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Widespread occurrence of non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase among gram-positive bacteria

Summary. The non-phosphorylating glyceraldehyde 3-phosphate dehydrogenase (GAPDHN, $NADP^+$-specific, EC 1.2.1.9) is present in green eukaryotes and some *Streptococcus* strains. The present report describes the results of activity and immunoblot analyses, which were used to generate the first survey of bacterial GAPDHN distribution in a number of *Bacillus, Streptococcus* and *Clostridium* strains. Putative *gapN* genes were identified after PCR amplification of partial 700-bp sequences using degenerate primers constructed from highly conserved protein regions. Alignment of the amino acid sequences of these fragments with those of known sequences from other eukaryotic and prokaryotic GAPDHNs, demonstrated the presence of conserved residues involved in catalytic activity that are not conserved in aldehyde dehydrogenases, a protein family closely linked to GAPDHNs. The results confirm that the basic structural features of the members of the GAPDHN family have been conserved throughout evolution and that no identity exists with phosphorylating GAPDHs. Furthermore, phylogenetic trees generated from multiple sequence alignments suggested a close relationship between plant and bacterial GAPDHN families. [**Int Microbiol** 2005; 8(4):251-258]

Key words: non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase · *Bacillus* · *Streptococcus* · *Clostridium* · *gapN* genes

Introduction

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is an enzyme involved in central pathways of carbon metabolism. The most common form of GAPDH is the NAD^+-dependent enzyme (EC 1.2.1.12) found in all organisms so far studied and located in the cytoplasm. This enzyme plays a role in the Embden-Meyerhoff pathway not only in glycolysis but also in gluconeogenesis [8]. $NADP^+$-dependent GAPDH (EC 1.2.1.13), located in the chloroplast stroma and the cyanobacterial cytoplasm, is involved in photosynthetic CO_2 assimilation [3,5,33]. The non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPDHN; EC 1.2.1.9) is encoded by the nuclear gene *gapN* and is ubiquitous among photosynthetic eukaryotes. While the enzyme is thought to metabolize trioses exported from the chloroplast, its precise function remains to be established [25,30]. It does, however, catalyze the oxidation of glyceraldehyde-3-phosphate (G3P) to 3-phosphoglycerate (3-PGA) with the reduction of $NADP^+$ to NADPH. No inorganic phosphate is required and the reaction is irreversible under physiological conditions. This is in

contrast to the reversibility of NAD^+- and $NADP^+$-dependent phosphorylating GAPDH reactions, which require inorganic phosphate to oxidize G3P into diphosphoglyceric acid [5,30]. GAPDHN was originally reported to be exclusively present in green eukaryotes, and GAPDHN activity was first found in the cytosolic protein fraction of leaf tissues, endosperm, and the cotyledons of plants [21]. This was followed by the discovery of GAPDHN activity in other photosynthetic eukaryotes, e.g. in different algae [18,25]. However, early reports of a non-phosphorylating GAPDH activity in *Streptococcus mutans* [4] were confirmed later by molecular data [2]. This strain lacks the two oxidative enzymes of the hexose monophosphate pathway, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase [4], and as a consequence must use alternative mechanisms, implicating GAPDHN, to generate NADPH for reductive biosynthetic reactions. Recently, we have cloned the *gapN* genes from *Clostridium acetobutylicum* and *Streptococcus pyogenes* and expressed them in *Escherichia coli*. The recombinant GAPDHNs produced were purified and their physical and catalytic properties investigated [15,16]. The results showed that these genes effectively encoded GAPDHN proteins with enzymatic characteristics similar to those previously described.

The GAPDHN of higher plants [10,11] and bacteria [2] consists of a subunit of about 490 amino acids. The active enzyme in plants is a homo-tetramer of about 190 kDa, as determined from beet, *Chlamydomonas reinhardtii*, and *Hevea brasiliensis* [18,19,27]. The enzyme from *S. mutans*, *C. acetobutylicum*, and *S. pyogenes* is a homotetramer with subunit molecular masses of 51, 50, and 55 kDa, respectively [15,16,22].

