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MINI-REVIEW

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New ubiquitous translocators: amino acid export by *Corynebacterium glutamicum* and *Escherichia coli*

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Abstract Molecular access to amino acid excretion by *Corynebacterium glutamicum* and *Escherichia coli* led to the identification of structurally novel carriers and novel carrier functions. The exporters LysE, RhtB, ThrE and BrnFE each represent the protoype of new transporter families, which are in part distributed throughout all of the kingdoms of life. LysE of *C. glutamicum* catalytes the export of basic amino acids. The expression of the carrier gene is regulated by the cell-internal concentration of basic amino acids. This serves, for example, to maintain homoeostasis if an excess of L-lysine or L-arginine inside the cell should arise during growth on complex media. RhtB is one of five paralogous systems in *E. coli*, of which at least two are relevant for L-threonine production. A third system is relevant for L-cysteine production. It is speculated that the physiological function of these paralogues is related to quorum sensing. ThrE of *C. glutamicum* exports L-threonine and L-serine. However, a ThrE domain with a putative hydrolytic function points to an as yet unknown role of this exporter. BrnFE in *C. glutamicum* is a two-component permease exporting branched-chained amino acids from the cell, and an orthologue in *B. subtilis* exports 4-azaleucine.

Keywords Carrier · Exporter · Efflux · Topology · Metabolite control · Amino acids · L-Cysteine · L-Threonine · L-Lysine · Biotechnology

Introduction

The view that amino acid efflux in industrial processes is artificial and therefore specific mechanisms for amino acid excretion are unlikely originally prevented investigations on the efflux of amino acids as occurs, for instance, with mutant strains of *Escherichia coli* and *Corynebacterium glutamicum*. However, Krämer and coworkers succeeded in demonstrating that the excretion of L-threonine from *E. coli* and the excretion of L-glutamate, L-lysine, L-isoleucine, and L-threonine from *C. glutamicum* is an active process (Krämer 1994). Based on these seminal studies, it has recently become possible to identify specific export carriers at the molecular level. This has led to the identification of novel transporter families and to basic insights into the bacterial amino acid balance. This mini-review summarizes what is known on these new types of carriers of *C. glutamicum* and *E. coli*. As yet, functional identifications of the carriers identified are only available for these two organisms since they are the main bacteria used for amino acid production. As evident from the numerous genome sequences now available, export carriers are widespread. Recent reviews are available on the production of amino acids by *C. glutamicum* and *E. coli* and on aspects of amino acid export by these bacteria (Burkovski and Krämer 2002; Eggeling and Sahm 2001; Eggeling et al. 2001a).

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The cell wall *of C. glutamicum*

While *E. coli* is probably quite familiar to the reader, this may not be the case with *C. glutamicum*; therefore, the special features of the cell wall structure of these two organisms will be briefly compared here. This is meaningful since several components of the cell wall are of significance for metabolite transport (Eggeling and Sahm 2001). Within the actinomycetes, *C. glutamicum* and closely related *Corynebacterium* species such as *C. melassecola*, *C. lactofermentum, C. flavum*, *C. efficiens*, and *Brevibacterium thiogenitalis* belong to the suborder *Corynebacterianeae* (Stackebrandt et al. 1997). This taxon also includes, for example, *Mycobacterium tuberculosis*. The *Corynebacterianeae* are characterized by an unusual cell wall (Brennan and Nikaido 1995; Puech et al. 2001). Whereas the classification according to Gram-staining only recognizes two types of bacterial cell walls, various biochemical analyses have led to a much more differentiated picture (Fig. 1). Thus, in addition to the cytoplasmic membrane, bacteria

