Nitrogen is essential to extend the fermentative growth of *Saccharomyces cerevisiae* in the presence of isoproturon

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Abstract

In nature and in industrial musts, pollutants are available in a complex mixture where some specific microorganisms are able to mineralize or degrade them in more basic and less harmful compounds to the environment. Isoproturon, a phenylurea herbicide, is an environmental contaminant poorly degraded in soils and aquifers, reaching levels considered toxic by European legislation. Thus, it is urgent to discover microorganisms able to degrade or eliminate these pollutants in situations of accidental or systematic contamination, establishing the best culture conditions. Therefore, in this work it was intended to evaluate if peptone, a nitrogen source, essential for yeast growth, amends the extension of fermentative phase of a wild-type strain *Saccharomyces cerevisiae* UE-ME$_3$ caused by isoproturon. The results show that this wild-type yeast grown in peptone starvation conditions, in presence of 100 µM isoproturon, started early the fermentative-respiratory transition.

Keywords: peptone; phenylurea; yeast; glutathione; malate dehydrogenase; alcohol dehydrogenase

1. Introduction

The isoproturon (IPU), 3-(4-isopropylfenyl)-1,1-dimethylurea (IUPAC), is a phenylurea herbicide used in autumn-winter crops which persists in South Europe soils reaching high toxic levels according with the directives of European Union [1-3]. This fact occurs because the IPU is a compound poorly degraded in soils and aquifers. In the literature, IPU is described as a photosynthesis inhibitor, by blocking this process at the level of photosystem II on the electron transport chain of chloroplast, inducing oxidative stress (4,5). Thus, we presume that this phenylurea can also generate oxidative stress, when reach other eukaryotic cells, at level of respiratory chain or electron transport chain of endoplasmic reticulum, increasing the cell content in reactive oxygen species (ROS) causing cell damages [6,7]. Therefore, it is urgent to identify new microorganisms that may contribute to degrade or eliminate phenylurea compounds, establishing the best growth conditions [8,9].

The glutathione (GSH) is present in high concentrations in most living cells. Inside the cells, this tripeptide assumes a critical role in bioreduction and protection against oxidative stress, acting as a radical scavenger through its cell cycle, where glutathione peroxidesase (GPx), detoxifies hydrogen peroxide and lipid hydroperoxides in their respective alcohols and water. On the other hand, the oxidized product of GSH, the glutathione disulfide (GSSG) is regenerated by glutathione reductase (GR) enzyme which uses the reducing equivalents as NADPH provided by glucose-6-P-desidrogenase (G6PD), a key enzyme of the pentose phosphate pathway. The effect of redox buffer is also ensured by cytoplasmic and peroxissomal catalase activity, enzymes involved in removing the hydrogen peroxide in these cell compartments [10,11].

Saccharomyces cerevisiae as facultative respiratory microorganism uses the fermentative pathway for obtaining energy to assist their growth when glucose concentration in culture medium is higher than 0.2%. Under glucose starvation conditions, the yeast fermentation starts a fermentative-respiratory transition increasing the expression of citrate cycle enzymes and multiprotein complexes of respiratory chain as well as malate dehydrogenase (MDH2), a key enzyme involved in the pyruvate regeneration, that keep the citrate cycle running. Thus, changes in energy metabolism of the pyruvate can block its regeneration and increase the fermentation rate modulated by alcohol dehydrogenase activity (ADH) or in lesser extension by lactic fermentation, modulated by lactate dehydrogenase (LDH), even in the presence of O_2, leading to NAD^+ regeneration [10,11].