The amino acid sequences of GAPDHNs align well with those of aldehyde dehydrogenases (ALDH), demonstrating that they are members of the large ALDH superfamily, sharing an amino acid identity of 20–30%. Thus, GAPDHNs clearly differ from phosphorylating GAPDHs both in primary structure and molecular mass [10,18,17]. Bacterial and plant enzymes of the GAPDHN family have a much closer affiliation among each other than with other enzymes of the ALDH superfamily. For example, *S. mutans* GAPDHN shows about 50% amino acid identity with the enzyme of photosynthetic eukaryotes [11]. By contrast, the archaeon *Methanococcus jannaschii* has a non-phosphorylating GAPDH with ferredoxin-dependent activity, but the sequence of the enzyme is distinctly separate from that of $NADP^+$-dependent GAPDHNs [11]. To clarify the distribution of GAPDHN in bacteria, we surveyed the occurrence and activity of the enzyme in several bacterial strains, applying a molecular genetic approach to gather more information on the enzyme's distribution.

Material and methods

Strains and culture conditions. *Clostridium acetobutylicum* ATCC 824 and ATCC 859, *C. perfringens* ATCC 13124, *C. pasteurianum* ATCC 6013, *C. difficile* ATCC 11011 and *C. sporogenes* CIP 79.39 strains were grown in trypticase-yeast-extract-glucose (TYA) broth [26] at 37ºC in an anoxic chamber within a nitrogen atmosphere. *Streptococcus pyogenes* [16], *S. agalactiae* ATCC 13813 and isolated *Streptococcus sp.* strains were grown in Todd Hewitt broth (THY) medium containing 0.2% (w/v) yeast extract [1]. *Bacillus megaterium* ATCC 14945, *B. subtilis* ATCC 6633, *B. thuringiensis* ATCC 10792, *Staphylococcus aureus* ATCC 25923, *Mycobacterium tuberculosis* ATCC 27294, *Pseudomonas aeruginosa* ATCC 9027, *Bacteroides fragilis* ATCC 25285, *Enterococcus faecium* CIP 54.32, and *E. hirae* ATCC 10541, and isolated *Bacillus sp., B. licheniformis*, and *B. cereus* [14], were grown at 37ºC in Luria-Broth (LB) medium [29]. *Neisseria meningitidis* M13 was grown at 37ºC on GCB medium (Difco Laboratories, USA) [20]. *Lactobacillus brevis* ATCC 14869, *L. paracasei* ATCC 25598, *L. plantarum* ATCC 8014, and *L. lactis* CNRZ 548 were grown at 25°C in MRS medium [7].

Cell-free extract preparation. Liquid culture cells were harvested by centrifugation at 8,000 × *g* for 15 min at 4ºC. Cell pellets were washed twice in 25 mM Tris-HCl (pH 7.5) and resuspended in the same buffer supplemented with 2 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF), and 10% (v/v) glycerol. Cells were then disrupted by ultrasonic treatment in a chilled water bath using a Branson 25U sonifier at medium strength. The resulting suspension was centrifuged at 20,000 × *g* for 20 min to obtain the cell-free extract.

Enzyme assays. GAPDHN activity was measured as described elsewhere [34]. The reaction was started by the addition of 10 µg of cell-free extract to an assay mixture containing 50 mM Tricine buffer (pH 8.5), 1 mM $NADP^+$, and 1 mM D-glyceraldehyde-3-phosphate at 25ºC. Absorbance at 340 nm was followed in a spectrophotometer (model 6405, Jenway, Dunmow, UK). Phosphorylating NAD^+-dependent GAPDH activity was measured using the same procedure but employing NAD^+ (1 mM) and inorganic phosphate or arsenate (10 mM) in the reaction solution.

Protein immunodetection. Immunoblot assays of protein samples were carried out after SDS-PAGE [12% (w/v) polyacrylamide slab gels] as described in [15] for *C. acetobutylicum*.

PCR methodology and DNA sequencing. Amplification of ca. 0.7-kb fragments of *gapN* genes from diverse bacterial genomic DNA samples was carried out by PCR using degenerate primers from two highly conserved regions at the N-terminal and C-terminal ends of GAPDHN proteins (NPCO1: 5′-C(T)TA(G)GCT(CAG)ATT(CA)T(A)C(G)T(C)CCT(CAG)TT T(C)AAT(C)-3′, and NPCO2: 5′-CCT(CAG)GGT(CAG)TTT(C)CCT(CA G)GAA(G)GAA(G)TGG-3′). Amplification conditions were: cycle 1, 92°C for 2 min; cycles 2–36, 92°C for 1 min, 45°C for 1 min, and 72°C for 1 min; cycle 37, 72°C for 30 min. Chromosomal DNA was isolated using a Wizard Kit (Promega, Madison, USA). The amplified reaction was visualized on 0.8 % (w/v) agarose gels with the addition of ethidium bromide according to [29]. PCR-amplified DNA fragments were purified by selective adsorption/desorption on glass beads (Gene Clean, Bio101, La Jolla, CA, USA). Sequence analysis was carried out employing the DNA Strider program (version 1.2 for Macintosh).

