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Induction of the Aerobic Methylation of Arsenic by *Candida humicola*

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COX and ALEXANDER (1973) have shown that growing cultures of the yeast Candida humicola methylate arsenite, arsenate, methylarsonate, and dimethylarsinate to trimethylarsine. We have extended these studies (CULLEN et al. 1977) and find that the CD_3 label from added L-methionine-methyl-d_3 is incorporated into the evolved arsine to a considerable extent indicating that the mechanism of the methylation involves S-adenosylmethionine or some related 'onium compound acting as a source of the methyl carbonium ion. A model chemical system involving trimethylsulfonium ion as the methyl donor and SO_2 as the reducing agent has been shown to reproduce the proposed pathway for biological arsenic methylation (CHALLENGER 1951, CHOPRA et al. 1977).

It is important to establish if any enzyme systems are involved in these alkylation reactions and some evidence for this is available. For example, phosphate inhibits the formation of trimethylarsine from arsenite, arsenate, and methylarsonate but not from dimethylarsinate by growing cultures of C. humicola and resting cells of C. humicola prepared from cultures grown in the presence of arsenate produce trimethylarsine more rapidly than cells prepared from cultures proliferating in media without arsenate (COX and ALEXANDER 1973b).

This last result is of interest in view of the possibility that the production of trimethylarsine is a detoxifying mechanism and that a tolerance to arsenic can be built up. This communication describes some experiments which show that growing cells can be induced to produce trimethylarsine from arsenate and dimethylarsinate by preconditioning with dimethylarsinate.

EXPERIMENTAL

The medium (pH 5) described by COX and ALEXANDER (1973a) was used and when necessary it was made 5 mM in one of the arsenicals (CULLEN et al. 1977).

A 100 ml inoculum was added to 1ℓ of medium which was 5 mM in dimethylarsinate and a further 100 ml of inoculum from the same growth was added to 1ℓ of medium. Both new growths were harvested after 40 hours at ∿20° and each was resuspended in 20 ml of medium, 20 ml of medium which was 5 mM in arsenate, and 20 ml of medium 5 mM in dimethylarsinic acid. The optical

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density of each culture was adjusted to 2.5 (455 nm) and each was added to an Erlenmeyer flask fitted with a side arm and septum plug, and capped with a ground glass joint which had two openings to the air protected by sterile cotton plugs. One ml gas samples were removed periodically from the head space to monitor trimethylarsine production using a Varian 1520 gas chromatograph (CULLEN et al. 1977).

RESULTS AND DISCUSSION

Figure 1 shows the effect of preconditioning C. humicola in dimethylarsinate. It is apparent that the organism produces trimethylarsine at a greater rate from both arsenate and dimethylarsinate after this treatment. Figure 2 shows the results of a similar series of experiments where the production of trimethylarsine is plotted over a shorter time scale. In this case no arsine was detected from the control growth to which arsenate had been added. The essential features of these curves were reproducible in other experiments. Thus the effects are real.

Figure 3 shows the effect of preconditioning with arsenate. In this case a dramatic reduction of trimethylarsine production from dimethylarsinate is seen, none being produced in three hours. Compare curves A and C in Figure 2 with A alone in Figure 3. (It is worth noting that these curves A are almost identical indicating the reproducibility of the techniques.) However, trimethylarsine production from arsenate seems to be slightly stimulated by arsenate preconditioning but not to as great an extent as is indicated in Figure 2.

In order to establish if a cell wall transferase is responsible for the observed induction by dimethylarsinate some experiments with ^{74}As labeled arsenate were conducted. C. humicola was grown in the presence of media alone or dimethylarsinate, harvested, and fed the labeled compound. The continuous line (Figure 4) shows the counts of ^{74}As label per 0.25 ml sample of culture as a function of time for the media grown organism whilst the other points present similar results obtained from two different experiments with the preconditioned cells. (Washing with prefiltered media was sufficient to remove background counts due to the labeled growth media.) The data show that no dramatic effect in the ability of the cell to assimilate arsenate has occurred as a result of dimethylarsinate preconditioning. These results also show that the concentration of labeled arsenic builds up in the cell during growth. Yet, in none of our experiments was trimethylarsine produced by preconditioned C. humicola when it was suspended in media which did not contain an arsenical. This seems to point to a permanent build up of the arsenic concentration in the cell at the same time that part of it is being metabolized to trimethylarsine and perhaps this build up is responsible for the induction effects. Further experiments with labeled arsenicals are planned to elucidate these interesting results.

