Secondary metabolic energy generating pathways in bacteria

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Bachelor thesis for the pre-master Molecular Biology and Biotechnology

Under the supervision of Dr. J.S. Lolkema

7th of January 2018

Preface

Beforehand, I want to thank my supervisor Dr. J.S. (Juke) Lolkema for his knowledge and guidance during my bachelor thesis. After a long career in the research profession, I wish him the best for his retirement in the near

Summary

Bacteria use catabolic mechanisms to obtain energy for energy requiring processes and biosynthesis. This energy is stored in forms of ATP and ion gradients. These forms are interconvertible through ion pumps. These pumps are primary transporters and energy obtained via this way is called primary metabolic energy generation. In addition to this, bacteria use secondary transporters to obtain energy via short, autonomous, catabolic pathways, which is called secondary metabolic energy generation. Secondary metabolic energy pathways are of importance in the manufacturing process of food and drink products, where fermenting lactic acid bacteria are used. In bacteria three types of secondary transporters are involved, uniporters, antiporter and symporters. In this essay, several decarboxylation- and direct level phosphorylation pathways that use secondary transporters to generate secondary metabolic energy are discussed.

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Introduction

Bacteria use nutrients as their source of matter and energy, which are essential for live. By degrading these nutrients, they can be used as building blocks for biosynthesis or as a source of free energy for energy requiring processes. Bacteria use catabolic mechanisms to degraded substrates in multiple steps to release free energy. This energy is termed metabolic energy and is stored in two major forms, ATP and ion (H^+ or Na^+) gradients across the membrane. In ATP, chemical energy is stored (ΔG_{ATP}), and is released by hydrolysis yielding ADP + P_i. With ion gradient, the free energy is stored in an electrochemical gradient of ions ($\Delta\mu_{H^+/Na^+}$). The gradient exerts force on the ions resulting in an ''ion motive force''. This ion (proton or sodium) motive force consist of a chemical gradient (ΔpH/Na) and the electrochemical potential (ΔΨ) described in the equation below. Where Z is the charge of the ion.

$$\Delta\tilde{\mu}_{H/Na^+} = -Z\Delta pH/Na + \Delta\Psi$$

Free energy stored in ATP and proton/sodium motive force (PMF/SMF) is available to the organism for energy consuming processes. By generating metabolic energy, free energy in these forms increases. The total energy free energy can increase by ion translocation using chemiosmotic pumps or by ATP synthesis via substrate level phosphorylation. Furthermore, these pools of energy are interconvertible by the use of reversible ATPase/synthase proteins that couple ion gradients to the hydrolyse or synthesise ATP. By for instance the membrane bound F_0F_1 ATPase complex (17). Na^+ and H^+ gradients are convertible through the use of H^+/Na^+ antiporters. These conversions are important, because if there is a shortage of one form it can be interconverted into the other. These conversions do not generate energy but require energy in the form of chemical energy or light. The Ion pumps, that use chemical energy, are primary transporters and this form of energy generation is referred to as primary metabolic energy generation. In addition to this primary metabolic energy generation, mostly in anaerobic bacteria, another system has evolved. This system uses secondary transporters to generate proton motive force indirectly or in combination with substrate level phosphorylation for direct ATP synthesis (fig 1). These forms of metabolic energy generation, which uses secondary transporters, is called secondary metabolic energy generation and consist of autonomous, short, catabolic pathways, which are the subject of this essay. Furthermore, these fermentation pathways are present in many lactic acid bacteria that have practical applications in the manufacturing process of food and drink products (11) (8) (9).