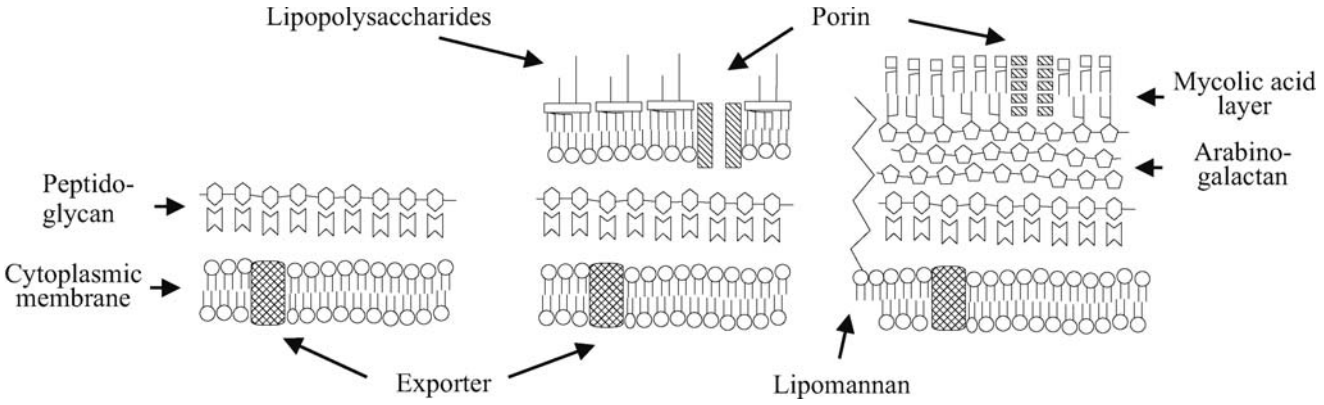

Fig. 1 The cell envelope of *Corynebacterianeae* (*right*) differs substantially from the canonical cell-wall structures of gram-positive (*left*) and gram-negative (*middle*) bacteria. The models do not contain all components. In most of the gram-positive bacteria (*left*), the cytoplasmic membrane is covered by a porous peptidoglycan layer which does not represent a permeability barrier. Gram-negative bacteria (*center*) are surrounded by two membranes. The outer membrane functions as an efficient permeability barrier and contains lipopolysaccharides and porins. In *Corynebacterianeae* (*right*) the peptidoglycan is linked to the heteropolysaccharide arabinogalactan, which in turn is attached to the mycolic acids. Together with soluble mycolic acid derivatives, they form an outer lipid bilayer that in mycobacteria serves as an exceptionally efficient permeability barrier. Since there are considerable variations of this outer lipid layer within the *Corynebacterianeae* (Guerardel et al. 2002), the influence of this barrier on the export of metabolites in *C. glutamicum* is not yet well defined. The structure of the glycolipid (lipomannan) anchored in the cytoplasmic membrane is also unknown

belonging to the *Corynebacterianeae* have a second lipid layer representing a pseudo outer membrane. In this respect, they resemble bacteria such as *E. coli*. The major fraction of this outer lipid layer consists of special branched fatty acids, the mycolic acids. The cytoplasmic membrane represents the major barrier to amino acid export, but the outer lipid layer can additionally control the efflux of certain amino acids. This will be discussed below in connection with L-glutamate efflux. At least for *M. tuberculosis* and *Mycobacterium smegmatis*, the outer lipid layer is regarded as a considerable permeation barrier that, in this case, presents a serious obstacle to the diffusive access of antibiotics (Brennan and Nikaido 1995). Current research on amino acid transport concerns novel carriers from *E. coli* and *C. glutamicum* that mediate export across the cytoplasmic membrane.

The L-lysine exporter

The cloning of the L-lysine export carrier gene *lysE* was a breakthrough in the molecular analysis of amino acid export (Vrljic et al. 1996). In contrast to the cloning of drug exporters that confer resistance, cloning of *lysE* from *C. glutamicum* required inducible lysine synthesis (Vrljic et al. 1995). The *lysE*-encoded polypeptide is a small membrane protein of 25.0 kDa that exhibits five transmembrane-spanning helices. By analogy with other small transporter proteins, e.g. Emr or AQP1 (Murata et al. 2000), LysE might be active as an oligomer, in this case a dimer. In addition to the transmembrane-spanning helices, a sixth hydrophobic segment is present in LysE that may dip into the membrane or be surface-localized (Vrljic et al. 1999). The energy source driving L-lysine export is the proton motive force (H^+ antiport or OH^- symport). The presence of this type of exporter obviously requires strict control of the export process. There are two mechanisms that serve this purpose. At the genetic level, the regulator LysG only drives transcription of the carrier gene at an elevated intracellular L-lysine concentration (≥35 mM) (Bellmann et al. 2001), and, at the protein level, the carrier exhibits a rather weak affinity for L-lysine ($K_m \approx 20$ mM) (Bröer and Krämer 1991). Both features ensure that, under normal conditions, where the intracellular concentration of L-lysine is about 5 mM, no substantial excretion occurs.