DNA alignment and phylogenetic analyses. Multiple sequence alignment of GAPDHN protein regions corresponding to the PCR-amplified DNA fragments of the bacteria studied were done with the Clustal X v.1.8 program [32]. Using this alignment, phylogenetic trees were constructed employing the distance (neighbor-joining, Kimura distance calculations),

maximum likelihood, and maximum parsimony methods and using the programs Clustal X v.1.8, Tree-Puzzle v.5.0 [31] and Protpars v.3.573c (PHYLIP package v.3.5c w1993x Felsenstein, J., Dept. of Genetics, Univ. of Washington, Seattle, USA), respectively. Bootstrap analyses (values presented on a percentage basis) were computed with 1000 replicates for the distance and maximum parsimony trees; for maximum likelihood analysis, estimations of support were assigned to each internal branch using the quarter puzzling algorithm [31]. Published amino acid sequences of GAPDHNs used in this work come from bacteria (*Bacillus anthracis,* accession number AAP24851; *Bacillus halodurans*, E83929; *Streptococcus mutans,* NP721104; *Streptococcus pneumoniae TIGR4,* NP345590*; Streptococcus pneumoniae R6,* NP358622*; Mycoplasma capricolum,* CAA 83756; *Ureaplasma urealyticum*, AAF30771), higher plants (*Pisum sativum,* P81406; *Nicotiana plumbaginifolia,* P93338*; Zea mays,* Q43272), and a microalga (*Scenedesmus vacuolatus,* CAC81014). Ferredoxin-dependent GAPDHN sequences of archaea (*Pyrococcus furiosus*, NP578193 and *Methanococcus jannaschii,* NP248149) were also used. Bacterial GAPDH encoded by the *Escherichia coli gap1* gene (accession number P06977) was used as out-group. PCR-amplified partial *gapN* sequences from bacteria were submitted to EMBL/GeneBank databases and assigned accession numbers as follows: AJ880320 (*Clostridium acetobutylicum* ATCC859), AJ880322 (*C. pasteurianum*), AJ880325 (*C. difficile*), AJ880321 (*C. perfringens*), AJ880323 (*C. sporogenes*), AJ8800317 (*Bacillus* sp.), AJ880318 (*B. cereus*), AJ880319 (*B. licheniformis*), AJ880324 (*B. thuringiensis*), AJ880316 (*Streptococcus* sp.), AJ880315 (*S. agalactiae*), and AJ880326 (*S. pyogenes*).

Results and Discussion

GAPDHN activity and immunoblot analyses. Table 1 shows the enzymatic activities (U/mg) of phosphorylating NAD$^+$-dependent and non-phosphorylating NADP$^+$-dependent glyceraldehyde-3-phosphate dehydrogenase (GAPDH and GAPDHN) in the strains studied. Phosphorylating NAD$^+$-dependent GAPDH was present universally and showed high specific activity values, in the range of 0.2–2.5 U/mg, in all bacterial strains examined. By contrast, GAPDHN activity (specific activity 0.01–0.1 U/mg) was found only in a number of gram-positive strains of the gen-

Table 1. Phosphorylating NAD$^+$-dependent (GAPDH) and non-phosphorylating NADP$^+$-dependent glyceraldehyde-3-phophate dehydrogenase (GAPDHN) activities in different bacterial strains

