Electronic Journal of Biotechnology ISSN: 0717-3458
http://www.ejbiotechnology.info
DOI: 10.2225/vol16-issue3-fulltext-9 *RESEARCH ARTICLE*

Levans production by *Gluconacetobacter diazotrophicus*

María Laura Molinari[1] · José Luis Boiardi[1] ✉

1 Universidad Nacional de La Plata, Facultad de Ciencias Exactas, Centro de Investigación y Desarrollo en Fermentaciones Industriales, La Plata, Argentina

✉ Corresponding author: boiardi@quimica.unlp.edu.ar
Received December 10, 2012 / Invited Article
Published online: May 15, 2013

Abstract

Background: Growth of *Gluconacetobacter diazotrophicus* with glucose as carbon an energy source has been extensively studied. However, there are no reports in the literature describing growth of *G. diazotrophicus* in cultures containing sucrose as carbon source. The first step in sucrose pathway and production of levans was investigated. Biomass, levans, gluconic acid and keto gluconic acids production and levansucrase activity were determined in cultures with different sucrose concentration and nitrogen sources.
Results: The biomass production was maximal in cultures containing 100 g x L^{-1} sucrose and inorganic nitrogen. Gluconic acid production was observed under all conditions tested, at levels up to 9 g x L^{-1} in cultures with sucrose excess and biological N_2-fixation (BNF). Keto gluconic acids were detectable only in cultures with sucrose excess and supplemented with organic nitrogen sources. Levans production, although observed in all cultures, was maximal in batch culture with 100 g x L^{-1} of sucrose and BNF, concomitant with a significant expression of extracellular levansucrase.
Conclusions: Ours results not only describe some unknown aspects of *G. diazotrophicus* physiology, but open up the possibility of developing a technology of levans production by this organism using culture media with sucrose (or some cheaper substitute, like molasses) and without the addition of any N-source because of its ability of fixing atmospheric N_2.

Keywords: gluconic acid, keto gluconic acids, levans, levansucrase, sucrose metabolism.

INTRODUCTION

G. diazotrophicus is an acid tolerant, nitrogen fixing, endophytic bacterium that has been isolated from sucrose-rich plants (Cavalcante and Döbereiner, 1988). This bacterium lacks a sucrose transport system and depends on the secretion of a constitutively expressed levansucrase (LsdA, EC 2.4.1.10) to utilize plant sucrose (Álvarez and Martínez-Drets, 1995; Velázquez-Hernández et al. 2011). Bacterial LsdA catalyses the transfructosylation from sucrose to a variety of acceptors such as: 1) water, leading to sucrose hydrolysis; 2) sucrose, producing fructooligosacharides (FOS); 3) fructans, reaction of polymerization; and 4) glucose, forming sucrose (Trujillo-Toledo et al. 2004, Velázquez-Hernández et al. 2011). Extracellular oxidation of glucose to gluconate is the first step of glucose metabolism in this organism (Luna et al. 2006). This oxidation reaction occurs in the periplasm by action of a pyrroloquinoline quinine-linked glucose dehydrogenase (PQQ-GDH) (Flores-Encarnación et al. 2004; Raspor and Goranovic, 2008). PQQ-GDH expression is stimulated under conditions of BNF and/or C-limitation (Luna et al. 2006). Although *G. diazotrophicus* is present in nature in sucrose-rich plants (Saravan et al. 2008), only few studies have been carried out on the growth of this organism in sucrose containing media.

Fructans are fructose polymers of microbial origin composed of β-(2,6)-fructosyl-fructose linked molecules and side chains (Mussatto and Mancilha, 2007). They can be classified according to their degree of polymerization into oligosaccharides (FOS) or polysaccharides. FOS contain from 3 to 10 monosaccharide units. Consequently, they are low molecular weight carbohydrates and are considered digestible. On the other hand, polysaccharides (called levans) contain more than 11 monosaccharide units. Consequently, they are high molecular weight carbohydrates and are classified as non-digestible (Mussatto and Mancilha, 2007). Fructans are considered functional food ingredients since they affect physiological and biochemical processes in human beings, promote the growth of beneficial bacteria in the colon, mainly *Bifidobacteria* (Mussatto and Mancilha, 2007) resulting in better health and risk reduction of many diseases. So, these polysaccharides are recognized as prebiotics. Moreover, levan polysaccharides have other potential applications in food technology as emulsifiers, stabilizers and coating materials (Maiorano et al. 2008; Singh and Singh, 2010).