In addition to L-lysine, LysE also exports L-arginine. Both amino acids are exported at a rate of 0.75 nmol min^{-1} (mg dry wt)$^{-1}$. Therefore, LysE is in fact an exporter of basic amino acids. L-Histidine, L-citrulline, and L-ornithine are not substrates of the carrier. Interestingly, in addition to L-lysine and L-arginine, also L-histidine and L-citrulline induce *lysE* expression, although the latter two amino acids do not serve as a substrate for the carrier (Bellmann et al. 2001).

Why amino acid export?

In environments rich in peptides, such as complex medium, peptide uptake may result in increased intracellular L-lysine concentrations. Since *C. glutamicum* is not able to degrade this amino acid, it must be pumped out of the cell to reduce the intracellular level. Consequently, when a *lysE* deletion mutant is grown on salt medium with glucose there is no observable phenotype. However, when a low concentration of the peptide Lys-Ala is added to the same medium (3 mM is sufficient), the intracellular L-lysine concentration accumulates to more than 1 M, resulting in growth arrest (Vrljic et al. 1996; Bellmann et al. 2001). Therefore, as a general conclusion, both the regulation of the synthesis of amino acids and also their export are mechanisms for achieving homoeostasis of the intracellular amino acid concentration. It may be anticipated that many

bacteria will prove to exhibit this type of intracellular amino acid control. Indeed, amino acid export has been observed, for instance, with *Streptococcus faecalis* (Nisbet and Payne 1982) and with *Lactococcus lactis* during growth on milk (Juillard et al. 1995), although the exporters responsible for this activity have not yet been identified.

The LysE family of translocators

At the time LysE was identified, only one other corresponding sequence was known. In the meantime, however, genome sequencing projects have shown that LysE proteins occur in many bacteria. At present, more than 30 functionally uncharacterized proteins with identities to LysE are known. They are found, for example, in *Bacillus subtilis*, *Aeromonas salmonicida*, *Helicobacter pylori*, *Vibrio cholerae*, and *Yersinia pestis* (Vrljic et al. 1999). Thus, LysE of *C. glutamicum* is the prototype of a new large family of translocators. Since members of a single transporter family seldom catalyze the transport of structurally divergent types of compounds (i.e. amino acids versus sugars), and, moreover, function with strongly preferential polarity of transport direction (i.e. outward versus inward) (Saier 2000), it is assumed that the majority of members of the LysE family export small positively charged molecules. In addition to L-lysine and L-arginine, these could also be structurally related substances or derivatives of basic amino acids. In fact, it has recently been shown that in the *att* locus of *Rhodococcus fasciens*, which is essential for the synthesis of an unknown compound, the LysE homologue AttX is present; this protein is associated with the virulence of the bacterium (Maes et al. 2001). Although the substance causing the virulence has not yet been identified, the *attA*, *attB* and *attH* genes, which are involved in its synthesis, share identities with L-arginine biosynthesis genes. This scenario makes it possible for AttX of *R. fasciens* to extrude an arginine derivative as the virulence-inducing factor.

The LysE superfamily

The CadD and the RhtB families are structurally very closely related to the LysE family. Together, these three families constitute the LysE superfamily of translocators (Vrljic et al. 1999). Members of the CadD family are present in gram-positive bacteria, e.g. *Staphylococcus* species, where they function in cadmium resistance (Chaouni et al. 1999). Another member of this family is QacF of *Bacillus firmus,* which possibly functions as an exporter of quaternary ammonium ions.