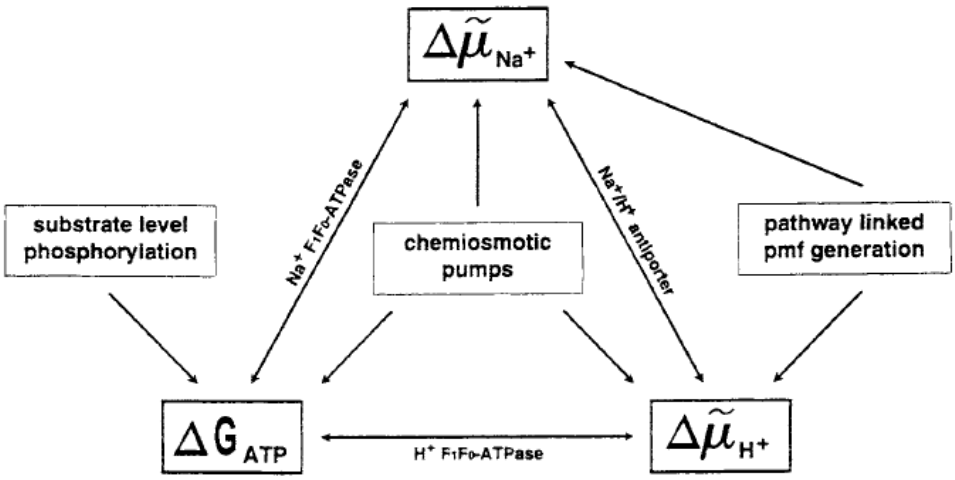

Figure 1: Schematic view of the generation and interconversion of metabolic energy in form of ATP and ion gradients by primary and secondary metabolic energy generation and chemiosmotic pumps (11).

1. Secondary transport systems

Secondary transport is a form of transport across biological membranes. A transporter protein couples the movement of one solute (or ion) down its electrochemical gradient to the upwards movement of another solute against its electrochemical or concentration gradient. Energy is thus stored from the electrochemical gradient of one solute (or ion) into the electrochemical gradient of another solute to drive its transport against the gradient. Secondary transporters can also use facilitated diffusion to transport a solute down its electrochemical gradient without the coupling of another. Which is different from primary transport. There the chemical energy released by ATP hydrolysis drives the transport of the solute against the gradient. Three types of secondary transporters are found in bacteria: *Antiporters* exchanges one solute for another, *symporters* couple the transport of two or more solutes in the same direction and *uniporters* catalyse transport of a single solute across the cytoplasmic membrane by facilitated diffusion (fig 2). Usually, because accumulation of solutes is favoured, electrochemical energy in the form of ions is used to drive transport of solutes. Nevertheless, this translocation reaction is reversible and the electrochemical energy of a solute can therefore also drive ion transport. Fermenting bacteria have large amount of metabolic end-products from the glycolysis, like lactate, that accumulate in the cell and may exceed the electrochemical gradient of ion. Thus, the electrochemical energy of the solute can be converted into electrochemical energy of an ion gradient in the form of proton motive force, which the cell can use as a source of metabolic energy. Transporters that use solute gradients to drive ion transport are electrogenic transporters (20) (21).

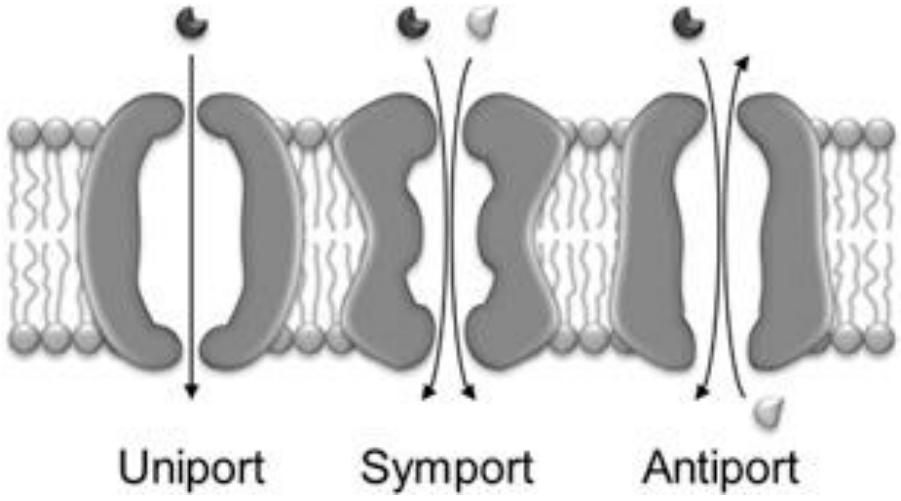

Figure 2: Illustration of the secondary transporter types in bacteria: *Antiporters* exchanges one solute for another, *symporters* couple the transport of two or more solutes in the same direction and *uniporters* catalyse transport of a single solute across the cytoplasmic membrane by facilitated diffusion (7).