In this study the growth behaviour of *G. diazotrophicus* growing with sucrose in batch cultures is reported, focusing in biomass, gluconic acids, LsdA and levan production under different nutritional conditions.

MATERIALS AND METHODS

Bacteria. *G. diazotrophicus* strain PAL5 (ATCC49037/LMG7603) was maintained on agar slopes on a potato medium (potato extract 500 ml, sucrose 20 g, $NaMoO_4$ x $2H_2O$ 2 mg H_3BO_3 2.8 mg, $CuSO_4$ x $5H_2O$ 0.08 mg, $ZnSO_4$ x H_2O 0.24 mg, biotin 0.01 mg, pyridoxine phosphate 0.2 mg, agar 14 g, H_2O 500 ml) and stocked on a defined minimal medium (LGI) described by Stephan et al. (1991) with 25% glycerol at -80ºC (Stephan et al. 1991).

Cultures conditions. Batch cultures of *G. diazotrophicus* were obtained using the chemically defined medium LGIM described by Stephan et al. (1991), varying initial sucrose concentration from 10 to 150 g x l^{-1}. Different N-sources were used: $(NH_4)_2SO_4$ (3 g x l^{-1}) for NoBNF condition, no added N-source for BNF and mixtures of either yeast extract and tryptone (1.5 g x l^{-1} each) or $(NH_4)_2SO_4$ (6 g x l^{-1}) and yeast extract (1.5 g x l^{-1}). Media were sterilized by filtration through a 0.22 µm membrane (Millipore). Cultures were grown at 30ºC in a 1.5-liter working volume fermentation unit (LH, Incelltech 210) and aerated at a rate of 15-20 l x h^{-1}. The dissolved oxygen concentration was continuously measured using a polarographic probe and maintained at the desired level of air saturation (≥ 15% for NoBNF conditions). To attain BNF conditions, the level of air saturation was maintained below 2% as described elsewhere (Luna et al. 2000). The pH was maintained at the desired value (6.00 ± 0.1) by automatic addition of either 0.5 N NaOH or H_2SO_4.

Analyses. At selected times, samples were withdrawn to determine biomass concentration, enzyme activity, and levan, gluconic acid and keto-gluconic acid production.

Biomass dry weight. It was determined in culture samples of 10 mL, centrifuged at 10000 x g for 30 min, washed twice with deionized water and dried at 105ºC.

Quantification of levan. Levans were analyzed from culture supernatant. The levans were precipitated with 2 volumes of 96% (v/v) ethanol, collected after centrifugationat 10000 x g for 20 min, washed twice with deionized water and dried at 60ºC.

HPLC analyses: Gluconic acid and 2- or 5-ketogluconic acids concentrations in the supernatant were analyzed by high-performance liquid chromatography (HPLC) with diode array detector, using a Hamilton PRP-X300 250 x 4.1 mm column. The mobile phase was H_2SO_4 (pH 1.7) at a flow rate of 1.0 ml min^{-1}. Column temperature was maintained at 25ºC.

Levansucrase activity was determined in a reaction using 250 mM sucrose in 100 mM sodium acetate buffer pH 5.2, at 40ºC. Glucose released from sucrose was determined using a glucose oxidase-peroxidase-coupled colorimetric kit (Wiener, Argentina). One unit of LsdA activity (IU) was defined as the amount of enzyme releasing 1 µmol of glucose x min^{-1} based on initial rate measurements at the above mentioned conditions.

RESULTS

Growth

Growth of *G. diazotrophicus* PAL5 in sucrose containing media, either with ammonium or N_2 as N-source, is shown in Figure 1 and Figure 2. Under C-limitation, no biphasic growth was observed, contrary to reported for cultures with glucose as carbon source (Luna et al. 2006). However, under sucrose-excess two phases of logarithmic growth were observed (Figure 2). Growth phase was prolonged in time with increasing initial sucrose concentration. Cultures under BNF did not show an extended lag phase, but showed a lower specific growth rate (Figure 1) with a concomitant decrease in sucrose consumption rate (data not shown).

