

## **Oral Session IV**

# **Physiology and Biochemistry of Acetic Acid Bacteria**

## IV-1: Cellulose biosynthesis in *Gluconacetobacter xylinus* – its Biochemistry, Genetics and Applications.

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Cellulose is the most abundant biopolymer on earth, and the vast majority of the production originates from the green land-plants. However, the studies of the biochemistry and genetics of its biosynthesis were pioneered in bacteria, and *Gluconacetobacter xylinus* (previously *Acetobacter xylinum*) has historically been the favorite model system. Cellulose biosynthesis in *G. xylinus* was first reported by Brown<sup>1</sup> as far back as in 1886. For a long time this was also the only model system that was seriously studied, but in the last decade there has been an enormous increase in the number of reported investigations of the process also in other bacterial species. Based on bio-informatics analyses of the genome sequences now available in international databases we have recently shown<sup>2</sup> that the capacity to produce cellulose is almost certainly very wide-spread among bacteria, and include many of the most familiar and otherwise well-studied bacteria such as *Escherichia coli*.

Cellulose biosynthesis in *G. xylinus* is special in that the quantities produced are very large, and this feature in combination with the unusual physicochemical properties of this cellulose has also resulted in very serious attempts to establish large-scale industrial production of the polymer based on the bacterium<sup>2</sup>. The chemical structure of cellulose is simple, as it is composed of glucose only, linked together by  $\beta$ -(1,4)-linkages. However, the linear chains form very big aggregates (fibers) in which the chains are organized in a parallel fashion with respect to each other, leading to the structure known as cellulose I. Another form, cellulose II (anti-parallel chains) is not found in nature even though it is more thermodynamically stable and can easily be prepared in the laboratory.

The origin of the cellulose I form can be found in the biosynthetic apparatus that is responsible for making the polymer, and in *G. xylinus* it is known that a series of linearly arranged pores on the cell surface represent the extrusion points for the individual glucan chains, each pore extruding about 15 chains oriented in parallel<sup>3</sup>. Cellulose is therefore produced as an exopolysaccharide in *G. xylinus*, and also in other bacteria. The parallel orientation of the extruded chains is undoubtedly the result of the mechanism of polymerization, which is now believed to occur by the chains growing from the non-reducing end<sup>4</sup>. It is also now well established, both biochemically and genetically that the precursor for polymerization is UDP-glucose, and a four-membered operon (*bcsA-D*) is responsible for synthesis of proteins involved in polymerization and transport out of the cells<sup>5</sup>. A number of other proteins also affect the process in as yet only partly understood ways. In conclusion, the history of studies of cellulose synthesis in *G. xylinus* is very long, but many of its mysteries still remain to be explored.

1. Brown, A.J. **J. Chem. Soc.** **49**, 432-439 (1886)
2. Valla, S. et al., **Horizon Press, in press**
3. Brown, R.M. et al. **Proc. Natl. Acad. Sci., USA.** **73**, 143-147 (1976)
4. Saxena et al. **Phytochemistry** **57**, 1135-1148 (2001)
5. Wong et al. **Proc. Natl. Acad. Sci., USA** **87**, 8130-8134 (1990)

## IV-2: Identification and characterization of lipopolysaccharide of acetic acid bacteria

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Lipopolysaccharide (LPS), a major component of cell wall of gram-negative bacteria and one of the main components of Coley's vaccine<sup>1</sup>, is known to have strong adjuvanticity. While acetic acid bacteria (gram-negative bacteria) produces many fermented foods which contribute to health, the substance contributing to health in these foods is not clarified yet. As the existence of LPS in acetic acid bacteria is not clarified yet. The existence and function of LPS in acetic acid bacteria are investigated in this study.

Extracts were obtained from acetic acid bacteria and from that used for the actual production of vinegar, and underwent limulus test. Next, nitric oxide (NO) and tumor necrosis factor (TNF) production were observed by the addition of the extracts to murine monocyte-macrophages cell line (RAW264.7), with or without polymyxin B. TNF production in peritoneal macrophages derived from LPS low responsive mice (C3H/HeJ) was studied by the addition of the extracts.