2. Decarboxylation pathways

In decarboxylation pathways, the secondary metabolic energy generation pathways use the free energy released in decarboxylation in combination with secondary electrogenic transporters to generate metabolic energy. The energy transduction does not drive ion transport directly but by two separate steps. First by the conversion of substrate to product. The decarboxylation reaction takes up a proton, making the environment inside the cell more alkaline, creating a gradient of protons (ΔpH). Furthermore, by exchanging the product for its precursor, by a electrogenic secondary transporter, either one nett positive charge is transported out or one nett negative charge is transported into the cell. The translocation is driven by an inwardly directed substrate concentration gradient and outwardly directed product concentration gradient, maintained by the decarboxylation reaction. Due to the translocation a membrane potential is generated (ΔΨ), which is a counteractive force that works against the translocation. These two factors together generate a PMF that can be used for energy consuming processes. The pathways use amino acids such as histidine and tyrosine or di- and tri-carboxylic acids such as oxalate, malate and citrate as substrates. In this section the different pathways and the involvement of decarboxylation and secondary transporters are discussed (3) (4).

2.1. Tyrosine pathway

Tyrosine is an amino-acid that is present in relatively high concentrations in cheese products. The tyrosine pathway is only present in some lactic acid bacteria. In *Lactobacillus brevis,* the tyrosine pathway was observed. In this pathway the amino acid tyrosine is unimported into the cell by the membrane transport protein TyrP. In the cell, the decarboxylase enzyme TyrDC decarboxylates tyrosine to monovalent tyramine and CO_2. Due to the reaction, a proton is taken up, which yields a more alkaline environment inside the cell and a positively charged product. The secondary transporter TyrP also functions as a electrogenic precursor/product exchanger. The transporter catalyses the exchange of the monovalent tyramine inside for the electroneutral precursor, driven by the concentration gradients of precursor and product. During the exchange one nett positive charge is translocated out of the membrane generating a membrane potential. Consequently, a PMF is generated, which the cell can use for other energy consuming processes. An illustration of the pathway is shown in figure 3 (24).

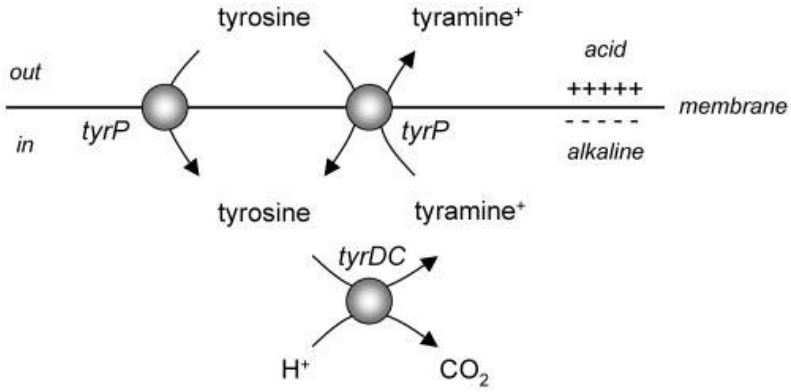

Figure 3: The tyrosine pathways in L. brevis illustrated. Tyrosine is uniported by TyrP and decarboxylated by TyrDC to monovalent tyramine yielding a proton gradient. Furthermore, tyramine is exchanged for its precursor tyrosine by TyrP, yielding a membrane potential and generating a PMF (24).

