 5×10^{-4} M. With the lower iron concentration a smaller amount of riboflavin was produced. This shows that the effect of cobalt is more specific than the effect of iron deficiency. Obviously cobalt replaces iron at the site where the latter is necessary for the binding of the riboflavin in the cell. At an iron concentration of 0.5 μg/l cobalt has no effect but the iron deficiency also retards the general metabolism of the cell and hence the amount of riboflavin released is smaller.

It was suspected that the small effect of cobalt at an iron concentration of 0.5 μg/l was due to a general toxic effect. In order to test this hypothesis an experiment was performed with nickel, which, as was shown by earlier experiments [4], has no specific effect on riboflavin but exerts a general toxic effect leading to autolysis of the yeast and consequently to a higher riboflavin concentration in the medium. In this experiment it was found that the effects of cobalt and nickel were of the same order of magnitude (Fig. 3). I can be concluded that cobalt had no specific effect on the riboflavin production of the yeast when only 0.5 μg of Fe per litre was present in the medium.

It has thus been proved that the effect of cobalt on the production of riboflavin by *C. guilliermondii* is due to an interaction with iron and that cobalt produces an iron deficiency in the yeast.

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