DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
156

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
156

RADI TEGOVA

The role of specialized DNA polymerases in mutagenesis in *Pseudomonas putida*

TARTU UNIVERSITY
PRESS

Department of Bioinformatics, Institute of Molecular and Cell Biology, University of Tartu, Estonia

Dissertation is accepted for the commencement of the degree of *Doctor philosophiae* (in genetics) on 06.02.2009 by the Council of the Institute of Molecular and Cell Biology, University of Tartu.

Supervisors: Prof. Maia Kivisaar, PhD (University of Tartu)
Teadusdirektor Andres Tover, PhD (Quattromed Cell Factory OÜ)

Opponent: Doctor Jesús Blázquez, PhD (Centro Nacional de Biotecnología, Cantoblanco)

Commencement: Room No 217, 23 Riia Str., Tartu, on April 17th 2009, at 12.15.

The publication of this dissertation is granted by the University of Tartu.

ISSN 1024–6479
ISBN 978–9949–19–068–3 (trükis)
ISBN 978–9949–19–069–0 (PDF)

Tartu Ülikooli Kirjastus
www.tyk.ee
Tellimus nr. 54

CONTENTS

2

LIST OF ORIGINAL PUBLICATIONS

I **Tegova R, Tover A, Tarassova K, Tark M, Kivisaar M**: Involvement of error-prone DNA polymerase IV in stationary-phase mutagenesis in Pseudomonas putida. J Bacteriol 2004, 186:2735–2744.

II **Koorits L, Tegova R, Tark M, Tarassova K, Tover A, Kivisaar M**: Study of involvement of ImuB and DnaE2 in stationary-phase mutagenesis in Pseudomonas putida. DNA Repair (Amst) 2007, 6:863–868.

III **Tark M, Tover A, Tarassova K, Tegova R, Kivi G, Hõrak R, Kivisaar M**: A DNA polymerase V homologue encoded by TOL plasmid pWW0 confers evolutionary fitness on Pseudomonas putida under conditions of environmental stress. J Bacteriol 2005, 187:5203–5213.

Contribution of the author of present dissertation to journal articles is following: Ref. I – partcipation in creating experimental designs, construction of plasmids and strains, performing *in vivo* experiments, contribution to preparing the manuscript.

Ref. II – contribution to designing and performing *in vivo* experiments, construction of plasmids and strains, participation in preparing the manuscript.

Ref. III – contribution to creating experimental designs, construction of plasmids, participation in preparing the manuscript.

ABBREVATIONS

pol	DNA polymerase
pol III HE	DNA polymerase III holoenzyme
ssDNA	single-stranded DNA
BER	base excision repair
NER	nucleotide excision repair
ROS	reactive oxygen species
8-oxo-G	8-oxoguanine
8-oxo-dGTP	8-oxodeoxyguanosine triphosphate
2-oxo-dATP	2-oxodeoxyadenosine triphosphate
εC	3,N^4-ethenocytosine
N^2-G adduct	N^2-guanine adduct
N^2-furfuryl-G	N^2-furfuryl-guanine
N^2-CE-G	N^2-carboxyethyl-guanine
γ-HO-G	γ-hydroxypropano-guanine
MG	methylglyoxal
NFZ	nitrofurazone
4-NQO	4-nitroquinoline 1-oxide
AAF-G adduct	*N*-2-acetylaminofluorene guanine adduct
ENNG	N-ethyl-N´-nitro-N-nitrosoguanine
AP-site	apurinic or apyrimidinic site
L	C1´-oxidized AP-site
C4-AP	C4´-oxidized AP-site
C2-AP	C2´-oxidized AP-site
UV	ultraviolet
CPD	cyclobutane pyrimidine dimmer
(6–4) PP	pyrimidine-pyrimidone (6–4) photoproduct
Rif	rifampicin
Rif^R	Rif resistant
ROSE	"Resting Organisms in a Structured Environment"
MAC	"Mutagenesis in Aging Colonies"
MMR	mismatch repair system
cAMP	cyclic adenosine monophosphate
CRP	cAMP receptor protein
DSB	double-strand break
DSBR	DSB repair
Lac^+	lactose utilizing
GASP	"Growth Advantage in Stationary Phase"
PBP	penicillin binding protein
Phe^+	phenol utilizing

INTRODUCTION

Basic chromosomal reproduction required for the transmission of genetic information during cell division proceeds in participation of DNA polymerases that replicate DNA accurately and efficiently. Yet, every autonomous organism encodes several additional DNA polymerases. Biological functions of these DNA polymerases have been under intense investigation during the last decade.

Genomic DNA of all living organisms is continuously exposed to DNA damaging agents, generated endogenously during normal cellular processes or originating from surrounding environment. Many DNA alterations are intolerable to replicative high-fidelity DNA polymerases impeding elongation of daughter-strand and leading to pause in replication. DNA polymerases unessential for basic replication generally lack error-correcting activity that results in low-fidelity DNA synthesis. This property together with more open structure of catalytic centre of these proteins enables to bypass a number of DNA lesions that are unsurpassable obstacles for replicative DNA polymerases. Consequently, translesion DNA synthesis performed by specialized DNA polymerases often results in changes in initial DNA sequence. Moreover, specialized DNA polymerases exhibit usually high level of inaccuracy also while replicating undamaged DNA template. To avoid harmful effects of specialized DNA polymerases on stability of genetic material, the DNA synthesis by particular enzymes is restricted to occur in emergency situations and is regulated at different levels from transcription initiation to access to replication.

Expression of specialized DNA polymerases is induced in addition to DNA-damage also by other stress conditions. For example, several environmental stresses including nutrient deprivation enhance genetic variability of bacteria, thereby facilitating adaptive evolution. There are several lines of evidences for contribution of specialized DNA polymerases to stress-induced mutagenesis of stationary phase bacterial populations. Involvement of different specialized DNA polymerases in mutagenic processes has been revealed for *Escherichia coli* stationary phase populations suffering from carbon-source limitation (Bjedov *et al*., 2003; Yeiser *et al*., 2000; McKenzie *et al*., 2001) as well as for populations of *Bacillus subtilis* starving for certain amino acids (Sung *et al.,* 2003).

Pseudomonas genus represents one of the most diverse and ecologically widely distributed groups of bacteria. In addition to many environmental species it also includes an opportunistic human pathogen *P. aeruginosa* and a plant pathogen *P. syringae.* Extensive diversity and distribution of pseudomonads is indication of their efficient physiological and genetic adaptability. *P. putida* is a soil-bacterium present in very different soil and water habitats. Therefore, long periods of nutrient limitation are common situations for this bacterium to be faced with. After exhaustion of available energy sources, any mutation with beneficial value should be under selective pressure. Hence,

several mechanisms providing transient and moderate increase of mutation frequency in response to nutrient limitation have been described.

Literature review of my thesis is focused on characterizing specialized bacterial DNA polymerases and their biological functions, including the involvement in stress-induced mutagenesis. Experimental part of the thesis is dedicated to the role of several specialized DNA polymerases in mutagenesis occurring in long term starving population of *P. putida*.

3

1. REVIEW OF LITERATURE

1.1. Introduction

DNA metabolism of a living cell comprises in addition to basal chromosomal replication, enabling cell division and transmission of genetic information, also many other processes, including translesion synthesis, DNA reparation and recombination, providing accurate and/or complete reproduction of genetic information. Although all these processes include DNA synthesis, DNA polymerases of specific properties are needed for particular processes.

DNA polymerases are grouped into five families: A, B, C, X, and Y, based on their sequence homology and phylogenetic relationships. Among these, DNA polymerases of A, B, C, and Y families are present in bacteria. Basic bacterial chromosomal replication employs one or two C-family DNA polymerases for majority of DNA synthesis during genome reproduction, and an A-family polymerase assisting in lagging-strand synthesis. *Escherichia coli* pol II as the only bacterial B family DNA polymerase characterized so far is involved in replication restart downstream single-stranded DNA gap and in translesion synthesis across certain types of damaged nucleotides in template DNA. Bacterial Y family polymerases characterized by the lack of exonucleolytic proofreading activity and a more open conformation of the active site if compared to other DNA polymerases, are able to bypass a wide variety of DNA lesions and/or contribute to spontaneous mutagenesis due to their intrinsic low fidelity. Additionally, Y family polymerases have been shown to be involved in cell cycle regulation. The expression of pol II and the two Y-family polymerases present in *E. coli*, pol IV and V, is induced by DNA damage, being thereby restricted to emergency situations and thus avoiding harmful effects of error-prone DNA synthesis.

The role of different bacterial DNA polymerases in chromosomal replication, in translesion synthesis at different DNA alterations and in involvement in generation of genetic variability as a response to various stress conditions will be discussed below to hypothesize the biological relevance of such a diversity of DNA polymerases.

1.2. Chromosomal replication

Replication of bacterial circular chromosome occurs bidirectionally and continuously from the replication origin to the terminus. The chromosomal replication is accomplished by a multiprotein complex called replisome. The composition and mechanics of bacterial replication machinery have been mainly investigated in a bacterial model organism *E. coli*. Therefore, description of bacterial replisome herein is based on this particular bacterium. *E. coli*

replisome is composed of three basic components: DnaB, DnaG and DNA polymerase III holoenzyme (pol III HE). DnaB is a major replicative DNA helicase unwinding DNA duplex in front of the moving replication fork, whereas DnaG serves as a primase which interacts with the DnaB helicase and synthesizes short RNA molecules to prime the DNA chain elongation, catalyzed by pol III HE. Replication carried out by pol III HE is a complex dynamic process, which enables concurrent elongation of both daughter strands. The polymerases catalyzing leading- and lagging-strand synthesis are attached to each other in pol III HE. As the DNA chain can be extended only in 5'→3'direction, replication of the leading strand is continuous. Meanwhile, extension of the lagging strand causes lagging-strand folding into a loop, and occurs discontinuously resulting in formation of 1–3 kb length RNA-primed nascent DNA pieces called Okazaki fragments after their discoverer. RNA primers of Okazaki fragments are later removed and resulting gaps are filled in by the DNA polymerase I (pol I). Nicks between adjacent DNA segments are sealed by DNA ligase (Johnson and O'Donnell, 2005; McHenry, 2003).

Concurrent elongation of leading and lagging-strands assumes the presence of at least a couple of DNA polymerase cores (pol III core) in composition of the pol III HE complex, one β-clamp (processivity factor), for each pol III core located on primer-template DNA duplex, and a multisubunit clamp loader γ/DnaX complex. β-clamp is a ring-shaped homodimeric protein which encircles the DNA duplex and while attached to the pol III core enhances its processivity by holding the polymerase on the DNA (Stukenberg *et al*., 1991; Johnson and O'Donnell, 2005).The γ/DnaX clamp loader assembles the β-clamp onto the DNA, but is also serving as a scaffold of the replisome by keeping pol III cores together and connecting pol III HE and DnaB helicase at the replication fork (McHenry, 2003). In Gram positive bacteria e.g. *Bacillus subtilis* and *Streptococcus pyogenes,* replisome is composed of two different C family polymerases for leading and lagging-strand synthesis (Dervyn *et al*., 2001; also reviewed by McHenry, 2003).

pol III core is a heterotrimer composed of α, ε and θ subunits. α subunit encoded by *dnaE* is responsible for DNA polymerase activity. ε (*dnaQ,* also known as *dnaA* or *mutD*) subunit possesses proofreading (3'→5' exonuclease) activity and is responsible for the accuracy of DNA synthesis carried out by replicative DNA polymerase. The ε subunit is also important for the processivity of pol III HE by stimulating the interaction of the α subunit with the β-clamp (Johnson and O'Donnell, 2005; Studwell and O'Donnell, 1990; Lamers *et al*., 2006) whereas the θ subunit (*holE*) expresses no other known function but a slight stimulation of ε (Taft-Benz and Schaaper, 2004). As pol III has a relatively low affinity for primer-provided DNA, a high catalytic efficiency of replicative polymerase depends on interaction of the pol III α subunit with the β-clamp (Stukenberg *et al*., 1991, also reviewed in Johnson and O'Donnell, 2005). α subunit of pol III core interacts with the β-clamp through two β-clamp interaction motifs located in the end of C terminal part of α.

Region between these two β-clamp interaction motifs is also important for processivity of replicative polymerase (Lamers *et al*., 2006). In addition, interaction between the pol III core and the β-clamp is stimulated by ε (Studwell and O'Donnell, 1990; also reviewed in Johnson and O'Donnell, 2005; Lamers *et al*., 2006).

β-clamp, which is considered a key participant in switchingof the replication to translesion synthesis conducted by specialized DNA polymerases, is assumed to serve as a "tool belt". It has been shown *in vitro* that the β-clamp is able to bind simultaneously two polymerases: the replicative and the specialized one. Moreover, switch from one polymerase to the other during the DNA synthesis does not inflict release of the replicative polymerase from the β-clamp (Indiani *et al*., 2005; also referred by Lehmann, 2006). These results support the hypothesis of sequential translesion synthesis during the DNA replication, according to which stall of replication fork causes coupling to a specialized DNA polymerase, enabling movement of the replication machinery after the bypass of the DNA lesion. Recently, more evidence has been received to support alternative hypothesis set up already in 1968 by Rupp and Howard-Flanders (referred by Lehmann and Fuchs, 2006). According to their hypothesis, DNA lesions inflict replication restart downstream of DNA lesion, leaving single-stranded DNA gaps in between. Therefore, the gap processing and translesion synthesis may occur simultaneously with ongoing chromosomal replication (Heller and Marians, 2006; also referred by Lehmann and Fuchs, 2006). Furthermore, a model has been proposed, according to which a specialized DNA polymerase, pol IV, mediates dissociation of the replicative polymerase from the primer-template DNA as well as from the β-clamp. The polymerase exchange is supposed to depend on two distinct interactions between pol IV and the β-clamp, and between pol IV and pol III (Furukohri *et al*., 2008; Uchida *et al*., 2008). There are also models concerning regression of replication fork, which facilitates translesion synthesis (Schlacher and Goodman, 2007) or permits the bypass of DNA damage without immediate replication over the lesion by using nascent lagging-strand as a template (Postow *et al*., 2001, also referred by Schlacher and Goodman, 2007). It is very likely that different models of translesion synthesis do not exclude each other and may concur in a living cell. Even if translesion synthesis does not occur in a sequential manner, the idea of β-clamp as a "tool belt" is still convenient to explain other models of translesion synthesis. For example, carrying a specialized DNA polymerase as a reserve tool for replicative machinery would predispose the translesion synthesis also in case of dissociation of the replicative DNA polymerase from the β-clamp, followed by replication restart downstream of DNA damage.

1.3. Bacterial specialized DNA polymerases

Bacterial genomic DNA is continuously damaged by reacting with compounds present in normal intracellular environment, and it is also insulted by exogenous physical and chemical agents. Even though different repair systems such as base excision repair (BER), nucleotide excision repair (NER) and recombination repair eliminate most of the DNA lesions, there is still requirement for specialized DNA polymerases to operate in lesion bypass when replication fork encounters a damaged nucleotide in the template. The replicative high-fidelity DNA polymerase is haltered when confronted by certain types of damaged template bases. Therefore, temporary switch to some low-fidelity polymerase enables to resume the DNA replication.

In addition, several lines of evidence indicate to contribution of specialized DNA polymerases in stress-induced mutagenesis. Due to relatively low fidelity of DNA synthesis carried out by these polymerases, induction of expression of specialized DNA polymerases and regulation of their access to DNA synthesis in response to different stress stimulus increase genetic diversity and therefore adaptability of bacterial population.

1.3.1. Translesion synthesis

The ability to replicate across a damaged nucleotide in the template DNA or incorporate a damaged nucleotide into the nascent DNA chain depends on the structure of particular DNA polymerase, the type of the lesion (nucleotide modification) and the context of adjacent DNA sequence.

Although cocrystallization of bacterial replicative DNA polymerase III with different imperfect base pairs in its active center is not accomplished yet, there is much information available about some other high-fidelity DNA polymerases such as *Bacillus stearothermophilus* DNA polymerase I and bacteriophage T7 polymerase (A family DNA polymerases) acting on translesion synthesis. The active site of high-fidelity DNA polymerases cannot tolerate base pairing much different from the canonical Watson-Crick ones resulting in transmission of primer terminus to the exonuclease subunit (Hogg *et al*., 2004; Hogg *et al*., 2005). Therefore, it is presumable that similar progress of events takes place when any replicative DNA polymerase encounters certain types of DNA lesions. Transmission of primer terminus to the exonuclease subunit may promote dissociation of the replicative polymerase from the DNA giving an opportunity to a specialized DNA polymerase to replace the replicative one and take over the synthesis of the nascent daughter-strand. Sterically less restrictive active sites of specialized DNA polymerases (Ling *et al*., 2001; also reviewed by Yang, 2003) and the absence of exonucleolytic activity in case of Y-family polymerases allows formation of base pairs different from canonical ones (Goodman, 2002). Probability of gaining the access to primer-provided DNA

for specialized DNA polymerases is enhanced by the induction of expression of these particular DNA polymerases as a response to DNA damage known as SOS-response (Hanaoka, 2001; Goodman, 2002). Obstruction of replicative polymerase by DNA lesion is followed by the generation of single-stranded DNA which forms a nucleoprotein filament by interacting with RecA. The RecA-ssDNA nucleoprotein filament mediates autocatalytic proteolysis of transcriptional repressor LexA, thus increasing the expression of many proteins required in the case of DNA damage e. g. enzymes involved in DNA reparation pathways including recombinational enzymes, cell division inhibitors and specialized DNA polymerases.

Several chemical agents which cause the DNA damage are generated during normal processes in a living cell, reactive oxygen species and alkylating agents serving as an example. Reactive oxygen and nitrogen species are also produced in during the host immune response stimulated by infection of pathogenic bacteria. In addition, the DNA damage can be inflicted by other exogenous sources like UV radiation and different chemical agents.

1.3.1.1. Oxidative lesions

Reactive oxygen species (ROS) are generated endogenously as by-products of normal aerobic metabolism, therefore being a major source of spontaneous damage to proteins, lipids, carbohydrates, and nucleic acids. In addition, pathogenic bacteria are confronted with exogenous ROS, produced by the host defense system. Among all primary reactive oxygen species such as superoxide radical ($\bullet O_2^-$), hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH), •OH is the most reactive one oxidizing almost every type of organic molecules. Due to its extremely high reactivity, •OH can diffuse only one or two molecular diameters at most before reacting with some cellular compound. Hence, to oxidize nucleotides in the composition of DNA, •OH has to be generated at immediate proximity of it.

Main source of oxidative DNA damage is thought to be •OH produced in Fenton reaction between H_2O_2 and Fe^{2+} (Fig. 1A). $\bullet O_2^-$ has been shown to be involved in generation of both components of the Fenton reaction. H_2O_2 is generated in spontaneously occurring dismutation reaction of $\bullet O_2^-$ (Fig. 1B) or catalyzed by superoxide dismutases. Also, Fe^{2+} is liberated by $\bullet O_2^-$ from iron-sulfur clusters. In addition, superoxide anion can also liberate Fe^{3+} from ferritin and reduce it to Fe^{2+} (Friedberg *et al*., 2006).

A. $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + {}^-OH$

B. $2 \cdot O_2^- + 2H^+ \rightarrow O_2 + e^{3+} + H_2O_2$

Fig. 1. A. Generation of hydroxyl radical in Fenton reaction. **B.** Generation of hydrogen peroxide in superoxide radical dismutation reaction.

During innate immune response of the host against a pathogen invasion, H_2O_2, $\bullet O_2^-$ and nitric oxide (NO•) are produced by macrophages and neutrophils as cytotoxic agents. $\bullet O_2^-$ reacts with NO• to form peroxynitrite ($ONOO^-$) (Fig. 2), another extremely reactive oxidant of organic molecules, which differently from •OH is able to diffuse further in the cell (Burney, Caulfield, Niles Wishnok and Tannenbaum, 1999).

$$\cdot O_2^- + NO\cdot \rightarrow ONOO^-$$

Fig. 2. Superoxide radical reacting with nitric oxide leads to the generation of peroxynitrite.

Hydroxyl radicals and peroxynitrite damage nitrogenous bases of DNA and deoxyribose sugar units in the DNA backbone, resulting in single-strand breaks. Although over 80 different types of the DNA base damage are known to be produced by ROS (Bjelland and Seeberg, 2003), the biologically most significant base lesion seems to be 8-oxoguanine (8-oxoG) (Fig. 3). Therefore, several repair pathways have been evolved to avoid generation of mutations in the translesion synthesis across the 8-oxoG of the template DNA. Mutagenicity triggered by of the incorporation of oxidatively damaged deoxynucleoside triphosphates, such as 8-oxo-dGTP and 2-oxo-dATP into the DNA, has been observed as well (Friedberg et al., 2006).

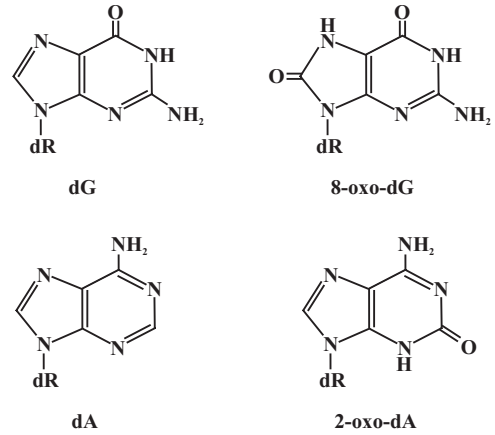


Fig. 3. Oxidatively damaged guanine and adenosine compared with appropriate undamaged nucleotides.

Incorporation of dCTP opposite to the template 8-oxoG distorts the template strand, whereas at the incorporation of dATP, Hoogsteen pair (which resembles a cognate base pair) is constituted, and therefore evades the proofreading. Thus, incorporation of dATP opposite to 8-oxoG is favored by high-fidelity and most specialized DNA polymerases resulting in generation of G:C to T:A transversions (Brieba *et al*., 2004; Hsu *et al*., 2004; also referred by Hogg *et al*., 2005). Presumably, similar distortion in the DNA backbone takes place when oxidized guanine is incorporated into the nascent DNA-chain opposite to C. Thus it has been observed that both *E. coli* replicative DNA polymerase pol III (Kamiya and Kasai, 2000) and also the Y-family polymerase pol IV (Yamada *et al*., 2006) incorporate 8-oxo-dGTP opposite to A, thereby promoting A:T to C:G transversions. Incorporation of 2-oxo-dATP into the daughter-strand is shown to be mutagenic as well. In case of both, replicative pol III and specialized pol IV, 2-oxo-dATP is preferentially inserted opposite to G, giving rise to G:C to T:A mutations (Kamiya and Kasai, 2000; Yamada *et al*., 2006). While 8-oxoG does not promote occurrence of frameshift mutations, it has been observed that other unspecified oxidative lesions trigger –1 and –2 frameshift mutations in SOS-inducible polymerases-dependent reaction. Dependent on the sequence context, participation of pol V together with pol IV or pol II in this process is needed (Wagner and Fuchs, 1997; Wagner *et al*, 2002).

8-oxoG is unstable due to its low redox potential and its susceptibility to further oxidation (Burney *et al*., 1999; also reviewed by Neeley *et al*., 2007). Oxidation of 8-oxoG by peroxynitrite ($ONOO^-$) induces formation of DNA lesions like guanidinohydantoin, spiroiminodihydantoin, oxaluric acid and urea **(**Fig. 4). $ONOO^-$ also oxidizes G nucleotide giving rise to 5-guanidino-4-nitroimidasole (Fig. 4) (Neeley and Essigmann, 2006).