2.2. Histidine pathway

A familiar system of amino acid catabolism is the histidine pathway. Histidine is present in high protein containing food like eggs and meat. In Lucas et al 2005 it was reported that the pathway functions as an acidic stress relieve in acidic environment next to the metabolic energy generation. The pathway is, among others, present in *Lactobacillus hilgardii* strain IOEB 0006. In this bacterium a plasmid was found, which contains the sequence for a transporter protein and a decarboxylase enzyme. Inside the cell, histidine is decarboxylated by the enzyme(s) HdcA and/or HdcB to monovalent histamine and CO_2. Due to the decarboxylation, a proton is taken up and the environment becomes more alkaline, yielding a proton gradient. The transporter protein HdcP functions as a electrogenic precursor/product exchanger. The transporter, exchanges histidine outside for monovalent histamine inside the membrane, driven by the concentration gradients of the precursor and product. Thereby, translocating one nett positive charge to the outside and generating a membrane (inside negative). An illustration of the pathway is shown in figure 4. The factors combined result in a PMF, which can be utilized for energy consuming processes (12) (16).

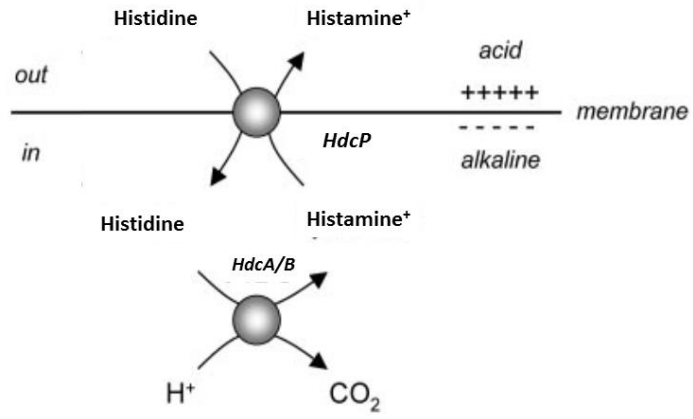

Figure 4: The histidine decarboxylation pathway in *L. hilgardii* illustrated. Histidine is decarboxylated in the cytoplasm by HdcA/B yielding an alkaline environment inside the cell. The secondary transporter HdcP exchanges the positively charged histamine for its precursor, thereby generating a membrane potential (12) (16).

2.3. Oxalate pathway

The oxalate pathway is one of the first and well-known decarboxylation pathways and was observed in *Oxalobacter formigenes*, a gram-negative bacterium found in mammalian intestine. Oxalic acid is found in many foods and readily available in the intestine. The oxalate pathway in this bacterium is an exception compared to other pathways. Decarboxylation pathways usually are an addition to the primary metabolic energy generation. Nevertheless*, O. formigenes* is able to grow solely on oxalate using the decarboxylation pathway and obtains all its ATP via PMF driven ATP synthesis. In this pathway the secondary transporter OxIT is involved. OxIT catalyses the electrogenic exchange of divalent oxalate for its decarboxylation product monovalent formate, driven by the concentration gradients of the precursor and product, thereby generating a membrane potential. After transport into the cell, oxalate is decarboxylated by a cytoplasmic decarboxylation enzyme to yield CO_2 and formate. During the decarboxylation a proton is taken up making the environment inside the cell more alkaline. Both processes together yield a PMF, which is utilized by the F_0F_1 ATPase to synthesize ATP (1) (14 An illustration of the pathway is shown in figure 5.