Bacteria	GAPDH activity (U/mg)	GAPDHN activity (U/mg)
Clostridium acetobutylicum ATCC824	1.010	0.013
Clostridium perfringens	0.192	0.011
Clostridium pasteurianum	0.190	0.060
Clostridium difficile	0.265	0.040
Clostridium sporogenes	2.300	0.037
Streptococcus pyogenes	2.510	0.017
Streptococcus agalactiae	0.282	0.090
Streptococcus sp.	0.400	0.102
Bacillus licheniformis	0.188	0.099
Bacillus cereus	0.200	0.040
Bacillus thuringiensis	0.312	0.010
Bacillus sp.	1.000	0.038
Staphylococcus aureus	1.229	nd*
Bacillus megaterium	1.420	nd
Bacillus subtilis	1.030	nd
Bacteroides fragilis	0.500	nd
Neisseria meningitidis	0.750	nd
Enterococcus faecium	1.006	nd
Enterococcus hirae	2.240	nd
Lactobacillus brevis	0.543	nd
Lactobacillus paracasei	0.465	nd
Lactobacillus plantarum	0.733	nd
Lactococcus lactis	0.312	nd
Mycobacterium tuberculosis	0.900	nd
Pseudomonas aeruginosa	1.000	nd

*nd, not detected.

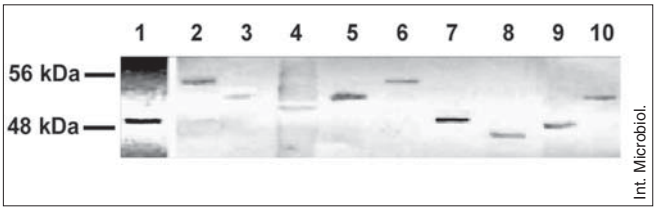

Fig. 1. Immunodetection of non-phosphorylating glyceraldehyde 3-phosphate dehydrogenase (GAPDHN) in crude extracts of various bacteria. *Lane 1 Clostridium acetobutylicum* ATCC 824, *lane 2 C. difficile*, *lane 3 Streptococcus agalactiae*, *lane 4 Streptococcus* sp., *lane 5 C. sporogenes*, *lane 6 S. pyogenes*, *lane 7 C. perfringens*, *lane 8 Bacillus cereus*, *lane 9 B. thuringiensis*, *lane 10 B. licheniformis*. Approximately 50 µg of crude extracts were loaded per lane. Apparent molecular masses are indicated on the left.

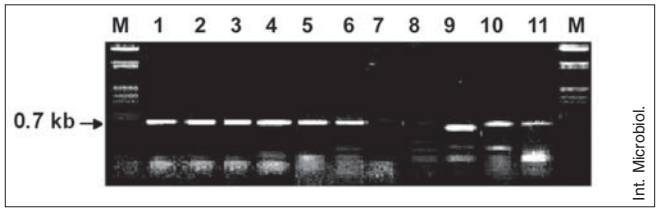

Fig. 2. PCR amplification of the 700-bp fragment from the *gapN* gene, employing chromosomal DNA of diverse bacteria. *Lane 1 Clostridium acetobutylicum* ATCC859, *lane 2 C. pasteurianum*, *lane 3 C. difficile*, *lane 4 C. perfringens*, *lane 5 C. sporogenes*, *lane 6 B. thuringiensis*, *lane 7 Neisseria meningitidis*, *lane 8 Lactobacillus brevis*, *lane 9 B. cereus*, *lane 10 Streptococcus pyogenes*, *lane 11 Streptococcus* sp. Bands were visualized on 0.8% (w/v) agarose gels in the presence of ethidium bromide. *Hin*dIII-restricted lambda DNA was used as the molecular size marker (M). The arrow indicates the amplified *gapN* fragments.

era *Clostridium, Streptococcus*, and *Bacillus.* Surprisingly, despite some cases of close evolutionary linkage to those bacteria, activity was absent from *B. megaterium, B. subtilis, E. faecium, E. hirae, S. aureus, L. brevis, L. paracasei, L. plantarum, L. lactis, M. tuberculosis*, and the gram-negative bacteria *N. meningitidis*, *Ba. fragilis*, and *P. aeruginosa.*

Figure 1 shows the immunoblot containing single-protein bands of ca. 48–54 kDa, corresponding to the GAPDHN subunits, in soluble protein extracts from the species mentioned above that have non-phosphorylating dehydrogenase activity Similar bands were detected by immunoblots and using the same antibodies in cell extracts from other bacteria, including *Clostridium pasteurianum* and *C. sporogenes* (data not shown). The observed cross-reaction suggests common epitopes between the *C. acetobutylicum* protein and the other bacterial GAPDHNs. As expected, no bands were immunodetected in cell extracts of those bacteria in which activity had not been recorded.

