Influence of carbon and nitrogen sources on growth, nitrogenase activity, and carbon metabolism of *Gluconacetobacter diazotrophicus*

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Abstract: The effects of different carbon and nitrogen sources on the growth, nitrogenase activity, and carbon metabolism of *Gluconacetobacter diazotrophicus* were investigated. The amino acids asparagine, aspartic acid, and glutamic acid affected microbial growth and nitrogenase activity. Several enzymatic activities involved in the tricarboxylic acid cycle were affected by the carbon source used. In addition, glucose and gluconate significantly increased the oxygen consumption (respiration rate) of whole cells of *G. diazotrophicus* grown under aerobic conditions. Enzymes responsible for direct oxidation of glucose and gluconate were especially active in cells grown with sucrose and gluconate. The presence of amino acids in the apoplastic and symplastic sap of sugarcane stems suggests that these compounds might be of importance in the regulation of growth and nitrogenase activity during the symbiotic association. The information obtained from the plant–bacterium association together with the results of other biochemical studies could contribute to the development of biotechnological applications of *G. diazotrophicus*.

Key words: Gluconacetobacter diazotrophicus, endophyte, metabolism, nitrogen fixation.

Résumé : Le travail consiste à étudier l'effet des différentes sources de carbone et d'azote sur la croissance, l'activité nitrogenase et la metabolisme du carbone sur les cellules de *Gluconacetobacter diazotrophicus* en milieu de culture. Les acides aminés, asparagine, l'acide aspartique et l'acide glutamique ont affecté la croissance microbienne et la fixation d'azote (l'activité nitrogenase). Aussi, plusieurs activités enzymatiques impliquées dans le cycle des acides tricarboxyliques ont été affectées par la source carbonique utilisée. De plus, le glucose et le gluconate ont significativement augmenté la consommation d'oxygène (le taux respiratoire) des cellules entieres de *G. diazotrophicus* cultivé dans des conditions aérobics. Les enzymes responsables de l'oxydation directe du glucose et du gluconate étaient spécialement actifs dans les cellules en croissance avec le saccharose et le gluconate. La présence d'acides aminés dans l'apoplaste et le jus de symplaste de tiges de canne à sucre suggèrent que ces composés puissent avoir de l'importance dans le règlement de la croissance et de l'activité nitrogenase pendant l'association symbiotique. L'information obtenue de l'association plantes–bactéries ensemble avec d'autres études biochimiques pourrait contribuer pour trouver les demandes biotechnologiques de *Gluconacetobacter diazotrophicus*.

Mots clés : Gluconacetobacter diazotrophicus, endophyte, métabolisme, fixation d'azote.

Introduction

Sugarcane (*Saccharum officinarum*) is an important crop in different tropical regions of the world, such as Brazil, Cuba, Australia, and Hawaii. Several reports have shown that this crop can obtain much of its nitrogen from biological nitrogen fixation (BNF) (Boddey et al. 1995; Döbereiner 1997) through *Gluconacetobacter diazotrophicus*, an obligate endophyte of sugarcane that seems to have an important role in this process. This recently discovered bacterium (Cavalcante and Döbereiner 1988) is a small, Gram-negative, aerobic rod exhibiting pellicle formation in N-free semisolid medium amended with 10% sucrose (Reis et al. 1994), which grows in the presence of sucrose concentration up to 30% (Cavalcante and Döbereiner 1988). The exact location of *G. diazotrophicus* within the sugarcane plant has not been yet established satisfactorily. The bacterium has been found in the roots, stems, and aerial parts of several Australian and Brazilian sugarcane cultivars (Li and McRae 1992; Reis et al. 1994). Also, the xylem sap (Reis et al. 1994) and the intercellular spaces (Dong et al. 1994) of sugarcane stems have been suggested as a possible suitable location for the bacterium (Sprent and James 1995) because of the low pO_2 within them that should allow for the nitrogenase expression. *G. diazotrophicus* grows well over a range of pH values (Burris 1994) on different carbon sources, such as glucose, fructose, galactose, glycerol, etha-

Received 26 January 2004. Revision received 2 June 2004. Accepted 9 June 2004. Published on the NRC Research Press Web site at http://cjm.nrc.ca on 10 November 2004.

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 doi: 10.1139/W04-066