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MICROBIOLOGY

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EFFECTS OF CARBON SOURCES ON SECONDARY METABOLITES BIOSYNTHESIS

BY *Fusarium verticillioides*

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Fusarium verticillioides is one of the most important fungal pathogens of maize, being responsible of major economic losses worldwide due to its effect on grain quality and mycotoxincontamination, particularly fumonisin B₁ (FB₁), which is extremely harmful to human and animal health. Secondary metabolites (SMs) production often occurs during stationary phase. *F. verticillioides* SMs include toxins such as FB₁, volatile organic compounds such as sesquiterpenes (SQT) and pigments (naphthoquinones), among other metabolites. The aim of the present study was to determine the effect of different carbon sources, consisting of glucose, sucrose, lactose, fructose or xylose, on vegetative growth and secondary metabolism in *F. verticillioides*. For evaluation of growth, *F. verticillioides* M3125 was incubated at 28°C in Czapek Dox Agar (CDA) supplemented with different carbon sources. For evaluation of secondary metabolites, liquid GYAM medium was used (adjusted to pH 3) and cultures were incubated with shaking at 25°C for 7 days. At the end of the incubation period, pH value was registered, FB₁ quantification was performed using a HPLC, SQT quantification was carried out using a GC-MS and total naphthoquinones were quantified with a spectrophotometer. Lower lag phase values were achieved with lactose and sucrose as carbon sources (both disaccharides), compared to glucose, xylose and fructose (monosaccharides). This may be explained by the development of more extended and poor branched hyphae when the fungus grows with disaccharides as carbon sources. This may be a strategy to explore the surrounding media for areas with simpler carbohydrates. The growth rate was higher with fructose and conidiation was higher with lactose. FB₁ biosynthesis was statistically higher with sucrose and glucose (both with pH 3.5) and lower with xylose and fructose. On the other hand, naphthoquinone biosynthesis was statistically higher with fructose (pH 3.7) followed by xylose (pH 4.8), while lower values were achieved with glucose and sucrose. Lactose did not support the biosynthesis of these SMs. On the other hand, SQT biosynthesis was statistically higher with xylose compared to the other carbon sources. According to our results, growing parameters, conidiation and biosynthesis of secondary metabolites are regulated by both environmental (pH) and nutritional factors (carbon source). Also, our results suggest an inverse relationship between FB₁ and naphthoquinones biosynthesis. It is well documented that both metabolites proceed via the polyketide route by formation of a common precursor. In addition, there are global and specific regulators that respond to environmental signals and activate one of the metabolites while repressing the other. SQT production does not show a pattern with the other SMs studied. In fact, its biosynthesis proceeds through a different pathway, the mevalonic acid pathway. It has been proposed that trichodiene synthase activity (the enzyme involved in the first step of SQT biosynthesis) is optimum at pH values near 6, which could explain the higher amount of SQT produced with xylose as carbon source (pH 4.8). However, xylose itself could represent a signal that induces SQT biosynthesis.

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EFFECT OF GLUCOSE CONCENTRATION ON GROWTH AND FATTY ACIDS PROFILE OF TWO *Rhodotorula glutinis* STRAINS

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Certain oleaginous yeasts as *Rhodotorula glutinis* can accumulate neutral lipids up to 70% of dry biomass, consisting mainly in triacylglycerols (TAG), under appropriate culture conditions. Microbial TAG represents a valuable alternative feedstock for biodiesel production. Lipid accumulation occurs under nutrient limitations, mainly nitrogen, with simultaneous excess of carbon source. The lipid synthesis and fatty acids (FA) composition are influenced by several factors as carbon source (type and concentration), aeration, growth, temperature, C/N ratio, pH, among others parameters. Effect of initial glucose concentration (30, 40 and 100 g L⁻¹) on growth, lipid synthesis and FA profile of two strains of *R. glutinis* (R4 and R48) isolated from Antarctica was investigated using a nitrogen-limited medium (MI). Yeasts were incubated aerobically on a rotary shaker during 120 h at 250 rpm and 25 °C. Analytical determinations (biomass, lipid production, lipid content and residual glucose) were performed after 120 h of culture time. Glucose affected the growth of the strains and the FA profile of the microbial TAG. According to the results, the growth exhibited by R48 (12.33-13.83 g L⁻¹) were higher than R4 (8.42-10.83 g L⁻¹) for all glucose concentrations assayed. An increase of the lipid content (from 50.6 to 60.2%) and a decrease of the biomass were exhibited by R4 when the glucose concentration increased from 30 to 100 g L⁻¹. However, significant differences on lipid accumulation and growth were not observed for R48 (p > 0.05, Tukey test). The FA profile of microbial lipids was analyzed by GC-FID. FA with chain length between 14 and 18 carbons dominated the lipidic profile of