Pharmacology
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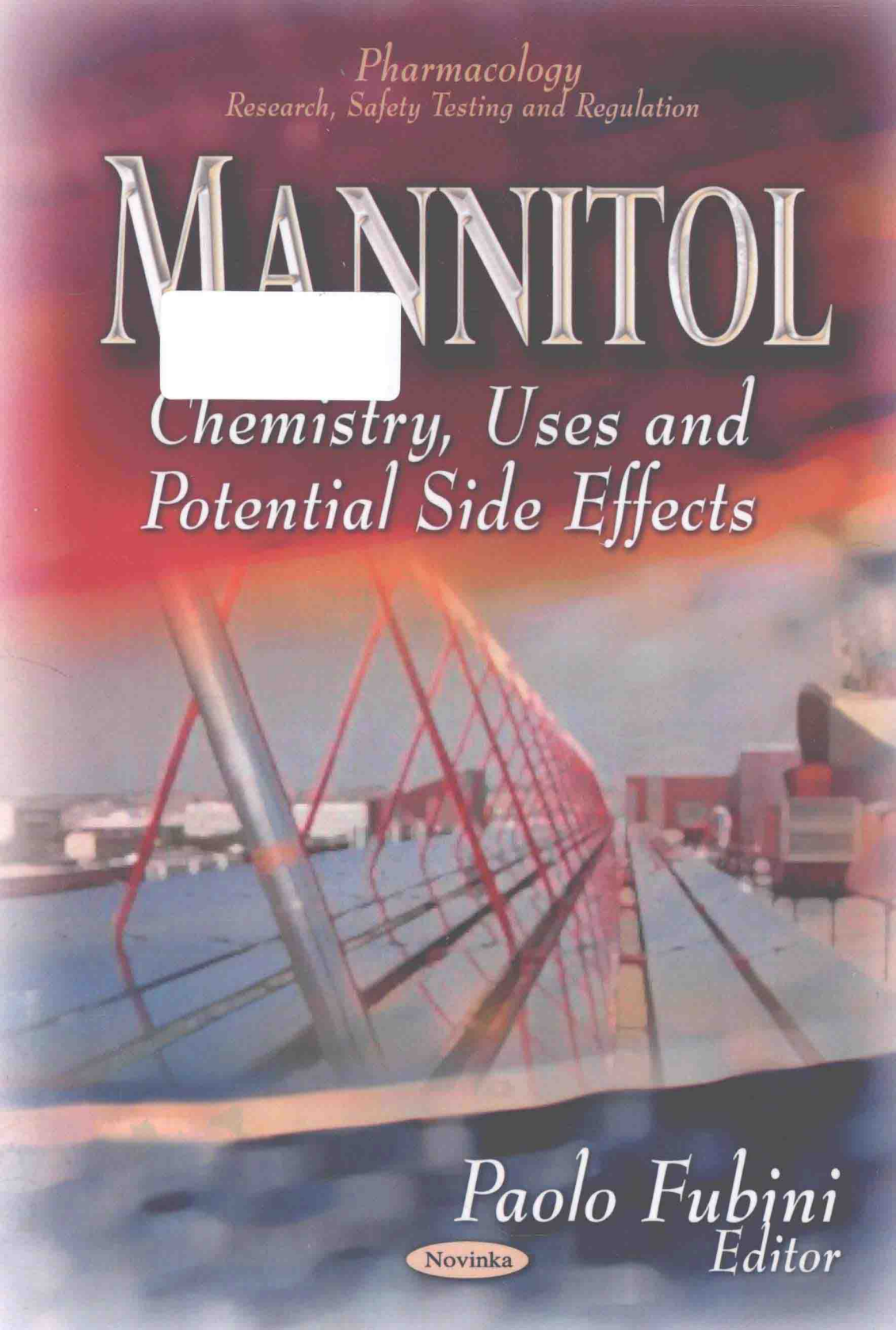
MANNITOL