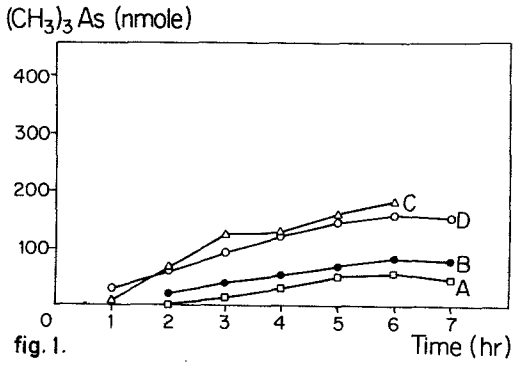

fig. 1.

Figure 1. Amount of $(CH_3)_3As$ in the headspace above growing cultures of C. humicola preconditioned in dimethylarsinate (C and D) and not preconditioned (A and B). In curves A and C the arsenical substrate is arsenate, in B and D it is dimethylarsinate.

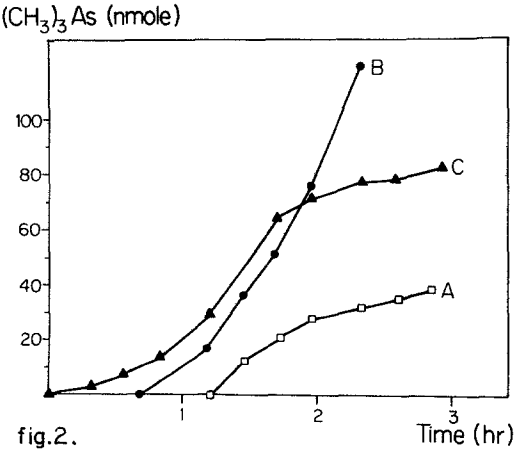

fig.2.

Figure 2. Amount of $(CH_3)_3As$ in the headspace above growing cultures of C. humicola preconditioned in dimethylarsinate (B and C) and not preconditioned (A). In curves A and C the arsenical substrate is dimethylarsinate, in B it is arsenate. No $(CH_3)_3As$ was produced from the control experiment with arsenate substrate.

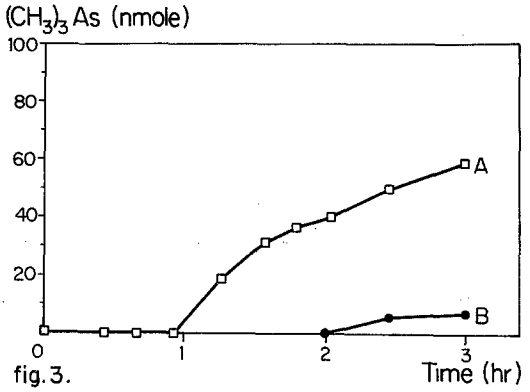

fig. 3.

Figure 3. Amount of $(CH_3)_3As$ in the headspace above growing cultures of C. humicola preconditioned with arsenate (B) and not preconditioned (A). In curve A the arsenical substrate is dimethylarsinate and in curve B it is arsenate. There was no production from the preconditioned cells growing in dimethylarsinate.

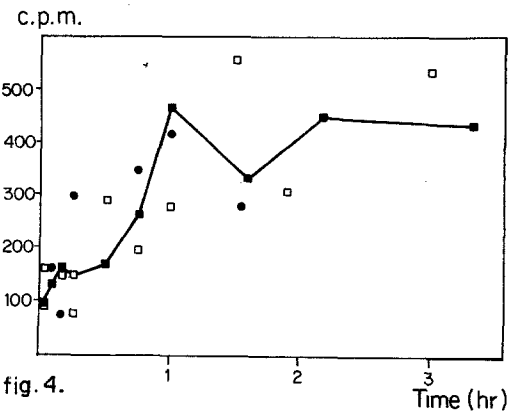

fig. 4.

Figure 4. Counts of ^{74}As incorporated in growing cultures of C. humicola preconditioned in dimethylarsinate (□ and ●) and not preconditioned (■). The arsenical substrate in the three experiments was labeled arsenate.

A series of experiments were conducted to establish if the concentration of arsenicals we used resulted in any inhibition of growth of C. humicola. Inhibition occurred at ∿ 100 mM arsenate (OD monitored) but at even this concentration, dimethylarsinate had no effect.

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