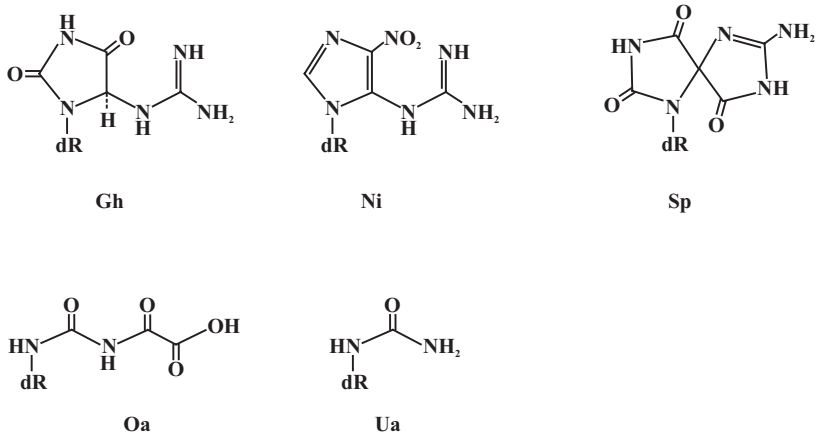


Fig. 4. 8-oxoG (7,8-dihydro-8-oxoguanine) oxidation products: Gh – guanidino-hydantoin, Sp – spiroiminodihydantoin, Oa – oxaluric acid, Ua – urea; and Ni – 5-guanidino-4-nitroimidasole, a product of guanine oxidation by peroxynitrite.

In *E. coli* cells, the oxidation products of 8-oxoG and 5-guanidino-4-nitro-imidasole are mainly bypassed at translesion synthesis by pol V in an error-prone manner, generating a variety of base substitution mutations, depending on the nature of the particular lesion. Although simultaneous action of pol V, IV and II has been shown to be important for translesion synthesis over 8-oxoG further oxidation products as well. The modest effect on translesion synthesis across some of 8-oxoG oxidation products was observed also in pol II- and pol IV-deficient strains. Differently from further oxidation products of 8-oxoG, 5-guanidino-4-nitroimidasole can be exceeded by pol II instead of pol V as well. In this case it occurs in error-free manner (Neeley *et al*., 2007).

1.3.1.2. DNA lesions caused by chemical agents other than ROS

Studies about DNA synthesis at lesions inflicted by chemical agents in *E. coli* have shown, that selection of certain specialized polymerase for the lesion bypass depends on the type of DNA damage and the adjacent sequence context, whereas the outcome of translesion synthesis, error-prone or not, mostly depends on the DNA polymerase involved.

In addition to the aforementioned mechanisms by which reactive oxygen species generate the DNA damage by attacking deoxynucleotides directly, the oxidative stress may trigger occurrence of DNA lesions indirectly as well. As pointed out earlier, oxygen radicals attack a variety of cellular macromolecules, whereas oxidation of nucleic acids is probably less significant for the survival of a bacterial cell compared to the oxidative damage of other cellular compounds. Peroxidation of polyunsaturated fatty acid residues of phospholipids in membranes may lead to the formation of reactive compounds, such as epoxides and aldehydes, which cause a variety of mutagenic exocyclic DNA lesions including propano-, etheno-, and malondialdehyde adducts and even DNA-protein cross-links (Marnett, 2000; also reviewed in Friedberg *et al*., 2006; Uchida *et al.*, 1998). Among the DNA lesions inflicted by lipid peroxidation only the translesion synthesis across 3,N^4-ethenocytosine (εC) and N^2-dG adducts has been investigated up to date.

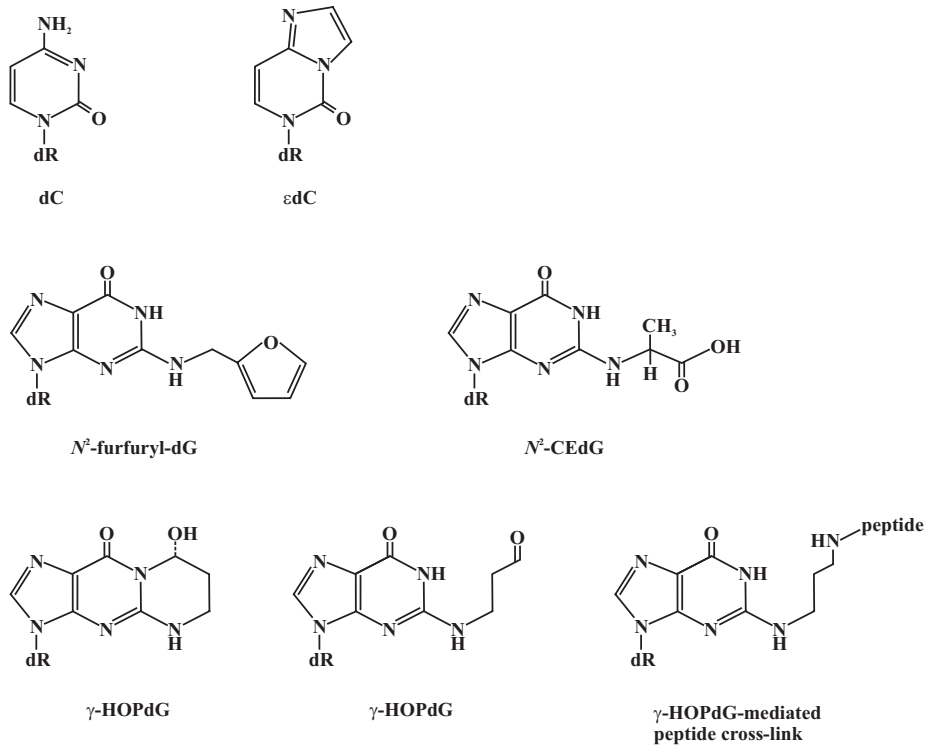


Fig. 5. εC (3,N^4-ethenocytosine) compared with undamaged cytosine and several N^2-G (N^2-guanine) adducts: N^2-furfuryl-G (N^2-furfuryl-guanine), N^2-CE-G (N^2-carboxyethyl-guanine), closed and open forms of γ-HO-G (γ-hydroxypropano-guanine), and DNA-peptide cross-link generated in further reaction of γ-HO-G.

3,N^4-ethenocytosine (εC) (Fig. 5) has been proved to be strongly genotoxic but only weakly mutagenic in *E. coli* (Marnett, 2000). Studies about the translesion synthesis at εC revealed, that pol II bypasses such type of DNA damage efficiently, but in an error-prone manner, favoring the insertion of dATP opposite to εC, thereby resulting in C:G to T:A transitions. However, this event seems to be quite rare under ordinary conditions, whereas replication across εC adduct occurs infrequently but mostly accurately in wild type *E. coli* cells and is proposed to be accomplished in most cases by the replicative DNA polymerase (Al Mamun and Humayun, 2006).

Similarly to 8-oxoG, N^2-G adducts are supposed to be frequent endogenously generated DNA lesions in a living cell (Marnett, 2002; Chung *et al*., 1999), therefore serving as a continuous subjects to cope with during chromosomal replication. In addition to lipid peroxidation byproducts, N^2-G adducts are also generated by reactive carbonyl species like methylglyoxal (MG) (Yuan *et al*., 2008), a common byproduct of glycolysis pathway being

produced in nonenzymatic fragmentation of triose phosphates as glyceraldehyde-3-phosphate and dihydroxyacetone phosphate (Thornalley, 1996). Recently, the *E. coli* pol IV was shown to perform accurate DNA synthesis at N^2-G adducts like N^2-furfuryl-G induced by nitrofurazone (NFZ) (Jarosz *et al*, 2006), N^2-carboxyethyl-G induced by MG (Yuan *et al*., 2008), and γ-hydroxypropano-guanine (γ-HO-G). pol IV-dependent accurate DNA synthesis also occurs across the DNA-peptide cross-links generated in further reaction of γ-HO-G with amines in the composition of peptides and proteins (Minko *et al*., 2008; Kurtz and Lloyd, 2003) (Fig. 5). Furthermore, pol IV exhibits increased proficiency at N^2-G adducts compared to undamaged G. The ability to catalyze the accurate translesion synthesis over N^2-G adducts depends on a single aromatic amino acid phenylalanine or tyrosine residue at particular position conserved among all orthologues of pol IV. These observations indicate that physiological role of pol IV and its orthologues is to perform accurate replication across ubiquitous N^2-G adducts that are severe obstacles to other DNA polymerases, especially for the replicative one (Jarosz *et al*, 2006). The notion that pol IV-deficient *Pseudomonas aeruginosa* is sensitive to N^2-G adducts, induced by NFZ and 4-nitroquinoline 1-oxide (4-NQO), also supports the consideration about the physical role of pol IV (Sanders *et al*., 2006).

Significant source of endogenous DNA damaging agents is nitrosation of amines e.g. amino acids, peptides and polyamines abundant in living cells leading to the formation of alkylating agents. Alkylating agents are able to directly attack nitrogenous bases of the DNA nucleotides by introducing methyl or ethyl groups to any available nitrogen or oxygen atom (Sedgwick, 1997; Taverna and Sedgwick, 1996; Nieminuszczy and Grzesiuk, 2007). Spontaneous generation of endogenous alkylating agents is increased in stationary phase cells of *E. coli,* elevating therefore the occurrence of base substitution mutations (Mackay *et al*., 1994; Taverna and Sedgwick, 1996). In case of *E. coli*, considerable amount of exogenous alkylating agents is also present in their natural habitat, the gastrointestinal tract of warm-blooded animals (Lijinsky, 1999; de Kok and van Maanen, 2000).

To reveal biological function of somewhat enigmatic specialized DNA polymerase, pol IV, Bjedov *et al*. (2007) have compared the mutation frequency in the absence of pol IV in genetic backgrounds in which certain types of DNA lesions were induced by elimination of different DNA repair systems. It turned out that pol IV had no effect on generation of mutations induced by oxidative DNA damage, abasic sites and bulky DNA adducts, whereas it participated in error-free processing of the DNA damage that accumulates in *E. coli* defective in 3-methyl adenine-DNA glycosylases. The absence of 3-methyl adenine-DNA glycosylases causes accumulation of cytotoxic alkyl-lesions such as 3-methyladenine and 3-methylguanine (Wyatt *et al*., 1999) (Fig. 6). Therefore, the accurate bypass of cytotoxic alkyl-lesions is proposed to be one of major biological roles of pol IV (Bjedov *et al*., 2007).

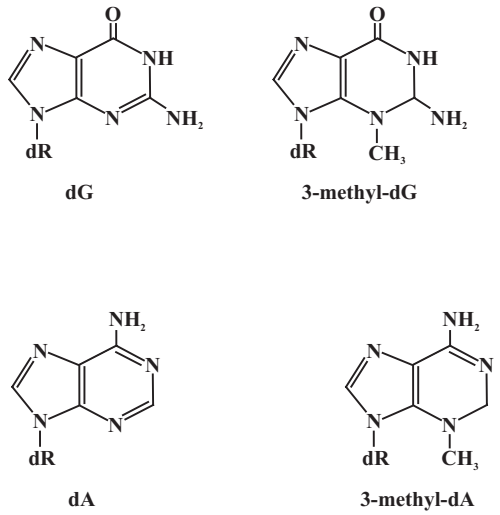


Fig. 6. Alkylatively damaged guanine and adenosine compared with appropriate undamaged nucleotides.

Subsequently described studies on the involvement of specialized DNA polymerases in generation of certain types of mutations due to bypassing DNA lesions generated by several different chemical agents, demonstrate a notable diversity but also overlap of the action of particular polymerases.

Translesion synthesis past *N*-2-acetylaminofluorene guanine (AAF-G) adduct was shown to be accomplished by pol II and pol V. *In vivo* experiments demonstrated that the translesion synthesis at AAF-G adduct in case of sequence context GCGC has absolute requirement for pol II to generate 2-bp deletions (Napolitano *et al*., 2000) while –2 frameshift mutagenesis in case of GCGCGC context exhibits functional redundancy for specialized polymerases and therefore can be carried out both by pol II or pol V (Wagner *et al*., 2002). In GCGC context, the pol V-dependent bypass of AAF-G adduct results in –1 frameshift mutation or occurs in an error-free manner (Napolitano *et al*., 2000). The ability to bypass AAF-G adduct has been ascribed also to DnaE of *B. subtilis*, a member of C-family polymerases. DnaE preferentially exceeds AAF-G by a slippage mechanism thereby resulting in generation of –1 or –2 bp deletions depending on particular sequence context (Le Chatelier *et al*., 2004). Bypass over benzo(*a*)pyrene (BaP) adduct requires the presence of both pol V and pol IV within the same process, leading to creation of 1-bp deletions or being accomplished in an error-free manner (Napolitano *et al*., 2000).

Involvement of either pol IV or pol V (or its homologues encoded by *samAB* or *mucAB*) in mutagenesis induced by a large set of different chemical mutagens was observed in different *Salmonella typhimurium* tester strains (Kokubo *et al*., 2005; Matsui *et al*., 2006). The specificity of lesion bypass by

pol IV and pol V homologues was shown to be partially overlapping. For instance, derivatives of BaP inflicted –2 frameshifts in pol IV-dependent manner were also bypassed by pol V homologues resulting in base substitution mutations. Yet, many structurally unrelated compounds such as 1-nitropyrene, aflatoxin B1 and acridine orange could give rise to –2 frameshifts in the presence of pol V homologue. An alkylating agent ENNG (N-ethyl-N'-nitro-N-nitrosoguanine) was shown to induce both pol IV- or pol V-dependent base substitution mutations and also 1- or 2-bp deletions, which shows that at translesion synthesis across this particular DNA lesion both polymerases can produce all three types of mutations (Kokubo *et al*., 2005; Matsui *et al*., 2006).

Still, mutagenicity of a number of chemicals in generation of 2-bp deletions does not depend on the presence or absence of SOS-induced polymerases. Hence, it is presumed that the replicative DNA polymerase, pol III, may participate in mutagenesis as well. There is also evidence of prevention of chemically induced mutations by pol I, most obviously by reducing the access of Y-family polymerases to the replication complex at the lesion bypass (Kokubo *et al*., 2005; Matsui *et al*., 2006).

1.3.1.3. Abasic sites

Creation of an abasic site (apurinic or apyrimidinic sites – AP-sites) (Fig. 7) by loss of nitrogenous base in the DNA, followed by hydrolytic cleavage of the N-glycosylic bond is frequent event in a living cell and may occur either spontaneously, due to the action of alkylating agents, or enzymatically, by DNA glycosylases as a repair intermediate (Lhomme *et al*., 1999). Though the mechanisms of depurination and depyrimidation are the same, the pyrimidine nucleosides are more stable than purine nucleosides. Therefore, adenine and guanine bases are lost more frequently compared to cytosine and thymine bases (Friedberg *et al*., 2006). Besides the alkali-labile nature of AP-sites promoting the strand breakage, AP-sites are also prone to oxidative stress (Lhomme *et al*., 1999; Friedberg *et al*., 2006). Oxidation of AP-sites has been recently shown to alter the translesion synthesis across the AP-site affecting the selection of incoming nucleoside triphosphates or the mode by which the lesion is bypassed.

Abasic sites strongly block the DNA synthesis due to the lack of the template base to instruct nucleotide incorporation. Also, the lesion-flanking DNA strand in complex with the DNA polymerase has been shown to adopt a conformation distinct from that with undamaged DNA strand. The DNA synthesis by a high-fidelity DNA polymerase stalls at the nucleotide incorporation step, whereas translocation of the DNA after the incorporation is impeded thereby precluding the extension of the nascent DNA strand. The stall of DNA synthesis is then followed by transmission of primer terminus to exonucleolytic subunit (Hogg *et al*., 2004).

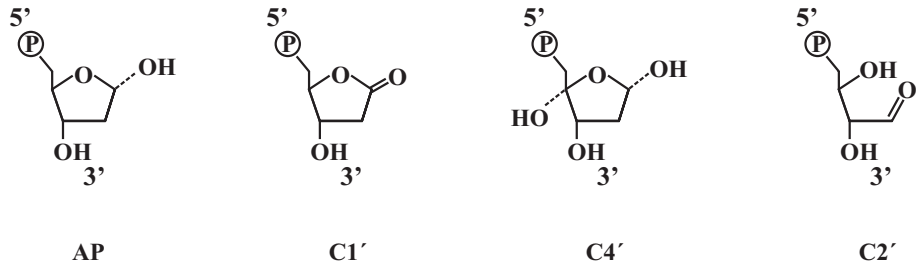


Fig. 7. AP-site (abasic site) and its oxidation products: C1' (C1'-oxidized AP-site), C4' (C4'-oxidized AP-site), and C2' (C2'-oxidized AP-site).

Although, abasic sites are obstacles to specialized DNA polymerases as well (Hogg *et al*., 2004), the more open conformation of an active site and the absence of exonucleolytic activity potentiate bypass of AP-sites by specialized polymerases. Studies of the crystal structure of an archaeal Y-family polymerase Dpo4 at the synthesis across abasic lesion revealed structural insights of mechanisms by which a lesion is bypassed, and also explicated the preference of a particular polymerase to generate –1 frameshifts (Ling *et al*., 2004). The generation of 1-bp deletion occurs when the abasic site of template DNA strand loops out, whereas the base 5' to the abasic lesion serves as a template to the incoming nucleotide, the phenomenon referred as the "5' rule" (Fleck and Schär, 2004). In some cases the template realignment during primer extension was also observed leading to nucleotide location opposite to the abasic site or even generation of +1 frameshifts (Ling *et al*., 2004).

In *E. coli*, which has two members of Y-family polymerases, the major biological significance in translesion synthesis across the abasic sites has previously been assigned rather to pol V than pol IV (Maor-Shoshani *et al*., 2003). Unlike archeal polymerase Dpo4, which generates preferentially –1 frameshifts when bypassing the AP-site, the pol V follows an "A-rule", first postulated by Strauss, 1991 according to which the A nucleotide is most frequently inserted opposite the noninstructural site in a template chain (Strauss, 1991; reviewed by Friedberg *et al*., 2006; Reuven *et al*., 1999; Tang *et al*., 1999). The efficiency of translesion synthesis of pol V of *E. coli* across the abasic site was shown to be 10–100-fold higher than that of *E. coli* DNA polymerases I, II or III (Reuven *et al*., 1999; Tang *et al*., 1999). Latter studies by Kroeger *et al*., (2004), although mainly focused on translesion synthesis across further oxidation products of abasic sites, have shown that, at least in some sequence context, both pol II and pol IV are involved in the bypass of the unoxidized AP-sites at similar extent as pol V. However, instead of generating base substitutions, these polymerases produced –1 frameshift mutations (Kroeger *et al*., 2004a). The results of Kroeger *et al*. indicate that pol II and pol IV act in cooperation. Therefore we can assume that the nucleotide insertion

step is probably carried out by pol IV according to the "5' rule", similarly to archeal Dpo4, whereas pol II may participate at the extension step. A replicative, but error-prone DNA polymerase DnaE of Gram-positive bacteria *Bacillus subtilis* and *Streptococcus pyogenes*, proposed to be responsible for the lagging strand synthesis, was also shown to bypass AP-site preferably according to the "5' rule" (Dervyn *et al*., 2001; Le Chatelier *et al*., 2004; Bruck *et al*., 2003).

Whether the "5' rule" or the "A-rule" is followed in translesion synthesis at abasic sites, depends on the structure of the little finger domain of the certain bypass polymerase (Fleck and Schär, 2004).

The selection of particular polymerases to perform the translesion synthesis at an abasic site and the outcome of the process is strongly altered by further oxidative modifications of abasic sites, resulting in disobedience of the A-rule. To date, the involvement of distinct specialized DNA polymerases in mutagenesis at translesion synthesis across different oxidized abasic site analogs including C1'-, C4'-, and C2'-oxidized abasic sites have been studied (Fig. 7).

The manner of replication over C1'-oxidized AP-site (usually referred to as 2'-deoxyribonolactone, L) was found to depend upon 5' locating nucleotide of the template sequence. 5'-adjacent T or C both resulted in incorporation of A and G nucleotide opposite to L with almost similar frequency, while 5'-C, but not 5'-T enabled generation of –1 frameshifts as well. Incorporation of A opposite to L was strongly supported by pol V, whereas the generation of 1-bp deletions depended upon the presence of pol II and pol IV (Kroeger *et al*., 2004a). C4'-oxidized abasic site (C4–AP) was shown to induce a significant level of 3-bp deletions, a rare type of mutations at translesion synthesis, whereas the presence of specialized polymerases pol II and pol IV was required for this process (Kroeger *et al*., 2004b). C2'-oxidized abasic site (C2–AP) produced single nucleotide deletions in concerted action of pol II and pol IV by dNTP-stabilized misalignment mechanism, similarly to unoxidized AP-site and probably L in some sequence context. If nucleotide incorporation opposite to C2–AP was carried through by pol II and pol IV, the inserted nucleotide was complementary to the template nucleotide, locating at 3' but not at 5' position to the lesion, presumably via primer looping out followed by the primer realignment (Kroeger *et al*., 2006). Nucleotide incorporation opposite the C2–AP and C4–AP by pol V occurred according to the A-rule (Kroeger *et al*., 2004b; Kroeger *et al*., 2006).

In vitro studies have revealed that both *E. coli* specialized polymerases, pol IV and Pol V, possess AP-lyase activity catalyzing cleavage of the phosphodiester backbone at 3'-side of an AP-site and removal of the 5'-deoxyribose phosphate. However, *in vivo* studies do not support relevance of either polymerase to repairing of the DNA damage (Shen *et al*., 2005; also reviewed by Jarosz *et al*., 2007).

Another intriguing finding emerged from *in vitro* replication studies of an archeal Y-family polymerase Dpo4, while using an AP-site-containing template

sequence. The DNA synthesis accomplished by Dpo4 was accurate upstream of the lesion, yet after passing an abasic site, replication became error-prone, resulting in numerous diverse mutations downstream of the lesion (Fiala and Suo, 2007). The mechanism of switch of Dpo4 to error-prone synthesis after the bypass of a basic site is yet unknown.

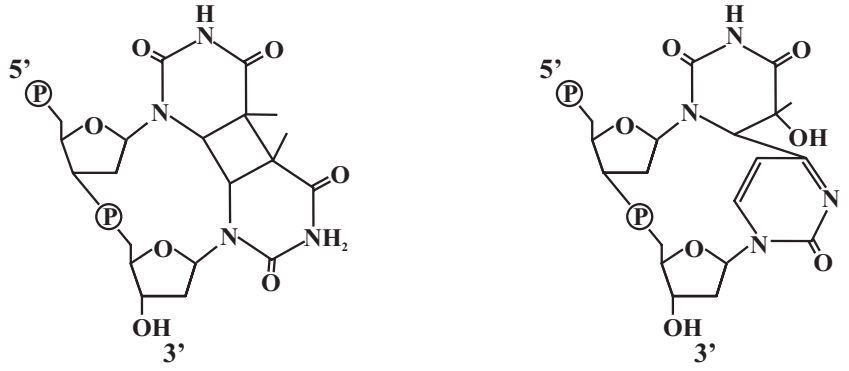
1.3.1.4. UV-induced DNA lesions

The ultraviolet (UV) light, an electromagnetic radiation with a wavelength shorter than that of the visible light, but longer than of the X-rays, is divided to UVC (100–295 nm), UVB (295–320 nm), and UVA (320–400 nm) ranges (Friedberg *et al*., 2006). UVA induces production of free radicals, thereby inflicting indirect DNA damage, whereas UVC and UVB cause direct DNA damage. Although the ozone layer of the atmosphere absorbs most of the high frequency ultraviolet radiation, such as UVC and UVB, and thereby 98.7% of the UV radiation that reaches the Earth's surface is UVA, there is still a high requirement for mechanisms to cope with direct DNA damage caused by high frequency UV. Thus, deficiency of one of the defence mechanisms e.g. nucleotide excision repair (NER) or particular specialized translesion synthesis polymerase leads to harsh consequences as seen in *Xeroderma pigmentosum* patients (Friedberg *et al*., 2006).