Chemistry, Uses and Potential Side Effects



Paolo Fubini
Editor

Novinka

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CHEMISTRY, USES AND POTENTIAL SIDE EFFECTS

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NOVINKA

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PREFACE

In this book, the authors present topical research in the study of the chemistry, uses and potential side effects of mannitol. Topics discussed include the utilization and production of D-mannitol by bacteria; concentration of mannitol and other soluble carbohydrates in the crustose lichen rhizocarpon geographicum; disease-related changes and mannitols use for clinical disorders; use of mannitol in thermal energy storage applications; and chiral phosphorous ligands derived from D-mannitol.

Chapter 1 - Certain plants, yeasts, algae, lichen and fungi produce large amounts of D-mannitol and many bacteria developed the capacity to utilize this naturally occurring carbon and energy source. For that purpose they use different transport systems and catabolic pathways. Some bacteria transport D-mannitol via ion-driven co-transport systems or ABC transporters without modification of their substrate. Intracellular D-mannitol is subsequently converted into fructose by the D-mannitol 2-dehydrogenase MtlD (l) and fructose is phosphorylated to the glycolytic intermediate fructose-6-P. Numerous other bacteria take up D-mannitol via the phosphoenolpyruvate (PEP): carbohydrate phosphotransferase system (PTS), which catalyzes the transport and concomitant phosphorylation of its substrates. D-mannitol transported by the PTS therefore arrives as D-mannitol-1-P in bacterial cells. The enzyme D-mannitol-1-P 5-dehydrogenase MtlD(s) converts D-mannitol-1-P into fructose-6-P. The genes encoding the different types of transport and catabolic enzymes are usually organized within the *mtl* operon. Transcription of the *mtl* operon is also controlled by various mechanisms, which allow the expression of the *mtl* genes only when mannitol is present in the growth medium. The *mtl* operon is usually also submitted to carbon catabolite repression, which prevents its expression when an efficiently metabolizable

carbon source, such as glucose, is present. Bacteria not only utilize mannitol, but some are also able to produce D-mannitol from fructose with the aid of the enzyme Mdh. This is of biotechnological interest and improvement of the yield of mannitol biosynthesis might allow a cheaper and more efficient production of D-mannitol compared to the industrial production via catalytic reduction of fructose.

Chapter 2 - In symbiotic lichens which have *Trebouxia* as the algal partner, photosynthesis by the algae results in the production of the soluble carbohydrate ribitol which is then transported to the fungus where it is converted to arabitol and mannitol. Within the fungus, arabitol may act as a short-term carbohydrate reserve while mannitol may have a more protective function and be important in stress resistance. The concentrations of ribitol, arabitol, and mannitol were measured, using gas chromatography, in the central areolae and marginal hypothallus of the crustose lichen *Rhizocarpon geographicum* (L.) DC. growing on slate rocks in north Wales, UK. The concentrations of all three soluble carbohydrates were greater in the central areolae than in the marginal prothallus. In addition, the ratio of mannitol in the prothallus to that in the areolae was least in July. The concentration of an individual carbohydrate in the prothallus was correlated primarily with the concentrations of the other carbohydrates in the prothallus and not to their concentrations in the areolae. Low concentration of ribitol, arabitol, and mannitol in the marginal prothallus compared with the central areolae suggests either a lower demand for carbohydrate by the prothallus or limited transport from areolae to prothallus and may explain the low growth rates of this species. In addition, soluble carbohydrates appear to be partitioned differently through the year with an increase in mannitol compared with arabitol in more stressful periods.