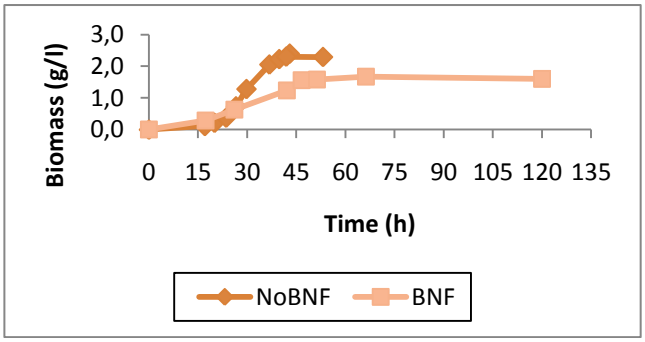

Fig. 1 Growth of *Gluconacetobacter diazotrophicus* PAL5 in sucrose-limited cultures (10 g x l^{-1}) with different N sources. Values in the figures are representative of two independent experiments. The standard deviation between them was lower than 10%.

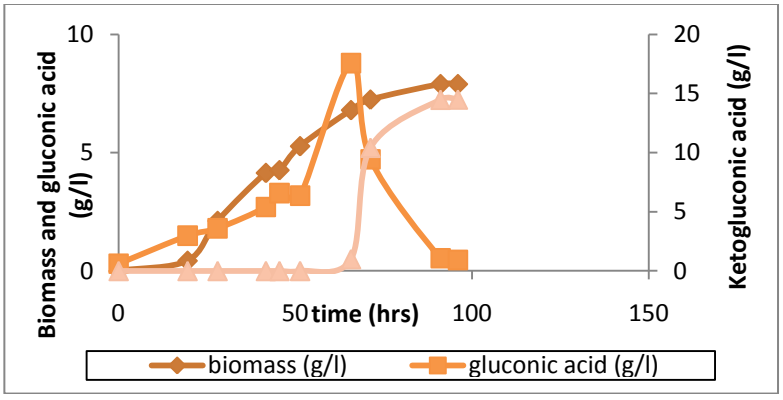

Fig. 2 Gluconic and ketogluconic acids and biomass production in sucrose-excess (100 g x l^{-1}) cultures of *Gluconacetobacter diazotrophicus* PAL5 with 3 g·l^{-1} of $(NH_4)_2SO_4$. Values in the figures are representative of two independent experiments. The standard deviation between them was lower than 10%.

Biomass production

Table 1 shows biomass yields obtained with *G. diazotrophicus* under different environmental conditions. At sucrose concentrations above 20 g x l^{-1} growth was limited by a nutrient different from the C-source. Under these conditions, limitation of cell growth could be ascribed to $(NH_4)_2SO_4$ when used as the N-source. Increasing sucrose concentration led to a decrease in cell yield indicating that the production of other carbon-containing metabolites increased with the concentration of C-source in the culture medium.

DOI: 10.2225/vol16-issue3-fulltext-9

Table 1. Maximum values obtained of biomass (g x l^{-1}), yield (g biomass g $sucrose^{-1}$), LsdA activity (IU mL^{-1}), LsdA specific activity (IU g $biomass^{-1}$) and levan (g x l^{-1}) in batch cultures by *Gluconacetobacter diazotrophicus* PAL5. Data are the mean of at least three repetitions. SD was always lower than 10%. Means followed by different letters are significantly different as determined by one-way ANOVA followed by LSD test ($P < 0.05$).

