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Oxidative Metabolism in *Saccharomyces cerevissiae* as Affected by Polychlorinated Biphenyls

Mª C. Tejedor, M. A. Murado[1], and G. Baluja
Instituto de Química Orgánica General, Juan de la Cierva, 3, Madrid, Spain

In spite of the concern about PCBs contamination in such aspects as environment, bioaccumulation and toxicology, particularly concerning mammalian species, relatively little is known on its mode of action. The toxicity of PCBs depends on the species differences in susceptibility to these foreign chemicals, but considering the scarcity of its toxic specificity it suggests that these xenobiotics are involved in some type of interference on general metabolic processes.

The purpose of this paper is to show some data from a study carried out with Saccharomyces cerevissiae cultures to know the response of the yeast grown in fermentable and nonfermentable substrates, in the presence of different dose of the five Aroclor-1232, A-1242, A-1248, A-1254 and A-1260, attending essentially to various parameters involved in the energetic metabolism of the microorganism.

MATERIAL AND METHODS

Culture conditions - 250 ml volume of Erlenmeyer flasks containing 50 ml of the liquid medium described by WALLACE et al. (1968) and supplemented with either 1 % glucose or 3 % ethanol for fermentable and nonfermentable media respectively were used for growing the yeast according to NELSON and WILLIAMS (1971). The inoculum was a suspension of cells obtained from a culture in the logarithmic phase, the incubation carried out at 27 - 28 ºC by shaking and the cell yield determined by measuring the absorbance of aliquots at 660 nm, compared to blanks of fresh medium. Cultures were dosed with 200 µl of solutions of Aroclor in acetone with appropriate concentrations to provide initial concentration levels of 5, 10, 25 and 50 ppm of PCBs in the medium. The

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[1] Present Address: Instituto de Investigaciones Pesqueras. Muelle de Bouzas. Vigo. Spain.

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same volume of pure solvent was added to the control medium. Duplicate assays were conducted for all experiments performed.

Oxygen consumption - The rate of oxygen consumption by yeast cells was determined following the Warburg manometric direct method (UMBREIT et al. 1972), using cell suspensions harvested by centrifugation of a culture in the logarithmic phase and the sediment resuspended in a sterile and fresh medium dosed with 25 ppm of the PCBs assayed.

Detection of respiratory deficient mutants - Series of experiments for assaying the possible mutagenicity of PCBs were made. In this sense the yeast was grown in fermentable liquid medium in the presence of 25 ppm of each of the Aroclor assayed. After a period of 20 hours the cells were harvested by centrifugation, washed twice with the sterile fresh medium, the sediment resuspended again in fresh medium and adjusted to 80 - 120 cells/ ml by dilution. Aliquots of 1 ml were inoculated in Petri dishes of 9 cm of diameter containing the fermentable medium solidified with agar 2 %.

After incubating for 60 - 80 hours at 26 ºC, the dishes were treated through the tetrazolium overlay technique according to OGUR et al. (1957). This technique is based on the fact that reduction of triphenyltetrazolium chloride (TTC, colorless) to formazan (red) is susceptible of coupling with the electron transport of the respiratory chain at the level of the ubiquinone-cytochrome b complex. This process permits to differentiate the colonies formed from normal cells (AER, red color uptaken from formazan) from those colonies developed from deficient mutants in some component of the chain (aer, colorless cells).

Electron transport activity estimation - Since the reduction of TTC is coupled with the limiting step of the electron transport velocity in the respiratory chain, the determination of the formazan developed by the TTC treated cell population provides a suitable method for estimating the electron transport activity.

To study the influence of PCBs on this activity the yeast was allowed to grow in a fermentable solid medium dosed with 8 $\mu g / cm^2$ of the assayed Aroclor. This concentration was achieved by vaporization of appropriate hexane solutions of each of the PCBs assayed on the surface of the solid substrates. After a period of 80 hours incubation at 26 ºC the content of the dishes were sub-

mitted to the TTC test. Once the color was well developed (3 - 4 hours at room temperature) the system formed by both agar plates, between which the cells are inserted, was homogenized in a Sorvall Omni-Mixer for 3 minutes with 10 ml of 0.067 M phosphate buffer, pH 7.5, and 40 ml of acetone : tetrachlorethylene mixture (3:2, v/v), and from the clear lower organic phase, aliquots were taken for measuring the absorbance at 490 nm. Blank controls of the extraction mixture were used.

Although this procedure, similar to another applied by PACKARD to phytoplankton filtrates (1971) does not allow high recovery rates in the agar extraction, it gave, however, highly reproducible values. Therefore, on the basis of comparing the electron transport activity on control and PCBs treated cultures appears to be consistent.