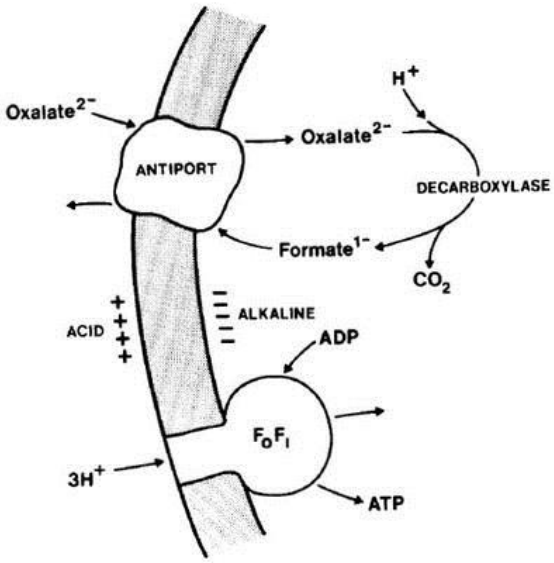

Figure 5: Illustration of the oxalate pathway in *O. formigenes*. Oxalate is decarboxylated in the cytoplasm, yielding a formate and a more alkaline environment inside. Formate is exchanged for its precursor oxalate by the OxIT transporter, translocating one net charge and generating membrane potential. The PMF generated by the 2 steps is used to synthesize ATP via the F_oF_1 ATPase complex (14).

2.4. Malate fermentation

Another secondary metabolic pathway that uses decarboxylation and a secondary transporter is the malate pathway. Malate is a di-carbocyclic acid and gives the sour taste to many fruits such as apples and is also found in grapes. The malate fermentation pathway is not generally present in lactic acid bacteria, but was only observed in strains of *Lactococcus*, *Lactobacillu*s, *Pediococcus*, and *Oenococcus*. Malate fermentation follows different pathways in these strains. In *L. lactis* the malate pathway consists of 2 enzymes, the malolactic enzyme MleS and the secondary transporter MleP. MleS facilitates the decarboxylation of malate yielding lactate. Afterwards the product lactate is exchanged for its precursor malate by the transporter MleP. MleP functions as an electrogenic precursor/product exchanger and the monovalent lactate from inside the cell is exchanged for divalent malate outside of the cell. Due to this exchange of malate for lactate by MleP, driven by the concentration gradients of precursor and product, one net negative charge is transported into the cell, which generates a

membrane potential. During the decarboxylation a proton is taken up, making the environment inside the cell becomes more alkaline and yielding a proton gradient. The decarboxylation and electrogenic exchange generate a PMF, which can be used for energy consuming processes. An illustration of the pathway is shown is figure 6.

In the non-alcoholic fermentation process of wine, the malate pathway follows a slightly different route. In wine, the bacterium *Oenococcus oeni* uses malate fermentation as a secondary metabolic energy generating pathway. But here, because wine is very acidic, malate and its decarboxylation product lactate are in a protonated state. Because of this protonation, lactate has no charge, leaves the cell by passive diffusion and cannot be used for the electrogenic exchange with its precursor malate. Here the pathway uses an electrogenic uniporter to import monovalent malate into the cell. The uptake of the solute is driven by the gradient of the substrate directed inwardly. Inside the cell malate is decarboxylated yielding lactate and resulting in an alkalization inside the cell generating a proton gradient. The electrogenic uniport of malate and the alkalization of cytoplasm generates a PMF (8) (22) (23). An illustration of the pathway is shown in figure 7.

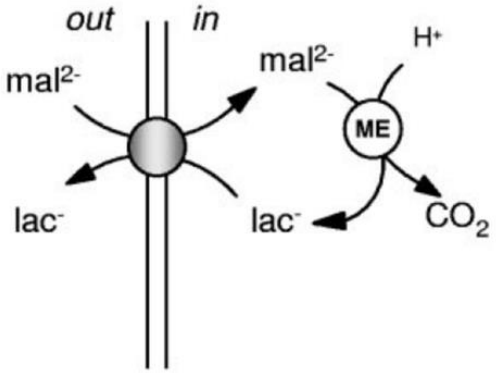

Figure 6: illustration of the malate fermentation pathway in, among others, *L. lactis*. Divalent malate is decarboxylated to monovalent lactate generating a proton gradient. Furthermore, precursor and product are exchanged generating a membrane potential (23).