Maximum values obtained at different initial sucrose concentration					
Sucrose concentration	biomass $g \cdot l^{-1}$	Yield g biomass g $sucrose^{-1}$	LsdA activity IU mL^{-1}	LsdA specific activity IU g $biomass^{-1}$	Levan $g \cdot l^{-1}$
10 g x L^{-1}	1.55^{a}	0.15^{a}	0.14	212	1.11
20 g x L^{-1}	3.50_{b}	0.18^{a}	0.11	132	2.01
40 g x L^{-1}	3.38^{b}	0.08^{b}	0.31	178	8.32
80 g x L^{-1}	5.61^{c}	0.07^{b}	0.51	128	14.15
100 g x L^{-1}	6.00^{c}	0.06^{b}	0.26	50	8.29
150 g x L^{-1}	3.40^{b}	0.02^{c}	0.24	77	11.76
Maximum values obtained under BNF					
20 g x L^{-1}	1.58^{a}	0.079^{a}	1.70	1495	3.38
100 g x L^{-1}	3.20^{b}	0.032^{b}	2.60	907	24.70
Maximum values obtained in cultures with 20 g x l^{-1} sucrose					
N-source					
3 g x l^{-1} $(NH_4)_2SO_4$	3.50^{a}	0.18^{a}	0.11	132	2.01
6 g x l^{-1} $(NH_4)_2SO_4$	3.56^{a}	0.18^{a}	2.55	743	1.90
BNF	1.58^{b}	0.08^{b}	1.70	1495	3.38
6 g x l^{-1} $(NH_4)_2SO_4$ + 1,5 g l^{-1} Y.E.	4.35^{a}	0.22^{a}	3.55	1090	2.24
1.5 g x l^{-1} Y.E. + 1.5 g x l^{-1} tryptone	2.48^{b}	0.12^{b}	2.75	1108	2.98

Free living *G. diazotrophicus* PAL5 was able to fix nitrogen under both C-excess and C-limitation conditions. BNF led to an important decrease in biomass yield compared to those obtained under NoBNF conditions.

No significant differences in biomass production were observed between cultures with 3 and 6 g x l^{-1} of $(NH_4)_2SO_4$. When the inorganic N-source was replaced by a mixture of yeast extract and tryptone biomass yield decreased (Table 1). When the inorganic N-source was supplemented with yeast extract biomass yield increased slightly. This culture was under N-excess and only 50% of initial $(NH_4)_2SO_4$ was consumed (data not shown).

Gluconic acid and ketogluconic-acids production

G. diazotrophicus produces gluconic acid in the first step of glucose catabolism (Álvarez and Martínez-Drets, 1995; Luna and Boiardi, 2008). In sucrose-containing cultures, gluconic acid was produced in all conditions studied, but production did not take place until free glucose was detected in the medium (data not shown). Gluconic acid production was significant during logarithmic growth of the cultures (Figure 2). Maximum gluconic acid concentration obtained was 9.0 g x l^{-1} in cultures with an initial sucrose concentration of 100 g x l^{-1} and BNF. In cultures with 20 g x l^{-1} of sucrose, gluconic acid concentration was around 3 g x l^{-1} independently of the N-source employed. On the other hand, in cultures with excess sucrose (100 g x l^{-1}), either under BNF or NoBNF, gluconic acid production was affected by further oxidation to ketogluconic-acids. Production of ketogluconic acids occurred in the

stationary phase of growth. Oxidation of gluconic acid did not involve a significant increase in biomass concentration (Figure 2).

Levansucrase activity

Table 1 shows a maximum for volumetric activity of LsdA in cultures with 80 g x l^{-1} of sucrose and NoBNF. However, specific enzyme activity increased with the decrease in initial sucrose concentration. Under BNF conditions, LsdA activities, either volumetric or specific, increased both in C-excess and C-limited cultures. The presence of a complex N-source like yeast extract or tryptone (or a mixture of them) also led to a higher LsdA activity.

Levan production

Levan production was observed in all growth conditions tested with conversions of initial sucrose into polysaccharides varying from 8% to 25%. Under NoBFN, the highest levan production (14 g x l^{-1} corresponding to 17.5% sugar conversion) was observed in cultures with 80 g x l^{-1} of sucrose. However, levan production was maximal in cultures under BNF with 100 g x l^{-1} of sucrose when around 25% of initial sucrose had been converted into levan.

In cultures with 20 g x l^{-1} of sucrose up to 17% sugar conversion into levan was obtained under BNF, while around 10% sugar conversion was obtained under conditions of NoBNF. In cultures using complex nitrogen sources (yeast extract + tryptone) around 15% sugar conversion into levan was obtained.

Discussion

Growth of *G. diazotrophicus* with glucose as C-source is well documented (Álvarez and Martínez-Drets, 1995; Luna and Boiardi, 2008; Saravanan et al. 2008). The natural habitat of *G. diazotrophicus* is the internal tissues of sugarcane and other sucrose-rich plants (Saravanan et al. 2008). However, there are few studies on growth and metabolism of this organism with sucrose as C-source.