Most frequent direct DNA lesions caused by UV radiation are cyclobutane pyrimidine dimers (CPD) and pyrimidine-pyrimidone (6–4) photoproducts [(6–4) PP]. (6–4) PPs have been shown to be more mutagenic compared to CPDs whereas overall frequency of formation of (6–4) PPs is one third of that of CPDs (Svoboda *et al*., 1993; Friedberg *et al*., 2006).

CPDs emerge as a result of formation of covalent linkages between the adjacent pyrimidines resulting in creation of a four-member ring structure accompanied by saturation of the pyrimidine 5,6-double bonds. Generation of a CPD between the two thymidines occurs at least four times more frequently than between the other combinations of pyrimidines (Fig. 8).

(6–4) PP adducts arise from generation of a linkage between the C6 position of the 5' pyrimidine and the C4 position of the 3' pyrimidine of the adjacent pyrimidine pair. Among the formation of (6–4) PP photoproducts, the most abundant ones are pyrimidine-pyrimidone pairs, composed of either thymidine and cytidine or two cytidines (Fig. 8) (Svoboda *et al*., 1993; Friedberg *et al*., 2006).



CPD **(6-4)PP**

Fig. 8. UV-induced DNA lesions: CPD (cyclobutane pyrimidine dimer) generated between the two thymidines, and (6–4) PP (pyrimidine-pyrimidone (6–4) photoproducts) composed of thymidines.

UV-induced DNA lesions are severe replication blocks, that effectively impede resumption of the DNA synthesis carried out by a replicative DNA polymerase, as asserted by high cytotoxic effect of the UV radiation. A vast majority of UV-induced photoproducts are processed by NER and recombinational repair (Friedberg *et al*., 2006), still some specific situations require bypass of such lesions by translesion synthesis. In particular, two closely located UV-lesions on opposing strands of the DNA double-helix are troublesome subjects to repair (Svoboda *et al*., 1993), and replication across the lesion by a specialized DNA polymerase then becomes advantageous (Schlacher and Goodman, 2007). In *E. coli*, the translesion synthesis across UV-induced photoproducts is accomplished efficiently by pol V, whereas the second Y-family DNA polymerase of *E. coli*, pol IV, is unable to copy CPDs or (6–4) PPs (Tang *et al*., 2000).

In vitro and *in vivo* studies have revealed the manner of translesion bypass of CPDs and (6–4) PPs by pol V. At the translesion synthesis across the (6–4) PPs, pol V preferentially incorporates G, instead of A opposite to 3'-T of the template (6–4) PP constituted of two thymine residues [TT (6–4) PP] or a thymine-cytosine pair [TC (6–4) PP]. Therefore, pol V strongly induces UV radiation-dependently T to C transition mutations (Tang *et al*., 2000; LeClerc *et al*., 1991; Smith *et al*., 1996). At the same time, the bypass of CPDs occurs mainly by insertion of two A nucleotides opposite to the lesion, thereby possessing only a modest mutagenic effect (Smith *et al*., 1996).

Whereas crystal structures of *E. coli* pol V in action of translesion synthesis across the UV-induced DNA lesions are not yet available, some predictions can be made according to the studies of a Y-family DNA polymerase Dpo4 of *Sulfolobus solfataricus*. Crystal structures of Dpo4 captured at nucleotide

incorporation as well as *in vitro* experiments, allow suggesting that Dpo4 probably accommodates the whole thymine dimer. A hypothetical model of the lesion bypass supports skipping the first template base and replicating the second base, followed by switch of the incorporated adenine to pair with the thymine at first position. Thereafter, the polymerase would replicate again the second base, this time without the template translocation (Boudsocq *et al*., 2001; Ling *et al*., 2001).

Plant pathogenic bacteria inhabiting the phyllosphere are more exposed to UV radiation, compared to many other bacterial species. Therefore, any mechanism enhancing resistance to UV would wield a beneficial value. Although many bacteria, including phyllosphere-colonizing species, do not carry pol V encoding genes in their genomes, several different plasmid-prone *umuDC* homologs have been characterized (Perry *et al*., 1985; Lodwick *et al*., 1990; Nohmi *et al*., 1991; Kulaeva *et al*., 1995; Sundin *et al*., 1996). *rulAB* genes, initially isolated from the plasmid pPSR1 of *Pseudomonas syringae* pv. syringae A2, a pathogen of ornamental pear trees (Sundin *et al*., 1996), have been shown to contribute to UV-B survival as well as to UV-induced mutagenesis *in vivo* (Kim and Sundin, 2000).

In many bacterial species which do not carry pol V-encoding *umuDC* orthologs in their genomes, proteins encoded by another DNA damage-inducible operon functionally replace the pol V in UV-mutagenesis and –tolerance (Abella *et al*., 2004; Sanders *et al*., 2006; Erill *et al*., 2006). This particular DNA damage-inducible operon contains *imuB* and *dnaE2* genes, encoding orthologs of DNA polymerases, while composition of other genes varies between different species. *dnaE2* encodes a second copy of the catalytic subunit of replicative DNA polymerase pol III, whereas *imuB* encodes a Y-family polymerase-like protein (Abella *et al*., 2007; Galhardo *et al*., 2005). *In vivo* experiments have revealed that *imuAB-dnaE2* operon of *Caulobacter crescentus* is responsible for most of UV-induced mutations in the RNA polymerase β-subunit-encoding *rpoB* gene, resulting in resistance to rifampicin (Rif) (Galhardo *et al*., 2005). Though protein encoded by *imuA* of this particular operon exhibits some similarities to cell division inhibitor SulA and also to RecA/RadA recombinases, and was initially annotated as *sulA* (Weinel *et al*., 2002), ImuA was shown not to be involved in cell division suppression (Galhardo *et al*., 2005). Since UV-induced mutagenesis is diminished in the absence of any of these three genes of this particular operon, apparently these proteins cooperate in the same pathway. Moreover, instead of G:C to A:T transition, which is most prominent UV-induced mutation in the *rpoB* context in *E. coli* (Garibyan *et al*., 2003), a high proportion of *imuAB/dnaE2* dependent G:C to C:G transversions were observed in *C. crescentus* indicating different UV-lesion bypass mechanism compared to *E. coli* pol V (Galhardo *et al*., 2005). Similar DNA damage-inducible operon has also been identified in *Pseudomonas putida*. This operon contains the genes *lexA2, sulA2, dinP and dnaE2* that encode second copy of repressor LexA, a putative cell division inhibitor,

Y-family DNA polymerase similar to pol IV, and a protein similar to catalytic subunit of pol III, respectively (Abella *et al*., 2004). This operon is under the negative control of LexA2 repressor, which recognizes different DNA sequences than the LexA. Analogous operon of *Pseudomonas aeruginosa,* opportunistic human pathogen inflicting chronic infections of cystic fibrosis patients, contains *sulA, dinP and dnaE* genes, but not the *lexA2*. The *dinP* gene from this operon encodes a pol IV ortholog and the *dnaE* designates an ortholog of the *dnaE2*. Similarly to *P. putida*, this particular operon is SOS-inducible, but instead of LexA2, it is under negative control of LexA. UV-induced mutagenesis in *P. aeruginosa* is dependent on a DnaE2 ortholog and DNA polymerase I, encoded by *polA* (Sanders *et al*., 2006).

Gram-positive bacterium *Bacillus subtilis* possesses two Y-family polymerases, YqjH (known also as PolY1) and YqjW (known also as PolY2). Expression of YqjW is SOS-inducible, whereas that of YqjH's is not (Duigou *et al*., 2004). Previous studies of monitoring the appearance of His^+ revertants have demonstrated the involvement of both YqjH and YqjW in generation of UV-induced mutations, although the effect appeared to be rather modest (2–3 fold) (Sung *et al*., 2003). Later, based on experiments observing generation of Rif-resistant mutants, YqjW has been established to have a major role in UV-induced mutagenesis (Duigou *et al*., 2004). Surprisingly, YqjW did not require interaction with the β-clamp for UV-mutagenesis. Moreover, UV-induced mutagenesis was shown to depend upon an A-family DNA polymerase Pol I, whereas these two polymerases interact with each other and form ternary complex consisting of YqjW, Pol I and the β-clamp. Whereas the catalytic activity of Pol I is also required for YqjW-dependent UV-mutagenesis, pol I presumably acts at lesion bypass in targeting the YqjW to the template as well as extending the primer terminus following the YqjW action at insertion step (Duigou *et al*., 2005).

1.3.2. Involvement of specialized DNA polymerases in stationary-phase mutagenesis

Studies on the involvement of specialized DNA polymerases in translesion synthesis across various types of DNA damage enable to define the properties and biological functions of particular polymerases. However, these studies give little information about circumstances and environmental conditions which trigger such processes where the involvement of specialized polymerases is in practice feasible and/or needed.

In addition to previously described DNA damaging chemical compounds and radiation which directly induce mutagenic events, several environmental stresses *e.g.* long-term nutritional deficiency, temperature shifts, and exposure to antibiotics have been shown to enhance genetic variability and therefore adaptive evolution of microorganisms. Among number of different functions,

error-prone DNA synthesis carried out by specialized DNA polymerases appears to be involved in increasing mutational frequency under stressful conditions.

1.3.2.1. Mutagenesis in aging colony

Structured environments of bacterial populations like biofilms, cellular aggregates, and colonies possess several beneficial features compared to planktonic bacterial cultures. For example, increased infectivity and resistance to various antimicrobial agents is achieved under such conditions. Differences in mutational processes between bacterial populations, living on solid surface compared to the populations in unstructured environments, have been found as well.

In order to study the effect of structured environment on mutagenesis in starved bacteria, mutational frequency has been observed in aging *E. coli* colonies. Exponential growth of bacteria on solid rich medium during the colony formation is followed by exhaustion of nutrients leading to adaptive physiological changes. The mutation frequency in *E. coli* colonies aged for a week on rich agar medium was found to increase approximately 10-fold when compared to one-day-old colonies, whereas such increase in mutation frequency was not observed in aging liquid culture (Taddei *et al*., 1995; Bjedov *et al*., 2003). In addition to the requirement for solid growth substrate, mutagenesis in aging colonies was shown to depend upon carbon source starvation and aerobic conditions (Bjedov *et al*., 2003). The phenomenon appears to be widespread since among a broad spectrum of natural (*E. coli*) isolates, including both commensal and pathogenic strains from various environments and hosts, most exhibited an increase in frequency of Rif^R mutants in aged colonies after 7 days of incubation (Bjedov *et al*., 2003). A negative correlation was recorded between high constitutive mutation frequency and high inducibility of mutations, with pathogenic isolates showing more often high constitutive mutation frequencies, while nonpathogenic isolates possessing generally high inducible mutation rate.

Mutagenesis in aging colonies was first studied in laboratory strains of *E. coli* and was named ROSE mutagenesis for "Resting Organisms in a Structured Environment" (Taddei *et al*., 1995). To evaluate the mutation frequency, number of rifampicin resistant (Rif^R) mutants within the colonies was monitored. This particular method allows to estimate the frequency of mutations in *rpoB* gene that alter the amino acid sequence of RNA polymerase β subunit by interfering its interaction with rifampicin. Therefore mainly base substitutions are feasible to retain the functionality of the RNA polymerase. As laboratory strains may have become substantially diverse in comparison with their natural ancestors, mutagenesis in aging colonies was later investigated also in natural isolates, as mentioned above (Bjedov *et al*., 2003). Mutation frequency was estimated by appearance of Rif^R mutants and also by using

several *lacZ* reversion systems allowing to detect separately base substitutions and –1 and –2 frameshifts. The frequency of frameshift mutations in aging colonies was increased similarly to that of base substitutions. Genetic requirements for elevated mutation frequency in aging colonies of natural isolates were different from those observed in earlier studies, and were therefore referred as MAC for "Mutagenesis in Aging Colonies" (Bjedov *et al*., 2003). However, only one isolate that expressed a magnitude higher inducible mutation rate than the average was investigated for genetic requirements of elevated mutation frequency, and only 13% of all isolates exhibited that high induction of MAC. Thus genetic requirements for MAC may not be identical for all natural isolates.

Main differences in genetic requirements for the increase of mutation frequency in aging colonies between the ROSE mutagenesis and MAC lie in dependence of SOS induction for ROSE. No increase in mutation rate in aged colonies was observed in either RecA-deficient or in non-cleavable repressor LexA backgrounds in case of ROSE. Further studies revealed that ROSE mutagenesis did not require pol V, but surprisingly, nucleotide excision repair (NER) appeared to be involved instead. ROSE was completely dependent on UvrB (component of NER pathway) and pol I which has been shown to perform DNA synthesis during NER.

Although MAC does not depend directly on SOS induction, the functionality of some SOS regulon genes is still essential for elevated mutation frequency in MAC as well. Similarly to ROSE, RecA is needed for MAC. However, MAC is independent of LexA inactivation. Contrary to ROSE, MAC is independent either of NER enzymes or pol I, but is dependent on pol II, one of the SOS induced DNA polymerases.

The main regulator which mediates the induction of general stress response during the entry of cells into stationary phase and under the conditions of nutrient limitation is RpoS, an alternative sigma subunit of RNA polymerase. Therefore it is not surprising that RpoS was shown to participate in MAC down-regulating mismatch repair system (MMR), the key enzyme MutS. However, there is no data supporting the involvement of RpoS in ROSE mutagenesis.

Despite of dissimilarities between genetic requirements for MAC and ROSE mutagenesis, in both cases the decline in energy resources (nutrient deprivation) is serving as a signal mediated by cyclic AMP (cAMP). cAMP was shown to be required for both ROSE mutagenesis and SOS induction. Although MAC does not depend on SOS induction, cAMP and cAMP receptor protein (CRP) are needed for MAC as well (Taddei *et al*, 1995; Bjedov *et al*., 2003).

1.3.2.2. Mutagenesis in a layer of cells on solid surface

There are several test-systems that enable investigation of stationary phase mutagenesis in bacteria under non-lethal selective conditions such as starvation for a carbon source or a certain amino acid. Although the mechanisms of mutational processes in stationary-phase bacteria are very complex and depend on a number of factors, involvement of specialized DNA polymerases appears to be a general feature.

Like many other processes, stationary phase mutagenesis has been most intensively studied in a bacterial model, *E. coli.* The generation of a majority of 1-bp deletion mutations in *E. coli* population under carbon starvation, leading to the acquisition of lactose utilization ability, was shown to depend upon functionality of a Y-family polymerase pol IV (McKenzie *et al*., 2001a). Further investigations have revealed, that other factors facilitate directly or indirectly the DNA synthesis performed by pol IV, by increasing its expression or regulating its access to the DNA synthesis.

Particular test-system used for investigation of molecular mechanisms of generation of pol IV-dependent frameshift mutations in *E. coli* stationary phase population is based on conjugative F' episome. Even in the absence of active conjugation, single-strand DNA nicks are continuously generated at conjugal origin, leading to the formation of double-strand breaks (DSB). Importantly, the occurrence of pol IV-dependent 1-bp deletions depends on formation of DSBs (Ponder *et al*., 2005). Proceeding recombinational repair of DSBs enables generation of 1-bp deletions during the DNA synthesis stage. Earlier studies have revealed that requirement for recombinational repair of DSBs for generation of Lac^+ adaptive mutations accounts for dependence upon the RecA, RecBCD and RuvABC proteins (Cairns *et al*., 1991; Foster *et al*., 1996; Harris *et al*., 1994; Harris *et al*., 1996). Involvement of pol IV in DSB repair by homologous recombination is facilitated by stationary phase specific sigma factor RpoS which promotes a switch from high-fidelity DSBR to error-prone DSBR (Ponder *et al*., 2005), presumably mainly through upregulation of pol IV (Layton and Foster, 2003; Lombardo *et al*., 2004). As SOS DNA damage response is also required for the generation of Lac^+ mutants. Its supposed role of SOS response in particular process is enhancement of the expression of pol IV by derepression of transcription of the pol IV gene (McKenzie *et al*., 2000, 2001b).

In addition to stimulation of the transcription of pol IV-encoding gene *dinB* by RpoS and SOS response, there are more factors enhancing a cellular amount or activity of pol IV in stationary phase cells. For example, molecular chaperon GroE was shown to be required for pol IV-dependent mutagenesis, probably by preventing pol IV degradation (Layton and Foster, 2005). The expression of polyphosphate kinase (Ppk), which is responsible for the synthesis of polyphosphate (PolyP), is required for the activity of pol IV as well, but does not affect the cellular amount of pol IV (Stumpf and Foster, 2005). PolyP

accumulates in stationary phase cells and under conditions of nutritional depletion (Kuroda *et al*., 1997). As polyP mimics DNA, it is able to bind various DNA-binding proteins whereas in some cases polyP-binding serves a regulatory function (Kusano and Ishihama, 1997; also reviewed in Foster, 2007). Recently, UmuD and RecA were shown to suppress generation of pol IV-dependent –1 frameshift mutations by concerted binding to pol IV, hence enclosing its active site (Godoy *et al*., 2007). It has been hypothesized that malfunctioning of MMR in a subpopulation of cells is an additional factor, facilitating the occurrence of Lac^+ reversions under lactose selection. Specifically, a functional limitation of MMR enzyme MutL may magnify the effect of pol IV on generation of 1-bp deletions (Harris *et al*., 1997, 1999; also reviewed in Foster, 2007 and Galhardo *et al*., 2007).

Studies about the involvement of DNA polymerases other than pol IV in occurrence of Lac reversions have revealed, that pol V does not participate in generation of Lac^+ mutations in starved *E. coli* (Cairns *et al*., 1991; also referred McKenzie *et al*., 2000), whereas pol II exhibits rather a negative effect on appearance of these mutations, probably by competing with pol IV (Foster, 2007). The rest of Lac^+ revertants, which emerge independently from pol IV, are most likely produced by pol III (Foster, 2007; McKenzie *et al*., 2001b).

Starvation induced mutagenesis has also been studied in a Gram-positive soil bacterium *Bacillus subtilis* which, due to its ability to form endospores, has been used as a simplified model to investigate cell differentiation. To investigate mutagenic processes in stationary phase *B. subtilis*, the appearance of prototrophic revertants within an auxotrophic population (auxotrophy for histidine, methionine or leucine) has been detected (Sung and Yasbin, 2002). The revertants accumulated on selective minimal medium lacking a certain required amino acid. Particular chromosomal reversions systems allow monitoring the occurrence of variuos base substitution mutations (Sung and Yasbin, 2002).

Y-family polymerase YqjH, homologous to *E. coli* pol IV (Ohmori *et al*., 2001) was found to be involved in stationary phase mutagenesis in *B. subtilis,* whereas YqjH was responsible for the generation of at least half of prototrophic revertants. The second Y-family polymerase of *B. subtilis*, YqjW, did not contribute to the generation of (His^+) prototrophic revertants (Sung *et al*., 2003). However, differently from *E. coli* pol IV-dependent stationary phase mutagenesis, the appearance of prototrophic mutants in *B. subtilis* was independent of SOS response and recombinational processes, since RecA functionality was not required for mutagenesis in particular tester strains (Sung and Yasbin, 2002).

Interestingly, amino acid starvation-induced mutagenesis in *B. subtilis* is at least partially mediated by transcriptional regulators ComA and ComK (Sung and Yasbin, 2002). Com factors are involved in starvation-induced spore formation and competence development for natural transformation by the DNA taken up from a surrounding environment. The role of Com factors in stationary

phase mutagenesis in a particular *B. subtilis*' system is speculated to be analogous to that of RpoS and cAMP in *E. coli* F' episome-based system (Galhardo *et al*., 2007).

Similarly to *E. coli*, it is presumed that a subpopulation of cells of *B. subtilis* population suffers from MMR limitation under amino acid starvation conditions, since generation of prototrophic revertants in stationary phase population is increased in MMR deficiency. Moreover, overexpression of the MMR key-enzyme MutS reduced mutation frequency in *B. subtilis* cells experiencing amino acid starvation, but not in a growing culture (Pedraza-Reyes and Yasbin, 2004).

Stationary phase mutagenesis in *B. subtilis* was observed to be a transcription-associated process. The generation of prototrophic revertants was reduced by deficiency in Mfd, a protein which removes stalled RNA polymerase and recruits nucleotide excision repair (NER) enzymes to actively transcribed regions of DNA, which in most circumstances overlap with the genes under selection (Ross *et al*., 2006). The role of Mfd in stationary phase mutagenesis is probably to endow error-prone DNA synthesis during the process of transcription-coupled repair (Foster, 2007).

Although the involvement of polymerase I in appearance of prototrophic revertants has not been described to date, both the spontaneous and UV-induced mutagenesis carried out by YqjH and YqjW, respectively, require the pol I activity. Furthermore, both Y-family polymerases were shown to physically interact with pol I, suggesting functional coupling between the A- and Y-family polymerases in mutagenic or translesion synthesis in *B. subtilis* (Duigou *et al*., 2005).

Opportunistic pathogen *Pseudomonas aeruginosa* colonizes the respiratory track of cystic fibrosis patients forming biofilm communities. High oxidative stress in the biofilm, leading to the DNA damage and highly variable environment, facilitate evolvement of heterogeneous populations (Boles and Singh, 2008), contributing to the appearance of resisting mutants e.g. exopolysaccharide alginate overproducing mucoid variants among other genetic alterations. Alginate overproduction possesses many beneficial properties, like protection of bacterial cells from the host immune response (Mai *et al*., 1993; also referred in Moyano *et al*., 2007), and exhibiting a higher resistance to antibiotics (Ciofu *et al*., 2001). Hence, a positive selection favors the emergence of mucoid variants and chronic infection. Conversion to mucoidy in the lungs of CF patients occurs mainly by acquisition of mutations in the *mucA* gene, encoding for anti-sigma factor which represses the alginate production by sequestration of AlgT (also known as AlgU), an alternative sigma factor positively regulating transcription from the alginate biosynthetic operon (Mathee *et al*., 1997). MucA gene was shown to be inactivated as a result of base substitutions or frameshift mutations. Base substitutions lead to the formation of premature stop codon or other alterations. Frameshift mutations such as 1-bp deletions within homopolymeric sequences of five Gs are known

to be characteristic to pol IV-type bacterial DNA polymerases (Martin *et al*., 1993; Moyano *et al*., 2007). Since the occurrence of mutations within the *mucA* gene was strongly reduced in pol IV-deficient background, (Moyano *et al*., 2007) it is obvious that pol IV plays a significant role in the development of chronic infections in airways of CF patients. The deficiency of MMR system, another frequently-detected alteration in clinical isolates of *P. aeruginosa* present in chronic infections (Oliver *et al*., 2000, 2002; Hogardt *et al*., 2007), magnifies the mutagenic effect of pol IV even more (Moyano *et al*., 2007).

1.3.2.3. Appearance of mutants with growth advantage in a stationary phase in liquid culture

It has been observed that bacterial cells aged for at least ten days in liquid batch cultures are able to out-compete the cells from younger cultures when incubated in a co-culture (Zambrano *et al*., 1993; Finkel and Kolter, 1999; Smeulders *et al*., 1999). Hence, the competitive advantage of long-term stationary phase culture cells appeares to be defined by a genetic alterations and does not depend upon physiological adaptation to stationary phase environment (Zambrano *et al*., 1993). The genetic alterations leading to the expression of GASP ("Growth Advantage in Stationary Phase") phenotype are connected mainly with increased ability to assimilate or catabolize one or several amino acids as a carbon source (Zinser and Kolter, 2004). The most prominent GASP mutations reduce the activity of an alternative stationary phase sigma factor RpoS. The other characterized genetic alterations concerning the GASP phenotype have been located to *lrp* and *ybeJ-gltJKL* locus, encoding leucine responsive protein Lrp and a high-affinity aspartate and glutamate transporter, respectively (Zinser and Kolter, 1999; 2000; Zinser *et al*., 2003; Zinser and Kolter, 2004).

