The Effects of 2,4-Dichlorophenoxyacetic Acid on Growth and Respiration in Yeasts*

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The 2,4-dichlorophenoxyacetic acid has been widely used as an effective herbicide. Its effects on higher plants were studied by Fang and Butts¹⁾, Akers and Fang²⁾, Morre and Rogers³⁾ and Key *et al.*⁴⁾. Concerning the effect of 2,4-D upon the respiratory activity of higher plants, Brown⁵⁾ reported that 2,4-D stimulated the respiration of seedlings of bean plants. Kelly⁶⁾ found that the respiration of pea stem tissues was accelerated by low concentrations of this substance. Further, Humphreys and Dugger^{7,8)} studied the effect of 2,4-D on respiration in pea, corn and oat seedling.

On the other hand, in lower plants such as algae and bacteria, the effect of 2,4-D on their respiratory activity has also been reported; it was proved that the respiration of *Spirogyra*⁹⁾, *Chlorella*¹⁰⁾, *Azotobacter* and *Rhizobium*^{11,12)} was stimulated by low concentrations of 2,4-D.

As far as the author is aware, yeasts have never been used in the study of 2,4-D. The present study deals with the effect of 2,4-D on the growth and respiratory activity of yeasts.

Materials and Methods

Three kinds of yeasts were used: *Rhodotorula glutinis* (wild yeast), *Saccharomyces cerevisiae* (baker's yeast) and *Saccharomyces carlsbergensis* (bottom brewer's yeast).

The yeasts were inoculated on 50 ml. of Henneberg solution contained in 300 ml. Erlenmeyer flasks and cultured at 30°. The details of culture methods used were identical with those described in a previous paper¹³⁾. In the present studies both the shaking culture (aerobic condition) and the static culture (relatively anaerobic condition) were used. Yeasts were grown in a solution containing 2,4-D (final concentration between 0.004 M and 0.046 M), and were collected by centrifugation at 4,000 r.p.m. at the end of the growth period. Then they were washed three times with distilled water, dried in an air bath, and their dry weights were measured.

In the test of respiration of yeasts, the cells were collected at logarithmic phase and the usual Warburg procedures were employed. The materials and their final molar concentration in each reaction chamber were as follows: cells suspension, 0.5 ml.; glucose as substrate, 0.025 M; phosphate buffer, pH 5.4, 0.025 M; 2,4-D as sodium salt, from 0.01 M to 0.05 M. The volume of solution in the reaction chamber was adjusted with distilled water to make 2 ml. in total. The center well contained 0.5 ml. of 20 per cent KOH and a fluted filter paper strip. The gas phase was air and the temperature was 30°.

Results

Effects of 2,4-D on the growth of yeasts.

The inhibitory effects of 2,4-D on the growth of three kinds of yeasts are shown

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in Figs. 1, 2 and 3. As will be seen in Fig. 1, the wild yeast, *Rhodotorula glutinis*, is more sensitive to 2,4-D than other yeasts, *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*. The growth of *R. glutinis* under the shaking culture was generally greater than that under static condition whether in the presence of 2,4-D or in its absence. In Fig. 2, it is indicated that baker's yeast is more sensitive to 2,4-D, if the yeast is cultivated under the shaking condition. Fig. 3 shows that brewer's yeast, *S. carlsbergensis*, is the most resistant species to 2,4-D among the three kinds of yeasts.

Effects of 2,4-D on the respiration of yeasts.

Effects of 2,4-D on the respiration of the three kinds of yeasts are shown in Figs. 4, 5 and 6. The respiration of *R. glutinis* was stimulated by low concentrations of 2,4-D. On the contrary, high concentrations of 2,4-D inhibited the respiration. On *S. cerevisiae* the respiration of exogenous glucose was stimulated fairly by 2×10^{-2} and 3×10^{-2} M of 2,4-D, but only a little effect was observed at higher

Fig. 1. The effects of various concentrations of 2,4-D on the growth of *Rhodotorula glutinis*. Solid bar: shaking culture. Clear bar: static culture.

Fig. 2. The effects of various concentrations of 2,4-D on the growth of *Saccharomyces cerevisiae*. Solid bar: shaking culture. Clear bar: static culture.

Fig. 3. The effects of various concentrations of 2,4-D on the growth of *Saccharomyces carlsbergensis*. Solid bar: shaking culture. Clear bar: static culture.

Fig. 4. The effects of various concentrations of 2,4-D on the respiration of resting *Rhodotorula glutinis* cells. Closed circles: respiration of glucose. Open circles: endogenous respiration.

Fig. 5. The effects of various concentrations of 2,4-D on the respiration of resting *Saccharomyces cerevisiae* cells. Closed circles: respiration of glucose. Open circles: endogenous respiration.

Fig. 6. The effects of various concentrations of 2,4-D on the respiration of resting *Saccharomyces carlsbergensis* cells. Closed circles: respiration of glucose. Open circles: endogenous respiration.

Fig. 7. The effects of various concentrations of 2,4-D on the respiration of dried *Saccharomyces cerevisiae* cells.

Fig. 8. The stimulatory effect of 2,4-D on the respiration of resting *Rhodotorula glutinis* cells.

concentrations. However, the endogenous respiration was not stimulated in any concentration tested but it was inhibited. The respiration of exogenous glucose on *S. carlsbergensis* was inhibited slightly by 2,4-D. Its endogenous respiration was almost unaffected.