Biomass yields obtained in this study with *G. diazotrophicus* growing in sucrose-containing media were significantly lower than those commonly reported for other heterotrophic organisms (Roels, 1983). This low efficiency in the C-source utilization for biomass production by *G. diazotrophicus* has already been reported in cultures with glucose (Luna et al. 2006; Luna and Boiardi, 2008). Heterotrophic microorganisms growing under C-excess conditions are not very efficient in the use of the carbon and energy source and show biomass yields much lower than those obtained under C-source limitation. In accordance to this general physiological characteristic, Table 1 shows that the higher the sucrose concentration in the culture medium the lower the biomass yields in cultures of *G. diazotrophicus* PAL5 with more than 20 g x l^{-1} of sucrose.

Free-living G*. diazotrophicus* PAL5 was able to fix nitrogen either under C-excess or C-limiting conditions. In cultures with glucose, in spite of the high energetic cost of N_2-fixation, biomass yield was not significantly affected by BNF, which was ascribed to the ability of *G. diazotrophicus* of expressing alternative branches of the respiratory chain as an adaptation mechanism to different environmental conditions (Tejera et al. 2004). This behaviour was not observed in cultures with sucrose where biomass yields were indeed significantly decreased under BNF (Table 1). It seems that the expression of better coupled branches of the respiratory chain by *G. diazotrophicus* is linked to some specific environmental conditions.

Under C-limitation, no significant differences in biomass production were observed between cultures containing yeast extract and tryptone as N-source and those with ammonium sulphate. A complex N-source does not seem to represent a metabolic advantage for biomass generation in *G. diazotrophicus.* Nevertheless, biomass concentration and yield were increased when adding yeast extract to a medium containing excess of ammonium sulphate (6 g x l^{-1}). The result suggests that part of the amino acids carbon framework from yeast extract could have been incorporated to the biomass leading to the observed increase in yield.

G. diazotrophicus is able to produce gluconic acid via a membrane-bound PQQ-GDH (Lery et al. 2008; Raspor and Goranovic, 2008). Gluconate can be metabolized in the cytoplasm through the pentose phosphate pathway or further oxidized in the periplasm by a gluconate dehydrogenase (GaDH) producing extracellular ketogluconic acids (Luna et al. 2006). In glucose containing cultures, gluconic acid is mostly generated in the lag and early growth phase. Then, microbial growth occurs at the expense of the produced acid. Ketogluconic-acids are produced in the late exponential growth phase only under C-excess conditions (Luna et al. 2006). In cultures with sucrose, gluconic acid could be detected late during the growth phase (Figure 2). The hydrolysis of sucrose by LsdA was necessary as a previous step. The free glucose released was then oxidized by PQQ-GDH. However, the levels of gluconic acid accumulation in these cultures were of a magnitude not comparable to those of *G. diazotrophicus* growing with glucose as carbon source (Luna et al. 2006). In sucrose-limited cultures, the maximum gluconic acid concentration observed was 3 g x l^{-1} and no ketogluconic acids were detected. In cultures with excess sucrose (100 g x l^{-1}), either under BNF or NoBNF conditions, gluconic acid was further oxidized to ketogluconic acids. The production of ketogluconic acids occurred in the stationary phase of bacterial growth and the increase in their concentration paralleled the decrease in gluconic acid concentration. Consumption of gluconic acid did not involve a significant change in biomass concentration. It seems that gluconate oxidation by GaDH is not linked to the generation of available energy for growth. The production of extracellular substances not growth- associated generally takes place in the final stages of bacterial cultures, mainly at conditions of excess C-source.

As reported by Velázquez-Hernández et al. (2011), *G. diazotrophicus* expresses the enzyme LsdA constitutively, being directly responsible for the hydrolysis of sucrose and the generation of available glucose. Sucrose concentration in the culture medium seems to regulate LsdA expression, but the nature of the N-source seems to exert the most significant influence on LsdA synthesis (Table 1). Similar behaviour was observed for the expression of PQQ-GDH in glucose-containing culture media (Luna et al. 2006). It was proposed that the synthesis of this enzyme is stimulated by the need of an efficient utilization of carbon and energy sources under conditions of high energetic demand, as occurs under C-limitation and BNF, which is a behavior commonly observed for other enzymes involved in substrate scavenging (Luna et al. 2006). Clearly, our results on the expression of LsdA in cultures under either sucrose-limitation or BNF conditions are in agreement with such reported behaviour.