The extracts were shown to be positive only in LPS specific Limulus test, and be negative in (1,3)- $\beta$ -D-glucan specific Limulus test. Both extracts were observed to induce NO and TNF production in RAW264.7 cells, which were inhibited by the presence of polymyxin B, indicating that the extracts revealed inhibition by polymyxin B. The activity to induce TNF production was inhibited in peritoneal macrophages from C3H/HeJ.

These results showed that LPS exists in acetic acid bacteria, and that fermented foods such as vinegar and yogurt have a possibility of containing LPS which has the macrophage activity, indicating that the LPS in fermented foods derived from acetic acid bacteria contribute to health as one of biological activators.

1. Starnes CO, *Nature*, **360**, 23 (1992)

### IV-3: Control of Acetic Acid Fermentation by Quorum Sensing via *N*-Acylhomoserine Lactones in *Gluconacetobacter intermedius*

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Many Gram-negative bacteria use *N*-acylhomoserine lactones (AHLs)-dependent quorum-sensing systems to regulate gene expression in concert with cell density. Quorum sensing is used to regulate diverse physiological functions, including secondary metabolite production, motility, biofilm formation, and virulence. Two important proteins are involved in AHL-dependent quorum sensing: a LuxR-type protein, which is an AHL-dependent transcriptional regulator, and a LuxI-type protein, which directs the synthesis of AHLs. In general, LuxR-type proteins bind their cognate AHLs once the concentration of the AHL reaches a critical level, and the resulting complex activates the transcription of specific target genes by binding the promoter recognition sequences termed a *lux* box.

Acetic acid bacteria are Gram-negative bacteria with the ability to oxidize ethanol and sugars into their corresponding organic acids. *Acetobacter* and *Gluconacetobacter* are used to commercially produce vinegar because of their high ability to oxidize ethanol into acetic acid and their strong resistance to acetic acid and ethanol. We found that several acetic acid bacteria, including *Gluconacetobacter intermedius* NCI1051, contain a pair of *luxI* and *luxR* homologues. The LuxI/LuxR homologue, named GinI/GinR, comprises a typical quorum-sensing system, in which GinI directs the synthesis of three AHLs with different acyl chains and GinR serves as a transcriptional regulator using the AHLs as its ligands to control the *lux* box-containing promoter of *ginI*. Downstream of *ginI* is encoded a small protein consisting of 89 amino acids, named GinA, and is co-transcribed with *ginI*. GinA, showing no homology with any known proteins, represses (i) oxidative fermentation, including acetic acid and gluconic acid fermentation, (ii) growth in the presence of ethanol, and (iii) antifoam activity of cells. These findings clearly show that the *ginI/ginR* quorum sensing system in *G. intermedius* controls the phenotypes that are characteristic of acetic acid bacteria.

In order to reveal how GinA affects these phenotypes characteristic of acetic acid bacteria and what target genes other than *ginA* are under the control of the *ginI/ginR* regulatory system, we performed 2D-PAGE analysis for comparison of protein profiles of the wild-type strain and a mutant deficient in the *ginI/ginR* regulatory system. As a result, we found that an OmpA family protein, named GmpA, was induced in response to the GinI/GinR quorum sensing system via GinA. GmpA was found to repress oxidative fermentation including acetic acid and gluconic acid fermentation. GmpA, an outer membrane protein possibly specific to acetic acid bacteria and their related genera, appears to play an important role in oxidative fermentation by this unique group of bacteria.

#### IV-4: Oxidative stress resistance systems in *Acetobacter pasteurianus* NBRC3283

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*Acetobacter pasteurianus* is a species belonging to the acetic acid bacteria used for the traditional method of vinegar production (static fermentation) in Japan. This bacterium is an obligate aerobe and requires a sufficient amount of oxygen for its energy generation.

When ethanol is the substrate, this bacterium acquires ATP by oxidizing it with alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) on the cell membrane with the resultant transmission of electrons to the electron transfer system consisting of CoQ and ubiquinol oxidase, which results in the accumulation of acetic acid outside of the cells. After oxidizing all the ethanol molecules, the cells then take-in acetic acid to convert it into acetyl-CoA for the generation of ATP via the general aerobic pathway consisting of the TCA cycle and electron transfer system (peroxidation of acetic acid). Thus, the cells grow by changing these two types of energy generating pathways that both require oxygen molecules, which has been confirmed by real-time monitoring of ATP generation in the cells using a <sup>31</sup>P-NMR method.

