# Errata

**Introduction**

| it is | it should be | Page | Line |
|---|---|---|---|
| Mycoplasma | *Mycoplasma* | page 9 | line2 |
| Ureaplasma | *Ureaplasma* | page 9 | line2 |
| Razin 1978 | Razin 1998 | page 9 | line16 |
| Methabolism | metabolism | page 11 | line 18 |
| *Thermaplasma* | *Thermoplasma* | page 16 | line 1 |
| *Chlymadia* | *Chlamydia* | page 16 | line 1 |
| Yoon 1989 | Yoon 1998 | page 18 | line 35 |
| Domingues 2003 | Domingues 2002 | page 18 | line 39 |
| *Chalymadia* | *Chlamydia* | page 23 | line 29 |
| *Fermaplasma* | *Ferroplasma* | page 23 | line 28, 30 |
| Recombiant | Recombinant | page 26 | line 11 |

In this thesis, the term of mycoplasma refer to both *mycoplasma* and *ureaplasma*, but in correct way, the term of *mycoplasma* is used only for *mycoplasma*, and should be italic throughout the thesis. Mollicutes refer to both *mycoplasma* and *ureaplasma*.

Mollicutes possess a single circular chromosome of double stranded DNA and have a G+C content of 24 -33 mol% which is lower than other Gram-positive prokaryotes. The G+C content distribution along the genome is uneven (Altschul *et al*., 1997; Fraser & Fleischmann, 1997; Razin *et al*., 1998). Their genome sizes range from 580 kb of *Mycoplasma genitalium* to 1,358 kb of *Mycoplasma penetrans* (Fraser *et al*., 1995; Sasaki *et al*., 2002).

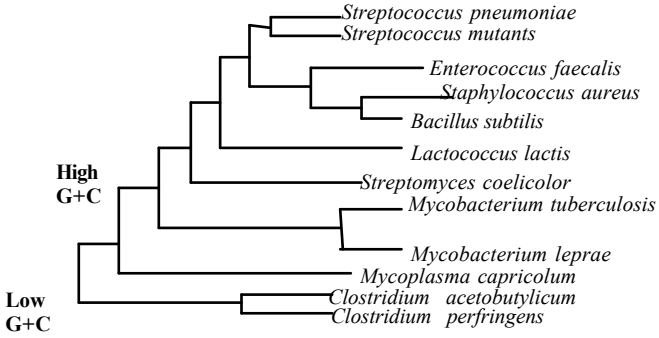


Figure 1. Figure 1. Phylogenetic relationships among Gram-positive bacteria deduced from 16S rRNA. This tree was produced using BLAST pairwise alignments. http://www.ncbi.nlm.nih.gov/blast/treeview.

The genome sizes are variable, not only within the same genus but even among strains of the same species. One of the reasons for this variability is the frequent occurrence of repetitive elements, consisting of segments of protein coding genes, differing in size and number of insertion sequences (Citti *et al*., 1997; Razin *et al*., 1998; Dandekar *et al*., 2002; Mrazek, 2006). Based on the analysis of the 16S rRNA sequences, Mycoplasmas have been classified into different groups (Fig. 2).

Mycoplasmas utilize the codon UGA to encode Trp (Dybvig, 1990), and in *E.coli* this codon is recognized as a stop signal, which will result in truncation of mycoplasma proteins expressed from cloned genes. By introducing the opal suppressor allele trpT176 the tRNA produced from this allele is charged with Trp; therefore, it has been possible to express Mycoplasma genes containing UGA codons in *E.coli* (Minion *et al*., 1995). This fact has been an important methodological tool used in this thesis work.