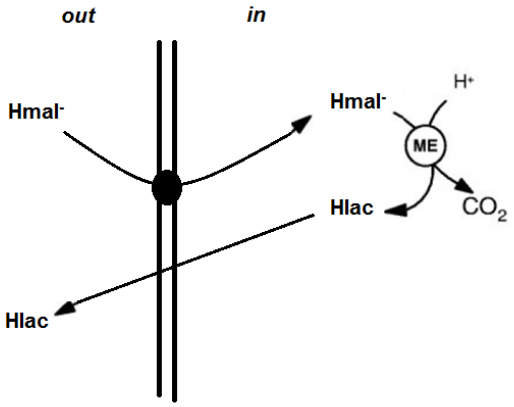

Figure 7: illustration of the malate fermentation in *O. oeni,* where malate and lactate are both protonated. Monovalent malate is uniported, generating membrane potential. Malate is decarboxylated generating a proton gradient, the end-product lactate leaves the cell by passive diffusion (23).

2.5. Citrate fermentation

The citrate pathway is a more complex pathway with multiple metabolic steps. Citrate is a di-carbocyclic acid, present in milk in relatively high concentrations. Many lactic acid bacteria use citrate fermentation as a source of energy, but cannot grow solely on citrate. These bacteria ferment citrate in co-metabolism with carbohydrates to obtain metabolic energy. There are multiple citrate fermentation pathways but here is the specific pathway of *L. lactis* mentioned, where it generates secondary metabolic energy. In this pathway the electrogenic exchanger CitP and the decarboxylase enzyme CitM are involved. CitP exchanges the end-product of the pathway, monovalent lactate, for its precursor divalent citrate, driven by the concentration gradient of precursor and product. During the electrogenic exchange, one net negative charge is transported into the cell, yielding a membrane potential. The citrate that is transported into the cell is cleaved by citrate lyase (CL) to yield oxaloacetate and acetate. Furthermore, acetate leaves the cell by passive diffusion and oxaloacetate is decarboxylated by the CitM decarboxylase, yielding pyruvate and CO_2. In the decarboxylation step, a proton is taken up yielding a more alkaline environment inside the cell generating a proton gradient.

The end-product lactate is obtained by dehydrogenation by LDH, which in the process reduces NADH to NAD^+. Lactate is exchanged for citrate and in combination with the decarboxylation, a PMF is generated (2) (23) (15). A schematic overview of the reaction is shown in figure 8.

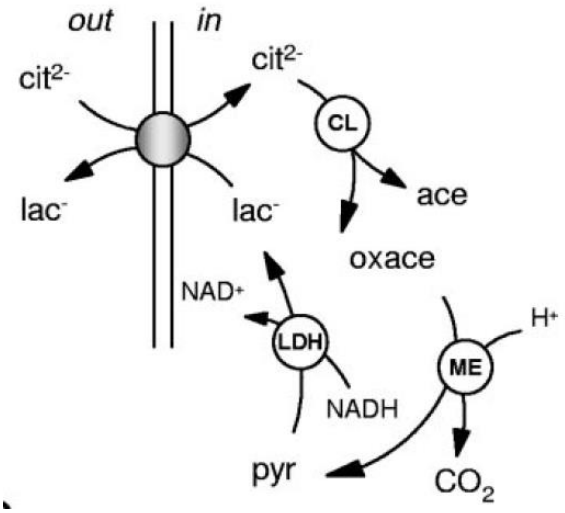

Figure 8: Steps of the citrate fermentation pathway in *L. lactis*. Citrate is cleaved by CL to oxaloacetate, and is decarboxylated to pyruvate. Pyruvate is dehydrogenated by LDH to lactate generating a proton gradient. Furthermore, monovalent lactate is exchanged for divalent citrate, generating a membrane potential (23).