In general, aerobic metabolism causes the formation of reactive oxygen species (ROS) in the process of electron transfer, which damages cells by reacting with many cellular components. Aerobic cells generally possess mechanisms to resist ROS-induced damage, and the representative factors involved in the resistance system are superoxide dismutase (SOD) and catalase. Therefore, these two factors were investigated in *A. pasteurianus*, and it was found that this strain expressed only one type each of SOD (SodB type) and catalase (KatE type), which are both growth dependent. These enzymes were shown to be involved in the resistance to oxidative stress, but were not significantly induced by these compounds. A mutant strain disrupted in the catalase gene showed a delay in growth compared to the parental strain at the peroxidation phase although little effect was observed in the ethanol oxidation phase. These results suggest that these enzymes might be involved in the oxidative resistance caused in the general aerobic pathway but not the ethanol oxidation pathway.

The detection of quinol peroxidase activities in the cells was attempted, which is first reported to delete H<sub>2</sub>O<sub>2</sub> by connecting to the respiratory chain in *Actinobacillus actinomycetemcomitans* in the year 2007.<sup>1</sup> The enzyme activity was detected in the cell membrane fraction of *A. pasteurianus* with higher activities in the log phase than in the stationary phase, while catalase showed stronger activity at the stationary phase. This result could indicate that hydrogen peroxide generated during substrate oxidation at the cell membrane might be detoxified by this peroxidase also located on the cell membrane.

From these results, it was suggested that this bacterium might use multiple types of oxidative-stress resistance systems and changes them depending on the type of energy generating system being utilized.

1. Yamada, H. et al., *FEBS Journal*, **274**, 853-866 (2007)

## **IV-5: A Specialized Citric Acid Cycle Requiring Succinyl-Coenzyme A (CoA):Acetate CoA-Transferase (AarC) Confers Acetic Acid Resistance on the Acidophile *Acetobacter aceti***

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Microbes tailor macromolecules and metabolism to overcome specific environmental challenges. Acetic acid bacteria perform the aerobic oxidation of ethanol to acetic acid and are generally resistant to high levels of these two membrane-permeable poisons. The citric acid cycle (CAC) is linked to acetic acid resistance in *Acetobacter aceti* by several observations, among them the oxidation of acetate to CO<sub>2</sub> by highly resistant acetic acid bacteria and the previously unexplained role of *A. aceti* citrate synthase (AarA) in acetic acid resistance at low pH.

Here we assign specific biochemical roles to the other components of the *A. aceti* strain 1023 *aarABC* region. AarC is succinyl-coenzyme A (CoA):acetate CoA-transferase, which replaces succinyl-CoA synthetase in a variant CAC. This new bypass appears to reduce metabolic demand for free CoA, reliance upon nucleotide pools, and the likely effect of variable cytoplasmic pH upon CAC flux. The putative *aarB* gene is reassigned to SixA, a known activator of CAC flux.

Carbon overflow pathways are triggered in many bacteria during metabolic limitation, which typically leads to the production and diffusive loss of acetate. Since acetate overflow is not feasible for *A. aceti*, a CO<sub>2</sub> loss strategy that allows acetic acid removal without substrate-level (de)phosphorylation may instead be employed. All three *aar* genes, therefore, support flux through a complete but unorthodox CAC that is needed to lower cytoplasmic acetate levels.

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## IV-6: OmpR Is Involved to Ethanol Resistance in Acetic Acid Bacteria

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Acetic acid bacteria have strong ability to oxidize ethanol and accumulate high concentration of acetic acid in the culture medium. The environment surrounding acetic acid bacteria during acetic acid fermentation contains high concentration of acetic acid and ethanol, and could survive under those conditions. But other microorganisms could not survive at the same conditions. These phenomena suggested that acetic acid bacteria have the resistance mechanism of both acetic acid and ethanol during acetic acid fermentation. Thus far, we have approached the resistance to acetic acid of *Acetobacter aceti* by genetic and biochemical approaches. But the mechanism conferring ethanol resistance in acetic acid bacteria has been hardly understood despite its importance in vinegar-manufacturing.