Chapter 3 - Mannitol, a white, crystalline alcohol, is derived from sugar by reduction. The pathway to obtain mannitol from natural products is through hydrogenation of fructose, which is formed from either starch or sugar. This substance is present in a wide variety of natural products, and in almost all plants. It is used as an osmotic diuretic agent and a weak renal vasodilator. Aqueous solutions of mannitol are mildly acidic and sometimes such solutions are used to decrease the pH. Mannitol is clinically used in osmotherapy to temporarily reduce acute intracranial pressure while waiting for the definitive treatment. It is also used to treat patients with oliguric renal failure. Consequently, mannitol increases water and Na^+ excretion, thereby decreasing extracellular fluid volume. It can also be used as a facilitating agent for transporting drug agents directly into the brain. This chapter reviews the

possible mechanisms of mannitol and its metabolite that are involved in common clinical disorders. Moreover, the analytical techniques and biochemical markers used to monitor mannitol and its metabolite are described in this recompilation.

Chapter 4 - Nowadays thermal energy storage (TES) systems are proposed as one of the most powerful technologies to be charged with heat (or cold) and hold energy over time by shifting demand over time to reduce peak loads and facilitating the greater use of renewable energy by storing the energy produced so it can coincide with demand. TES systems are able to store energy as sensible heat leading with temperature increment of the storage medium, as latent heat storing energy using the latent heat produced when a phase change state occur using phase change materials–PCM, and as chemical reaction energy storing energy using a exothermic/endothermic reversible reaction by thermochemical materials (TCM). Solar cooling and air-conditioning is a technology that allows coincidence of solar gains with cooling loads reducing peak loads created by air-conditioning. TES systems can be coupled between absorption chillers and solar collectors in order to use the energy stored when the there is a peak load or the system is practically discharged. In addition, phase change materials – PCM candidates must fulfill several conditions to be used as storage materials: melting point of PCM must be closed to selected work temperature range, high latent heat and high specific heat, elevate thermal conductivity (solid and liquid state) to support charging and discharging processes inside the storage system. Additionally, the change volume during phase change transformation must be minimum, as well as the pressure vapor, allowing the use of conventional containers. Moreover, it must melt congruently with minimum subcooling and it must be chemically stable. D-mannitol has a phase change temperature at 167 °C and a phase change enthalpy is around 316 $kJ \cdot kg^{-1}$. These thermophysical properties turn d-mannitol as a perfect candidate to be used as PCM and it was studied with this purpose. D-mannitol was characterized performing differential scanning calorimetry under dynamic mode using a 0.5 $K \cdot min^{-1}$ heating rate between 25 °C and 200 °C. This substance was cycled several times and results shows 3 different thermal behavior: The first one was a single peak at 167 °C, the second has double peak at 156 °C and 167 °C, and the third thermal behavior is a single peak at 157 °C. Accordingly, there is a polymorphic transformation which was studied with FT-IR and two different phases were identified: β-phase and δ-phase. Then, a temperature range was established to work with this substance as PCM between 135 °C and 175 °C. This working range includes the phase change transformation of the two phases under analysis (δ

and β). To test the d-mannitol thermal behaviour at pilot plant scale, 150 kg of d-mannitol were introduced in a storage tank which was designed as shell-and-tubes heat exchanger. Results show that applying different cooling conditions produces d-mannitol polymorphic changes. Moreover, it has been shown that the working range (between 135°C and 175°C) is adequate for pilot plant experiments.

Chapter 5 - As a kind of readily available carbohydrate, D-mannitol has been widely used in the synthesis of chiral phosphorous ligands. This chapter reviews the design and synthesis of various mono- and diphosphorous ligands based on D-mannitol backbone. Also, the successful applications of these ligands in asymmetric catalysis, such as enantioselective hydrogenation and enantioselective conjugate addition, are summarized.