The calibration curve was made by measuring a dilution series of a standard solution of TTC in the buffer above mentioned. Once extracted with the acetone : tetrachlorethylene mixture, after the reduction of TTC with an excess of sodium dithionite, the rate obtained between absorbance and concentration

$$31.8\ A_{1\ cm}^{490\ nm} \gtrless 2.09\ \mu mol/\ ml$$

becomes homologous with that obtained by PACKARD and HEALEY (1968) through estimations by coulombimetric reduction

$$31.8\ A_{1\ cm}^{490\ nm} \gtrless 2.00\ \mu mol/\ ml.$$

RESULTS AND DISCUSSION

As it is shown in Fig. 1 and 2 the response of the microorganism, on the basis of dry weight, in the presence of 25 ppm of each of the Aroclor assayed becomes significantly conditioned by the carbon source in the culture medium. In fermentable media, which produced a rapid growth of yeast, all Aroclor tested developed an inhibitory effect whose intensity follows an order inversely related to the chlorine content of the Aroclor. In nonfermentable media, with a slower growth but higher yield, the two lesser chlorinated Aroclor inhibited much more than the same in fermentable substrates. The higher chlorinated PCBs stimulate the yeast growth however, even when this fact must not be attributed to the PCBs as a carbon source. This was confirmed, on the other hand, by the unchanged PCBs recovered at the end of a series of incubations (TEJEDOR et al., in press).

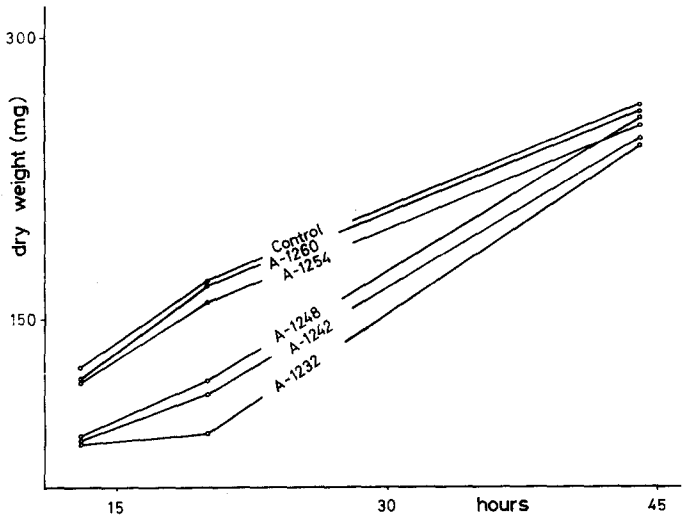

Fig. 1 - Growth rate of S. cerevissiae cultivated in fermentable medium containing 25 ppm of the assayed Aroclor.

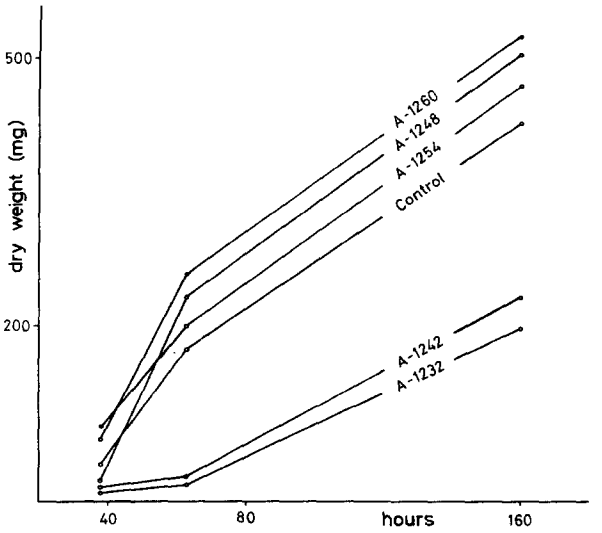

Fig. 2 - Growth rate of S. cerevissiae cultivated in nonfermentable medium containing 25 ppm of the assayed Aroclor.