3. Arginine/agmatine deiminase pathways

3.1. ADI

The arginine deiminase (ADI) pathway is the most frequent pathway for arginine degradation in anaerobic bacteria. It functions as an energy source and survival in acidic environments. This pathway was observed in *L. lactis* and is co-induced by the presence of arginine and repressed by the presence of glucose or aerobic growth conditions (5). The pathway consists of the activities of three enzymes and a transport step. Just as in the decarboxylation pathways, a secondary transporter is responsible for the combined uptake and excretion of substrate and product (13). However, generates metabolic energy by direct substrate level phosphorylation to synthesise ATP instead of PMF. In the pathway arginine is converted to ornithine, ammonia and CO_2 with a yield of 1 mol ATP per mol arginine. The conversion consists of three metabolic steps. First, ADI converts L-arginine to citrulline and ammonia. The functional carbamoyl group of citrulline is transferred to carbamoyl-phosphate by ornithine-transcarbamylase (OTC) yielding ornithine. The third step is the phosphorylation of ADP with carbamoyl-phosphate by the enzyme carbamate kinase(CK), yielding ATP, CO2 and ammonia. Furthermore, ornithine (product) inside the membrane is exchanged for arginine (substrate) with the electroneutral exchanger ArcD. The transport of arginine and ornithine is electroneutral and uses no metabolic energy. Inside the cytoplasm arginine is degraded fast to ornithine, generating a gradient of product directed outward. Simultaneously creating a gradient of the precursor, arginine directed inward, which can be exchanged without energy for the product ornithine. All energy released in the form of ATP can be used for other energy requiring processes (6) (18).

3.2. AgDI

Some lactic acid bacteria use the decarboxylated product of arginine, agmatine, for direct substrate level phosphorylation to synthesise ATP. The agmatine demininase (AgDI) pathway was observed in *LB. higardii* and *Entrococcus faecalis*. *E. faecalis* is able to use both arginine and its decarboxylated product agmatine as an energy source for growth. The pathway is co-induced by growth on agmatine and repressed by glucose and arginine. As with the ADI pathways, the agmatine pathway uses three enzymes and one electroneutral transport step of precursor and product, agmatine and putrescine respectively. First agmatine is deiminated by AgDI to yield carbamoyl-putrescine. Furthermore, a catabolic putrescine carbamoyl-transferase (PTC) catalyses the phosphorolysis of carbamoyl-putrescine to carbamoyl-phosphate and putrescine. The final step is the same as with the arginine pathway, where the energy-rich phosphate bond is transferred to ADP to yield ATP, NH_3 and CO_2. Putrescine is exchanged for the precursor agmatine by the electroneutral transporter AgmP. In the agmatine pathway, the energy yield is the same as with arginine. 1 mol agmatine yields 1 mol ATP. The transport requires no metabolic energy, because of the inward directed gradient of the substrate agmatine and outward directed gradient of the product putrescine. All energy released in the form of ATP can be used for other energy requiring processes (5) (13).

Both the ADI- and AgDI pathways follow the same steps, after the decarboxylation of arginine to agmatine. Consisting of a deiminase step followed by the transfer of the functional carbamoyl group to carbamoyl-phosphate. After which ADP is phosphorylated with carbamoyl-phosphate yielding ATP. The steps of the ADI and AgDI pathways both leading to ATP are shown in figure 9 (6).