The RhtB family and amino acid export by *E. coli*

The RhtB family consists of at least 30 proteins. These proteins are present in gram-negative and gram-positive bacteria as well as in archaea (Aleshin et al. 1999). Interestingly, *E. coli* has as many as five paralogues, which were discovered and investigated in connection with L-threonine production using *E. coli* mutants (Aleshin et al. 1999; Zakataeva et al. 1999). We systematically studied all five paralogues using deletion mutants and overexpressing strains made from *E. coli* MG1655. The assay consisted of growth response on plates with a gradient of increasing metabolite concentrations. As shown in Table 1 and in accordance with the data of Zakataeva et al. (1999), overexpression of *rhtB* confers resistance to L-threonine, L-homoserine, and L-homoserine lactone, and a deletion mutant exhibits increased sensitivity to these compounds. A similar response is present with *yeaS* (Table 1). With *yfiK* and *rhtC* a growth response was observed only in the presence of L-threonine. However, it has recently been shown that YfiK exports L-cysteine and *O*-acetylserine (Franke et al. 2003). YfiK together with YdeD, a major facilitator protein, augment L-cysteine production by *E. coli* (Daßler et al. 2000). The specificity of the RhtB exporters and their true substrates are not known. The effects found with L-homoserine lactone led to the suggestion that they may be involved in quorum sensing (Aleshin et al. 1999). The recently completed genome sequence of *Shewanella oneidensis* revealed that this organism contains genes for seven RhtB paralogues and one LysE protein (Heidelberg et al. 2002).

Although it is probable that none of the five RhtB carriers of *E. coli* is the major carrier for active L-threonine export (Kruse et al. 2002), they are nevertheless relevant for the excellent properties of producer strains. Producers

Table 1 Growth response of ten isogenic *Escherichia coli* strains with altered gene expression to the presence of increasing concentrations of L-threonine, L-homoserine, and L-homoserine lactone. The response of clones with the denoted genes deleted (*Δ*) or overexpressed in pUC18 (*pUC*) is given in relation to the parental strain MG1655. The concentrations were 0–100 mM for L-threonine, 0–20 mM for L-homoserine, and 0–20 mM for L-homoserine lactone. ● Growth better than control (strain MG1655), ○ less growth, – no effect, *nd* not done

Compound added to the growth medium	Gene deleted/overexpressed									
	yahN		*yeaS*		*yfiK*		*rhtC*		*rhtB*	
	Δ	pUC	Δ	pUC	Δ	pUC	Δ	pUC	Δ	pUC
L-Threonine	–	–	○	●	–	●	○	●	○	●
L-Homoserine	–	○	–	●	–	○	–	nd	○	●
L-Homoserine lactone	–	○	○	●	–	○	–	nd	○	●

Fig. 2 Depiction of the domain structure of selected proteins of the ThrE family of exporters. *Arrows* indicate the C-termini. ThrE of *C. glutamicum* is given at the *top*; the hydrophobic part is shown as two *hatched rectangles*, which represent the carrier part with 10-transmembrane helices and illustrate its origin by duplication. In *Campylobacter jejeuni*, two adjacent genes encode the exporter, in which the two polypeptides, each with 5-transmembrane helices, are not fused. A hydrophilic domain probably not involved in export is indicated as a *black box*. *Mycobacterium tuberculosis* has two such hydrophilic domains. For further details see text

accumulate L-threonine concentrations in the medium ranging from more than 100 g l^{-1} up to the limit of solubility (Debabov 2002). However, the addition of 5 mM L-threonine to a culture of *E. coli* wild-type (MG1655) reduces the growth rate from 0.42 to 0.37 h^{-1}. Thus, at the exceptionally high concentrations that producer strains have to tolerate even a small difference in the movement of L-threonine over the membrane might contribute to the stability of strains and their accumulation properties. This view is supported by the fact that a producer strain has been found to be impaired in its L-threonine uptake (Okamoto et al. 1997), and that upon *rhtB* or *rhtC* overexpression the specific productivities in producer strains were slightly increased (Kruse et al. 2002).