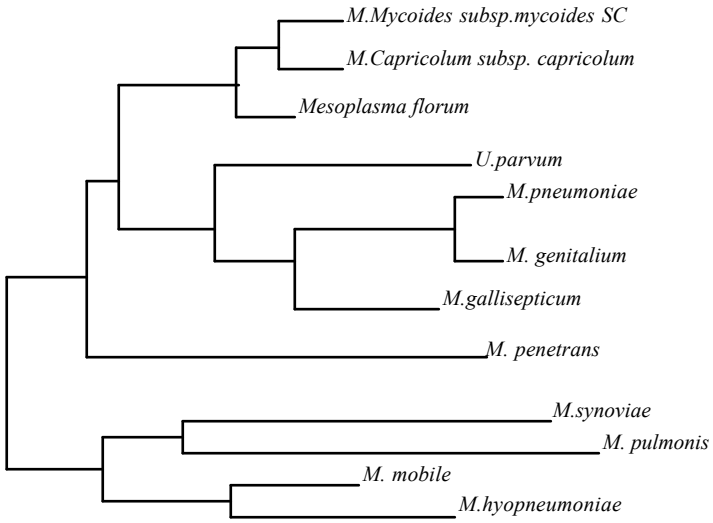


Figure 2. Phylogenetic tree of Mycoplasmas based on the 16S rRNA genes.
This tree was produced using BLAST pairwise alignments.
http://www.ncbi.nlm.nih.gov/blast/treeview

## *Energy metabolism*

Mollicutes are divided into two groups, based on their ability to metabolize carbohydrates: fermentative and non-fermentative organisms. Members of the fermentative group produce acids from carbohydrates, decreasing the pH of the medium. Most mollicutes have the ability to use glucose and arginine for ATP synthesis. During their evolution, Mycoplasmas appear to have lost all of the genes involved in amino acid and cofactor biosynthesis, as well as synthesis of the cell wall and lipid metabolism, resulting in a requirement for the full spectrum of substrates and cofactors taken up from the host or from a complex artificial culture medium. (Miles *et al.*, 1991; Himmelreich *et al*., 1996; Razin *et al.*, 1998). The majority of Mycoplasmas is deficient in genes coding for components of intermediary and energy metabolism and thus are dependent mostly on glycolysis as an ATP-generating pathway (Frey, 2002).

The energy metabolism of *Ureaplasmas* presents a special case. *Ureaplasmas*, unique among the Mollicutes, possess a very potent urease, which hydrolyses urea. The resulting intracellular accumulation of ammonia/ammonium ion is coupled to ATP synthesis through a chemiosmotic type of mechanism (Blanchard et *al.*, 1988; Neyrolles *et al.*, 1996; Razin *et al*., 1998).

## *Nucleotide metabolism*

Nucleotides have a central role in the physiology of all organisms as building blocks of nucleic acids, storage of chemical energy, carriers of activated metabolites for biosynthesis, structural moieties of coenzymes, and metabolic regulators.

homologues of *Thermoplasma* and *Chlamydia* species are much longer and contain two fused ThyX domains in the same polypeptide and share very little homology to other ThyX sequences (Mathews *et al*., 2003; Sampathkumar *et al*., 2005; Griffin *et al*., 2005).

The difference of the target site that can be defined most clearly is the covalent inactivation of ThyA by FdUMP. This fluorinated deoxyuridylate analog is formed via the reduction of FUDP by ribonucleotide reductase and dephosphorylation. Alternatively, it can be formed directly from 5-FdUrd by thymidine kinase when this 5-FUrd is regionally infused. However, the inhibition of 5-FdUMP to ThyX or FDTS enzymes is not via covalent complex between 5-FdUMP to ThyX or FDTS enzymes, rather competitive inhibition (Costi *et al.,* 2005).

ThyX proteins contain a flavin group as demonstrated spectroscopically in this thesis as well as by Myllkallio et al. (2002). Flavoproteins are highly reactive with free oxygen (Massey, 2000) and their biochemical reaction is normally studied under anaerobic conditions. The oxygen sensitivity observed in *E.coli ΔThyA* that expressing *thyX* might therefore be attributed to oxygenation of the flavin group by molecular oxygen (Giladi *et al*., 2002).

Many of the FDTS containing organisms are pathogenic or parasitic. ThyA and ThyX share no sequence or structural homology (Fig. 6) and this makes FDTS a promising target for drug design. Information regarding the FDTS reaction mechanisms, 3-D structures and the differences between the classical TS (e.g., human TS) and bacterial ThyX will facilitate future antibiotic development.