To analyze the ethanol resistance in acetic acid fermentation, the acetic acid and ethanol sensitive mutants were constructed from *A. aceti* AS5 by NTG mutagenesis. One of the NTG mutants exhibited a loss of acetic acid and ethanol resistance and a lower activity of NAD-dependent alcohol dehydrogenase (ADH), but *A. aceti* AS5 exhibited resistance to acetic acid and ethanol. The recombinant plasmid, pER1, that complemented the ethanol sensitive mutant was isolated by shot-gun cloning from the parental strain chromosomal DNA using *E. coli*-*Acetobacter* shuttle vector pMV24, and carrying 2.3-kb fragment that conferred ethanol resistance to the mutant. The nucleotide sequence of the cloned 2.3-kb fragment on pER1 revealed the presence of OmpR-EnvZ operon. To identify the gene involved ethanol resistance, the OmpR and EnvZ deficient mutants were constructed by two genes disruption, respectively, and examined the growth in the culture medium at various concentrations of ethanol. This result indicated that *ompR* gene was responsible to ethanol resistance in *A. aceti*, but *envZ* gene was not responsible to ethanol resistance.

In the *ompR* disruptant, the activity of NAD-dependent ADH markedly decreased when cultured in a medium containing ethanol compared the parental strain. To confirm that OmpR regulates the expression of NAD-dependent ADH, we were carried the Northern analysis. This result showed that OmpR regulated the transcriptions of two NAD-dependent ADH genes, *adh1* and *adh2*.

These results suggested that OmpR is involved in ethanol resistance in *A. aceti*, and regulates the expression of *adh1* and *adh2*.

## IV-7: The Aldehyde Dehydrogenase (ALDH) of *Gluconacetobacter diazotrophicus* PAL5 is a Quinohemoprotein containing PQQ, Cytochrome *b* and Cytochrome *c*. Structure and kinetics

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*Gluconacetobacter diazotrophicus* is a N<sub>2</sub>-fixing endophyte originally isolated from sugar cane<sup>1</sup>. The oxidation of ethanol to acetic acid is located in the periplasmic space and catalyzed by two membrane bound enzymes complexes, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). These enzymes are usually recognized as quinohemoproteins that contain PQQ and cytochromes *c* as prosthetic groups. This view of ADH is fully supported by experimental data<sup>2</sup>. Several aldehyde dehydrogenase (ALDH) complexes have been purified from the membrane of acetic acid bacteria. The enzyme structures and the chemical nature of the prosthetic groups associated with these enzymes remains a matter of debate. The presence of PQQ in the ALDH of *Acetobacter sp* BPR2001<sup>3</sup>, and *Ga. europaeus*<sup>4</sup>, has been questioned. Also, a purified preparation of ALDH that contains PQQ, but not cytochrome *c* has been reported in *A. polyoxogenes* sp. Nov.<sup>5</sup>. A further complication arose from a report in *Ga. europaeus*<sup>4</sup>, that suggested the existence of cytochrome *b*, a [2Fe:2S] cluster, and a molybdopterin centre, as additional prosthetic groups of ALDH. *A. aceti*<sup>5</sup> and *Ga. europaeus*<sup>4</sup> contain three subunits, while only two subunits are detected in the reported ALDH complexes of *G. suboxydans*<sup>6</sup>, *A. rances*<sup>6</sup> and *Ga. polyoxogenes*<sup>7</sup>.

Here we report on the molecular and catalytic properties of the membrane-bound ALDH complex, which was purified from the diazotrophic bacterium *Ga. diazotrophicus*. The ALDH complex is a heterodimer of two subunits with 79.7 and 50 kDa, and contains pyrroloquinoline quinone (PQQ). EPR spectroscopy revealed the presence of a narrow signal around  $g = 2$  which was assigned to the PQQ radical. Heme *b* was detected by UV/Vis spectroscopy and confirmed by reverse phase HPLC. The smaller subunit posses three cytochromes *c*. Aliphatic aldehydes, but not formaldehyde, are suitable substrates. Using ferricyanide as electron acceptor, the enzyme showed a pH optimum at 3.5 that shifted to pH 7.0 in the presence of phenazine methosulphate plus 2,6-dichlorophenolindophenol. Acetaldehyde did not reduce measurable levels of cytochrome *b* and *c* centres; however, the dithionite-reduced hemes were conveniently oxidized by ubiquinone-1; this finding suggests that cytochrome *b* and the cytochromes *c* constitute an intramolecular redox sequence that delivers electrons to the membrane ubiquinone.