PCR and sequence analysis of bacterial *gapN* genes. The bands resulting from separating the PCR products on agarose gel are shown in Fig. 2. After amplification of the ca. 0.7-kb single DNA fragments, these bands were excised from the gel, sequenced, and their deduced amino acid composition analyzed. All of the amino acid sequences showed good identity with that from the *gapN* fragment of *S. mutans*. In agreement with biochemical and immunochemical data, no band was amplified in the cases of *B. megaterium, B. subtilis, Ba. fragilis, S. aureus, N. meningitidis, E. faecium, E. hirae, L. brevis, L. paracasei, L. plantarum, L. lactis, M. tuberculosis*, and *P. aeruginosa.*

Figure 3 shows the alignment between the approximately 240 amino acids corresponding to the amplified 700-bp fragments and the deduced amino acid sequences of the corresponding *GapN* fragments from higher plants (*P. sativum, N. plumbaginifolia, Z. mays*) and algae (*S. vacuolatus*). The latter were also compared with other deduced amino acid sequences of bacterial GAPDHNs in databases (*B. anthracis, B. halodurans*, *S. mutans, M. capricolum, U. urealyticum*).

As expected, the non-phosphorylating GAPDHN showed no significant identity match with phosphorylating GAPDHs. Both non-phosphorylating GAPDH and phosphorylating GAPDHs showed high specificity towards D-glyceraldehyde-3-phosphate [13,18], which suggested that substrate specificity of the two GAPDH forms emerged by convergent evolution along independent lines [10]. Sequences of the archaeal ferredoxin-dependent GAPDHN, which requires a heavy-metal cofactor and is oxygen sensitive, were distinctly different from that of any other $NADP^+$-dependent GAPDHN (not shown).

The 12 deduced partial bacterial GAPDHN sequences determined in this study were compared using the CLUSTAL X (v. 1.8) program with other published amino acid sequences of eukaryotic and prokaryotic origin. The results showed significant similarities between the various prokaryote and eukaryote sources (Fig. 3). In addition to the conserved heptapeptide Ser-Gly-Glu-Arg-Cys-Thr-Ala (residues 294–300, following pea GAPDHN numbering [10], including residues Arg-297, necessary for phosphate group binding of the substrate, and Cys-298, involved in catalytic thioester formation), other residues involved in enzyme activity are strictly conserved in all GAPDHN proteins. These are the 191–192 dipeptides Lys-Pro, and the hexapeptide Glu-Leu-Gly-Gly-Lys-Asp at position 264–269. The latter includes the active site Glu-264, involved in deacylation through activation and orientation of the attacking water molecule [22,23,24] (Fig. 3). Thus, the basic structural features of the members of the GAPDHN family have been conserved over evolution. The highly conserved Gly-249 and Gly-295 are also present in the aldehyde dehydrogenase superfamily [10], showing that