The ThrE family and L-threonine export by *C. glutamicum*

The recently identified L-threonine exporter ThrE of *C. glutamicum* also represents the prototype of a new translocator family (Simic et al. 2001). This is in agreement with the notion that most eukaryotic transporters belong to already recognized families, whereas prokaryotic systems frequently belong to uncharacterized families of which no functionally characterized members are known (Saier 2000). The ThrE family is relatively small, with about 20 identified homologues. Members of the family are found in Bacteria, Archaea, and the fungal kingdom. It has been suggested that the carrier is active as a ten-transmembrane-spanning transporter. An interesting phylogenetic feature of the members of the ThrE family is that they exist either as a single long polypeptide chain or as two short polypeptides (Fig. 2). Together with the weak sequence similarities between the amino-terminal and carboxy-terminal parts of the large polypeptide, this is evidence that the proteins resulted from a gene duplication event (Yen et al. 2002). A similar situation is found with carriers of the drug-metabolite-exporter family and the major-intrinsic-protein family (Murata et al. 2000).

The ThrE exporters are characterized by another interesting structural feature. All of them show an extended hydrophilic N-terminal domain (black box in Fig. 2). This domain exhibits weak sequence similarity with portions of hydrolases (proteases, peptidases, and glycosidases). The occurrence of this domain is not likely to be accidental and must have functional significance. According to the topology prediction, it is located at the cytoplasmic side of the membrane. Thus, the hydrophilic domains of these exporters may be involved in generating the transport substrate by a hydrolytic activity present on the polypeptide. Furthermore, this domain supports the view that ThrE has an additional function and that L-threonine is not the true substrate but accepted due to a side activity of ThrE.

Nevertheless, at an intracellular L-threonine concentration of 170 mM, 59% of the L-threonine excretion in *C. glutamicum* is driven by ThrE activity. In addition, 22% of the total efflux is due to passive diffusion and the remaining 19% is due to at least one other as yet unidentified carrier (Simic et al. 2001). As far as biotechnology is concerned, it is interesting that increased export can be achieved in *C. glutamicum* by *thrE* overexpression, i.e. L-threonine accumulation is increased by up to 40 % upon *thrE* overexpression. Further increased accumulation is achieved by reduced cell-internal degradation of L-threonine. This degradation to glycine proceeds by aldol cleavage catalyzed by serine hydroxymethyl transferase (Simic et al. 2002).

The LIV-E family of translocators

As shown for *C. glutamicum* producer strains, L-isoleucine efflux is mediated both by diffusion and by active export. The export carrier involved has recently been identified (Kennerknecht et al. 2002). It exports L-isoleucine or L-leucine at comparable rates of about 7 nmol min^{-1} (mg dry wt)$^{-1}$, whereas L-valine is excreted at a significantly reduced rate. The exporter is a two-component permease encoded by *brnFE*, and similar proteins in *Bacillus subtilis* have been found to be related to 4-azaleucine resistance (Belitsky et al. 1997). The BrnF polypeptide is predicted to span the membrane seven times and the polypeptide of the smaller BrnE protein four times. The genes *brnF* and *brnE* are widespread in prokaryotes, but lacking in eukaryotes, and generally map together in the same order in operons. Together, BrnF and BrnE comprise the mem-

bers of the novel LIV-E family of exporters. A paralogue of this carrier is present in *C. glutamicum*. Some α-Proteobacteria, such as *Agrobacterium tumefaciens* and *Sinorhizobium meliloti* contain up to three paralogues. The fact that the phylogeny of LIV-E family members does not correlate to that of the organismal 16S rRNAs leads to the suggestion that these carriers do not play a role as essential "housekeeping" proteins. This suggestion is further supported by the fact that in both *C. glutamicum* and *B. subtilis* the LIV-E family carriers are nonessential.

Outlook

Novel families of export carriers have been identified in connection with bacterial amino acid excretion. The L-glutamate exporter of *C. glutamicum* may represent another new carrier family. However, this exporter has not yet been identified, although an average of almost 3,000 tonnes of sodium glutamate are produced daily using *C. glutamicum* and the process has been studied for almost 50 years. L-Glutamate excretion is a very special case, since additional treatments involving the entire cell wall, such as penicillin addition, are always necessary (Eggeling et al. 2001b). Also, the cell wall's outer lipid layer, consisting of mycolic acids, could in part limit L-glutamate efflux (Fig. 1). Mutants with a reduced mycolic acid content have altered permeability (Puech et al. 2000), and mutants defective in trehalose synthesis exhibit significantly increased L-glutamate production, from 40.2 to 45.6 g l^{-1} (Nakamura et al. 2002).