Levan may have a wide range of applications in medicine, food, printing, and cosmetics, thus optimization of levan production is an important research area. A central problem in levan production is that the activity of levansucrases is strongly inhibited by the glucose generated from sucrose hydrolysis (Singh and Singh, 2010).

Table 1 shows that *G. diazotrophicus* PAL5 was able to produce significant amounts of extracellular levan by the action of LsdA, which in turn released glucose that was not accumulated in the culture medium but oxidized to gluconic acid, which was further metabolized by bacteria for their own growth. Cultures with high concentrations of sucrose and BNF showed the highest levan production level converting around 25% of the initial sugar into the polysaccharide (which represents around 47% yield on the basis of available fructose). This level of sucrose conversion into levan is similar to that reported for other microorganisms (Moosavi-Nasab et al. 2010). The high levan production observed under BNF and sucrose-excess was accompanied by a significant expression of LsdA, which is responsible for the fructan synthesis. It was postulated that production of extracellular polysaccharides could be a strategy of diazotrophs as a physical barrier leading to the protection of nitrogenase inhibition by O_2 (Dixon and Kahn, 2004).

These results, not only describe some unknown aspects of *G. diazotrophicus* physiology, but open up the possibility of developing a technology of levan production by this organism using culture media with sucrose (or some cheaper substitute like molasses) and without the addition of any N-source because of its ability of fixing atmospheric N_2.

Financial support: This work was supported by grants from CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas) and UNLP (Universidad Nacional de La Plata).

REFERENCES

ÁLVAREZ, B. and MARTÍNEZ-DRETS, G. (1995). Metabolic characterization of *Acetobacter diazotrophicus*. *Canadian Journal of Microbiology,* vol. 41, no. 10, p. 918-924. **[CrossRef]**

CAVALCANTE, V.A. and DÖBEREINER, J. (1988). A new acid-tolerant nitrogen fixing bacterium associated with sugarcane. *Plant and Soil*, vol. 108, no. 1, p. 23-31. **[CrossRef]**

DIXON, R. and KAHN, D. (2004). Genetic regulation of biological nitrogen fixation. *Nature Reviews Microbiology*, vol. 2, no. 8, p. 621-631. **[CrossRef]**

FLORES-ENCARNACIÓN, M.; SÁNCHEZ-CUEVAS, M. and ORTIZ-GUTIÉRREZ, F. (2004). The PQQ-dehydrogenases. A novel example of bacterial quinoproteins. *Revista Latinoamericana de Microbiología*, vol. 46, no. 1-2, p. 47-59.

LERY, L.M.S.; COELHO, A.; VON KRUGER, W.M.A.; GONCALVES, M.S.M.; SANTOS, M.F.; VALENTE, R.H.; SANTOS, E.O.; ROCHA, S.L.G.; PERALES, J.; DOMONT, G.B.; TEIXEIRA, K.R.S. and BISCH, P.M. (2008). Protein expression profile of *Gluconacetobacter diazotrophicus* PAL5, a sugarcane endophytic plant growth-promoting bacterium. *Proteomics*, vol. 8, no. 8, p. 1631-1644. **[CrossRef]**

LUNA, M.F.; MIGNONE C.F. and BOIARDI, J.L. (2000). The carbon source influences the energetic efficiency of the respiratory chain of N_2-fixing *Acetobacter diazotrophicus*. *Applied Microbiology and Biotechnology*, vol. 54, no. 4, p. 564-569. **[CrossRef]**

LUNA, M.F.; BERNARDELLI, C.E.; GALAR, M.L. and BOIARDI, J.L. (2006). Glucose metabolism in batch and continuous cultures of *Gluconacetobacter diazotrophicus* PAL 3. *Current Microbiology,* vol. 52, no. 3, p. 163-168. **[CrossRef]**