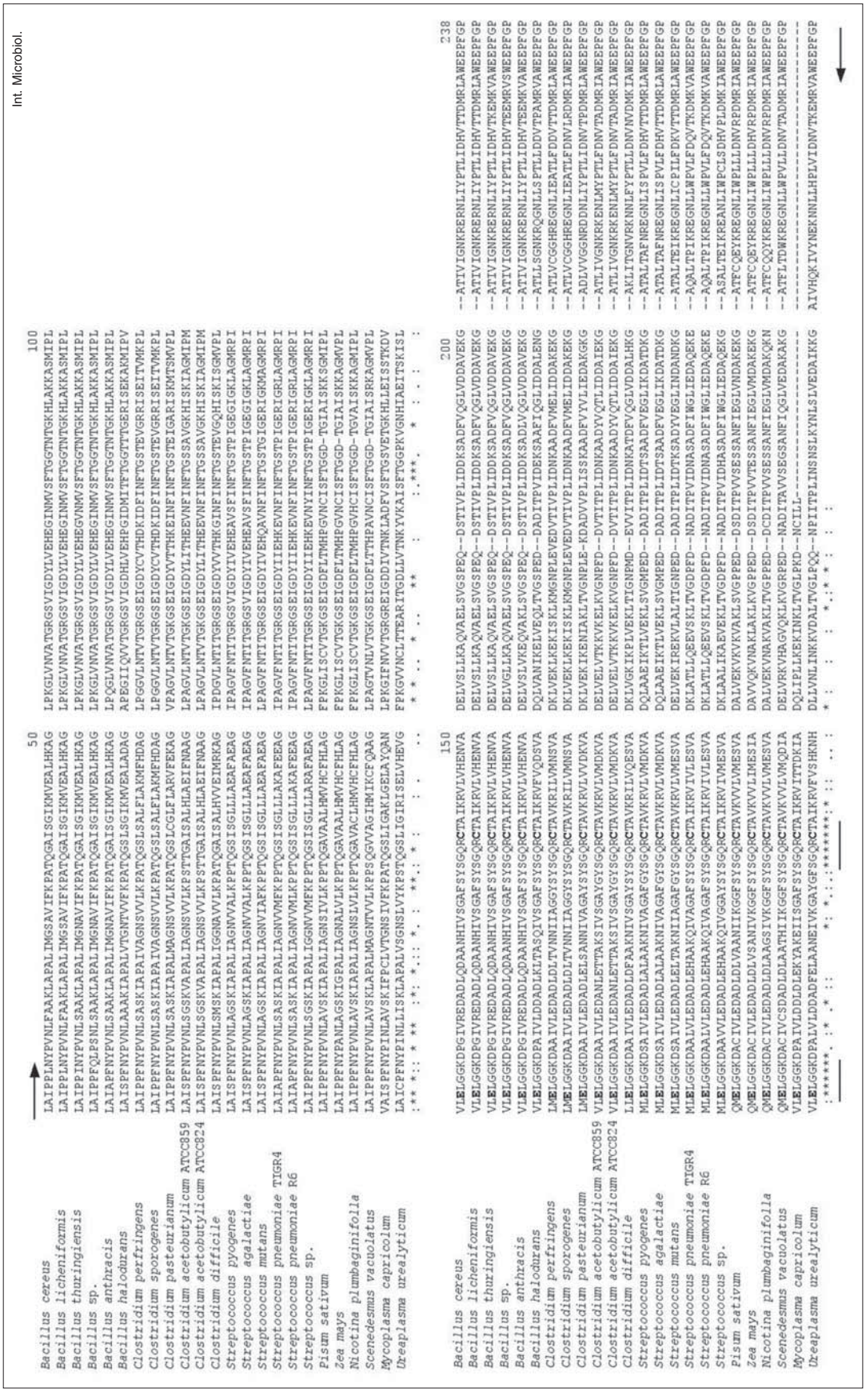

Fig. 3. Multiple sequence alignment using the CLUSTAL X v.1.8 program of the partial GAPDHN sequences from photosynthetic eukaryotes and bacteria. Sequences obtained in this work as well as published amino acid sequences of bacterial and plant sources, as described in Materials and methods, were included. The conserved motives including the catalytic important residues Glu 264 and Cys 298 (showed in bold) are underlined. The degenerate primers used in PCR experiments are indicated by arrows.