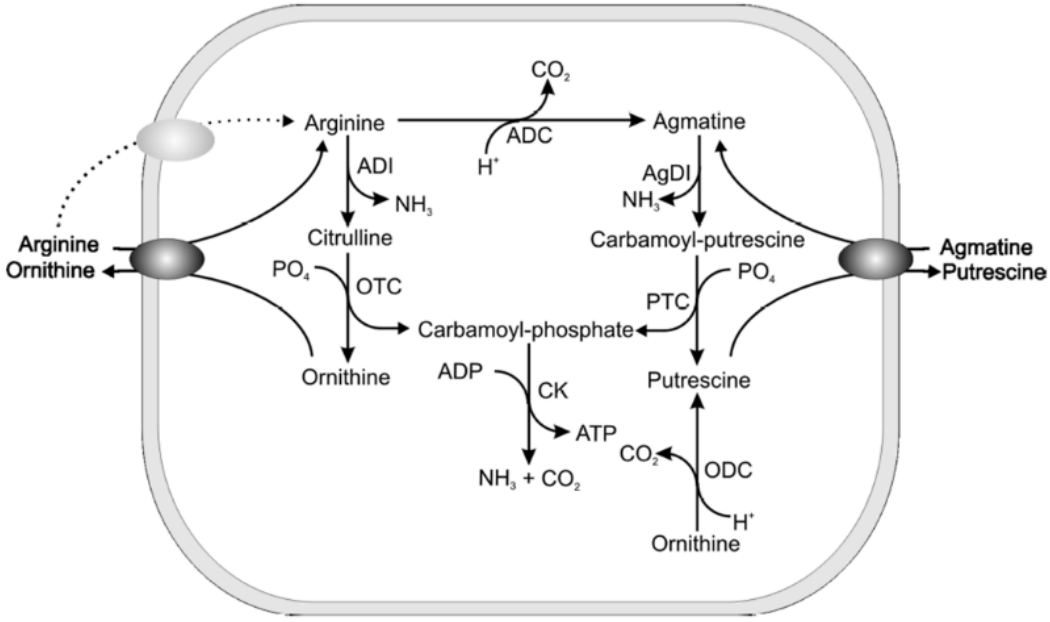

Figure 9: Steps of the ADI and AgDI pathways, with AgDI arginine is decarboxylated to agmatine. Furthermore, both pathways follow the same steps with different enzymes leading to carbamoyl-phosphate where its phosphate group is transferred to ATP. The end product of ADI, ornithine is exchanged for its precursor arginine. The end product of AgDI putrescine is exchanged for its precursor agmatine (6).

4. Discussion

Secondary metabolic energy generating pathways in response to acidic stress

Secondary metabolic energy generating pathways that use decarboxylase enzymes are usually activated in an acidic environment and can also function as a way to relieve acidic stress next to metabolic energy generation. In *L. brevis* for instance, which uses the tyrosine decarboxylation pathway, it was observed that the bacterium induced the pathway in response to acidic stress on the cell. In Pereira et al. 2009 was described how cells could survive at some time at pH 2.5 by inducing the tyrosine pathway. This acidic stress relieve was also observed with the histidine and ADI pathways. This shows the benefit for the cell of having these pathways in addition to metabolic energy generation.

Energy recycling by secondary transporters

Many fermenting bacteria, due to anaerobic conditions, are only able to use glycolysis. The end-products of the glycolysis accumulate rapidly inside the cell. The accumulation of these products results in an efflux driving the products outside the cell and can be used as a ''energy recycling'', depending on the pH. In Konings et al 1995 an example was given. In *E. coli* and *L. lactis* it was found that lactate, a product of the fermentation of lactose and glucose, accumulates inside the cell and is excreted via a secondary transporter. At high pH, lactate is in dianionic form and is electrogenicly symported with two H^+ ions and thereby generating metabolic energy. At low pH, lactate is protonated and the transport is electroneutral, generating no metabolic energy. By using a secondary transporter, the organism can use the excretion of end-product to obtain extra metabolic energy.

Secondary transporter family's

The malate pathways in *L. lactis* and *O. Oenii* use the same substrate, but differ in the transporters they use to transport malate. The electrogenic exchanger in *L. lactis* is part of the 2-Hydroxycarboxylate Transporter (2-HCT) family. 2-HCT transporters all have affinity for either malate, citrate and or lactate. The electrogenic uniporter in the malate pathway of *O. Oenii* is part of the auxin efflux carrier (AEC) family and has adapted to the acidic conditions in which the bacterium is present. Illustrating the adaptiveness of the secondary transporters in relation to the environment, that are not necessarily related to the substrates (23).

Divergent evolution of the ADI and AgDI pathways

The ADI and AgDI pathways both have one electroneutral antiporter and 3 enzymes involved in the pathway and more or less the same substrate, with agmatine being the decarboxylation product of arginine. One might expect the transporters from the ADI pathway to have somewhat affinity for the AgDI an vise versa. The opposite is true, Llacier et al. 2006 found that the transporter for arginine/ornithine and agmatine/putrescine have no affinity for each other and both pathways including the enzymes evolved independently.

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