Due to the selective pressure acting upon many different loci, the ascertainment of exact molecular mechanisms of generation of GASP mutations is complicated. Most obviously, several distinct molecular mechanisms are responsible for the appearance of GASP mutations, whereas the nature of mutations varies from point mutations up to large genomic rearrangements. Nevertheless, the involvement of specialized DNA polymerases is crucial for the generation of GASP mutants in *E. coli*, although the processes and pathways wherein those polymerases participate have remained unclear (Yeiser *et al*., 2002). Strains deficient for any of the SOS-inducible DNA polymerases pol II, pol IV, or pol V, exhibit remarkable decrease in relative fitness when co-cultured with wild-type strain. It leads to displacement of specialized DNA polymerase-deficient strains and population takeover by wild-type bacteria, whereas individually cultured SOS-inducible polymerase deficient populations show no alteration in viability compared to wild-type population in long-term stationary phase growth (Yeiser *et al*., 2002). When co-cultured with each other, every polymerase mutant strain possesses similar relative fitness which means

that virtually half of the times one polymerase-deficient strain out-competes the other from initially identical co-cultures and *vice versa* (Yeiser *et al*., 2002). The reduced ability of specialized DNA polymerase-deficient strains to express GASP phenotype is supposed to be inflicted by complications in replicating across certain DNA lesions and diminished genetic diversity on which the selection may act (Yeiser *et al*., 2002; Finkel, 2006).

1.3.2.4. Antibiotic-induced mutagenesis

Development of antibiotic resistant mutants of pathogenic bacteria is a serious problem in infection therapy, especially in case of chronic infections and immunocompromised patients. Therefore, the mechanisms by which the antibiotic resistance evolves are under intense investigation.

Exposure of bacteria to antibiotics, like other stressful conditions described above, induces mutagenic processes which in turn increase the probability of resistance acquisition. The presence of several different types of antibiotics in bacterial growth environment, whether involved directly in DNA metabolism or not, eventually leads to increased expression of specialized DNA polymerases.

The most prominent mechanism of up-regulation of specialized DNA polymerases due to the presence of antimicrobial agents appears to be the SOS response. So, the evolvement of ciprofloxacin-resistant *E. coli* mutants after the exposure to a particular antibiotic depends on activation of SOS response, whereby functionality of all three specialized DNA polymerases pol II, pol IV, and pol V is required (Cirz *et al*., 2005; Cirz and Romesberg, 2006). Ciprofloxacin-induced mutagenesis requires, in addition to specialized polymerases, also RecA, RecBC and RuvABC proteins, all involved in double-strand break repair by homologous recombination (Cirz *et al*., 2005). Therefore, the presumed model for the generation of ciprofloxacin-induced mutations resembles in molecular mechanisms to the generation of Lac reversions described hereinbefore. Though, instead of double-strand break formation due to conjugal functions in case of Lac reversion mutations, ciprofloxacin induces double-strand breaks by inhibition of topoisomerase-DNA complex after the cleavage and before the ligation of DNA strands (Galhardo *et al*., 2007).

Ciprofloxacin treatment inflicts SOS response-dependent upregulation of specialized DNA polymerases DnaE2 and ImuB in *P. aeruginosa* as well (Cirz *et al*., 2006). Located in same operon, the former encodes a C family DNA polymerase, known also as an error-prone DNA polymerase in other organisms, whereas the latter encodes a pol IV-type Y-family DNA polymerase. Some contrarieties have appeared in data available for transcription induction from pol IV-encoding *dinB* gene by ciprofloxacin in *P. aeruginosa*. Cirz *et al*. have observed ciprofloxacin-dependent, albeit SOS-induction independent, induction of pol IV, (Cirz *et al*., 2006), while Blazques *et al*. have not detected transcription induction from *dinB* by ciprofloxacin (Blázquez *et al*, 2006). The

latter observation is also supported by the results from global microarray studies of alterations in gene expression as a response to antibiotic treatment (Brazas and Hancock, 2005). Discrepancy in pol IV expression induction by ciprofloxacin is most probably derived from dissimilarities in experimental procedures applied by different authors.

SOS response-dependent induction of expression of a specialized Y-family DNA polymerase, UmuC-type polymerase (SACOL1400), occurs in response to ciprofloxacin treatment also in *Staphylococcus aureus*, a Gram-positive pathogen associated with diverse serious infections. Although the second Y-family DNA polymerase of *S. aureus* is not SOS-regulated, a slight up-regulation of pol IV (SACOL1955) by ciprofloxacin has also been observed (Cirz *et al*., 2007).

The appearance of rifampicin resistant *Mycobacterium tuberculosis* mutants during the infection is observed to depend on functionality of a specialized DNA polymerase DnaE2 (Boshoff *et al*., 2003). Whereas UV-induced mutagenesis provided by DnaE2 in *M. tuberculosis* (and also in *M. smegmatis*) is a RecA-dependent (Boshoff *et al*., 2003), it is very likely that rifampicin treatment inflicts SOS response-dependent induction of specialized DNA polymerases similarly to ciprofloxacin in *E. coli*. SOS-induction-dependent evolvement of rifampicin resistant *E. coli* mutants was also observed in a mouse infection model in response to rifampicin treatment (Cirz *et al*., 2005).

It is intriguing to note that SOS-induction is also generated as a response to the treatment of bacteria with a β-lactam antibiotic, an antimicrobial agent which interferes with cell wall synthesis by binding to penicillin binding proteins (PBPs) (Miller *et al*., 2004). Inactivation of PBP3 by β-lactams induces two-component signal transduction system-encoding operon *dpiBA*. In addition to transcription regulation, the response regulator DpiA also regulates the DNA replication by binding to the chromosomal replication origin and competing with replication proteins DnaA and DnaB, that leads to inhibition of replication and induction of SOS response (Miller *et al*., 2003). SOS response results in inhibition of cell division by SulA protein, thereby preventing the cell from harmful effects of β-lactam antibiotics, but also inflicting the induction of specialized DNA polymerases. However, the transcription from *E. coli* pol IV encoding *dinB* gene as well as pol IV-dependent increase in 1-bp frameshift mutations in *E. coli* FC40 system is induced by the exposure to β-lactam antibiotics in a SOS-independent manner as well (Perez-Capilla *et al*., 2005). The induction of expression of pol IV and increase in mutation frequency by β-lactam antibiotics is intrinsic also to *P. aeruginosa* (Blázquez *et al*., 2006).

2. RESULTS AND DISCUSSION

2.1. Construction of the test systems for investigation of stationary phase mutagenesis in *Pseudomonas putida* (Ref. I)

Study of the mechanisms of mutagenic processes as well as monitoring the occurrence of certain types of mutations, is restricted by availability and the nature of test systems. Depending on the test system employed, it is possible to detect either only single type of mutations or a broad range of changes simultaneously. Selection type of a particular mutagenesis assay defines the growth phase of bacterial population during which the mutagenic processes can be investigated. For example, presence of certain antibiotics in growth medium is lethal to bacteria, and therefore mutations generated only prior the selection are observed using the assay system based on the selection for resistance to antibiotics. At the same time, test systems based on either acquisition of carbon source utilization or appearance of prototrophic revertants within the auxotrophic population enable monitoring of the mutagenesis throughout long periods of starvation.

A plasmid-based test system employed in the studies of mechanisms of stationary phase mutagenesis in *P. putida* in our laboratory earlier (Kasak *et al.*, 1997; Saumaa *et al.*, 2002) permits observation of occurrence of several different types of mutations simultaneously, e. g. base substitutions, deletions, and insertions, but also larger DNA rearrangements such as transposition of an IS element and the transposon Tn*4652*. All these mutations allow generation of a functional promoter upstream of silent phenol degradation operon *pheBA* enabling utilization of phenol as a carbon source. In order to study the mechanisms of generation of different types of mutations separately, a set of novel test systems was constructed (Ref. I). These new assay systems are based on detection of reversion mutations (either framshift mutations or base substitutions) restoring functional phenol monooxygenase-encoding *pheA* sequence under the control of a constitutively expressed promoter on RSF1010-derived plasmid. Tester plasmid pKTpheA56+A, which was constructed to monitor the appearance of 1-bp deletions, contains a single A nucleotide insertion within the ACC codon of the reporter gene *pheA,* encoding threonine at the position 56 (Thr-56) of the amino acid sequence of phenol mono-oxygenase (Fig. 9; Ref. I, Fig. 1). Other test systems designed by the replacement of CTG codon for the leucine at the position 22 (Leu-22) of the amino acid sequence of PheA with the stop codons, TAG, TAA, or TGA (pKTpheA22TAG, pKTpheA22TAA, pKTpheA22TGA) allow the detection of different base substitution mutations (Fig. 9; Ref. I, Fig. 1).

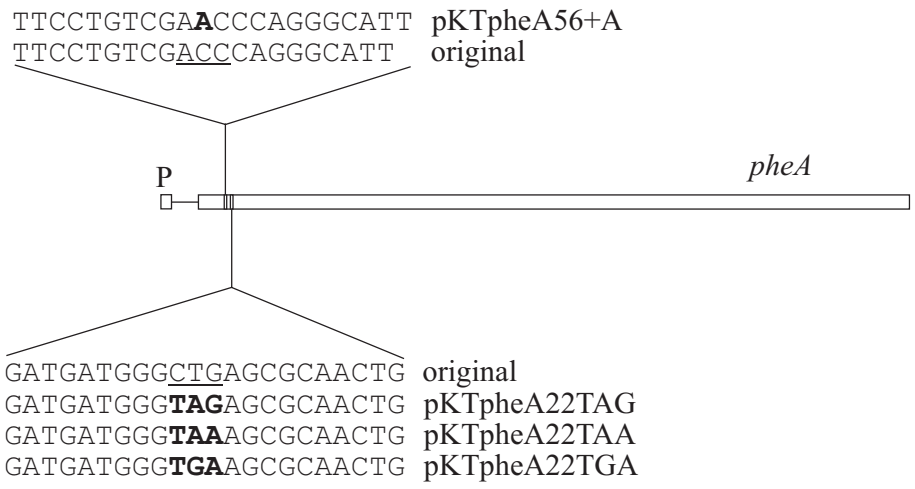


Fig. 9. Assay systems allowing detection of 1-bp deletions (pKTpheA56+A) or different base substitutions (pKTpheA22TAG, pKTpheA22TAA, pKTpheA22TGA) in *P. putida* stationary phase cells. Regions containing introduced alterations are shown aside with original sequence of the phenol monooxygenase tester gene *pheA*. The transcription of the *pheA* gene is initiated from a constitutively expressed promoter, indicated by P.

Observation and isolation of phenol-degrading Phe^+ mutants using the assay systems described herein was performed under nonlethal selection on solid minimal medium containing phenol as a sole carbon source. Approximately 5×10^8 to 1×10^9 tester plasmid-carrying cells of *P. putida* overnight grown culture are spread onto phenol minimal plates. Phe^+ mutants emerging in the growing culture form visible colonies on phenol minimal plates by second day after the plating, whereas Phe^+ colonies appearing on day 3 and later carry mutations generated under starvation conditions after the plating. These particular test systems enable to study mutagenic processes during at least 2 weeks of carbon source starvation. Mechanisms of stationary phase mutagenesis have mostly been investigated in experiments lasting less than 1 week. However, in natural environments, bacterial populations very likely face far longer periods of starvation. Our previously published results suggested that mutational processes in cells from different starvation periods are not entirely compatible (Saumaa *et al*., 2002). Therefore, in current study (Ref. I), the appearance of Phe^+ mutants onto phenol minimal plates was monitored throughout a longer period of starvation for the carbon source.

Sequence analysis of *P. putida* Phe^+ mutants carrying the test system pKTpheA56+A which measures the reversion of +1 frameshifts revealed that majority of 1-bp deletions had occurred within the homopolymeric sequence CCC flanked to the inserted A nucleotide whereas only few mutants contained a deletion of inserted A. The 1-bp deletion at the CCC repeat restored the *pheA*

reading frame by replacing the ACC codon for Thr-56 with the AAC encoding asparagine (Asn).

Approximately 100 Phe^+ revertants isolated using the test systems carrying different stop codons instead of the codon for Leu-22 were all generated by base substitutions which eliminated the stop codon by replacing it with codons for several different amino acids (Table 1; Ref. I, Table 3). These results excluded the appearance of supressor mutations. In addition, in case of every revertant studied, Phe^+ phenotype was conjugatively transferred to *P. putida* Phe^- recipient. Since the Phe^+ transconjugants carrying different mutant plasmids grew equally well on phenol minimal plates, amino acid Leu-22 of the PheA protein appeared to be replaceable with several other amino acids without changing the ability to utilize phenol compared to the wild-type enzyme. Analysis of Phe^+ revertants generated due to base substitutions revealed different spectra of mutations depending on the sequence of stop codon used in the particular test system (Table 1; Ref. I, Table 3).

Table 1. Base substitutions occurred in stop codons (TGA, TAA, TAG) within the *pheA* gene in Phe^+ revertants emerged in starving cell population of *P. putida*.

Target[a]	DNA change	Occurrences	Reversion amino acid change	
TGA	T → C	13 (54%)	CGA	Arg
	T → G	5 (21%)	GGA	Gly
	G → T	4 (17%)	TTA	Leu
	A → C	2 (8%)	TGC	Cys
TAA	T → C	38 (88%)	CAA	Gln
	T → G	5 (12%)	GAA	Glu
TAG	T → C	44 (61%)	CAG	Gln
	A → G	11 (15%	TGG	Trp
	G → T	8 (11%)	TAT	Tyr
	T → G	6 (8.5%)	GAG	Glu
	G → C	1 (1.5%)	TAC	Tyr
	A → T	1 (1.5%)	TTG	Leu
	T → A	1 (1.5%)	AAG	Glu

[a] In all cases the same codon, CTG for Leu 22, was altered in the wild type *pheA* sequence.

The spectrum of alterations leading to the appearance of Phe^+ mutants was most variable in case of the test system pKTpheA22TAG with seven different base substitutions represented. However, the dominant substitution in all three test systems appeared to be the T→C transition.

2.2. Pol IV is involved in generation of 1-bp deletion mutations in starving population of *Pseudomonas putida* (Ref. I)

Exhaustion of nutrients from the growth medium leads to the growth reduction of the bacterial population i. e. it enters the stationary phase. Although the number of viable cells in the culture remains constant, stationary phase population appears to be a highly dynamic system in which mutants possessing growth-advantage arise and multiply at the expense of the previous population (Zambrano *et al*., 1993). Moreover, genetic variability has been shown to increase under the growth-restricting conditions. Specialized DNA polymerases contribute to increased mutation frequency under stressful conditions. Involvement of specialized DNA polymerases in stationary phase mutagenesis has been demonstrated in aging *E. coli* colonies (Bjedov *et al*., 2003) as well as in appearance of mutants exhibiting growth advantage in liquid *E. coli* culture (Yeiser *et al*., 2002). Appearance of most Lac^+ reversions, generated due to 1-bp deletion mutations permitting utilization of lactose as a carbon source in *E. coli* starving population, was demonstrated to depend on a Y-family DNA polymerase IV (McKenzie *et al*., 2001). YqjH, a homologue of *E. coli* pol IV was shown to contribute to the emergence of prototrophic revertants within an auxotrophic population of *B. subtilis* under amino acid starvation conditions (Sung *et al.,* 2003).

In order to study the involvement of a specialized DNA polymerase pol IV in stationary phase mutagenesis in *P. putida*, generation of Phe^+ mutants in a population of pol IV-defective derivative of the wild-type strain PaW85 was monitored and compared with the parental strain, by using test systems described hereinbefore. Our results demonstrate that the occurrence of 1-bp deletions in *P. putida* cells starving for carbon source depends on the presence of functional pol IV since the frequency of appearance of Phe^+ revertants in *P. putida* carrying the tester plasmid pKTpheA56A was decreased by up to 10 times compared to the pol IV-proficient wild-type strain (Fig. 10A; Ref. I, Fig. 2A). Viability of both strains under starvation conditions throughout the period of evaluation of stationary phase mutagenesis was comparable (Fig. 10B; Ref. I, Fig. 2B). Intriguingly, the generation of pol IV-dependent frameshifts increased significantly as carbon source limitation had lasted longer than one week, thereby indicating that induction of pol IV-dependent mutagenesis occurs under the long-term environmental stress.

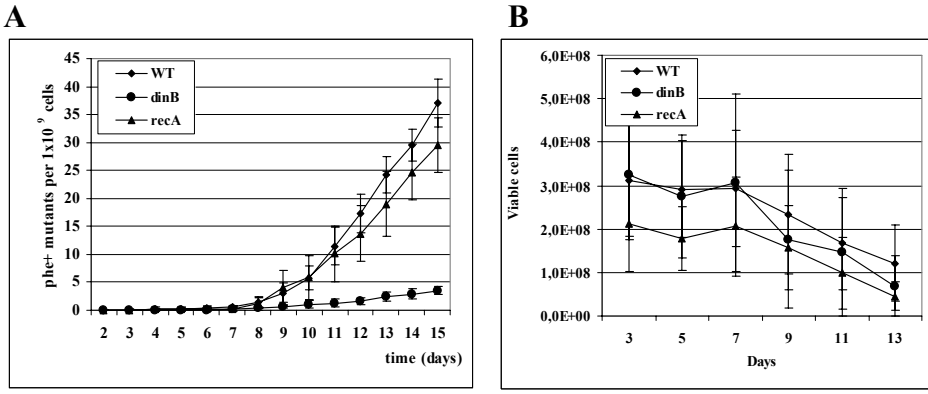


Fig. 10. A. Accumulation of Phe$^+$ mutants as a result of generation of 1-bp deletions in test system pKTpheA56+A throughout the period of two weeks of carbon source starvation in population of *P. putida* wild-type strain PaW85 (WT), its pol IV-deficient (dinB) and RecA-deficient (recA) derivatives. Data for at least five parallel experiments are presented. Means ± standard deviations for 10 plates calculated per 10^9 cells are shown. **B.** Viability of *P. putida* wild-type strain PaW85 (WT) and its pol IV-deficient (dinB) and RecA-deficient (recA) derivatives on phenol minimal plates. Means ± standard deviations for at least five cultures are shown. 1.0E+09 indicates 10^9 viable cells.

Chromosomal reversion systems employed in investigation of stationary phase mutagenesis in *B. subtilis* allow to estimate the occurrence of base substitution mutations. Involvement of a Pol IV homologue of *B. subtilis* YqjH in emergence of prototrophic revertants, shows the contribution of this particular DNA polymerase in generation of base substitutions in stationary phase cells (Sung *et al*., 2003). In contrast to that, no effect of pol IV was observed on generation of base substitution mutations in *P. putida* stationary phase cells: no difference between pol IV defective and wild-type strains was detected in frequency of appearance of Phe$^+$ mutants in populations of starving cells carrying the above-described test systems which measure base substitutions (data not shown).

Generation of pol IV-dependent base substitution mutations in *E. coli* overnight culture was shown under conditions of pol IV over-expression by monitoring the appearance of rifampicin (Rif)-resistant mutants and also by using a λ *c*II gene inactivation assay (Wagner and Nohmi, 2000). The acquisition of Rif-resistance is derived from alterations in amino acid sequence of the RNA polymerase *β*-subunit, which impede binding of rifampicin with the RNA polymerase. Mainly base substitution mutations occurring in the RNA polymerase *β*-subunit-encoding *rpoB* gene were responsible for the generation of Rif-resistance. Similarly to the results obtained by the others for *E. coli,* overproduction of pol IV in *P. putida* overnight grown cells increased the emergence of Rif-resistant mutants (Table 2; Ref. I, Table 2). Thus, the absence

of any effect of pol IV on generation of base substitutions under long-term starvation conditions in *P. putida* may be inflicted by differences in sequence context between the test systems used for the investigation of pol IV-dependent mutagenesis. Additionally, mutagenic processes involving the DNA synthesis by pol IV in actively dividing cells most likely differ from those occurring in stationary phase cells.

Table 2. The effect of *P. putida* pol IV overexpression on generation of Rif-resistant mutants in growing cells[a].

Strain	*dinB* overexpression	Control[b]
E. coli TG1	20.5	0.85
P. putida PaW85	1.70	0.30

[a] The frequency of mutation to Rif^R per 10^9 cells was calculated using the Lea-Coulson method of the median (Lea and Coulson, 1949; Rosche and Foster, 2000). The Mann-Whitney test (Sokal and Rohlf, 1981) for the effect of *dinB* overexpression gave significant *P* values: $P < 0.001$ for *P. putida*, and $P < 0.0001$ for *E. coli*.
[b] Bacteria carried the same plasmid except of lacking the *dinB* gene.

Several mechanisms have been shown to control mutagenic processes at different levels, starting from transcriptional repression of corresponding genes to posttranslational modifications, such as modulation of the functionality and access of the enzymes to DNA replication and repair maschinery. In addition to exposure to direct DNA damage-inflicting agents, other stresses (including nutrient limitation) also induce mutagenesis. For example, increase in mutation frequency in *E. coli* aging colonies is controlled by the SOS response and the catabolite repression system (Taddei *et al*., 1995; Bjedov *et al*., 2003). In case of one natural isolate, the dependence upon the alternative RNA polymerase sigma subunit RpoS was also observed (Bjedov *et al*., 2003). Additionally, the accumulation of prototrophic revertants in *B. subtilis* stationary phase population exhibited requirement for the activity of transcriptional regulators involved in starvation-induced development of spore formation and transformation competence (Sung and Yasbin, 2002).

The pol IV-encoding gene *dinB* belongs to SOS regulon in *E. coli*, being therefore subject to DNA damage-dependent transcription induction (Courcelle *et al*., 2001; Goodman, 2002). SOS response was shown to occur also in static bacterial populations without the presence of external DNA damaging agents (Taddei *et al.*, 1995). SOS response was also demonstrated to be required for the generation of pol IV-dependent stationary phase mutations (McKenzie *et al*., 2000, 2001).

The promoter region of *P. putida* pol IV-encoding *dinB* gene contains a sequence similar to *E. coli* LexA binding consensus CTG-N10-CAG (Walker, 1984; Wertman and Mount, 1985)(Fig. 11; Ref. I, Fig. 4B).

Fig. 11. Nucleotide sequence of *P. putida dinB* promoter region. Putative –35 and –10 hexamers of the promoter are boxed, and the 5' end of the *dinB* mRNA is indicated by an asterisk. The potential LexA binding site is underlined whereas LexA binding consensus nucleotides are in boldface and cursive. The translation start site of the *dinB* gene is indicated by an arrow.

This LexA binding consensus motif overlaps the –10 hexameric sequence of the *dinB* promoter by two nucleotides. Therefore we expected repression of this promoter by LexA and induction under different stressful conditions by DNA damage similarly to that observed in *E. coli* (Tang *et al*., 2000; Napolitano *et al*., 2000; McKenzie *et al*., 2000, 2001). However, despite the presence of putative LexA binding site within the *dinB* promoter region, addition of the DNA damage-inducing agent mitomycin C into the growth medium of bacteria had only a modest increasing effect (merely up to twofold) on the level of transcription from the *dinB* promoter (Fig. 12; Ref. I, Fig. 3).