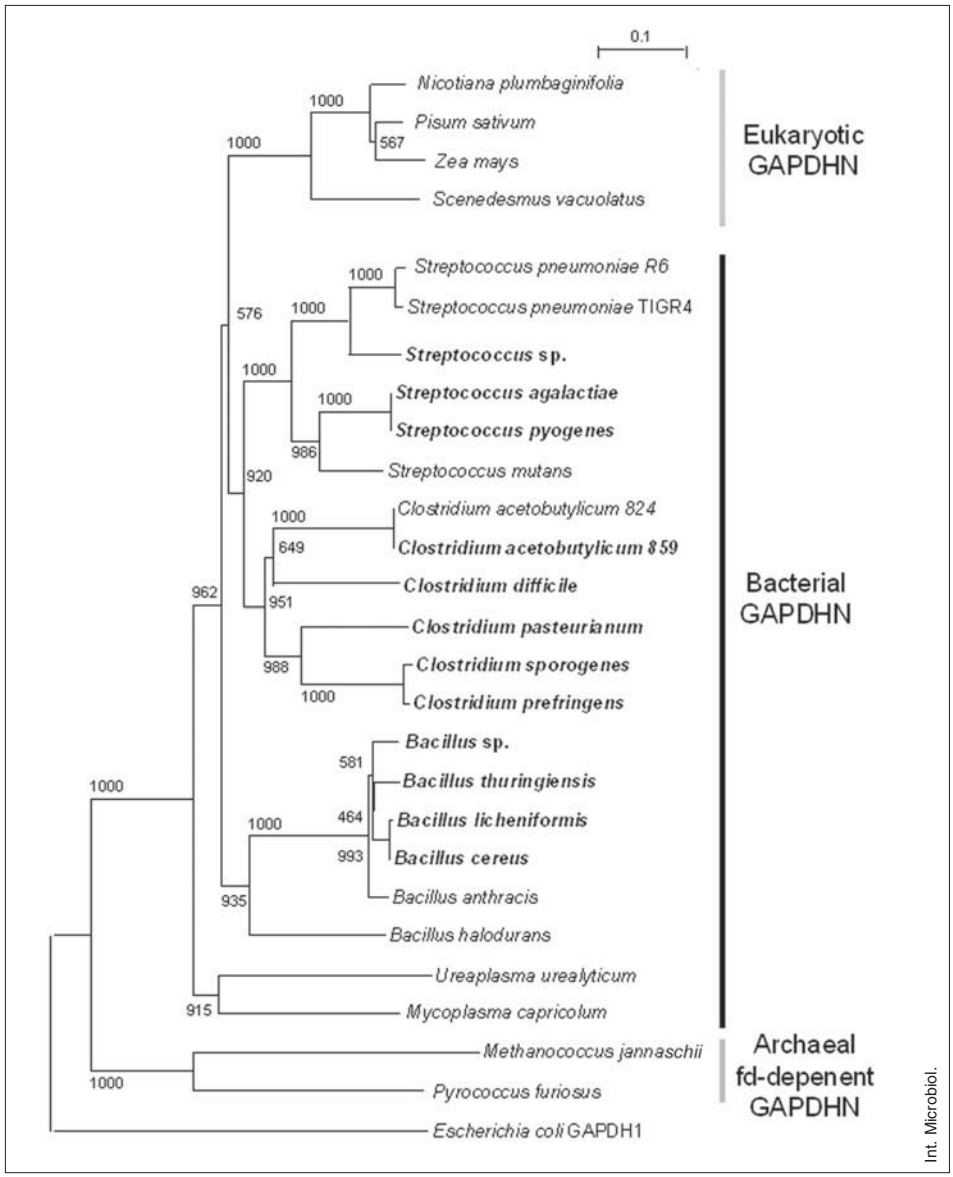

Fig. 4. Distance phylogenetic tree of the bacterial GAPDHN sequences deduced from the *gapN* fragments, as described in Materials and methods. Sequences of *Clostridium acetobutylicum* ATCC 859, *C. perfringens*, *C. pasteurianum*, *C. difficile*, *C. sporogenes*, *Streptococcus pyogenes*, *S. agalactiae*, *Streptococcus* sp., *Bacillus licheniformis*, *B. cereus*, *Bacillus* sp., were obtained in this work (**in bold**).

GAPDHNs and ALDHs probably shared a common ancestor. Nevertheless, the number of residues in which ALDHs differ from GAPDHN in the aligned sequences provides evidence that they actually belong to different enzyme families.

Phylogenetic analysis of GAPDHN sequences. The phylogenetic tree constructed using the above-described multiple sequence alignment and the distance (neighbor-joining) method [28] is shown in Fig. 4. Maximum likelihood and parsimony methods were also used, giving results (not shown) very similar to those in Fig. 4. *E. coli* GAPDH1 protein was used as out-group. The trees showed an analogous phylogenetic relationship among the GAPDHNs of the eukaryotic and prokaryotic groups. These groups are different from the archaeal ferredoxin-dependent GAPDHN, which probably diverged out first, before eukaryotic and bacterial GAPDHN separation. However, note that these results depict only the molecular phylogeny of the GAPDHN protein and do not necessarily represent phylogenetic relationships between species. A paraphyletic relationship is observed between bacterial GAPDHN sequences that appear in three clusters (Streptococcaceae, Clostridia, and Bacillaceae) together with the plant group, and a separate, early-branching group of mycoplasm sequences (*Urea-*

plasma urealyticum and *Mycoplasma capricolum*). This suggests that GAPDHNs of Mycoplasmataceae separated early in evolution. Indeed, mycoplasms include some of the smallest prokaryotic genomes (600 kb, about 500 genes), and the sequences of many genes are very divergent when compared to homologous sequences of other bacteria [9]. These phylogenetic relationships may be due to horizontal gene transfers and enzyme functional substitutions, such as those described for some GAPDHs [6,8,12].