CONTENTS

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Chapter 1

UTILIZATION AND PRODUCTION OF D-MANNITOL BY BACTERIA

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ABSTRACT

Certain plants, yeasts, algae, lichen and fungi produce large amounts of D-mannitol and many bacteria developed the capacity to utilize this naturally occurring carbon and energy source. For that purpose they use different transport systems and catabolic pathways. Some bacteria transport D-mannitol via ion-driven co-transport systems or ABC transporters without modification of their substrate. Intracellular D-mannitol is subsequently converted into fructose by the D-mannitol 2-dehydrogenase MtlD (l) and fructose is phosphorylated to the glycolytic intermediate fructose-6-P. Numerous other bacteria take up D-mannitol via the phosphoenolpyruvate (PEP): carbohydrate phosphotransferase system (PTS), which catalyzes the transport and concomitant phosphorylation of its substrates. D-mannitol

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transported by the PTS therefore arrives as D-mannitol-1-P in bacterial cells. The enzyme D-mannitol-1-P 5-dehydrogenase MtlD(s) converts D-mannitol-1-P into fructose-6-P. The genes encoding the different types of transport and catabolic enzymes are usually organized within the *mtl* operon. Transcription of the *mtl* operon is also controlled by various mechanisms, which allow the expression of the *mtl* genes only when mannitol is present in the growth medium. The *mtl* operon is usually also submitted to carbon catabolite repression, which prevents its expression when an efficiently metabolizable carbon source, such as glucose, is present. Bacteria not only utilize mannitol, but some are also able to produce D-mannitol from fructose with the aid of the enzyme Mdh. This is of biotechnological interest and improvement of the yield of mannitol biosynthesis might allow a cheaper and more efficient production of D-mannitol compared to the industrial production via catalytic reduction of fructose.

INTRODUCTION

The hexitol D-mannitol is by far the most abundant sugar alcohol in nature. This sugar alcohol has the same stereochemical configuration as mannose and therefore possesses a two-fold symmetry axis. Rotation of the hexitol by 180° provides a molecule with identical configuration and the 1- and 6-position are therefore indistinguishable. Nevertheless, phosphorylated mannitol is usually referred to as mannitol-1-P and not as mannitol-6-P. D-Mannitol is produced in relatively large quantities for example by certain marine algae. In the brown seaweed *Laminaria japonica* mannitol is the most abundant carbohydrate. *Enterobacter* sp. JMP3 was recently shown to efficiently convert D-manitol produced by the seaweed into bioethanol [1]. In algae the polyol exerts multiple functions, such as osmoregulation, storage, regeneration of reducing power, and scavenging of active oxygen species [2, 3]. In these organisms, D-mannitol is mainly produced from fructose-6-P, which is reduced to D-mannitol-1-P by the enzyme D-mannitol-1-P 5-dehydrogenase and subsequently dephosphorylated to D-mannitol [4]. When D-mannitol-producing algae are transferred from a saline medium (sea water) to fresh water most of the accumulated intracellular D-mannitol is released into the environment by a yet unknown efflux system [5, 6]. In higher vascular plants D-mannitol is also one of the major photosynthetic products [7] and also protects against osmotic pressure and stress [2]. In celery and some related plants biosynthesis does not seem to occur from fructose-6-P but from mannose-6-P, which is reduced by an NADPH-dependent mannose-6-P 1-reductase to D-mannitol-1-P [8, 9]. Finally, a D-mannitol-1-P-specific phosphatase dephosphorylates D-mannitol-1-P to D-mannitol [6, 10]. Some plants

contain in the roots a mannose 1-oxidoreductase, which converts D-mannitol into mannose and not fructose [11]. The resulting mannose is specifically used for growth of the roots. Similar to algae, fungi and mushrooms primarily use an NADH-dependent D-mannitol-1-P 5-dehydrogenase to convert fructose-6-P into mannitol-1-P. Similar as in plants, an acid phosphatase was identified in mushrooms that catalyzes the dephosphorylation of D-mannitol-1-P to D-mannitol [12]. To a lesser extent fungi and mushrooms produce D-mannitol also from fructose-6-P by the NADH-dependent D-mannitol dehydrogenase [10].