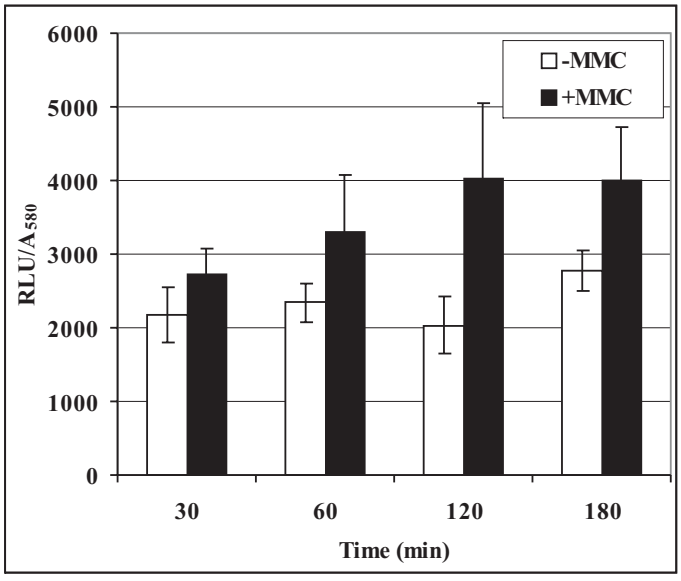


Fig. 12. The effect of DNA damage on transcription from the *dinB* promoter in *P. putida* PaW85. The promoter was cloned upstream of the luciferase encoding reporter genes *luxAB* into the pKT240-derived tester-plasmid. Transcription from the *dinB* promoter was assayed by measuring the luciferase activity (relative light units/optical density units at 580 nm) in cells grown in the presence or absence of a DNA-damaging agent mitomycin C (2 µg/ml).

At the same time, transcription from the *P. putida dinB* promoter had similar induction level in *E. coli* as the *E. coli dinB* promoter (data not shown). Latter indicates that there is a stronger repression of pol IV-encoding gene in *E. coli,* probably caused by a higher affinity of the *E. coli* LexA repressor to its binding site in the *P. putida dinB* promoter, compared to that of *P. putida* LexA. Hypothesis of lower binding affinity of *E. coli*-like SOS box by *P. putida* LexA is supported by similar results of DNA damage-inducibility of pol V-encoding *rulAB* genes on the plasmid pWW0 (Ref. III). Indeed, the uninduced level of transcription from the *dinB* promoter was higher in *P. putida* than in *E. coli*. It is possible, that the higher basal level of expression of pol IV in *P. putida* would possess beneficial value facilitating adaptation and survival in constantly changing environment, characteristic to natural habitats of soil bacteria (compared to relatively stable natural environment of an enterobacterium *E. coli*).

Besides functioning as a signal sensor and transducer molecule for SOS response, leading to stimulation of autoproteolysis of the LexA repressor, the involvement of RecA in mutational processes may also occur via recombinational repair of double-stranded DNA breaks (Ponder *et al*., 2005; He *et al*., 2006; also reviewed in Galhardo *et al.*, 2007). The generation of pol IV-dependent 1-bp deletions in F′ episome-based Lac reversion system was observed to depend on double-stranded DNA break formation exhibiting also a requirement for RecA, RecBCD and RuvABC proteins (Ponder *et al*., 2005; Cairns *et al*., 1991; Foster *et al*., 1996; Harris *et al*., 1994; Harris *et al*., 1996). Recombinational repair of double-stranded DNA breaks enables the generation of mutations during the DNA synthesis step.

In order to find out whether the occurrence of *dinB*-dependent stationary phase mutations is affected by the functionality of RecA in *P. putida*, generation of 1-bp deletions in a RecA defective strain was compared with respective frequency in the wild type. Our experiments showed that the rate of accumulation of –1 framshifts was comparable between the wild type *P. putida* and its RecA-deficient derivative (Fig. 10; Ref. I, Fig. 2). Hence, occurrence of pol IV-dependent mutations in *P. putida* stationary phase population observed in this study does not require the presence and functionality of RecA protein. Therefore, the requirement for SOS induction and also for recombinational double-strand break repair for the generation of pol IV-dependent 1-bp deletions within this particular system is excluded. Based on these results, one may hypothesize that the frequency of generation of pol IV-dependent 1-bp deletions in *P. putida* long-term-starved population may be increased as a result of pol IV-provided DNA repair synthesis initiated by DNA reparation pathway(s) other than recombinational repair. It is possible that the access of pol IV to DNA synthesis is controlled either by regulation of cellular amount of pol IV by some posttranscriptional/posttranslational mechanisms and/or by modification of the activity of this enzyme. Indeed, the regulation of cellular amount and activity of pol IV at several different levels has been described in

E. coli. For example, the pol IV-dependent mutagenesis in *E. coli* stationary phase cells has been shown to depend on a molecular chaperon GroE and a polyphosphate kinase Ppk. The effect of GroE on pol IV-dependent mutagenesis most probably lays in prevention of pol IV degradation, therefore increasing its cellular amount (Layton and Foster, 2005). Ppk responsible for synthesis of polyphosphate in stationary phase cells does not affect the cellular amount of pol IV but is required for its activity (Stumpf and Foster, 2005). pol II has been observed to exhibit a negative effect on the appearance of pol IV-dependent Lac^+ mutations in *E. coli* stationary phase population probably by competing with pol IV and limiting the mutational potential of pol IV in DSBR pathway (Foster, 2007). Additionally, UmuD and RecA were shown to suppress the –1 frameshift mutator activity of pol IV in *E. coli* by concerted binding with it, thus enclosing the relatively open active site of pol IV (Godoy *et al*., 2007).

2.3. Opposite effects of DNA polymerases DnaE2 and ImuB encoded by LexA2-regulated multiple gene cassette in stationary phase mutagenesis of *Pseudomonas putida* (Ref. II)

Several bacterial species lacking DNA polymerase V-encoding *umuDC* orthologs carry in their genomes a DNA damage-inducible multiple gene cassette. This multiple gene cassette encodes in most cases LexA-like repressor (LexA2), protein exhibiting some similarities to cell division inhibitor SulA and RecA/RadA recom enzyme similar to the catalytic subunit of replicative DNA polymerase (DnaE2) (Erill *et al*., 2006; Abella *et al*., 2004; Weinel *et al*., 2002). Transcription from the multiple gene cassette has shown to be regulated by its own-encoded LexA2 repressor which binds to cyanobacteria-like recognition sequence $GTACN_4GTGC$ different from the *E. coli*-like LexA binding consensus $CTGTN_8ACAG$ (Abella, *et al*., 2004). In a gamma proteobacterium *P. aeruginosa*, a beta proteobacterium *Ralstonia solanacearum* and many alpha proteobacteria species a three-gene cassette derivative of particular multiple gene cassette lacking a second LexA-encoding gene has been described (Erill *et al*., 2006; Abella *et al*., 2004). Phylogenetic analysis has revealed that the origin of particular gene cassette dates back to actinobacteria and has been dispersed across the bacterial domain through duplications, vertical inheritance, and lateral gene transfer (Erill *et al*., 2006). The absence of a second copy of LexA-encoding gene within the given operon in some bacterial species does not exclude the DNA damage-inducibility of the particular multiple gene cluster, whereas in these organisms adoption of their primal LexA-binding site in the promoter region of the first gene of the operon has been occurred (Abella *et al*., 2004). For example, the three-gene cassette *imuA-imuB-dnaE2* of *Caulobacter crescentus* was shown to be DNA damage-inducible and negatively regulated

by *C. crescentus* LexA protein which is similar to *E. coli* LexA. In addition, the *imuA-imuB-dnaE2* cassette was responsible for most UV-induced mutations in *C. crescentus* (Galhardo *et al*., 2005). The involvement of DnaE2 has been observed in DNA damage-induced mutagenesis in *Mycobacterium tuberculosis* as well (Boshoff *et al*., 2003). Furthermore, the contribution of DnaE2 has been observed also in development of *M. tuberculosis* drug resistant mutants (Boshoff *et al*., 2003). There are also data on dependence of UV-induced mutagenesis upon a DnaE2 homologue in *Pseudomonas aeruginosa* (Sanders *et al.*, 2006). The presence of a DNA damage-inducible gene cassette in genomes of bacterial species which does not carry pol V-encoding umuDC orthologs indicates at least partially overlapping functional replacement of pol V by specialized DNA polymerases of multiple gene cassette at translesion DNA synthesis in these species. Involvement of particular specialized DNA polymerases in error-prone translesion synthesis together with DNA damage-inducibility of the multiple gene cassette has given a reason for its designation as a "mutagenesis cassette".

Multiple gene cassette identified in *P. putida* genome encodes LexA-like repressor (*lexA2*), SulA (*sulA2/imuA*), Y-family DNA-polymerase similar to pol IV (*dinP/imuB*), and a protein similar to the alpha subunit of replicative DNA polymerase (*dnaE2*). Given operon has shown to be DNA damage-inducible and self-regulated by LexA2 (Abella *et al*., 2004). DNA damage-inducible DNA polymerases contribute to adaptive mutagenesis under stressful conditions including nutritional limitation (McKenzie *et al*., 2001; Bjedov *et al*., 2003; Yeiser *et al*., 2002). This raised the question about the role of this multiple gene cluster of DNA polymerases in mutagenic processes under long-term starvation of *P. putida*.

In order to investigate the involvement of ImuB and DnaE2 in stationary phase mutagenesis, the appearance of Phe^+ revertants in ImuB- and DnaE2 defective *P. putida* strains was monitored. To study the mechanisms of occurrence of 1-bp deletions and base substitution mutations separately, the assay systems pKTpheA56+A and pKTpheA22TAG (described in Ref. I) were used, . Surprisingly, the absence of functional DnaE2 enhanced the appearance of base substitution mutations (up to threefold) in a starving *P. putida* population (Fig. 13A; Ref. II, Fig. 1A). Hence, in contrary to our expectation, DnaE2 does not elevate mutation frequency but rather acts as an antimutator in stationary-phase *P. putida*. This is in strict contrast to functions of DnaE2 homologues in other organisms studied so far that possess under any circumstances an error-prone nature. Similar effect of DnaE2 deficiency was observed also in a growing *P. putida* culture. Additionally, negative effect of DnaE2 on the appearance of base substitutions in *P. putida* emerged also in the studies of UV-induced mutagenesis. DnaE2 deficiency resulted in about twofold increase in the frequency of appearance of Rif-resistant mutants compared to that of the parental strain after the exposure of bacteria to UV-radiation (Fig. 14; Ref. II, Fig. 3). Hence, the anti-mutator effect of DnaE2 in

12

generation of base substitution mutations is not restricted to a particular plasmidial test system employed but appears to be more general. These results indicate that a reducing effect of *P. putida* DnaE2 on mutagenesis may lie in accurate DNA synthesis across certain DNA lesions including UV-induced DNA damage like cyclobutane pyrimidine dimers and pyrimidine-pyrimidone (6–4) photoproducts. DnaE2 may also impede the access of error-prone DNA polymerases to DNA synthesis on undamaged template during genome duplication or DNA repair synthesis.

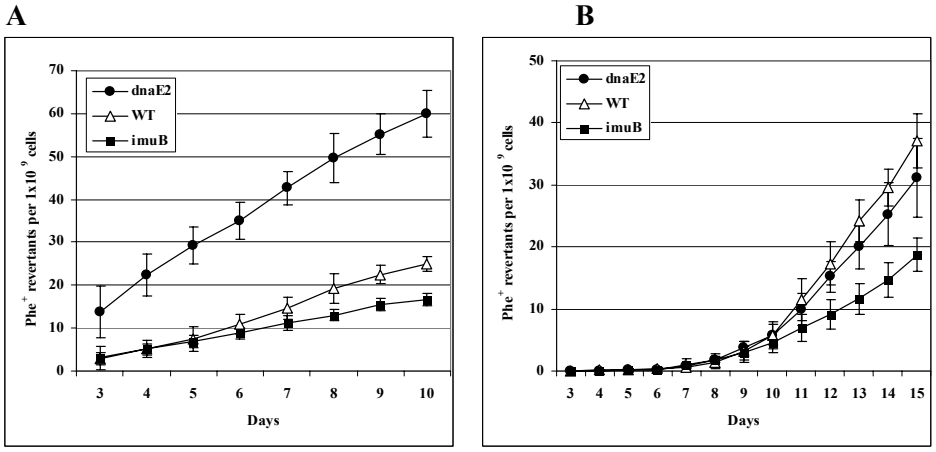


Fig. 13. The accumulation of Phe$^+$ revertants on phenol minimal plates in *P. putida* wild-type strain PaW85 (WT), and its DnaE2-deficient (dnaE2) and ImuB-deficient (imuB) derivatives. The effect of DnaE2 and ImuB deficiency on generation of base substitution mutations (**A**) and 1-bp deletions (**B**). Data for at least five parallel experiments are presented. In all cases, means ± standard deviations for at least 10 plates calculated per $1x10^9$ cells are shown.

The frequency of appearance of 1-bp deletions in a DnaE2-defective *P. putida* strain was comparable to that in the parental strain (Fig. 13B; Ref. II, Fig. 1B). Therefore, DnaE2 seems not to be involved either in generation of 1-bp frameshifts or prevention of the occurrence of this type of mutations in a long-term starved *P. putida* population.

At the same time, the deficiency of ImuB resulted in up to twofold lower frequency of generation of both base substitution mutations and 1-bp deletions in *P. putida* stationary phase population (Fig. 13; Ref. II, Fig. 1). In accordance with the results of stationary phase mutagenesis, the absence of functional ImuB reduced the UV-induced mutation frequency up to twofold (Fig. 14; Ref. II, Fig. 3). Hence, ImuB contributes to generation of different type of mutations and several distinct mutagenic processes in *P. putida*. Predisposition of mutagenesis by ImuB in *P. putida* correlates with the effects ascribed to an ImuB homologue

in *C. crescentus* in which contribution to UV-mutagenesis was observed (Galhardo *et al*., 2005). Thus, the homologues of DNA polymerases encoded by a DNA damage-inducible multiple gene cassette in *P. putida* possess opposite effects on mutagenesis.

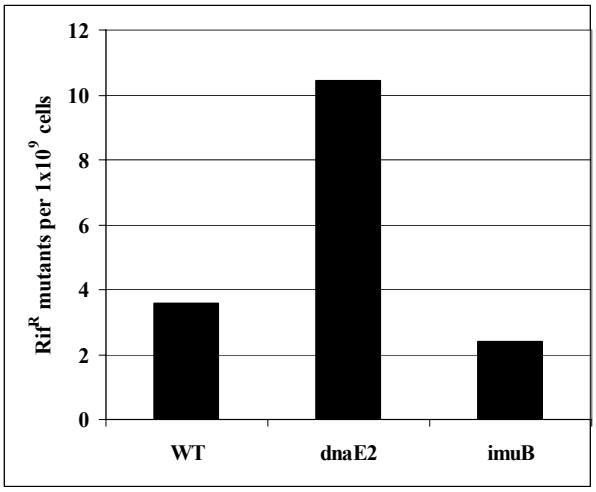


Fig. 14. The effect of DnaE2 and ImuB deficiency on UV-induced mutagenesis. The median value for frequency of Rif-resistant mutants per 10^9 UV-C-irradiated cells of *P. putida* wld-type strain PaW85 (WT), and its DnaE2-deficient (dnaE2) and ImuB-deficient (imuB) derivatives is shown. Differences between the frequencies of occurrence of Rif-resistant mutations in DnaE2-deficient and ImuB-deficient strains relative to wild-type strain were statistically significant (P-values less than 0.05) at the 95% confidence level based on the Mann-Whitney test (Sokal *et al*., 1981).

However, the exact role of ImuB in mutagenic processes became an intriguing question after its amino acid sequence analysis and protein structure prediction. Based on predicted structure of ImuB, the functioning of this protein as a DNA polymerase is very unlikely. The active site including the palm domain of Y-family DNA polymerases contains three conserved amino acid residues required for catalytic activity of all Y-family DNA polymerases studied so far. Crystal structure analysis of Y-family polymerase Dpo4 revealed the location of two aspartic acid residues at the positions 7 (D7) and 105 (D105), and a glutamic acid residue at the position 106 (E106) on adjacent β-strands 1 and 6 (Ling *et al*., 2001).

Prediction of the protein structure by Modbase database generated a highly scored model for *P. putida* ImuB using the structure of Y-family polymerase Dpo4 as a template (Fig. 15; Ref. II, Supplementary data S3).

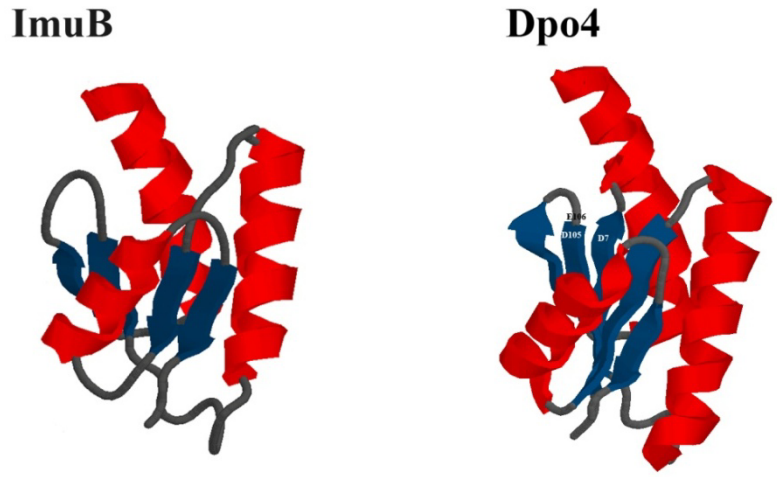


Fig. 15. The ImuB structure prediction. A ribbon diagram comparison of *P. putida* ImuB (amino acids 78–163) with *S. solfataricus* DNA polymerase IV Dpo4 (amino acids 1–10 and 78–166). ImuB structure was modeled in ModBase database (Pieper *et al*., 2004) using Dpo4 (PDB code 1jx4A) as a template (Ling *et al*., 2001). The β-strands forming a palm domain are indicated in blue. The conserved residues D7, D105 and E106 forming the catalytic center are indicated on Dpo4 structure. No amino acid identity exists between the ImuB and Dpo4 within first and 19th residues. Therefore the ImuB model was built without N-terminal residues. One of conserved residues of the catalytic center, D7, is located on β-strand 1, a region absent from the ImuB model. The predicted amino acid sequence of ImuB did not reveal any D residues at the N-terminus of this protein. Moreover, the other two conserved catalytic residues locating in the β-strand 6 of Dpo4 are also absent from ImuB.

As no amino acid identity was found within the first nineteen residues of *P. putida* ImuB and Dpo4, the β-strand 1 was excluded from the structure model of the ImuB protein. However, the predicted amino acid sequence of ImuB did not reveal the presence of any aspartic acid residues in N-terminus of the protein. Moreover, the other two conserved carboxylates locating on β-strands 6 in Dpo4 are also absent from ImuB. DNA polymerase activity of eukaryotic Y-family DNA polymerase Rev1 is restricted to the ability to incorporate one or two molecules of dCMP irrespectively of the template nucleotide, being therefore often referred to as a dCMP transferase. Catalytic activity of Rev1 is required for the replicative bypass of AP-sites but the requirement for Rev1 in UV-induced mutagenesis does not depend on its catalytic activity (Friedberg *et al*., 2005). At the same time, in addition to binding with PCNA, a functional homolog of the bacterial *β*-clamp, the interaction between Rev1 and the other Y-family DNA polymerases has been observed (Guo *et al*., 2003; Tissier *et al*., 2004; Ohashi *et al*., 2004). Therefore it has been proposed that the role of Rev1 in translesion DNA synthesis lies in serving as a scaffold protein at blocked

replication forks coordinating the polymerase switching between the replicative machinery and insertion polymerases, or between an insertion polymerase and an extension polymerase (Friedberg *et al*., 2005; also reviewed in Lehmann, 2006). The observed effect of ImuB on mutagenesis in *P. putida* in conjunction with the absence of catalytically essential residues in its active site indicates that ImuB may participate in polymerase traffic presumably by recruitment of specialized DNA polymerases, enabling their access to DNA synthesis.

2.4. Plasmid-encoded pol V homologue contributes to growth advantage in stationary phase population of *Pseudomonas putida* (Ref. III)

Harboring plasmids could have a beneficial value for bacteria in several different ways, depending on transient environmental conditions the bacterial population passing through. In their natural environment, bacteria are faced with various stresses, including nutritional limitation common for bacteria occupying water and soil habitats, but also temperature shifts, dehydration, and exposition to UV radiation most often experienced by bacteria colonizing the phyllosphere. Large low-copy-number plasmids carrying genes for catabolic pathways for the degradation of aromatic compounds permit environmental bacteria to extend their nutritional diversity. Many plasmids possess the ability to increase not only the survival but also the mutation frequency of their hosts exposed to UV-radiation due to the presence of genes exhibiting homology to *E. coli umuDC* genes encoding a specialized DNA polymerase pol V. However, the occasional undergo through growth inhibiting conditions due to exhaustion of available energy source resulting in entrance of bacterial populations to the stationary phase in their natural habitats is indispensable. Within starving bacterial populations, there is a strong selective pressure for acquisition of any mutation with selective advantage. Populations of stationary phase cells are highly dynamic while the fitter mutants with growth advantage (GASP mutants) emerge and constantly out-compete the previous population (Zambrano *et al*., 1993; Smeulders *et al*., 1999; Finkel and Kolter, 1999). Specialized DNA polymerases contribute to the appearance of GASP mutants probably by enhancing generation of mutations the selection may act on (Yeiser *et al*., 2002).

The toluene degradation plasmid pWW0 of *P. putida* strain mt-2 (Williams and Murray, 1974) carries the DNA polymerase V (pol V) homologue-encoding genes that were initially designated as *ruvAB* in the pWW0 sequence annotation (Greated *et al*., 2002), but were renamed by us according to their function as *rulAB*. pWW0 confers resistance to reactive oxygen species (Yano *et al*., 1981). The presence of *rulAB* in composition of pWW0 led us to speculate that *rulAB* encoded pol V may contribute to pWW0-provided resistance to reactive oxygen

13

species. The accumulation of oxidative DNA damage has been proposed to be a major factor enabling generation of stationary phase mutations in bacteria (Bridges, 1998). Based on these data we supposed that besides enabling catabolism of aromatic compounds, pWW0 may enhance the probability of plasmid-harboring cells to acquire beneficial mutations in during starvation.

The evolvement of growth advantage in stationary phase conditions is detectable in competition experiments, since cells that acquire advantageous mutations will increase in their number relative to the rest of the population as the culture ages. In order to distinguish the cells from the aged culture from the younger ones, *P. putida* strains used in this study were labeled with different antibiotic resistance markers (resistance to kanamycin or tetracycline) by inserting the antibiotic resistance genes into the same chromosomal location (for details, see Ref. III Materials and Methods). Cells from an 8-day-old culture were inoculated as a numerical minority into a 1-day-old culture. The relative proportion of both subpopulations was determined during the next 30 days by plating cells onto selective media containing appropriate antibiotics.

The competition between aged and young subpopulations within following pairs was monitored: the wild type *P. putida versus* itself, a *rulAB* containing *P. putida versus* itself, and a *rulAB* containing *P. putida versus* the wild type. Representative examples of GASP competition profiles observed during co-cultivation of different subpopulations are shown in Fig. 16 (Ref. III, Fig. 3). Results of the studies on the expression of GASP phenotype in mixed populations are summarized in Table 3 (Ref. III, Table 2).