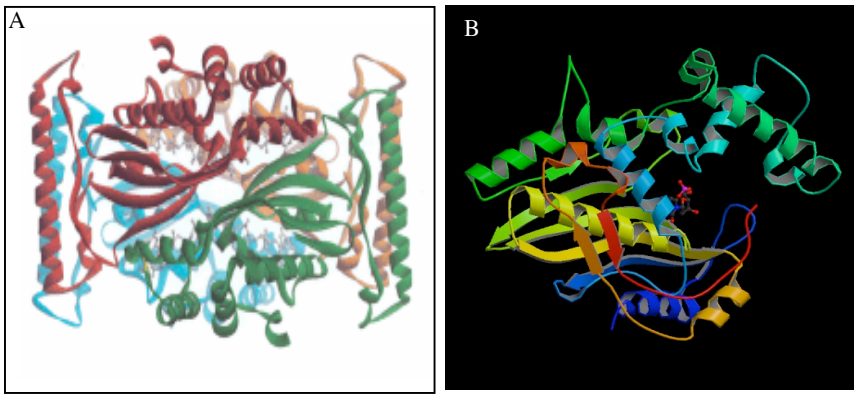


Figure 6. Crystal structure of *Thermotoga maritima* ThyX (A) and *E.coli* ThyA (B). (**A**, Leduc et al., 2004; **B**, Costi et al., 2005).

*Paper IV: A New family of flavin-dependent thymidylate synthase in pathogenic mycoplasmas.*

ThyA homologues are presence in many Mycoplasmas species e.g. *M. genitallium* (Table. 1). The ThyA sequences are conserved within Mycoplasmas, ~ 50-70% sequence identities, while sequence homology to other bacterial ThyA is only ~ 30-40%. As expected, Mycoplasmas, which have the *thyA* genes, possess also genes for DHFR, which is required for reduction of the $H_2$folate to $H_4$folate.

*M. pneumoniae and M. genitalium* share an identical set of pyrimidine metabolism pathways and other proteins (Inamine *et al*., 1989) as compared to other mycoplasmas and both *M. genitalium* (Mg) and *M. pneumoniae* (Mpn) possess *thyA* genes (Table. 1). However, studies by Glass *et al.* showed that Mg *thyA* gene is not essential since its interruption did not affect Mg growth (Glass *et al*., 2006). Using Uu FDTS sequence as query sequence, we identified an open reading frame in Mg (Mg255) and Mpn (MPN358), which showed significant homology to Uu FDTS, ≈ 26%.

We also identified homologous genes e.g. Mcap620 in Mcap genome and Mhp655 in Mhp genome by using MmmSC FDTS as query sequence. We cloned these four genes by using PCR method using genomic DNA as template. MPN358, Mcap612 and Mhp655 could functionally replace the *thyA* gene of an *E. coli* strain ISM612 that is TS deficient and suitable for expression of mycoplasma proteins. Recombinant Mhp655 protein was expressed and purified using anion exchange chromatography. Purified mhp655 protein showed FDTS activity and the elution profile of Mhp655 was similar to that of UU572 and MSC0676. Fractions containing TS activity displayed yellow color.

Thus, we have identified a new family of flavin dependent thymidylate synthase. Since these Mycoplasma favin dependent thymidylate synthases have no sequence and possibly structural similarity to ThyA proteins, and they are attractive targets for the design of new antibiotics against these pathogenic bacteria.

Table1. Thymidylate synthase genes (thyA and thyX) in Mollicutes.

| *Mollicutes* | *thyA* | *thyX* |
|---|---|---|
| *M.gallisepaticum R* | + | – |
| *M.synoviae* | + | – |
| *M.mobile* | + | – |
| *M. penetran* | + | – |
| *M.pneumoniae* | + | + |
| *Onion yellow phytoplasma* | + | – |
| *Mesoplasma florum* | + | – |
| *M.genitalium* | + | + |
| *M.pulmonis* | + | – |
| *M.mycoides SC* | – | + |
| *M.hyopneumoniae* | – | + |
| *M.capricolum* | – | + |
| *U.urealyaticum* | – | + |