References

Aleshin VV, Zakataeva NP, Livshits VA (1999) A new family of amino-acid-efflux proteins. Trends Biochem Sci 24:133–135

Belitsky BR, Gustafsson MC, Sonenshein AL, von Wachenfeldt C (1997) An Lrp-like gene of *Bacillus subtilis* involved in branched-chain amino acid transport. J Bacteriol 179:5548–5457

Bellmann A, Vrljić M, Pátek M, Sahm H, Krämer R, Eggeling L (2001) Expression control and specificity of the basic amino acid exporter LysE of *Corynebacterium glutamicum*. Microbiology 147:1765–1774

Brennan PJ, Nikaido H (1995) The envelope of mycobacteria. Annu Rev Biochem 64:29–63

Bröer S, Krämer R (1991) Lysine excretion by *Corynebacterium glutamicum*. 1. Identification of a specific secretion carrier system. Eur J Biochem 202:131–5

Burkovski A, Krämer R (2002) Bacterial amino acid transport proteins: occurrence, functions, and significance for biotechnological applications. Appl Microbiol Biotechnol 58:265–274

Chaouni LB, Etienne J, Greenland T, Vandenesch F (1996) Nucleic acid sequence and affilation of pLUG10, a novel cadmium resistance plasmid from *Staphylcococcus lugdunensis*. Plasmid 36:1-8

Daßler T, Maier T, Winterhalter C, Bock A (2000) Identification of a major facilitator protein from *Escherichia coli* involved in efflux of metabolites of the cysteine pathway. Mol Microbiol 36:1101–1112

Debabov VG (2002) The threonine story. Adv Biochem Eng/Biotechnol 79:113–136

Eggeling L, Sahm H (2001) The cell wall barrier of *Corynebacterium glutamicum* and amino acid efflux. J Bioscience Bioeng 92:201–213

Eggeling L, Pfefferle W, Sahm H (2001a)Amino acids. In: Ratledge C, Kristiansen B (eds) Basic biotechnology. Cambridge University Press, pp 281–302

Eggeling L, Krumbach K, Sahm H (2001b) L-Glutamate efflux with *Corynebacterium glutamicum*: why is penicillin treatment or Tween addition doing the same? J Mol Microbiol Biotechnol 3:67–68

Franke I, Resch A, Dassler T, Maier T, Böck A (2003) YfiK from *Escherichia coli* promotes export of O-acetylserine and cysteine. J Bacteriol 185:1161–6

Guerardel Y, Maes E, Elass E, Leroy Y, Timmerman P, Besra GS, Locht C, Strecker G, Kremer L (2002) Structural study of lipomannan and lipoarabinomannan from *Mycobacterium chelonae*. Presence of unusual components with alpha 1,3-mannopyranose side chains. J Biol Chem277:30635–48

Heidelberg JF, Paulsen IT, Nelson KE, Gaidos EJ, Nelson WC, Read TD, Eisen JA, Seshadri R, Ward N, Methe B, Clayton RA, Meyer T et al. (2002) Genome sequence of the dissimilatory metal ion-reducing bacterium *Shewanella oneidensis*. Nat Biotechnol 20:1118–23

Juillard V, Le Bars D, Kunji ER, Konings WN, Gripon JC, Richard J (1995) Oligopeptides are the main source of nitrogen for *Lactococcus lactis* during growth in milk. Appl Environ Microbiol 61:3024–30

Kennerknecht N, Sahm H, Yen MR, Patek M, Saier MH Jr, Eggeling L (2002) Export of L-isoleucine from *Corynebacterium glutamicum*: a two-gene-encoded member of a new translocator family. J Bacteriol 184:3947–56