Several bacteria, mostly heterofermentative lactic acid bacteria, are also able to produce D-mannitol when grown on specific media [13] and this biotechnological aspect will be discussed in detail. However, seen the abundance of D-mannitol in nature it is not surprising that most bacteria utilize the hexitol as a carbon and energy source. In this chapter we will describe the different bacterial transport systems used for the uptake of D-mannitol as well as the various catabolic routes and the enzymes catalyzing its catabolism. The synthesis of the enzymes required for the transport and metabolism of D-mannitol is tightly regulated. We will also describe the various mechanisms controlling induction and carbon catabolite repression of the various types of *mtl* operons.

UPTAKE AND METABOLISM OF D-MANNITOL

Most bacteria do not produce D-mannitol but use it as a carbon and energy source. For that purpose they developed transport systems allowing the uptake of the hexitol from the environment. Three major types of bacterial D-mannitol-specific transport systems can be distinguished: Uptake by ion gradient-driven transporters belonging to the major facilitator superfamily, uptake by ATP binding cassette (ABC) transporters and uptake via the phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS) (Figure 1). Facilitated diffusion of D-mannitol similar to that reported for the triol glycerol has so far not been reported. Uptake via ion gradient-driven transporters or ABC transporters occurs without any modification of the substrate. In contrast, during transport via the PTS D-mannitol is phosphorylated at the 1-position and therefore arrives as D-mannitol-1-P in the cytoplasm of bacterial cells. In bacteria taking up D-mannitol via an ion gradient-driven transporter (MtlT) or an ABC transport system (MtlEFGK) intracellular D-mannitol is usually first oxidized to D-fructose by the enzyme D-mannitol 2-dehydrogenase. The genes for the dehydrogenase and the ion gradient-driven transporter are organized in the *mtlTD* operon (Figure 2) [14]. This is also true for the genes encoding the D-mannitol ABC transporter

of the family pseudomonadaceae, where the *mtl* operon is formed by the *mtlEFGKDZY* genes (Figure 2), with *mtlZ* and *mtlY* encoding carbohydrate kinases. The MtlD proteins of corynebacteria and pseudomonadaceae have a similar length (about 500 amino acids) and exhibit significant sequence identity (more than 40%). They belong to the long chain alcohol dehydrogenases [15]. The ABC transporter of *Pseudomonas fluorescens* has a relatively broad substrate specificity and in addition to D-mannitol takes up also D-glucitol and arabitol [16]. Consequently, the dehydrogenase MtlD (l) of the members of this family oxidizes the two hexitols to fructose and the pentitol arabitol to xylulose [17].