1. Alvarez, B. and G. Martínez-Drest. *Jour. Syst. Bact.* **39**, 361-364 (1989).
2. Matsushita, K. et al. *Adv. Microbiol. Physiol.* **36**, 246-301 (1994).
3. Takemura, H. et al. *Biosci. Biotechnol. Biochem.* **58**, 2082-2083 (1994).
4. Thurner C. et al. *Archiv. Microbiol.* **168**, 81-82 (1997).
5. Ameyama M. and O. Adachi. *In Meth. Enzymol* **89** [82], 491-497.
6. Hommel, R. and P. Kleber. *J. Gen. Microbiol.* **136**, 1705-1711 (1990).
7. Fukaya, M et al. *Appl. Microbiol Biotech.* **32**, 176-180, (1989).

## IV-8: Quinoprotein and Flavoprotein Dehydrogenases Function Separately in *Gluconobacter* Respiratory Chain

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*Gluconobacter* strains are able to oxidize various kinds of sugars and sugar alcohols incompletely to accumulate corresponding oxidation products. Such oxidative fermentations are important and applied for industrial production of valuable substances such as L-sorbose, dihydroxyacetone, 2-keto-D-gluconate (2KGA), 5-keto-D-gluconate (5KGA), and so on. These reactions are performed by PQQ-quinoprotein or FAD-flavoprotein dehydrogenases linked to terminal oxidase(s) of the respiratory chain, which leads not only the accumulation of oxidative products but also the energy generation for cell growth.

Recent works<sup>1,2</sup> have shown that PQQ-glycerol dehydrogenase (GLDH), isolated 23 years ago<sup>3</sup>, is the central enzyme catalyzing versatile polyol oxidation, responsible to almost all oxidative fermentations of sugar alcohols. Another works<sup>4</sup> have shown that terminal ubiquinol oxidase in the respiratory chain, accepting electrons from the primary dehydrogenases, of *Gluconobacter* is branched with a cyanide-sensitive cytochrome *bo*<sub>3</sub> (BO3) and a cyanide-resistant bypass oxidase (CIO). Recently, a set of genes in *Gluconobacter* genome turned out to be highly homologous to *cioAB* in *Pseudomonas*, and actually shown to be responsible for the cyanide-resistant bypass oxidase.

Our recent studies on D-sorbitol or D-gluconate oxidation showed that a set of primary dehydrogenases, GLDH and FAD-sorbitol dehydrogenase (SLDH) or GLDH and FAD-gluconate dehydrogenase (GADH), respectively, is involved in the oxidation in *Gluconobacter* strains. The oxidized product of D-sorbitol by GLDH or SLDH is the same, that is, L-sorbose. Whereas, the oxidized product of D-gluconate by GLDH or GADH is different: 5KGA or 2KGA, respectively. It is revealed that a strain defective in GLDH exhibited a delayed L-sorbose production, decreased D-sorbitol oxidase activity, which became more resistant to cyanide, and generated less energy than either wild or SLDH-defective strain. For D-gluconate oxidation, it was shown that a mutant defective in GLDH converts D-gluconate only to 2KGA with a delayed but increased production, while GADH-defective mutant to produce only 5KGA but it is not so effective due to the slow growth rate. A mutant defective in CIO, though exhibited a slow growth, did increased 5KGA production in the resting cell reaction. Thus, 5KGA-producing gluconate oxidation with GLDH seems to be more closely linked to BO3 than CIO.

These results suggest that PQQ-GLDH connects efficiently to BO3 and plays a main role in the L-sorbose or 5KGA production; on the other hand, FAD-dependent SLDH or GADH links preferably to CIO, which may not produce so much energy, and work for additional production of L-sorbose or 2KGA which may be important for the cell growth.