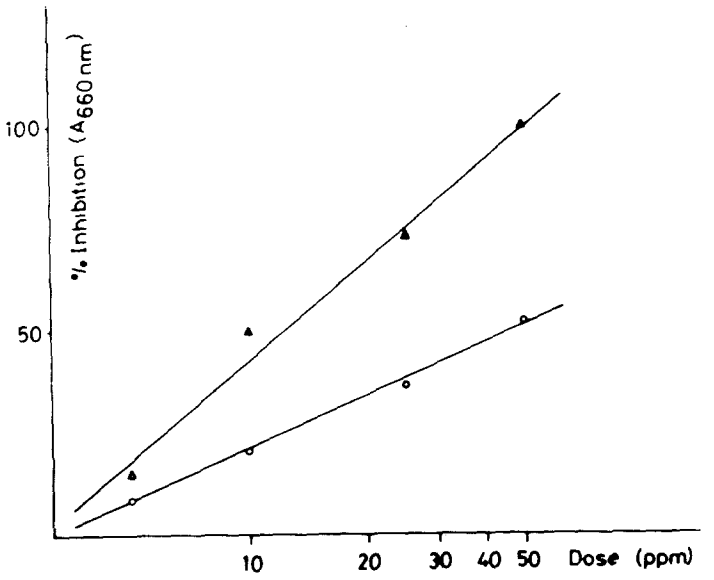

Fig. 3 - Dose-response relationship in S. cerevisiae cultures treated with Aroclor-1232. ▲ : fermentable medium; o : nonfermentable medium.

Furthermore, concerning the most active Aroclor-1232 after exposing periods of 20 and 40 hours in fermentable and nonfermentable media respectively, the dosage-response relationship (fig. 3) may be adjusted to the following equation (>99 % signification in both cases):

$$I = 43.02 \log D - 21.98 \quad (1\ \%\ \text{glucose})$$

$$I = 80.85 \log D - 38.08 \quad (3\ \%\ \text{ethanol})$$

where I = percentage of inhibition measured in absorbance units at 660 nm compared to the control, and D = initial concentration of Aroclor-1232 in ppm.

According to these equations ID_{50} values calculated were 46.7 ppm and 12.3 ppm for fermentable and nonfermentable substrates respectively.

The effects of the PCBs of higher chlorine content on cultures in nonfermentable media is unknown so far.

But the fact that Aroclor of higher toxic incidence be almost four times more active when the microorganism cannot apply the fermentative metabolism to cover its own energetic demand, may suggest, at least in this case, some type of interference in the respiratory mechanism. This hypothesis is conducted in the following demonstration.

Oxygen consumption and electron transport activity

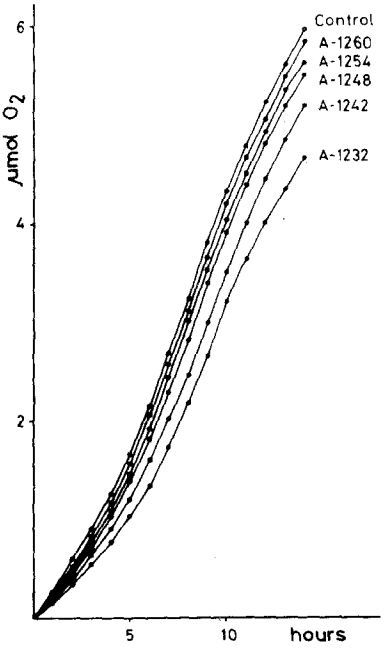

Fig. 4 - Oxygen uptake by suspensions of S. cerevissiae in nonfermentable medium Aroclor treated.

The accumulative consumption of oxygen by cells suspensions (2.5 ml; 4 x 10^6 cells/ml at the beginning of the test) for 14 hours at 28 º C in nonfermentable media and in the presence of 25 ppm of each of the Aroclor assayed is summarized in Figure 4. The sigmoidal curves show that the main phases of a conventional batch culture are accomplished during the period tested in each of the respirometer flasks, that initialy contain a high cell density. In the system formed the evolution of all the parameters directly involved with the biomass may be described by the well known logistic equation

$$\frac{dx}{dt} = rx \left(\frac{K - x}{K} \right)$$

where x = variable to be considered, t = time, r = exponential increase rate of the variable x, K = its maximun value or upper asymptote.

If the experimental values for the oxygen uptake are adjusted to the above equation the depressions pro-

duced by the PCBs are fundamentaly reflected in the upper asymptote (K = carry capacity) which take lower values the smaller the chlorine content of the Aroclor assayed is.

Regarding the hypothetic mutagenic activity of PCBs, no significant variations were found if compared to the frecuency of the natural appearance of respiratory deficiences, detectable by the TTC test. It was observed, however, that the formazan produced in nonfermentable media was sensibly stimulated by the PCBs, and this effect increased as the chlorine content of PCBs decreased (Table I).

TABLE I

Electron transport activity in S. cerevissiae cultures on solid nonfermentable medium containing 8 $\mu g / cm^2$ of various Aroclor. (a): estimated values calculated from manometric data during the period of maximun oxygen uptake.