In all cases studied, the titer of aged cell-population increased in the beginning of the experiment, becoming equal to the number of cells of the younger subpopulation. Thereafter, the patterns of GASP takeovers diverged, expressing a strong dependence upon the presence or absence of *rulAB* genes in bacteria. The co-cultivation of aged and young subpopulations of the wild type strain revealed mild expression of the GASP phenotype i. e. a relative proportion of cells derived from aged cultures became increased about 5 to 10 times, with a concomitant decrease of the younger subpopulation. Aged *P. putida* strain containing *rulAB* genes expressed mostly (in seven out of nine competition experiments) strong GASP phenotype, completely out-competing the young wild type strain, and in only few cases (in two experiments out of nine) a milder expression of GASP phenotype was observed. Moreover, young *rulAB* genes-containing cells were also able to completely or partially out-compete the aged wild type subpopulations whereas in only two cases both subpopulations exhibited equal fitness. The competition between aged and young subpopulations of *rulAB*-carrying *P. putida* strain expressed a stronger GASP phenotype compared with the population dynamics of cocultivated aged and young wild type subpopulations. Hence, we can conclude that *rulAB* genes from the TOL plasmid pWW0 increase the probability of *P. putida* cells to accumulate beneficial mutations providing growth advantage in a stationary phase population.

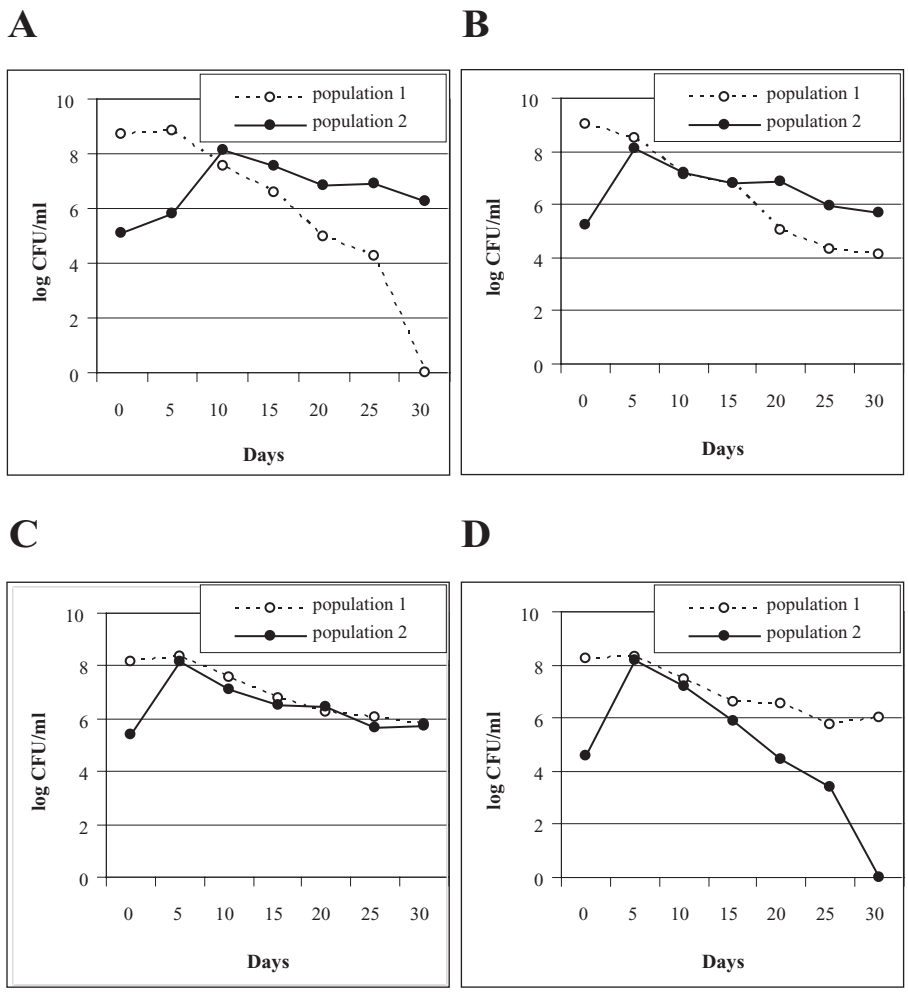


Fig. 16. Representative examples of GASP competition profiles which appeared during co-cultivation of aged (population 2) and nonaged (population 1) sub-populations of *P. putida*. Aged subpopulation consists of cells derived from an 8-days-old culture whereas cells of the nonaged subpopulation are derived from 1-day-old culture. Population 2 was able to out-compete population 1 either completely (strong GASP phenotype, **panel A**) or partially (mild GASP phenotype, **panel B**), or displayed fitness equal to that of population 1 (equal GASP phenotype, **panel C**). In some cases, population 2 was either partially or completely out-competed by population 1 (no GASP phenotype, **panel D**).

Table 3. The effect of the presence of pWW0-derived *rulAB* genes on expression of GASP phenotype in *P. putida*.

Competing sub-populations[a]	Growth advantage in stationary phase[b]							
	Cells from 8-day-old culture				Cells from 1-day-old culture			
	Strong	Mild	Equal	None	Strong	Mild	Equal	None
WT (8) + WT (1)	0	8	1	0	0	0	1	8
rulAB (8) + WT (1)	7	2	0	0	0	0	0	9
WT (8) + rulAB (1)	0	0	2	7	5	2	2	0
rulAB (8) + rulAB (1)	2	5	0	2	2	0	0	7

[a] Numbers 8 and 1 in parentheses indicate how many days sub-populations were grown separately in LB medium before starting the competition experiments with mixed populations. Cells from 8-day-old cultures were mixed with 1-day-old cultures at ratio 1:1000 vol/vol.

[b] Number of cases when particular sub-population expressed GASP phenotype in 9 parallel competition experiments if cells derived from 8-day-old and 1-day-old cultures co-evolved for 30 days in stationary-phase cultures. Expression of GASP phenotype in 9 parallel competition experiments is shown separately for both cells either derived from aged (8-day-old) or non-aged (1-day-old) cultures. Representative examples of expression of strong, mild or equal GASP phenotype or lack of expression of GASP phenotype are illustrated in Fig. 16

In all cases studied, the titer of aged cell-population increased in the beginning of the experiment, becoming equal to the number of cells of the younger subpopulation. Thereafter, the patterns of GASP takeovers diverged, expressing a strong dependence upon the presence or absence of *rulAB* genes in bacteria. The co-cultivation of aged and young subpopulations of the wild type strain revealed mild expression of the GASP phenotype i. e. a relative proportion of cells derived from aged cultures became increased about 5 to 10 times, with a concomitant decrease of the younger subpopulation. Aged *P. putida* strain containing *rulAB* genes expressed mostly (in seven out of nine competition experiments) strong GASP phenotype, completely out-competing the young wild type strain, and in only few cases (in two experiments out of nine) a milder expression of GASP phenotype was observed. Moreover, young *rulAB* genes-containing cells were also able to completely or partially out-compete the aged wild type subpopulations whereas in only two cases both subpopulations exhibited equal fitness. The competition between aged and young subpopulations of *rulAB*-carrying *P. putida* strain expressed a stronger GASP phenotype compared with the population dynamics of cocultivated aged and young wild type subpopulations. Hence, we can conclude that *rulAB* genes from the TOL plasmid pWW0 increase the probability of *P. putida* cells to accumulate beneficial mutations providing growth advantage in a stationary phase population.

CONCLUSION

Bacteria constitute ecologically most diverse and widespread domain of life inhabiting every environment one can imagine and exhibiting extremely efficient physiological and genetic adaptability. Generation of mutations is an important source of biological diversity and evolution. Several lines of evidence indicate that there is positive correlation between stressful growth-restrictive environmental conditions and genetic variability within bacterial populations. Among different processes, error-prone DNA synthesis by specialized DNA polymerases has been shown to contribute to stress-induced mutagenesis in stationary-phase bacteria. Thus, literature review of this thesis is focused on characterization of specialized DNA polymerases, their biological functions and involvement in stress-induced mutagenesis.

Broad investigation of mutagenic processes using *E. coli* as a model bacterium has provided large quantities of information of medical as well as scientific value. However, cellular mechanisms of different bacteria are highly diverse. So, dissimilarities between different bacterial phyla have become evident even at the level of the mechanisms of basic cellular processes such as chromosomal replication. Therefore, one cannot make conclusions about mutagenic processes in bacteria considering only data on *Escherichia coli*. Recently, more attention has been payed to mutagenic processes in other bacterial species such as *Bacillus subtilis*, *Mycobacterium tuberculosis*, *Caulobacter crescentus* and *Pseudomonas aeruginosa*.

In my thesis I have described the involvement of specialized DNA polymerases in mutagenic processes in *Pseudomonas putida*. *P. putida* colonizes diverse soil and water habitats, being therefore continuously exposed to various environmental stresses.

Long periods of nutrient limitation are the most common stress factor environmental bacteria have to face with. Investigation of mutagenic processes in different bacterial species under nutrient limitation conditions is limited by the availability of assay systems. We have constructed a set of novel test systems that allowing monitoring of 1-bp deletions and different base substitution mutations in *P. putida* separately throughout long-term period (at least 2 weeks) of carbon source starvation. From the experimental part of my work that is focused on the the role of *P. putida* specialized DNA polymerases in mutagenesis under carbon source starvation , the following conclusions can be drawn:

1. Occurrence of 1-bp deletions in late stationary-phase cells of *P. putida* depends on the presence of a Y-family DNA polymerase IV. Thereat, the generation of pol IV-dependent frameshifts is significantly elevated as carbon source limitation proceeds more than one week which indicates that pol IV-dependent mutagenesis is induced under conditions of long-term environmental stress.

2. The DNA polymerase DnaE2, a homologue of α-subunit of replicative DNA polymerase III and an Y-family DNA polymerase ImuB of *P. putida* that is encoded by DNA damage-inducible multiple gene cassette, have opposite effects on mutagenesis. Similarly other bacteria carrying this particular gene cassette, ImuB contributes to the generation of both base substitution mutations and 1-bp deletions in *P. putida* stationary phase population. The *P. putida* DnaE2, on the contrary, has a reducing effect on the occurrence of base substitutions which is in strict contrast with previously studied DnaE2 homologues of other organisms.
   The sequence analysis and protein structure prediction of ImuB revealed that functioning of this particular protein as a DNA polymerase is very unlikely due to the absence of conserved essential residues of the catalytic center. Observed effect of ImuB on mutagenesis in *P. putida* in conjunction with the absence of essential catalytic residues in its active site indicates that ImuB may participate in polymerase traffic presumably by recruitment of specialized DNA polymerases enabling their access to the DNA synthesis.
3. A DNA polymerase V homologue encoded by the *rulAB* genes of the toluene degradation plasmid pWW0 increases the probability of *P. putida* cells to accumulate beneficial mutations permitting to gain the growth advantage in stationary phase population.

REFERENCES

Abella, M., Campoy, S., Erill, I., Rojo, F. & Barbé, J. (2007). Cohabitation of two different *lexA* regulons in *Pseudomonas putida*. *J Bacteriol* 189, 8855–8862.

Abella, M., I. Erill, M. Jara, G. Mazón, S. Campoy, and J. Barbé. (2004). Widespread distribution of a *lexA*-regulated DNA damage-inducible multiple gene cassette in the Proteobacteria phylum. *Mol Microbiol* 54, 212–222.

Al Mamun, A. A. & Humayun, M. Z. (2006). *Escherichia coli* DNA polymerase II can efficiently bypass 3,N(4)-ethenocytosine lesions *in vitro* and *in vivo*. *Mutat Res* 593, 164–176.

Bjedov, I., Dasgupta, C. N., Slade, D., Le Blastier, S., Selva, M. & Matic, I. (2007). Involvement of *Escherichia coli* DNA polymerase IV in tolerance of cytotoxic alkylating DNA lesions *in vivo*. *Genetics* 176, 1431–1440.

Bjedov, I., Tenaillon, O., Gérard, B., Souza, V., Denamur, E., Radman, M., Taddei, F. & Matic, I. (2003). *Science* 300, 1404–1409.

Bjelland, S & Seeberg, E. (2003). Mutagenicity, toxicity and repair of DNA base damage induced by oxidation. *Mutat Res* 531, 37–80.

Blázquez, J., Gómez-Gómez, J. M., Oliver, A., Juan, C., Kapur, V. & Martín, S. (2006). PBP3 inhibition elicits adaptive responses in *Pseudomonas aeruginosa*. *Mol Microbiol* 62, 84–99.

Boles, B. R. & Singh, P. K. (2008). Endogenous oxidative stress produces diversity and adaptability in biofilm communities. *Proc Natl Acad Sci U S A* 105, 12503–12508.

Boshoff, H. I., Reed, M. B., Barry, C. E. 3rd. & Mizrahi, V. (2003). DnaE2 polymerase contributes to *in vivo* survival and emergence of drug resistance in *Mycobacterium tuberculosis*. *Cell* 113, 183–193.

Boudsocq, F., Iwai, S., Hanaoka, F. & Woodgate, R. (2001). *Sulfolobus solfataricus* P2 DNA polymerase IV (Dpo4): an archaeal DinB-like DN A polymerase with lesion-bypass properties akin to eukaryotic poleta. *Nucleic Acids Res* 29, 4607–4616.

Brazas, M. D. & Hancock, R. E. (2005). Ciprofloxacin induction of a susceptibility determinant in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 49, 3222–3227.

Bridges, B. A. (1998). The role of DNA damage in stationary phase ("adaptive") mutation. *Mutat Res* 408, 1–9.

Brieba, L. G., Eichman, B. F., Kokoska, R. J., Doublié, S., Kunkel, T. A. & Ellenberger, T. (2004). Structural basis for the dual coding potential of 8-oxoguanosine by a high-fidelity DNA polymerase. *EMBO* 23, 3452–3461.

Bruck, I., Goodman, M. F. & O'Donnell, M. (2003). The essential C family DnaE polymerase is error-prone and efficient at lesion bypass. *J Biol Chem* 278, 44361–44368.

Burney, S., Caulfield, J. L., Niles, J. C., Wishnok, J. S. & Tannenbaum, S. R. (1999). The chemistry of DNA damage from nitric oxide and peroxynitrite. *Mutat Res* 424, 37–49.

Burney, S., Niles, J. C., Dedon, P. C. & Tannenbaum, S. R. (1999). DNA damage in deoxynucleosides and oligonucleotides treated with peroxynitrite. *Chem Res Toxicol* 12, 513–520.

Cairns, J. & Foster, P. L. (1991). Adaptive reversion of a frameshift mutation in *Escherichia coli*. *Genetics* 128, 695–701.

Chung, F. L., Nath, R. G., Nagao, M., Nishikawa, A., Zhou, G. D. & Randerath, K. (1999). Endogenous formation and significance of 1,N2-propanodeoxyguanosine adducts. *Mutat Res* 424, 71–81.

Ciofu, O., Fussing, V., Bagge, N., Koch, C. & Høibi, N. (2001). Characterization of paired mucoid/non-mucoid *Pseudomonas aeruginosa* isolates from Danish cystic fibrosis patients: antibiotic resistance, beta-lactamase activity and RiboPrinting. *J Antimicrob Chemother* 48, 391– 396.

Cirz, R. T. & Romesberg, F. E. (2006). Induction and inhibition of ciprofloxacin resistance-conferring mutations in hypermutator bacteria. *Antimicrob Agents Chemother* 50, 220–225.

Cirz, R. T., Chin, J. K., Andes, D. R., de Crécy-Lagard, V., Craig, W. A. & Romesberg, F. E. (2005). Inhibition of mutation and combating the evolution of antibiotic resistance. *PloS Biol* 3, e176 (1024–1033).

Cirz, R. T., Jones, M. B., Gingles, N. A., Minogue, T. D., Jarrahi, B., Peterson, S. N. & Romesberg, F. E. (2007). Complete and SOS-mediated response of *Staphylococcus aureus* to the antibiotic ciprofloxacin. *J Bacteriol* 189, 531–539.

Cirz, R. T., O'Neill, B. M., Hammond, J. A., Head, S. R. & Romesberg, F. E. (2006). Defining the *Pseudomonas aeruginosa* SOS response and its role in the global response to the antibiotic ciprofloxacin. *J Bacteriol* 188, 7101–7110.

Courcelle, J., Khodursky, A., Peter, B., Brown, P. O. & Hanawalt, P. C. (2001). Comparative gene expression profiles following UV exposure in wild-type and SOS-deficient *Escherichia coli*. *Genetics* 158, 41–64.

de Kok, T. M. & van Maanen, J. M. (2000). Evaluation of fecal mutagenicity and colorectal cancer risk. *Mutat Res* 463, 53–101.

Dervyn, E., Suski, C., Daniel, R., Bruand, C., Chapuis, J., Errington, J., Jannière, L. & Ehrlich, S. D. (2001). Two essential DNA polymerases at the bacterial replication fork. *Science* 294, 1716–1719.

Dervyn, E., Suski, C., Daniel, R., Bruand, C., Chapuis, J., Errington, J., Jannière, L. & Ehrlich, S.D. (2001). Two essential DNA polymerases at the bacterial replication fork. *Science* 294, 1716–1767.

Duigou, S., Ehrlich, S. D., Noirot, P. & Noirot-Gros, M. F. (2004). Distinctive genetic features exhibited by the Y-family DNA polymerases in *Bacillus subtilis*. *Mol Microbiol* 54, 439–451.

Duigou, S., Ehrlich, S. D., Noirot, P. & Noirot-Gros, M. F. (2005). DNA polymerase I acts in translesion synthesis mediated by the Y-polymerases in *Bacillus subtilis*. *Mol Microbiol* 57, 678–690.

Erill, I., Campoy, S., Mazon, G. & Barbé, J. (2006). Dispersal and regulation of an adaptive mutagenesis cassette in the bacteria domain. *Nucleic Acids Res* 34, 66–77.

Fiala, K. A. & Suo, Z. (2007). Sloppy bypass of an abasic lesion catalyzed by a Y-family DNA polymerase. *J Biol Chem* 282, 8199–8206.

Finkel, S. E. & Kolter, R. (1999). Evolution of microbial diversity during prolonged starvation. *Proc Natl Acad Sci U S A* 96, 4023–4027.

Finkel, S. E. (2006). Long-term survival during stationary phase: evolution and the GASP phenotype. *Nat Rev Microbiol* 4, 113–120.

Fleck, O. & Schär, P. (2004). Translesion DNA synthesis: little fingers teach tolerance. *Curr Biol* 14, 389–391.

Foster, P. L. (2007). Stress-induced mutagenesis in bacteria. *Crit Rev Biochem Mol Biol* 42, 373–397.

Foster, P. L., Trimarchi, J. M. & Maurer, R. A. (1996). Two enzymes, both of which process recombination intermediates, have opposite effects on adaptive mutation in *Escherichia coli*. *Genetics* 142, 25–37.

Friedberg, E. C., Lehmann, A. R. & Fuchs, R. P. (2005). Trading places: how do DNA polymerases switch during translesion DNA synthesis? *Mol Cell* 18, 499–505.

Friedberg, E. C., Walker, G. C., Siede, W., Wood, R. D., Schultz, R. A. & Ellenberger, T. (2006) DNA Repair and Mutagenesis. Second edition, ASM Press, Washington, D.C.

Furukohri, A., Goodman, M. F. & Maki, H. (2008). A dynamic polymerase exchange with *Escherichia coli* DNA polymerase IV replacing DNA polymerase III on the sliding clamp. *J Biol Chem* 283(17), 11260–11269.

Galhardo, R. S., Hastings, P. J. & Rosenberg, S. M. (2007). Mutation as a stress response and the regulation of evolvability. *Crit Rev Biochem Mol Biol* 42, 399–435.

Galhardo, R. S., Rocha, R. P., Marques, M. V. & Menck, C. F. (2005). An SOS-regulated operon involved in damage-inducible mutagensis in *Caulobacter crescentus*. *Nucleic Acids Res* 33, 2603–2614.

Garibyan, L., Huang, T., Kim, M., Wolff, E., Nguyen, A., Nguyen, T., Diep, A., Hu, K., Iverson, A., Yang, H. & Miller, J. H. (2003). Use of the *rpoB* gene to determine the specificity of the base substitution mutations on the *Escherichia coli* chromosome. *DNA Repair* 2, 593–608.

Godoy, V. G., Jarosz, D. F., Simon, S. M., Abyzov, A., Ilyin, V. & Walker, G. C. (2007). UmuD and RecA directly modulate the mutagenic potential of the Y family DNA polymerase DinB. *Mol Cell* 28, 1058–1070.

Goodman, M. F. (2002). Error-prone repair DNA polymerases in prokaryotes and eukaryotes. *Annu Rev Biochem* 71, 17–50.

Greated, A., Lambertsen, L., Williams, P. A. & Thomas, C. M. (2002). Complete sequence of the IncP-9 TOL plasmid pWW0 from *Pseudomonas putida*. *Environ Microbiol* 4, 856–871.

Guo, C., Fischhaber, P. L., Luk-Paszyc, M. J., Masuda, Y., Zhou, J., Kamiya, K., Kisker, C. Friedberg, E. C. (2003). Mouse Rev1 protein interacts with multiple DNA polymerases involved in translesion DNA synthesis. *EMBO J* 22, 6621–6630.

Hanaoka, F. (2001). DNA replication. SOS polymerases. *Nature* 409, 33–34.

Harris, R. S., Feng, G., Ross, K. J., Sidhu, R., Thulin, C., Longerich, S., Szigety, S. K., Winkler, M. E. & Rosenberg, S. M. (1997). Mismatch repair protein MutL becomes limiting during stationary-phase mutation. *Genes Dev* 11, 2426–2437.

Harris, R. S., Feng, G., Ross, K. J., Sidhu, R., Thulin, C., Longerich, S., Szigety, S. K., Hastings, P. J., Winkler, M. E. & Rosenberg, S. M. (1999). Mismatch repair is diminished during stationary-phase mutation. *Mutat Res* 437, 51–60.

Harris, R. S., Longerich, S. & Rosenberg, S. M. (1994). Recombination in adaptive mutageneis. *Science* 264, 258–260.

Harris, R. S., Ross, K. J. & Rosenberg, S. M. (1996). Opposing roles of the holliday junction processing systems of *Escherichia coli* in recombination-dependent adaptive mutation. *Genetics* 142, 681–691.

He, A. S., Rohatgi, P. R., Hersh, M. N. & Rosenberg, S. M. (2006). Roles of *E. coli* double-strand-break-repair proteins in stress-induced mutation. *DNA Repair (Amst)* 5, 258–273.

Heller, R. C. & Marians, K. J. (2006). Replication fork reactivation downstream of a blocked nascent leading strand. *Nature* 439(7076), 557–562.

Hogardt, M., Hoboth, C., Schmoldt, S., Henke, C., Bader, L. & Heesemann, J. (2007). Stage-specific adaptation of hypermutable *Pseudomonas aeruginosa* isolates during chronic pulmonary infections in patients with cystic fibrosis. *J Infect Dis* 195, 70–80.

Hogg, M., Wallace, S. S. & Doublié, S. (2005). Bumps in the road: How replicative DNA polymerases see DNA damage. *Curr Opin Struct Biol* 15, 86–93.

Hogg, M., Wallace, S. S. & Doublié, S. (2004). Crystallographic snapshots of a replicative DNA polymerase encountering an abasic site. *EMBO J* 23, 1483–1493.

Hsu, G. W., Ober, M., Carell, T. & Beese, L. S. (2004). Error-prone replication of oxidatively damaged DNA by a high-fidelity DNA polymerase. *Nature* 431, 217–221.

Hubscher, U., Maga, G. & Spadari, S. (2002). Eucaryotic DNA polymerases. *Annu Rev Biochem* 71, 133–163.

Indiani, C., McInerney, P., Georgescu, R., Goodman, M.F. & O'Donnell, M. (2005). A sliding-clamp toolbelt binds high- and low-fidelity DNA polymerases simultaneously. *Mol Cell* 19, 805–815.

Jarosz, D. F., Beuning, P. J., Cohen, S. E. & Walker, G. C. (2007). Y-family DNA polymerases in *Escherichia coli*. *Trends Microbiol* 15, 70–77.

Jarosz, D. F., Godoy, V. G., Delaney, J. C., Essigmann, J. M. & Walker, G. C. (2006). A single amino acid governs enhanced activity of DinB DNA polymerases on damaged templates. *Nature* 439, 225–228.