Krämer R (1994) Secretion of amino acid by bacteria: physiology and mechanism. FEMS Microbiol Rev 13:75–94

Kruse D, Krämer R, Eggeling L, Rieping M, Pfefferle W, Tchieu JH, Chung YJ, Jr Saier MH, Burkovski A (2002) Influence of threonine exporters on threonine production in *Escherichia coli*. Appl Microbiol Biotechnol 59:205–10

Maes T, Vereecke D, Ritsema T, Cornelis K, Thu HN, Van Montagu M, Holsters M, Goethals K (2001) The *att* locus of *Rhodococcus fascians* strain D188 is essential for full virulence on tobacco through the production of an autoregulatory compound. Mol Microbiol 42:13–28

Murata K, Mitsuoka K, Hirai T, Walz T, Agre P, Heymann JB, Engel A, Fujiyoshi Y. (2000) Structural determinants of water permeation through aquaporin-1. Nature 407:599–605

Nakamura J, Izui H, Nakamatsu T (2002) Bacterium producing L-glutamic acid and method for producing L-glutamic acid. European Patent Application EP 1 174 508 A2

Nisbet TM, Payne JW (1982) The characteristics of peptide uptake in *Streptococcus faecalis*: studies on the transport of natural peptides and antibacterial phosphonopeptides. J Gen Microbiol 128:1357–64

Okamoto K Kino K, Ikeda M (1997) Hyperproduction of L-threonine by an *Escherichia coli* mutant with impaired L-threonine uptake. Biosci Biotechnol Biochem 61:1877–82

Puech V, Bayan N, Salim K, Leblon G, Daffé M (2000) Characterization of the in vivo acceptors of the mycoloyl residues transferred by the corynebacterial PS1 and the related mycobacterial antigens 85. Mol Microbiol 35:1026–41

Puech V, Chami M, Lemassu A, Laneelle MA, Schiffler B, Gounon P, Bayan N, Benz R, Daffé M (2001) Structure of the cell envelope of corynebacteria: importance of the non-covalently bound lipids in the formation of the cell wall permeability barrier and fracture plane. Microbiology 147:1365–82

Saier MH Jr (2000) Families of transmembrane transporters selective for amino acids and their derivatives. Microbiology 146: 1775–95

Simic P, Sahm H, Eggeling L (2001) L-threonine export: use of peptides to identify a new translocator from *Corynebacterium glutamicum*. J Bacteriol 183:5317–24

Simic P, Willuhn J, Sahm H, Eggeling L. (2002) Identification of *glyA* (encoding serine hydroxymethyltransferase) and its use together with the exporter ThrE to increase L-threonine accumulation by *Corynebacterium glutamicum*. Appl Environ Microbiol 68:3321–7

Stackebrandt E, Rainey FA, Ward-Rainey NL (1997) Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. Int J Syst Bacteriol 47:479–491

Vrljić M, Kronemeyer W, Sahm H, Eggeling L (1995) Unbalance of L-lysine flux in *Corynebacterium glutamicum* and its use for the isolation of excretion-defective mutants. J Bacteriol 177: 4021–7

Vrljić M, Eggeling L, Sahm H (1996) A new type of transporter with a new type of cellular function: L-lysine export from *Corynebacterium glutamicum*. Mol Microbiol 22:815–826

Vrljić M, GargJ, Bellmann A, Wachu S, Freudl R, Malecki MJ, Sahm H, Kozina VJ, Eggeling L, Saier MH Jr (1999) The LysE superfamily: Topology of the lysine exporter LysE of *Corynebacterium glutamicum*, a paradyme for a novel superfamily of transmembrane solute translocators. J Mol Microbiol Biotechnol 1: 327–336

Yen MR, Tseng YH, Simic P, Sahm H, Eggeling L, Saier MH Jr. (2002) The ubiquitous ThrE family of putative transmembrane amino acid efflux transporters. Res Microbiol 153:19–25

Zakataeva NP, Aleshin VV, Tokmakova LL, Troshin PV, Livshits VA (1999) The novel transmembrane *Escherichia coli* proteins involved in the amino acid efflux. FEBS Letters 452:228–232