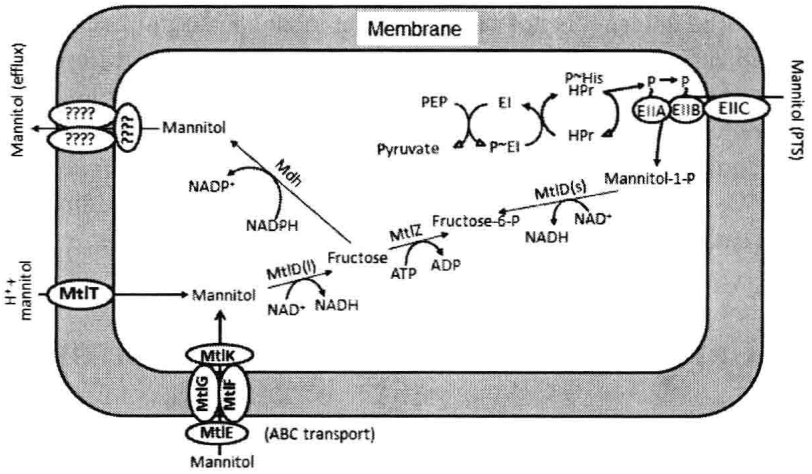

Figure 1. Schematic presentation of the three different known D-mannitol uptake systems, the catabolic pathways of D-mannitol and a potential D-mannitol efflux system present in some heterofermentative lactic acid bacteria. D-Mannitol can be transported by bacteria via ion-driven permeases (MtlT), ABC transport systems (MtlEFGK) or PTS permeases (MtlA or MtlA and MtlF). D-Mannitol transported by the PTS arrives as D-mannitol-1-P in the cell and is subsequently converted to the glycolytic intermediate D-fructose-6-P by a short-chain alcohol dehydrogenase MtlD(s). D-Mannitol taken up by an ion-driven permease or an ABC transport system is first oxidized to D-fructose by a long-chain alcohol dehydrogenase MtlD (l) and subsequently phosphorylated to D-fructose-6-P. MtlE of the ABC transport complex is a D-mannitol binding protein located in the periplasm, MtlK is an ATP hydrolyzing protein providing the energy for the transport process and MtlF and MtlG are two membrane-spanning proteins. Some heterofermentative lactic acid bacteria produce D-mannitol from D-fructose in an NADPH-requiring reaction catalyzed by the enzyme Mdh, another type of mannitol 2-dehydrogenase. D-Mannitol might be secreted into the medium via an ABC efflux system, the genes of which are frequently located in the vicinity of the *mdh* gene.

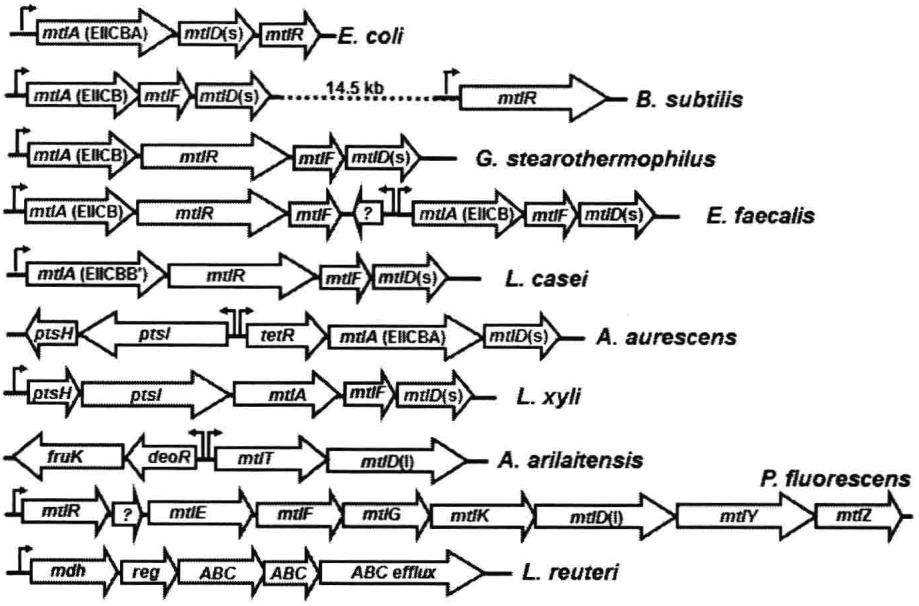

Figure 2. The gene organization of D-mannitol-specific regions in different bacteria. Shown are the D-mannitol-specific regions for organisms transporting D-mannitol via the PTS, ABC transport systems or ion-driven permeases. The genes encoding D-mannitol or D-mannitol-1-P dehydrogenases (both called *mtlD*) belonging either to the long- or the short-chain alcohol dehydrogenases, respectively, are distinguished by adding (l) or (s) to *mtlD*. The *tetR* gene encodes a TetR-like and *deoR* a DeoR-like transcription activator and *reg* an unknow regulator. The *mdh* gene codes for an $NADP^+$-dependent D-mannitol dehydrogenase, *fruK* as well as *mtlZ* for a fructokinase, *mtlY* for a xylulose kinase, and *mtlT* for an ion-driven D-mannitol permease of the major facilitator superfamily. The D-mannitol-specific ABC transport system of pseudomonodaceae is encoded by *mtlE* (D-mannitol binding protein), *mtlK* (ATP binding protein) and *mtlF* and *mtlG* (two transmembrane transport proteins). The transcription activator MtlR has been identified for strain DSM 50106 [42] and a similar gene is located upstream from *mtlE* in strain SBW25, in others quite distant from the *mtl* operon. The organization of D-mannitol PTS regions is highly variable and the examples presented here are not exhaustive. The most common operon organization in enterobacteriaceae is *mtlADR* and in firmicutes *mtlARFD*. Highly remarkable are the co-localization of the *ptsHI* (*ptsIH*) genes encoding the general PTS proteins EI and HPr with the D-mannitol-specific PTS genes in *L. xyli* and *A. arilaitensis*, the duplication of the EIIB domain in several homofermentative lactic acid bacteria and the duplication of all D-mannitol-specific PTS domains/proteins in several enterococci.