Johnson, A. & O'Donnell, M. (2005). Cellular DNA replicases: components and dynamics at the replication fork. *Annu Rev Biochem* 74, 283–315.

Kamiya, H. & Kasai, H. (2000). 2-Hydroxy-dATP is incorporated opposite G by *Escherichia coli* DNA polymerase III resulting in high mutagenicity. *Nucleic Acids Res* 28, 1640–1646.

Kasak, L., Hõrak, R. & Kivisaar, M. (1997). Promoter-creating mutations in *Pseudomonas putida*: a model system for the study of mutations in starving bacteria. *Proc Natl Acad Sci U S A* 94, 3134–3139.

Kim, J. J. & Sundin, G. W. (2000). Regulation of the *rulAB* mutagenic DNA repair operon of *Pseudomonas syringae* by UV-B (290 to 320 nanometers) radiation and analysis of *rulAB*-mediated mutability *in vitro* and *in planta*. *J Bacteriol* 182, 6137–6144.

Kokubo, K., Yamada, M., Kanke, Y. & Nohmi, T. (2005). Roles of replicative and specialized DNA polymerases in frameshift mutagenesis: mutability of *Salmonella typhimurium* strains lacking one or all of SOS-inducible DNA polymerases to 26 chemicals. *DNA Repair (Amst)* 4, 1160–1171.

Kroeger, K. M., Jiang, Y. L., Kow, Y. W., Goodman, M.F. & Greenberg, M. M. (2004). Mutagenic effects of 2-deoxyribonolactone in *Escherichia coli*. An abasic lesion that disobeys the A-rule. *Biochemistry* 43, 6723–6733.

Kroeger, K. M., Kim, J., Goodman, M. F. & Greenberg, M. M. (2004). Effects of the C4'-oxidized abasic site on replication in *Escherichia coli*. An unusually large deletion is induced by a small lesion. *Biochemistry* 43, 13621–13627.

Kroeger, K. M., Kim, J., Goodman, M. F. & Greenberg, M. M. (2006). Replication of an oxidized abasic site in *Escherichia coli* by a dNTP-stabilized misalignment mechanism that reads upstream and downstream nucleotides. *Biochemistry* 45, 5048–5056.

Kulaeva, O. I., Wootton, J. C., Levine, A. S. & Woodgate, R. (1995). Characterization of the *umu*-complementing operon from R391. *J Bacteriol* 177, 2737–2743.

Kuroda, A., Murphy, H., Cashel, M. & Kornberg, A. (1997). Guanosine tetra- and pentaphosphate promote accumulation of inorganic polyphosphate in *Escherichia coli*. *J Biol Chem* 272, 21240–21243.

Kurtz, A. J. & Lloyd, R. S. (2003). 1,N2-deoxyguanosine adducts of acrolein, crotonaldehyde, and trans-4-hydroxynonenal cross-link to peptides via Schiff base linkage. *J Biol Chem* 278, 5970–5976.

Kusano, S. & Ishihama, A. (1997). Functional interaction of *Escherichia coli* RNA polymerase with inorganic polyphosphate. *Genes Cells* 2, 433–441.

Lamers, M. H., Georgescu, R. E., Lee, S. G., O'Donnell, M. & Kuriyan, J. (2006). Crystal structure of the catalytic alpha subunit of *E. coli* replicative DNA polymerase III. *Cell* 126, 881–892.

Layton, J. C. & Foster, P. L. (2003). Error-prone DNA polymerase IV is controlled by the stress-response sigma factor, RpoS, in *Escherichia coli*. *Mol Microbiol* 50, 549–561.

Layton, J. C. & Foster, P. L. (2005). Error-prone DNA polymerase IV is controlled by the heat shock chaperon GroE in *Escherichia coli*. *J Bacteriol* 187, 449–457.

Le Chatelier, E., Bécherel, O. J., d'Alençon, E., Canceill, D., Ehrlich, S. D., Fuchs, R. P. & Jannière, L. (2004). Involvement of DnaE, the second replicative DNA polymerase from *Bacillus subtilis*, in DNA mutagenesis. *J Biol Chem* 279, 1757–1767.

Lea, D. E. & Coulson, C. A. (1949). The distribution of the numbers of mutants in bacterial populations. *J Genetics* 49, 264–285.

LeClerc, J. E., Borden, A. & Lawrence C. W. (1991). The thymine-thymine pyrimidine-pyrimidone(6–4) ultraviolet light photoproduct is highly mutagenic and specifically induces 3' thymine-to-cytosine transitions in *Escherichia coli*. *Proc Natl Acad Sci U S A* 88, 9685–9689.

Lecointe, F., Shevelev, I. V., Bailone, A., Sommer, S. & Hübscher, U. (2004). Involvement of an X family DNA polymerase in double-stranded break repair in the radioresistant organism *Deinococcus radiodurans*. *Mol Microbiol* 53, 1721–1730.

Lehmann, A. R. & Fuchs, R. P. (2006). Gaps and forks in DNA replication: Rediscovering old models. *DNA Repair* 5, 1495–1498.

Lehmann, A. R. (2006). New functions for Y family polymerases. *Mol Cell* 24, 493–495.

Lhomme, J., Constant, J. F. & Demeunynck, M. (1999). Abasic DNA structrure, reactivity, and recognition. *Biopolymers* 52, 65–83.

Lijinsky, W. (1999). N-Nitroso compounds in the diet. *Mutat Res* 443, 129–138.

Ling, H., Boudsocq, F., Woodgate, R. & Yang, W. (2001). Crystal structure of a Y-family DNA polymerase in action: a mechanism for error-prone and lesion-bypass replication. *Cell* 107, 91–102.

Ling, H., Boudsocq, F., Woodgate, R. & Yang, W. (2004). Snapshots of replication through an abasic lesion; structural basis for base substitutions and frameshifts. *Mol Cell* 13, 751–762.

Lodwick, D., Owen, D. & Strike, P. (1990). DNA sequence analysis of the imp UV protection and mutation operon of the plasmid TP110: identification of a third gene. *Nucleic Acids Res* 18, 5045–5050.

Lombardo, M. J., Aponyi, I. & Rosenberg, S. M. (2004). General stress response regulator RpoS in adaptive mutation and amplification in *Escherichia coli*. *Genetics* 166, 669–680.

Mackay, W. J., Han, S. & Samson, L. D. (1994). DNA alkylation repair limits spontaneous base substitution mutations in *Escherichia coli*. *J Bacteriol* 176, 3224–3230.

Mai, G. T., McCormack, J. G., Seow, W. K., Pier, G. B., Jackson, L. A. & Thong, Y. H. (1993). Inhibition of adherence of mucoid *Pseudomonas aeruginosa* by alginase, specific monoclonal antibodies, and antibiotics. *Infect Immun* 61, 4338–4343.

Maor-Shoshani, A., Hayashi, K., Ohmori, H. & Livneh, Z. (2003). Analysis of translesio replication across an abasic site by DNA polymerase IV of *Escherichia coli*. *DNA Repair (Amst)* 2, 1227–1238.

Marnett, L. J. (2000). Oxyradicals and DNA damage. *Carcinogenesis* 21, 361–370.

Marnett, L. J. (2002). Oxy radicals, lipid peroxydation and DNA damage. *Toxicology* 181–182, 219–222.

Martin, D. W., Schurr, M. J., Mudd, M. H., Govan, J. R., Holloway, B. W. & Deretic, V. (1993). Mechanism of conversion to mucoidy in *Pseudomonas aeruginosa* infecting cystic fibrosis patients. *Proc Natl Acad Sci U S A* 90, 8377–8381.

Mathee, K., McPherson, C. J. & Ohman, D. E. (1997). Posttranslational control of the *algT* (*algU*)-encoded sigma22 for expression of the alginate regulon in *Pseudomonas aeruginosa* and localization of its antagonist proteins MucA and MucB (AlgN). *J Bacteriol* 179, 3711–3720.

Matsui, K., Yamada, M., Imai, M., Yamamoto, K. & Nohmi, T. (2006). Specificity of replicative and SOS-inducible DNA polymerases in frameshift mutagenesis: mutability of *Salmonella typhimurium* strains overexpressing SOS-inducible DNA polymerases to 30 chemical mutagens. *DNA Repair (Amst)* 5, 465–478.

McHenry, C. S. (2003). Chromosomal replicases as asymmetric dimers: studies of subunit arrangement and functional consequences. *Mol Microbiol* 49(5), 1157–1165.

McKenzie, G. J. & Rosenberg, S. M. (2001b). Adaptive mutations, mutator DNA polymerases and genetic change strategies of pathogens. *Curr Opin Microbiol* 4, 586–594.

McKenzie, G. J., Harris, R. S., Lee, P. L. & Rosenberg, S. M. (2000). The SOS response regulates adaptive mutation. *Proc Natl Acad Sci U S A* 97, 6646–6651.

McKenzie, G. J., Lee, P. L., Lomberdo, M. J., Hastings, P. J. & Rosenberg, S. M. (2001a). SOS mutator DNA polymerase IV functions in adaptive mutation and not adaptive amplification. *Mol Cell* 7, 571–579.

Miller, C., Ingmer, H., Thomsen, L. E., Skarstad, K. & Cohen, S. N. (2003). DpiA binding to the replication origin of the *Escherichia coli* plasmids and chromosomes destabilizes plasmid inheritance and induces the bacterial SOS response. *J Bacteriol* 185, 6025–6031.

Miller, C., Thomsen, L. E., Gaggero, C., Mosseri, R., Ingmer, H. & Cohen, S. N. (2004). SOS response induction by beta-lactams and bacterial defence against antibiotic lethality. *Science* 305, 1629–1631.

Minko, I. O., Yamanaka, K., Kozekov, I. D., Kozekova, A., Indiani, C., O'Donnell, M. E., Jiang, Q., Goodman, M. F., Rizzo, C. J. & Lloyd, R. S. (2008). Replication bypass of the acrolein-mediated deoxyguanine DNA-peptide cross-links by DNA polymerases of the DinB family. *Chen Res Toxicol* 21, 1983–1990.

Moyano, A. J., Luján, A. M., Argaraña, C. E. & Smania, A. M. (2007). MutS deficiency and activity of the error-prone DNA polymerase IV are crucial for determining *mucA* as the main target for mucoid conversion in *Pseudomonas aeruginosa*. *Mol Microbiol* 64, 547–559.

Napolitano, R., Janel-Bintz, R., Wagner, J. & Fuchs, R. P. (2000). All three SOS-inducible DNA polymerases (Pol II, Pol IV and Pol V) are involved in induced mutagenesis. *EMBO* 19, 6259–6265.

Neeley, W. L. & Essigman, J. M. (2006). Mechanisms of formation, genotoxicity, and mutation of guanine oxidation products. *Chem Res Toxicol* 19, 491–505.

Neeley, W. L., Delaney, S., Alekseyev, Y. O. Jarosz, D. F., Delaney, J. C., Walker, G. C. & Essigman, J. M. (2007). DNA polymerase V allows bypass of toxic guanine oxidation products *in vivo*. *J Biol Chem* 282, 12741–12748.

Nieminuszczy, J. & Grzesiuk, E. (2007). Bacterial DNA repair genes and their eukaryotic homologues: 3.AlkB dioxygenase and Ada methyltransferase in the direct repair of alkylated DNA. *Acta Biochim Pol* 54, 459–468.

Nohmi, T., Hakura, A., Nakai, Y., Watanabe, M., Murayama, S. Y. & Sofuni T. (1991). *Salmonella typhimurium* has two homologous but different *umuDC* operons: cloning of a new *umuDC*-like operon (*samAB*) present in a 60-megadalton cryptic plasmid of *S. typhimurium*. *J Bacteriol* 173, 1051–1063.

Ohashi, E., Murakumo, Y., Kanjo, N., Akagi, J., Masutani, C., Hanaoka, F. & Ohmori, H. (2004). Interaction of hREV1 with three human Y-family DNA polymerases. *Genes Cells* 9, 523–531.

Ohmori, H., Friedberg, E. C., Fuchs, R. P., Goodman, M. F., Hanaoka, F., Hinkle, D., Kunkel, T. A., Lawrence C.W., Livneh, Z., Nohmi, T., Prakash, L., Prakash, S., Todo, T., Walker, G. C., Wang, Z. & Woodgate, R. (2001). The Y-family of DNA polymerases. *Mol Cell* 8, 7–8.

Oliver, A., Baquero, F. & Blázquez, J. (2002). The mismatch repair system (*mutS*, *mutL*, and *uvrD* genes) in *Pseudomonas aeruginosa*: molecular characterization of naturally occurring mutants. *Mol Microbiol* 43, 1641–1650.

Oliver, A., Cantón, R., Campo, P., Baquero, F. & Blázquez, J. (2000). High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. *Science* 288, 1251–1254.

Pedraza-Reyes, M. & Yasbin, R. E. (2004). Contribution of the mismatch DNA repair system to the generation of stationary-phase-induced mutants of *Bacillus subtilis*. *J Bacteriol* 186, 6485–6491.

Pérez-Capilla, T., Baquero, M. R., Gómez-Gómez, J. M., Ionel, A., Martín, S. & Blázquez, J. (2005). SOS-independent induction of *dinB* transcription by beta-lactam-mediated inhibition of cell wall synthesis in *Escherichia coli*. *J Bacteriol* 187, 1515–1518.

Perry, K. L., Elledge, S. J., Mitchell, B. B., Marsh, L. & Walker, G. C. (1985). *umuDC* and *mucAB* operons whose products are required for UV light- and chemical-induced mutagenesis: UmuD, MucA, and LexA proteins share homology. *Proc Natl Acad Sci U S A* 82, 4331–4335.

Pieper, U., Eswar, N., Braberg, H., Madhusudhan, M. S., Davis, F. P., Stuart, A. C., Mirkovic, N., Rossi, A., Marti-Renom, M. A., Fiser, A., Webb, B., Greenblatt, D., Huang, C. C., Ferrin, T. E. & Sali, A. (2004). MODBASE, a database of annotated comparative protein structure models, and associated resources. *Nucleic Acids Res* 32, D217–D222.

Ponder, R. G., Fonville, N. C. & Rosenberg, S. M. (2005). A switch from high-fidelity to error-prone DNA double-srand break repair underlies stress-induced mutation. *Mol Cell* 19, 791–804.

16

Postow, L., Crisona, N. J., Peter, B. J., Hardy, C. D. & Cozzarelli, N. R. (2001). Topological challenges to DNA replication: conformations at the fork. *Proc Natl Acad Sci U S A* 98, 8219–8226.

Reuven, N. B., Arad, G., Maor-Shoshani, A. & Livneh, Z. (1999). The mutagenesis protein UmuC is a DNA polymerase activated by UmuD', RecA, and SSB and is specialized for translesion replication. *J Biol Chem* 274, 31763–31766.

Rosche, W. A. & Foster, P. L. (2000). Determining mutation rates in bacterial populations. *Methods* 20, 4–17.

Ross, C., Pybus, C., Pedraza-Reyes, M., Sung, H. M., Yasbin, R. E. & Robleto, E. (2006). Novel role of *mfd*: effects on stationary-phase mutagenesis in *Bacillus subtilis*. *J Bacteriol* 188, 7512–7520.

Sanders, L. H., Rockel, A., Lu, H., Wozniak, D. J. & Sutton, M. D. (2006). Role of *Pseudomonas aeruginosa dinB*-encoded DNA polymerase IV in mutagenesis. *J Bacteriol* 188, 8573–8585.

Saumaa, S., Tover, A., Kasak, L. & Kivisaar, M. (2002). Different spectra of stationary-phase mutations in early-arising *versus* late-arising mutants of *Pseudomonas putid*: involvement of the DNA repair enzyme MutY and the stationary-phase sigma factor RpoS. *J Bacteriol* 184, 6957–6965.

Schlacher, K. & Goodman, M. F. (2007). Lessons from 50 years of SOS DNA-damage-induced mutagenesis. *Nat Rev Mol Cell Biol* 8(7), 587–594.

Sedgwick, B. (1997). Nitrosated peptides and polyamines as endogenous mutagens in O6-alkylguanine-DNA alkyltransferase deficient cells. *Carcinogenesis* 18, 1561–1567.

Shen, X., Woodgate, R. & Goodman, M. F. (2005). Lyase activities intrinsic to *Escherichia coli* polymerases IV and V. *DNA Repair* 4, 1368–1373.

Smeulders, M. J., Keer, J., Speight, R. A. & Williams, H. D. (1999). Adaptation of *Mycobacterium smegmatis* to stationary phase. *J Bacteriol* 181, 270–283.

Smith, C. A., Wang, M., Jiang, N., Che L., Zhao, X. & Taylor, J. S. (1996). Mutation spectra of M13 vectors containing site-specific Cis-Syn, Trans-Syn-I, (6–4), and Dewar pyrimidone photoproducts of Thymidylyl-(3'-->5')-thymidine in *Escherichia coli*. *Biochemistry* 35, 4146–4154.

Sokal, R. R. & Rohlf, F. J. (1981). Biometry. Freeman, W. H., San Francisco, Calif.

Strauss, B. S. (1991). The 'A-rule' of mutagen specificity: a consequence of DNA polymerase bypass of non-instructional lesions? *Bioessays* 13, 79–84.

Studwell, P. S. & O'Donnell, M. (1990). Processive replication is contingent on the exonuclease subunit of DNA polymerase III holoenzyme. *J Biol Chem* 265, 1171–1178.

Stukenberg, P. T., Studwell-Vaughan, P. S. & & O'Donnell, M. (1991). Mechanism of the sliding beta-clamp of DNA polymerase III holoenzyme. *J Biol Chem* 266(17), 11328–11334.

Stumpf, J. D. & Foster, P. L. (2005). Polyphosphate kinase regulates error-prone replication by DNA polymerase IV in *Escherichia coli*. *Mol Microbiol* 57, 751–761.

Sundin, G. W., Kidambi, S. P., Ullrich, M. & Bender, C. L. (1996). Resistance to ultraviolet light in *Pseudomonas syringae*: sequence and functional analysis of the plasmid-encoded *rulAB* genes. *Gene* 177, 77–81.

Sung, H. M. & Yasbin, R. E. (2002). Adaptive, or stationary-phase, mutagenesis, a component of bacterial differentiation in *Bacillus subtilis*. *J Bacteriol* 184, 5641–5653.

Sung, H. M., Yeamans, G., Ross, C. A. & Yasbin, R. E. (2003). Roles of YqjH and YqjW, homologs of the *Escherichia coli* UmuC/DinB or Y superfamily of DNA polymerases, in stationary-phase mutatgenesis of *Bacillus subtilis*. *J Bacteriol* 185, 2153–2160.

Svoboda, D. L., Smith, C. A., Taylor, J. S. & Sancar, A. (1993). Effect of sequence, adduct type, and opposing lesions on the binding and repair of the ultraviolet photodamage by DNA photolyase and (A)BC excinuclease. *J Biol Chem* 268, 10694–10700.

Taddei, F., Matic, I. & Radman, M. (1995). cAMP-dependent SOS induction and mutagenesis in resting bacterial populations. *Proc Natl Acad Sci U S A* 92, 11736–11740.

Taft-Benz, S. A. & Schaaper, R. M. (2004) The theta subunit of *Escherichia coli* DNA polymerase III: a role in stabilizing the epsilon proofreading subunit. *J Bacteriol* 186, 2774–2780

Tang, M., Pham, P., Shen, X., Taylor, J. S., O'Donnell, M., Woodgate, R. & Goodman, M. F. (2000). Roles of *E. coli* DNA polymerases IV and V in lesion-targeted and untargeted SOS mutagenesis. *Nature* 404, 1014–1018.

Tang, M., Shen, X., Frank, E. G., O'Donnell, M., Woodgate, R. & Goodman, M. F. (1999). UmuD'(2)C is an error-prone DNA polymerase, *Escherichia coli* pol V. *Proc Natl Sci U S A* 96, 8919–8924.

Taverna, P. & Sedgwick, B. (1996). Generation of an endogenous DNA-methylating agent by nitrosation in *Escherichia coli*. *J Bacteriol* 178, 5105–5111.

Thornalley, P. J. (1996). Pharmacology of methylglyoxal: formation, modification of proteins and nucleic acids, and enzymatic detoxification—a role in pathogenesis and antiproliferative chemotherapy. *Gen Pharmacol* 27, 565–573.

Tisser, A., Kannouche, P., Reck, M. P., Lehmann, A. R., Fuchs, R. P. & Cordonnier, A. (2004). Co-localization in replication foci and interaction of human Y-family members, DNA polymerase pol eta and REVI protein. *DNA Repair (Amst)* 3, 1503–1514.

Uchida, K., Furukohri, A., Shinozaki, Y., Mori, T., Ogawara, D., Kanaya, S., Nohmi, T., Maki, H. & Akiyama, M. (2008). Overproduction of *Escherichia coli* DNA polymerase DinB (Pol IV) inhibits replication fork progression and is lethal. *Mol Microbiol* 70(3), 608–622.

Uchida, K., Kanematsu, M., Morimitsu, Y., Osawa, T., Noguchi, N. & Niki, E. (1998). Acrolein is a product of lipid peroxidation reaction. Formation of free acrolein and its conjugate with lysine residues in oxidized low density lipoproteins. *J Biol Chem* 273, 16058–16066.

Wagner, J. & Fuchs, R. P. (1997). Frameshift mutagenesis induced in *Escherichia coli* after *in vitro* treatment of double-stranded DNA with methylene blue plus white light: evidence for the involvement of lesion(s) other than 8-oxo-7,8-dihydro-2'-deoxyguanosine. *Chem Res Toxicol* 10, 568–574.

Wagner, J. & Nohmi, T. (2000). *Escherichia coli* DNA polymerase IV mutator activity: genetic requirement and mutational specificity. *J Bacteriol* 182, 4587–4595.

Wagner, J., Etienne, H., Janel-Bintz, R. & Fuchs, R. P. (2002). Genetics of mutagenesis in *E. coli*: various combinations of translesion polymerases (Pol II, IV and V) deal with lesion/sequence context diversity. *DNA Repair* 1, 159–167.

Wagner, J., Etienne, H., Janel-Bintz, R. & Fuchs, R. P. (2002). Genetics of mutagenesis in *E. coli*: various combinations of translesion polymerases (Pol II, IV and V) deal with lesion/sequence context diversity. *DNA Repair* 1, 159–167.