1. Adachi, O. et al., *Biosci. Biotechnol. Biochem.*, **65**, 2755-2762 (2001)
2. Matsushita, K. et al., *Appl. Environ. Microbiol.*, **69**, 1959-1966 (2003)
3. Ameyama, M. et al., *Agric. Biol. Chem.*, **49**, 1001-1010 (1985)
4. Matsushita, K. et al., *Advances in Microbial Physiology*, **36**, 247-301 (1994)

## IV-9: DEXTRAN DEXTRINASE AND (OLIGO)DEXTRAN SYNTHESIS BY *GLUCONOBACTER OXYDANS*

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Certain strains of *Gluconobacter oxydans* have been known since the 1940's to produce the enzyme dextran dextrinase (DDase; E.C.2.4.1.2), which is a transglucosidase, converting maltodextrins into (oligo)dextran. The enzyme catalyses the transfer of an  $\alpha$ 1,4 linked glucosylunit of a donor to an acceptor molecule, forming an  $\alpha$ 1,6 linkage: consecutive glucosyl transfers result in the formation of high molecular weight dextran from maltodextrins. This *Gluconobacter* dextran differs in many aspects from *Leuconostoc* dextran, which is produced only from sucrose by dextransucrase action (1,2).

The Japanese group of Yamamoto (3) revived in the early 1990's research on dextran dextrinase, focussing on the purification and characterisation of the intracellular DDase produced by *Gluconobacter oxydans* ATCC 11894. This was taken further by Suzuki and coworkers (4), who investigated more recently the properties and kinetics of the extracellular DDase, formed by the same strain.

Our group further elaborated on fermentation processes to optimise DDase synthesis, on DDase characterisation and on its use as a biocatalyst. Surprisingly, the intracellular DDase level decreased significantly in fermentations in the presence of (iso)maltooligosaccharides, while the extracellular enzyme level increased. Specific assays for measuring DDase activity were devised. The general characteristics, substrate specificity and mode of action of DDase as a transglucosidase will be discussed. The purified enzyme displayed transglucosylation as well as hydrolytic activity. Applications of DDase will be summarized ie. to produce (oligo)dextran, transglucosylated products and speciality oligosaccharides such as panose.

*Gluconobacter* dextran, recovered from a submerged fermentation, displayed unusual rheological shear thinning flow behaviour. Production of dextran via solid state fermentation yielded a maltodextrin-free polysaccharide of eight-fold higher molecular weight (7000 kDa). The polysaccharide displayed lower viscosity than *Leuconostoc* dextran of similar molecular weight, due to its higher degree of branching. *Gluconobacter* dextran might thus be suitable for food use applications, not associated with thickening functionality: examples include use as a source of dietary fibre, as a cryostabiliser, as a fat substitute, or as a low calorie bulking agent for sweeteners (1,5).

1. Naessens M, Cerdobbel A, Soetaert W, Vandamme EJ, Dextran dextrinase and dextran of *Gluconobacter oxydans*. *J. Ind. Microbiol. Biotechnol.*, **32**,323-334 (2005)
2. Naessens M, Cerdobbel A, Soetaert W, Vandamme EJ, *Leuconostoc* dextran sucrose and dextran: production, properties and applications. *J. Chem. Technol. Biotechnol.*, **80**, 845-860 (2005)
3. Yamamoto K, Yoshikawa K, Kitahata S, Okada S, Purification and some properties of dextran dextrinase from *Acetobacter capsulatum* ATCC 11894. *Biosci. Biotechnol. Biochem.* **58**,330-333 (1992)
4. Suzuki M, Unno T, Okada G, A kinetic, study of an extracellular dextran dextrinase from *Acetobacter capsulatum* ATCC 11894. *J. Appl. Glycosci.*, **47**,27-33 (2000)
5. De Muynck C, Pereira C.S.S., Naessens M, Parmentier S, Soetaert W, Vandamme EJ, The Genus *Gluconobacter oxydans*: comprehensive overview of biochemistry and biotechnological applications. *Crit. Rev. Biotechnol.*, **27**,147-171 (2007)