LUNA, M.F. and BOIARDI, J.L. (2008). Growth yields and glucose meabolism of N_2-fixing *Gluconacterbacter diazotrophicus* at different culture pH values. *World Journal of Microbiology and Biotechnology*, vol. 24, no. 4, p. 587-590. **[CrossRef]**

MAIORANO, A.E.; PICCOLLI, R.M.; DA SILVA, E.S. and DE ANDRADE RODRÍGUES, M.F. (2008). Microbial production of fructosyltransferases for synthesis of pre-biotics. *Biotechnology Letters*, vol. 30, no. 11, p. 1867-1877. **[CrossRef]**

MOOSAVI-NASAB, M.; LAYEGH, B.; AMINLARI, L. and HASHEMI, M.B. (2010). Microbial production of levan using date syrup and investigation of its properties. *World Academy of Sciences*, *Engineering and Technology*, vol. 44, p. 1248-1254.

MUSSATTO, S.I. and MANCILHA, I.M. (2007). Non-digestiblee oligosaccharides: A review. *Carbohydrate Polymers*, vol. 68, no. 3, p. 587-597 **[CrossRef]**

RASPOR, P. and GORANOVIC, D. (2008). Biotechnological applications of acetic acid bacteria. *Critical Reviews in Biotechnology*, vol. 28, no. 2, p. 101-124. **[CrossRef]**

ROELS, J.A. (1983). Energetics and kinetics in biotechnology. Amsterdam: Elsevier Biomedical Press. 330 p. ISBN 0444804420.

SARAVANAN, V.S.; MADHAIYAN, M.; OSBORNE, J.; THANGARAJU, M. and SA, T.M. (2008). Ecological occurrence of *Gluconacetobacter diazotrophicus* and nitrogen-fixing *Acetobacteraceae* members: Their possible role in plant growth promotion. *Microbial Ecology*, vol. 55, no. 1, p. 130-140. **[CrossRef]**

SINGH, R.S. and SINGH, R.P. (2010). Production of fructooligosaccharides from inulin by endoinulinases and their prebiotic potential. *Food Technology and Biotechnology*, vol. 48, no. 4, p. 435-450.

STEPHAN, M.P.; OLIVEIRA, M.; TEIXEIRA, K.R.S.; MARTÍNEZ-DRETS, G. and DÖBEREINER, J. (1991). Physiology and dinitrogen fixation of *Acetobacter diazotrophicus*. *FEMS Microbiology Letters,* vol. 77, no. 1, p. 67-72. **[CrossRef]**

TEJERA, N.A.; ORTEGA, E.; RODÉS, R. and LLUCH, C. (2004). Influence of carbon and nitrogen sources on growth, nitrogenase activity, and carbon metabolism of *Gluconacetobacter diazotrophicus*. *Canadian Journal of Microbiology*, vol. 50, no. 9, p. 745-750. **[CrossRef]**

TRUJILLO-TOLEDO, L.E.; GÓMEZ-RIERA R.; BANGUELA-CASTILLO, A.; SOTO-ROMERO, M.; ARRIETA-SOSA, J.G. and HERNÁNDEZ-GARCÍA, L. (2004). Catalytical properties of N-glycosylated *Gluconacetobacter diazotrophicus* levansucrase produced in yeast. *Electronic Journal of Biotechnology*, vol. 7, no. 2. **[CrossRef]**

VELÁZQUEZ-HERNÁNDEZ, M.L.; BAIZABAL-AGUIRRE, V.M.; CRUZ-VÁZQUEZ, F.; TREJO-CONTRERAS, M.J.; FUENTES-RAMÍREZ, L.E.; BRAVO-PATIÑO, A.; CAJERO-JUÁREZ, M.; CHÁVEZ-MOCTEZUMA, M.P. and VALDÉZ-ALARCÓN, J.J. (2011). *Gluconacetobacter diazotrophicus* levansucrase is involved in tolerance to NaCl, sucrose and desiccation, and in biofilm formation. *Archives of Microbiology*, vol. 193, no. 2, p. 137-149. **[CrossRef]**

How to reference this article:

MOLINARI, M.L and BOIARDI, J.L. (2013). Levans production by *Gluconacetobacter diazotrophicus*. *Electronic Journal of Biotechnology*, vol. 16, no. 3. http://dx.doi.org/10.2225/vol16-issue3-fulltext-9