Effect of 2,4-D on respiration of dried yeast.

Fig. 7 indicates the effect of 2,4-D on the respiration of dried baker's yeast. The respiration was fairly inhibited by a low concentration of 2,4-D.

Durability of stimulated respiration.

Fig. 8 shows the durability of stimulated respiration of wild yeast, *R. glutinis*, which demonstrates that the effect of 2,4-D continued for about 10 hours.

Discussion

The effect of 2,4-D on the growth of yeast used in these investigations was inhibitory rather than stimulatory at any concentrations tested (from 4×10^{-3} to 4.6

$\times 10^{-2}$ M). Especially, the growth of *R. glutinis* was inhibited greatly as compared with the other two kinds, *S. cerevisiae* and *S. carlsbergensis*.

According to Johnson *et al.*^{11,12}), the respiration of *Azotobacter vinelandii* was inhibited perfectly with 9.2×10^{-3} M of 2,4-D. Wedding *et al.*¹⁰) reported that the respiration of *Chlorella* was inhibited by 5×10^{-4} M or higher concentrations of 2,4-D. Suematsu⁹) also observed that the respiration of *Chlorella* was inhibited perfectly by 1 per cent (about 2×10^{-2} M) of 2,4-D.

In the present study, it was indicated that the respiration of yeasts was more resistant to 2,4-D than are *Azotobacter* and *Chlorella*. On *R. glutinis* the respiratory activity was stimulated even by 1×10^{-2} , 2×10^{-2} and 3×10^{-2} M of 2,4-D, and never inhibited. The inhibition occurred only at 4×10^{-2} M. Moreover, the respiration of *S. cerevisiae* and *S. carlsbergensis* was more resistant to 2,4-D than that of *R. glutinis*. The resistance of yeast against 2,4-D may be attributed to the non-permeability of cell membrane, however, further investigation is necessary for elucidation of this problem. There appears a relationship between respiratory activity and respiratory inhibition by 2,4-D in these yeasts. Those with stronger respiration seem more susceptible to the inhibitory action of 2,4-D in higher concentrations than the yeasts with lower respiratory activities.

In relatively low concentration (4×10^{-3} M) of 2,4-D the growth of all yeasts is inhibited. This effective concentration is higher than the concentration (9.2×10^{-3} , 5×10^{-4} M) effective in *Azotobacter* as reported by Johnson^{11,12}) and Wedding¹⁰), but is lower than the concentration (from 1×10^{-2} to 5×10^{-2} M) effective in respiratory inhibition. 2,4-D in 1×10^{-3} M inhibited the growth of *R. glutinis*, on the other hand, 2×10^{-3} M of 2,4-D stimulated its respiratory activity.

With regard to growth and respiration of yeasts, it was found that *R. glutinis* and *S. cerevisiae* were more sensitive to 2,4-D than *S. carlsbergensis*. And it was concluded that the growth of yeasts is more sensitive to 2,4-D than respiration is.

An unexpected result was obtained on the effect of 2,4-D upon respiration of dried baker's yeast, *S. cerevisiae*, in the present study. In the case of intact yeast the respiration of this yeast was stimulated by 2×10^{-2} to 3×10^{-2} M of 2,4-D. Nevertheless, there was no stimulatory effect of the dried cells in these concentrations. It is considered that intact cell-structure may be necessary for the stimulation of respiration.

Summary

The effects of 2,4-D on growth and respiration of three kinds of yeasts were studied.

The growth of *R. glutinis* and *S. cerevisiae* was more sensitive against 2,4-D than that of *S. carlsbergensis*.

A stimulatory effect on the respiration of *R. glutinis* and *S. cerevisiae* was observed in relatively high concentrations. On the contrary, the endogenous respiration of *S. cerevisiae* was not stimulated but inhibited. The respiration of *S. carlsbergensis* was slightly affected.

On dried baker's yeast, the respiration was not stimulated at all but rather it was inhibited.

The respiration of intact cells of *R. glutinis* was stimulated for about 10 hours.

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摘 要

西上一義： 酵母の生長と呼吸とにおよぼす 2,4-D の影響

3 種類の酵母, *Rhodotorula glutinis*, *Saccharomyces cerevisiae* および *Saccharomyces carlsbergensis* の生長と呼吸におよぼす 2,4-D の影響をしらべた。

4×10^{-3} M ないし 4.6×10^{-2} M の 2,4-D を与えた生長実験では酵母はつねに阻害を受けた。なかでも呼吸能の高い酵母ほど阻害率が高かった。

呼吸に対する影響では、呼吸能の比較的に高い酵母 *R. glutinis* は比較的低濃度の 2,4-D で呼吸促進の効果がみられ、高濃度では阻害を受けた。呼吸能の比較的に低い酵母 *S. carlsbergensis* ではあまり影響がみられなかった。

乾燥酵母では呼吸促進効果がみられなかった。

R. glutinis に対する呼吸促進効果は、約 10 時間続いた。

一般に酵母に対して影響を与えるためには、*Azotobacter* や *Chlorella* と比べて高濃度の 2,4-D を必要とする。(島根大学文理学部生物学教室)