In this work, we demonstrated that the *gapN* gene is present in various gram-positive bacteria with a characteristic low G + C content, including Bacillaceae, Streptococcaceae, and Clostridiaceae. A possible explanation for this distribution among some gram-positive bacteria and for the absolute absence of *gapN* in gram-negative bacteria could be an early divergence in basic metabolic enzymes. Nevertheless, a wider survey of this protein among other microorganisms would provide better knowledge of its distribution and, especially, its relation with GAPDHs.

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Amplia distribución de la gliceraldehído-3-fosfato deshidrogenasa no-fosforilante entre las bacterias gram-positivas

Resumen. La gliceraldehído-3-fosfato deshidrogenasa no-fosforilante (GAPDHN, $NADP^+$-específica, EC 1.2.1.9) está presente en organismos eucariotas fotosintéticos y en algunas cepas de *Streptococcus* y *Clostridium*. En este trabajo se presentan los resultados de los análisis de actividad e inmunotransferencia, que se utilizaron para la primera prospección de la distribución de GAPDHN bacteriana en diversas cepas de *Bacillus*, *Streptococcus* y *Clostridium*. Se han identificado genes putativos *gapN* mediante amplificación por PCR de secuencias parciales de 700 bp utilizando cebadores degenerados construidos a partir de regiones proteínicas muy conservadas. Las secuencias de aminoácidos de estos fragmentos se alinearon con las de otras secuencias conocidas de GAPDHN eucarióticas y procarióticas, lo que demuestra la presencia de residuos conservados que participan en la actividad catalítica y que no se han conservado en las aldehído deshidrogenasas, una familia de proteínas estrechamente relacionadas con las GAPDHN. Los resultados confirman que las características estructurales básicas de los miembros de la familia GAPDHN se han conservado durante la evolución y que no existe identidad con las GAPDH fosforilantes. Además, los árboles filogenéticos generados a partir de alineaciones de secuencia múltiples sugieren una estrecha relación entre las familias GAPDHN en plantas y bacterias. [**Int Microbiol** 2005; 8(4):251-258]

Palabras clave: gliceraldehído-3-fosfato deshidrogenasa no fosforilante · *Bacillus* · *Streptococcus* · *Clostridium* · genes *gapN*

Ampla distribuição da gliceraldeído-3-fosfato desidrogenase não fosforiladora entre as bactérias gram-positivas

Resumo. Indicou-se a presença da gliceraldeído-3-fosfato desidrogenase não fosforiladora (GAPDHN, $NADP^+$-específica, EC 1.2.1.9) em organismos eucariotas fotossintéticos e em algumas cepas de *Streptococcus* e *Clostridium*. Neste trabalho apresenta-se os resultados da atividade en imunotransferência, usados para a primeira prospecção da distribuição da GAPDHN bacteriana em diversas cepas de *Bacillus*, *Streptococcus* e *Clostridium*. Se identificaram genes putativos *gapN* mediante amplificação por PCR de seqüências parciais de 700 bp utilizando iniciadores degenerados construídos a partir de regiões proteicas altamente conservadas. As seqüências de aminoácidos destes fragmentos se alinharam com as de outras seqüências desconhecidas de GAPDHNs eucarióticas e procarióticas, o que demonstra a presença de resíduos conservados que participam da atividade catalítica que não estão conservados nas aldeído desidrogenases, uma família de proteínas estreitamente relacionados com as GAPDHN. Este trabalho confirma que as características estruturais básicas dos membros da família GAPDHN se conservaram durante a evolução e que não existe identidade com as GAPDH fosforilantes. Além disso, as árvores filogenéticas geradas a partir de alinhamentos de seqüência múltiplas indicam uma estreita relação entre as famílias de GAPDHN de plantas e bactérias. [**Int Microbiol** 2005; 8(4):251-258]

Palavras chave: gliceraldeído-3-fosfato desidrogenase não fosforiladora · *Bacillus* · *Streptococcus* · *Clostridium* · genes *gapN*