Corynebacterium glutamicum was reported to lack a fructokinase converting fructose into fructose-6-P and fructose formed from D-mannitol was therefore found to be secreted into the medium and subsequently taken up and phosphorylated by a fructose-specific PTS [14]. One of the carbohydrate kinases

encoded by the *mtl* operon, MtlZ, strongly resembles fructokinase from *Vibrio alginolyticus* and has indeed been shown to convert fructose formed from D-mannitol or D-glucitol by the D-mannitol dehydrogenase MtlD(l) into fructose-6-P [16]. The other kinase MtlY phosphorylates xylulose formed by MtlD(l) from arabitol into xylulose-5-P [16].

Gram-positive organisms including firmicutes and actinobacteria as well as enterobacteriaceae take up D-mannitol via a PTS and phosphorylate it already during its transport to D-mannitol-1-P, which is subsequently converted to fructose-6-P by the enzyme D-mannitol-1-P 5-dehydrogenase (MtlD). Unfortunately, the nomenclature is ambiguous because both, the D-mannitol 2-dehydrogenase of bacteria transporting D-mannitol via ion-driven transporters or ABC transport systems as well as D-mannitol-1-P 5-dehydrogenase of bacteria transporting D-mannitol via a PTS were called MtlD. D-Mannitol-1-P 5-dehydrogenases and D-mannitol 2-dehydrogenase have a different length (about 370 and 500 amino acids, respectively) and belong to the short-chain and long-chain alcohol dehydrogenases [15]. In this article they will be distinguished as MtlD(l) for the long-chain and MtlD(s) for the short-chain alcohol dehydrogenases. The two types of alcohol dehydrogenases probably have the same evolutionary origin, because despite their different lengths the C-terminal part of mannitol-1-P 5-dehydrogenases from firmicutes and enterobacteriaceae exhibits significant sequence similarity (about 40%) to the central part of mannitol dehydrogenases from corynebacteria and pseudomonadaceae.

In order to phosphorylate its substrate during the transport step four of the ususally five PTS components form a phosphorylation cascade (Figure 3). Enzyme I (EI) autophosphorylates with PEP and transfers the phosphoryl group to the histidyl residue of the second general PTS protein HPr. P~His-HPr phosphorylates one of usually several sugar-specific EIIA components and P~EIIA donates its phosphoryl group to the cognate EIIB. In the last step, P~EIIB phosphorylates the carbohydrate (sugar, sugar alcohol, amino sugar and many other sugar derivatives) bound to the corresponding membrane integral EIIC. Phosphorylation of the carbohydrate lowers the affinity for its EIIC and the phosphorylated substrate is released into the cytoplasm (Figure 3) [18]. The EII components are frequently fused together providing one single protein. This is the case for the D-mannitol-specific MtlA protein of *E. coli* and other enterobacteriaceae, which is composed in the following order of the EIIC, EIIB and EIIA domains. Several actinobacteria, such as *Arthrobacter aurescens*, also possess an $EIICBA^{Mtl}$ protein composed of the three mannitol-specific domains. This is also true for *Corynebacterium durum*, while, as mentioned above, most other corynebacteria possess an ion-driven transporter MtlT [14]. In contrast, in