	Formazan (μmol/culture)	E.T.A. % control	(a): depression O_2 uptake (%)
CONTROL	0.213	100	--
A-1232	0.498	232	35.7
A-1242	0.437	204	24.6
A-1248	0.382	178	14.4
A-1254	0.314	147	8.8
A-1260	0.291	136	3.7

These results suggest that PCBs disturb the electron transport of respiratory chain, as it was also found in other biological entities, particularly on mitochondria isolated from mammalian tissues (PARDINI 1971). It is also worth noting that the oxidative phosphorylation is uncoupled from such a process by some organochlorine pesticides (NELSON and WILLIAMS 1971, SIVALIGAN et al. 1973).

Therefore, the Aroclor that show the higher incidence in the reduction of TTC also decreases the oxygen uptake more intensively as it shown in Table I. Furthermore, the curves shown in Figure 5 reveal that between these two effects there is a very significant linear relationship. If the electron system is normaly trans-

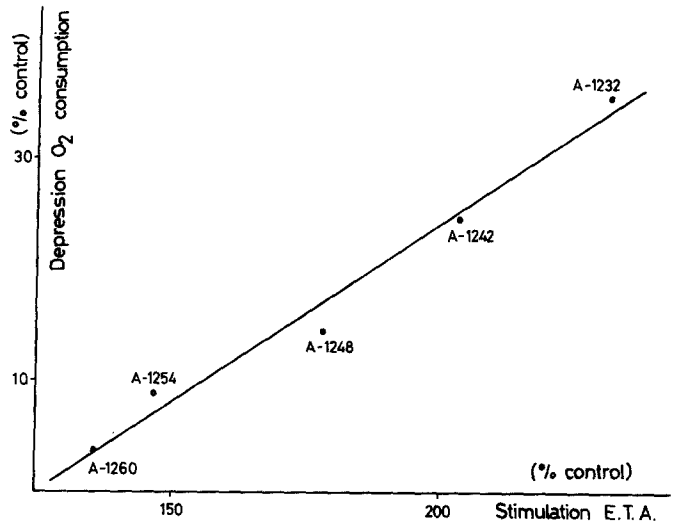

Fig. 5 - Relationship between the oxygen uptake depression and the electron transport activity stimulation in nonfermentable medium Aroclor treated (>99 % signification).

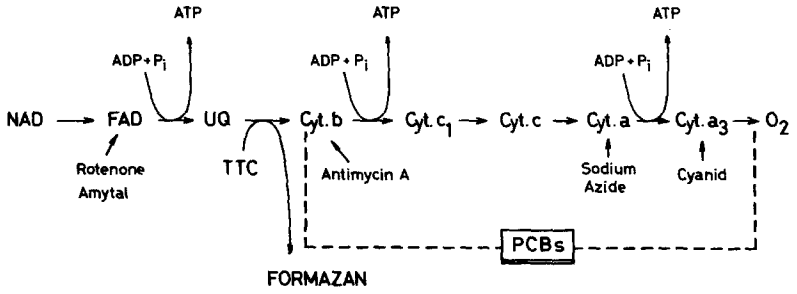

Fig. 6 - Chain of the mitochondrial electron transport system. Coupling sites of some inhibitors and TTC reduction are shown. PCBs effect appears to be localized between the cytochrome b and the oxygen molecule in the cytochromic chain.

ported as far as the ubiquinone-cytochrome b level (coupling site of the TTC reduction), in spite of the delay in the reduction of the oxygen molecule, the PCBs effects appear to be consistent with an inhibition of the electron transport at the cytochrome level such as it is shown in Figure 6.

An ecological consideration - In view of these results, it is interesting to remark that the response of a species to a chemical "stress" leads to reproduce the displacement towards what is called by ecologists "r strategies" which has been once and again appointed as different kinds of community response to environmental disturbances.

Taking into account the differences of the PCB effects on yeast both in fermentable and nonfermentable media, the logic of evolution make us to suppose that if these two kinds of sustrata would be equally available to a Saccharomyces with a mean fermentative capacity as the one studied here, in natural conditions the xenobiotic presence would favour the displacement of the energetic metabolism of the microorganism to the fermentative ways, ignoring the role of the respiratory ones.

This tendency to ignore one of the metabolic possibilities undoubtedly means a regressive tendency K → r. Moreover, in this case the favoured process is a fermentation which, even supposing a faster grow (r manifestation), has a less efficiency than oxidation through Krebs cycle, since the substratum is only partially oxidated. The loss of this efficiency determines also another displacement K → r.

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