Walker, G. C. (1984). Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. *Microbiol Rev* 48, 60–93.
Weinel, C., Nelson, K. E. & Tümmler, B. (2002). Global features of the *Pseudomonas putida* KT2440 genome sequence. *Environ Microbiol* 4, 809–818.
Wertman, K. F. & Mount, D. W. (1985). Nucleotide sequence binding specificity of the LexA repressor of *Escherichia coli* K-12. *J Bacteriol* 163, 376–384.
Williams, P. A. & Murray, K. (1974). Metabolism of benzoate and the methylbenzoates by *Pseudomonas putida* (*arvilla*) mt-2: evidence for the existence of a TOL plasmid. *J Bacteriol* 120, 416–423.
Wyatt, M. D., Allan, J. M., Lau, A. Y. Ellenberger, T. E. & Samson, L. D. (1999). 3-methyladenine DNA glycosylases: structure, function, and biological importance. *Bioessays* 21, 668–676.
Yamada, M., Nunoshiba, T., Shimizu, M., Gruz, P., Kamiya, H., Harashima, H. & Nohmi, T. (2006). Involvement of Y-family DNA polymerases in mutagenesis caused by oxidized nucleotides in *Escherichia coli*. *J Bacteriol* 188, 4992–4995.
Yang, W. (2003). Damage repair DNA polymerases Y. *Curr Opin Struct Biol* 13, 23–30.
Yano, K., Homma, S. & Takagi, M. (1981). Degradative plasmid TOL provided singlet oxygen resistance for *Pseudomonas* strains. *Biochem Biophys Res Commun* 99, 1245–1250.
Yeiser, B., Pepper, E. D., Goodman, M. F. & Finkel, S. E. (2002). SOS-induced DNA polymerases enhance long-term survival and evolutionary fitness. *Proc Natl Acad Sci U S A* 99, 8737–8741.
Yuan, B., Cao, H., Jiang, Y., Hong, H. & Wang, Y. (2008). Efficient and accurate bypass of N2-(1-carboxyethyl)-2'-deoxyguanosine by DinB DNA polymerase *in vitro* and *in vivo*. *Proc Natl Acad Sci U S A* 105, 8679–8684.
Zambrano, M. M., Siegele, D. A., Almirón, M., Tormo, A. & Kolter, R. (1993). Microbial competition: *Escherichia coli* mutants that take over stationary phase cultures. *Science* 259, 1757–1760.
Zinser, E. R. & Kolter, R. (1999). Mutations enhancing amino acid catabolism confer a growth advantage in stationary phase. *J Bacteriol* 181, 5800–5807.
Zinser, E. R. & Kolter, R. (2000). Prolonged stationary-phase incubation selects for *lrp* mutations in *Escherichia coli* K-12. *J Bacteriol* 182, 4361–4365.
Zinser, E. R. & Kolter, R. (2004). *Escherichia coli* evolution during stationary phase. *Res Microbiol* 155, 328–336.
Zinser, E. R., Schneider, D., Bolt, M. & Kolter, R. (2003). Bacterial evolution through the selective loss of beneficial Genes. Trade-offs in expression involving two loci. *Genetics* 164, 1271–1277.

SUMMARY IN ESTONIAN

Spetsialiseerunud DNA polümeraaside roll *Pseudomonas putida* rakkudes toimuvates mutatsiooni protsessides

Baktereid võib leida kõikjalt: mullast, veest, samuti ka kõikidest elusorganimidest ja nende jäänustest. Bakteritele on üldiselt iseloomulik võime nii füsioloogiliselt kui ka geneetiliselt kiiresti muutunud keskkonnatingimustega kohaneda. Geneetilise adapteerumise ja evolutsioneerumise aluseks on erinevad mutatsiooniprotsessid. Ebasobivad, bakterite kasvu pärssivad keskkonnatingimused nagu näiteks antibiootiliste ja reaktiivsete hapniku- ning lämmastikuühendite olemasolu kasvukeskkonnas, aga ka toitainete limitatsioon soodustavad mutatsioonide tekkimist.

Mutatsioonide tekkes on oluline roll spetsialiseerunud DNA polümeraaside poolt läbiviidaval vigaderohkel DNA sünteesil. Käesoleva töö kirjanduse ülevaates kirjeldan erinevaid bakteriaalseid spetsialiseerunud DNA polümeraase ja nende funktsioone mutatsiooniprotsessides, sealhulgas ka stressi poolt indutseeritud mutageneesis.

DNA polümeraaside osa mutatsiooniprotsessides on peamiselt kirjedatud *Escherichia coli* mudelil. Kuna bakterite domeen on kõige mitmekesisem elusloodus domeenidest, on selge, et erinevusi bakteriliikide vahel esineb ka mutatsioonide tekkeprotsessides. Meie töögrupis uuritakse mutatsiooniprotsesse bakteris *Pseudomonas putida. P. putida* on mullabakter, mis asustab erinevaid vee ja pinnase keskkondi. Mullabakterite looduslikus elukeskkonnas on pikki perioode kestev toiteainete puudus tavaline.

Pikaajalise süsinikunälja tingimustes viibivas bakteripopulatsioonis toimuvate mutatsiooniprotsesside uurimist takistab sageli sobivate testsüsteemide puudumine. Selle doktoritöö valmimise käigus oleme konstrueerinud komplekti uusi, fenooli süsinikuallikana kasutuselevõtul põhinevaid testsüsteeme, mis võimaldavad jälgida eraldi nii asendusmutatsioonide kui ka ühenukleotiidsete deletsioonide teket pseudomonaadide populatsioonis pikaajalise süsinikunälja tingimustes.

Doktoritöös esitatud tulemused, mis käsitlevad erinevate spetsialiseerunud DNA polümeraaside osalust *P. putida* nälgivates rakkudes toimuvates mutatsiooni protsessides, võib kokku võtta järgnevalt:

1. *P. putida* rakkudes osaleb Y perekonna DNA polümeraas IV ühenukleotiidsete deletsioonide tekkes. Ühenukleotiidsete deletsioonide teke toimub kasvavas *P. putida* populatsioonis ja esimese nälgimisnädala jooksul väga madala sagedusega. Alates teisest nädalast süsinikunälja tingimustes suureneb DNA polümeraas IV sõltuvate ühenukleotiidsete deletsioonide tekkesagedus olulisel määral.

2. Paljud väga erinevad bakteriliigid on omandanud lateraalse geeniülekande teel algselt aktinobakteritest pärineva geenikasseti, milles leiduvate geenide ekspressioon on DNA kahjustuste poolt indutseeritav. Kõnealune geenikassett kodeerib kahte DNA polümeraasi: DnaE2 ja ImuB. DnaE2 on replikatiivse DNA polümeraas III α-subühiku homoloog. ImuB sarnaneb Y perekonda kuuluvatele DNA polümeraasidele. *P. putida* rakkudes omavad DnaE2 ja ImuB mutageneesile vastupidist efekti. Sarnaselt teistes bakteriliikides kirjeldatule soodustab ImuB *P. putida* statsionaarse faasi populatsioonis nii asendusmutatsioonide kui ka ühenukleotiidsete deletsioonide teket. DnaE2 olemasolu aga, vastupidiselt kõigile senikirjeldatud DnaE2 homoloogidele, vähendab *P. putida* rakkudes asendusmutatsioonide tekkesagedust.
   ImuB aminohappelise järjestuse ja ennustatava valgustruktuuri analüüs näitas, et selle valgu funktsioneerimine DNA polümeraasina on väga ebatõenäoline, kuna selles puuduvad kõikides DNA polümeraasides konserveerunud katalüütlise aktiivtsentri moodustumiseks vajalikud aminohapped. Kuna aga ImuB mõjutab mutatsioonisagedust, võib oletada, et ImuB vahendab ja reguleerib teiste spetsialiseerunud DNA polümeraaside juurdepääsu DNA sünteesile.

3. DNA polümeraas V homoloog, mis on kodeeritud tolueeni degradatsiooni võimaldavas plasmiidis pWW0 asuvate rulAB geenide poolt, suurendab statsionaarse faasi populatsioonis geneetiliselt enam kohastunud mutantide tekkesagedust.

ACKNOWLEDGEMENTS

First of all, I thank my supervisors Maia Kivisaar and Andres Tover for guidance, advice, and for introducing me to scientific way of thinking. I am especially thankful to Andres Tover for all the help in the experimental part of the work. I would also like to thank all of my colleagues for scientific discussion and co-authors of my publications for productive collaboratio. I also owe my thanks to Milvi Siniroht, Ene Põldroos, and Annely Kukk whose contribution to practical work in laboratory has been indispensable. I thank Jüri Parik for providing very useful technical tips (accompanied with spicy blue jokes)and Eeva Heinaru, Andres Mäe and Aivar Torp for good company and exciting conversations. I am also very grateful to Tiiu Rootslane for all the help with administrative work as well as for patience and care. I thank Tiina Sedman, Tiina Alamäe, Mariliis Tark, Monika Hõim and Maarja Adojaan for reading and commenting on my thesis.

Finally, I am really thankful to my family and friends for always supporting me despite the subject I have decided to deal with.

CURRICULUM VITAE

Radi Tegova

Date of birth:	21.11.1977. Tartu
Citizenship:	Estonian
Marital status	single
Address, telephone, e-mail:	Elva 33A, Tartu; tel. 56638233; radi.tegova@quattromed.ee
Current position:	Quattromed Cell Factory, scientist
Education:	1996 – Tartu Tamme Gymnasium, 2002 – Tartu University, BSc 2004 – Tartu University, MSc 2004 – doctoral studies
Language skills:	Estonian, English, Japanese and Spanish in basic level
Working experience:	from the 2006 Tartu University, Instutute of Molecular and Cell Biology, scientist (0.5 half time basis) Tartu University, Instutute of Molecular and Cell Biology, scientist (0.5 half time basis) from 2007 Tartu University, Institute of Molecular and Cell Biology extraordinary scientist (0.5 half tibe basis) from the 2008 Tartu University, Institute of Molecular and Cell Biology, scientist (full time basis) from the 2009 Quattromed Cell Factory, scientist

Scientific activities

1. Main research interests: Mechanisms of genetic adaptation in pseudomonades
2. List of publications:

Tegova R, Tover A, Tarassova K, Tark M, Kivisaar M: Involvement of error-prone DNA polymerase IV in stationary-phase mutagenesis in Pseudomonas putida. J Bacteriol 2004, 186:2735–2744.

Tark M, Tover A, Tarassova K, Tegova R, Kivi G, Hõrak R, Kivisaar M: A DNA polymerase V homologue encoded by TOL plasmid pWW0 confers evolutionary fitness on Pseudomonas putida under conditions of environmental stress. J Bacteriol 2005, 187:5203–5213.

Koorits L, Tegova R, Tark M, Tarassova K, Tover A, Kivisaar M: Study of involvement of ImuB and DnaE2 in stationary-phase mutagenesis in Pseudomonas putida. DNA Repair (Amst) 2007, 6:863–868.

Saumaa S, Tarassova K, Tark M, Tover A, Tegova R, Kivisaar M. Involvement of DNA mismatch repair in stationary-phase mutagenesis during prolonged starvation of Pseudomonas putida. DNA Repair 2006 8: 505–14.

Putrins M, Tover A, Tegova R, Saks U, Kivisaar M. Study of factors which negatively affect expression of the phenol degradation operon pheBA in Pseudomonas putida. Microbiology. 2007 153: 1860–71.

Saumaa S, Tover A, Tark M, Tegova R, Kivisaar M. Oxidative DNA damage defense systems in avoidance of stationary-phase mutagenesis in Pseudomonas putida. J Bacteriol. 2007 189: 5504–14.

Tark M, Tover A, Koorits L, Tegova R, Kivisaar M. Dual role of NER in mutagenesis in Pseudomonas putida. DNA Repair. 2008 1: 20–30.

3. Fellowships:
   none
4. Other organizational and professional activities:
   1. Member of Estonian Society for Microbiologists.
   2. Participating in supervising of practical course in genetics (GBMR 02.036) in The University of Tartu and also in The Gifted and Talented Development Centre
5. International courses attended:
   Participation in cource **„Bacterial Adaptation Mechanisms: Biofilms, Hypermutability and Antibiotic Resistance (BAMBHAR)",** ESCMID Postgraduate Education Course and Symposium, 7–9 November 2007, Palma de Mallorca, Spain

CURRICULUM VITAE

Radi Tegova

1. Sünniaeg ja koht:	21.11.1977. Tartu
2. Kodakondsus:	Eesti
3. Perekonnaseis:	vallaline
4. Aadress, telefon, e-post:	Elva 33A, Tartu; tel. 56638233; radi.tegova@quattromed.ee
5. Praegune töökoht, amet:	Quattromed Cell Factory, teadur
6. Haridus:	1996 – Tartu Tamme Gümnaasium, keskharidus 2002 – Tartu Ülikool,BSc 2004 – Tartu Ülikool, MSc Alates 2004 – Tartu Ülikool, doktorantuur
7. Keelteoskus:	eesti, inglise, hispaania ja jaapani keel algtasemel.
8. Töökogemus (teenistuskäik):	alates 2006 Tartu Ülikooli molekulaar- ja rakubioloogia instituut, teadur (0,5k) alates 2007 Tartu Ülikooli molekulaar- ja rakubioloogia instituut, erak. teadur (0,5k) alates 2008 Tartu Ülikooli molekulaar- ja rakubioloogia instituut, teadur (1,0k) alates 2009 Quattromed Cell Factory, teadur

Teaduslik ja arendustegevus

1. Peamised uurimisvaldkonnad
   Geneetilise adaptatsiooni molekulaarsed mehhanismid pseudomonaadises.
2. Publikatsioonide loetelu
   Tegova R, Tover A, Tarassova K, Tark M, Kivisaar M: Involvement of error-prone DNA polymerase IV in stationary-phase mutagenesis in Pseudomonas putida. J Bacteriol 2004, 186:2735–2744.
   Tark M, Tover A, Tarassova K, Tegova R, Kivi G, Hõrak R, Kivisaar M: A DNA polymerase V homologue encoded by TOL plasmid pWW0 confers evolutionary fitness on Pseudomonas putida under conditions of environmental stress. J Bacteriol 2005, 187:5203–5213.
   Koorits L, Tegova R, Tark M, Tarassova K, Tover A, Kivisaar M: Study of involvement of ImuB and DnaE2 in stationary-phase mutagenesis in Pseudomonas putida. DNA Repair (Amst) 2007, 6:863–868.

Saumaa S, Tarassova K, Tark M, Tover A, Tegova R, Kivisaar M. Involvement of DNA mismatch repair in stationary-phase mutagenesis during prolonged starvation of Pseudomonas putida. DNA Repair 2006 8: 505–14.

Putrins M, Tover A, Tegova R, Saks U, Kivisaar M. Study of factors which negatively affect expression of the phenol degradation operon pheBA in Pseudomonas putida. Microbiology. 2007 153: 1860–71.

Saumaa S, Tover A, Tark M, Tegova R, Kivisaar M. Oxidative DNA damage defense systems in avoidance of stationary-phase mutagenesis in Pseudomonas putida. J Bacteriol. 2007 189: 5504–14.

Tark M, Tover A, Koorits L, Tegova R, Kivisaar M. Dual role of NER in mutagenesis in Pseudomonas putida. DNA Repair. 2008 1: 20–30.

3. Saadud uurimistoetused ja stipendiumid

4. Muu teaduslik organisatsiooniline ja erialane tegevus:
    1. Osalemine geneetika praktikumi (BGMR 02.036) läbiviimises
    2. Kuulun Eesti Mikobioloogide Ühendusse
5. Erialane enesetäiendus:
   Osalemine kursusel „**Bacterial Adaptation Mechanisms: Biofilms, Hypermutability and Antibiotic Resistance (BAMBHAR)**", ESCMID Postgraduate Education Course and Symposium, 7–9 November 2007, Palma de Mallorca, Spain

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS

1. **Toivo Maimets**. Studies of human oncoprotein p53. Tartu, 1991, 96 p.
2. **Enn K. Seppet**. Thyroid state control over energy metabolism, ion transport and contractile functions in rat heart. Tartu, 1991, 135 p.
3. **Kristjan Zobel**. Epifüütsete makrosamblike väärtus õhu saastuse indikaatoritena Hamar-Dobani boreaalsetes mägimetsades. Tartu, 1992, 131 lk.
4. **Andres Mäe**. Conjugal mobilization of catabolic plasmids by transposable elements in helper plasmids. Tartu, 1992, 91 p.
5. **Maia Kivisaar**. Studies on phenol degradation genes of *Pseudomonas* sp. strain EST 1001. Tartu, 1992, 61 p.
6. **Allan Nurk**. Nucleotide sequences of phenol degradative genes from *Pseudomonas sp.* strain EST 1001 and their transcriptional activation in *Pseudomonas putida.* Tartu, 1992, 72 p.
7. **Ülo Tamm**. The genus *Populus* L. in Estonia: variation of the species biology and introduction. Tartu, 1993, 91 p.
8. **Jaanus Remme**. Studies on the peptidyltransferase centre of the *E.coli* ribosome. Tartu, 1993, 68 p.
9. **Ülo Langel**. Galanin and galanin antagonists. Tartu, 1993, 97 p.
10. **Arvo Käärd**. The development of an automatic online dynamic fluorescense-based pH-dependent fiber optic penicillin flowthrought biosensor for the control of the benzylpenicillin hydrolysis. Tartu, 1993, 117 p.
11. **Lilian Järvekülg**. Antigenic analysis and development of sensitive immunoassay for potato viruses. Tartu, 1993, 147 p.
12. **Jaak Palumets**. Analysis of phytomass partition in Norway spruce. Tartu, 1993, 47 p.
13. **Arne Sellin**. Variation in hydraulic architecture of *Picea abies* (L.) Karst. trees grown under different enviromental conditions. Tartu, 1994, 119 p.
13. **Mati Reeben**. Regulation of light neurofilament gene expression. Tartu, 1994, 108 p.
14. **Urmas Tartes**. Respiration rhytms in insects. Tartu, 1995, 109 p.
15. **Ülo Puurand**. The complete nucleotide sequence and infections *in vitro* transcripts from cloned cDNA of a potato A potyvirus. Tartu, 1995, 96 p.
16. **Peeter Hõrak**. Pathways of selection in avian reproduction: a functional framework and its application in the population study of the great tit (*Parus major*). Tartu, 1995, 118 p.
17. **Erkki Truve**. Studies on specific and broad spectrum virus resistance in transgenic plants. Tartu, 1996, 158 p.
18. **Illar Pata**. Cloning and characterization of human and mouse ribosomal protein S6-encoding genes. Tartu, 1996, 60 p.
19. **Ülo Niinemets**. Importance of structural features of leaves and canopy in determining species shade-tolerance in temperature deciduous woody taxa. Tartu, 1996, 150 p.

30

20. **Ants Kurg**. Bovine leukemia virus: molecular studies on the packaging region and DNA diagnostics in cattle. Tartu, 1996, 104 p.
21. **Ene Ustav**. E2 as the modulator of the BPV1 DNA replication. Tartu, 1996, 100 p.
22. **Aksel Soosaar**. Role of helix-loop-helix and nuclear hormone receptor transcription factors in neurogenesis. Tartu, 1996, 109 p.
23. **Maido Remm**. Human papillomavirus type 18: replication, transformation and gene expression. Tartu, 1997, 117 p.
24. **Tiiu Kull**. Population dynamics in *Cypripedium calceolus* L. Tartu, 1997, 124 p.
25. **Kalle Olli**. Evolutionary life-strategies of autotrophic planktonic microorganisms in the Baltic Sea. Tartu, 1997, 180 p.
26. **Meelis Pärtel**. Species diversity and community dynamics in calcareous grassland communities in Western Estonia. Tartu, 1997, 124 p.
27. **Malle Leht**. The Genus *Potentilla* L. in Estonia, Latvia and Lithuania: distribution, morphology and taxonomy. Tartu, 1997, 186 p.
28. **Tanel Tenson**. Ribosomes, peptides and antibiotic resistance. Tartu, 1997, 80 p.
29. **Arvo Tuvikene**. Assessment of inland water pollution using biomarker responses in fish *in vivo* and *in vitro*. Tartu, 1997, 160 p.
30. **Urmas Saarma**. Tuning ribosomal elongation cycle by mutagenesis of 23S rRNA. Tartu, 1997, 134 p.
31. **Henn Ojaveer**. Composition and dynamics of fish stocks in the gulf of Riga ecosystem. Tartu, 1997, 138 p.
32. **Lembi Lõugas**. Post-glacial development of vertebrate fauna in Estonian water bodies. Tartu, 1997, 138 p.
33. **Margus Pooga**. Cell penetrating peptide, transportan, and its predecessors, galanin-based chimeric peptides. Tartu, 1998, 110 p.
34. **Andres Saag**. Evolutionary relationships in some cetrarioid genera (Lichenized Ascomycota). Tartu, 1998, 196 p.
35. **Aivar Liiv**. Ribosomal large subunit assembly *in vivo*. Tartu, 1998, 158 p.
36. **Tatjana Oja**. Isoenzyme diversity and phylogenetic affinities among the eurasian annual bromes (*Bromus* L., Poaceae). Tartu, 1998, 92 p.
37. **Mari Moora**. The influence of arbuscular mycorrhizal (AM) symbiosis on the competition and coexistence of calcareous crassland plant species. Tartu, 1998, 78 p.
38. **Olavi Kurina**. Fungus gnats in Estonia (*Diptera: Bolitophilidae, Keroplatidae, Macroceridae, Ditomyiidae, Diadocidiidae, Mycetophilidae*). Tartu, 1998, 200 p.
39. **Andrus Tasa**. Biological leaching of shales: black shale and oil shale. Tartu, 1998, 98 p.
40. **Arnold Kristjuhan.** Studies on transcriptional activator properties of tumor suppressor protein p53. Tartu, 1998, 86 p.

41. **Sulev Ingerpuu.** Characterization of some human myeloid cell surface and nuclear differentiation antigens. Tartu, 1998, 163 p.
42. **Veljo Kisand.** Responses of planktonic bacteria to the abiotic and biotic factors in the shallow lake Võrtsjärv. Tartu, 1998, 118 p.
43. **Kadri Põldmaa.** Studies in the systematics of hypomyces and allied genera (Hypocreales, Ascomycota). Tartu, 1998, 178 p.
44. **Markus Vetemaa.** Reproduction parameters of fish as indicators in environmental monitoring. Tartu, 1998, 117 p.
45. **Heli Talvik.** Prepatent periods and species composition of different *Oesophagostomum* spp. populations in Estonia and Denmark. Tartu, 1998, 104 p.
46. **Katrin Heinsoo.** Cuticular and stomatal antechamber conductance to water vapour diffusion in *Picea abies* (L.) karst. Tartu, 1999, 133 p.
47. **Tarmo Annilo.** Studies on mammalian ribosomal protein S7. Tartu, 1998, 77 p.
48. **Indrek Ots.** Health state indicies of reproducing great tits (*Parus major*): sources of variation and connections with life-history traits. Tartu, 1999, 117 p.
49. **Juan Jose Cantero.** Plant community diversity and habitat relationships in central Argentina grasslands. Tartu, 1999, 161 p.
50. **Rein Kalamees.** Seed bank, seed rain and community regeneration in Estonian calcareous grasslands. Tartu, 1999, 107 p.
51. **Sulev Kõks.** Cholecystokinin (CCK) — induced anxiety in rats: influence of environmental stimuli and involvement of endopioid mechanisms and erotonin. Tartu, 1999, 123 p.
52. **Ebe Sild.** Impact of increasing concentrations of O_3 and CO_2 on wheat, clover and pasture. Tartu, 1999, 123 p.
53. **Ljudmilla Timofejeva.** Electron microscopical analysis of the synaptonemal complex formation in cereals. Tartu, 1999, 99 p.
54. **Andres Valkna.** Interactions of galanin receptor with ligands and G-proteins: studies with synthetic peptides. Tartu, 1999, 103 p.
55. **Taavi Virro.** Life cycles of planktonic rotifers in lake Peipsi. Tartu, 1999, 101 p.
56. **Ana Rebane.** Mammalian ribosomal protein S3a genes and intron-encoded small nucleolar RNAs U73 and U82. Tartu, 1999, 85 p.
57. **Tiina Tamm.** Cocksfoot mottle virus: the genome organisation and translational strategies. Tartu, 2000, 101 p.
58. **Reet Kurg.** Structure-function relationship of the bovine papilloma virus E2 protein. Tartu, 2000, 89 p.
59. **Toomas Kivisild.** The origins of Southern and Western Eurasian populations: an mtDNA study. Tartu, 2000, 121 p.
60. **Niilo Kaldalu.** Studies of the TOL plasmid transcription factor XylS. Tartu 2000. 88 p.

61. **Dina Lepik.** Modulation of viral DNA replication by tumor suppressor protein p53. Tartu 2000. 106 p.
62. **Kai Vellak.** Influence of different factors on the diversity of the bryophyte vegetation in forest and wooded meadow communities. Tartu 2000. 122 p.
63. **Jonne Kotta.** Impact of eutrophication and biological invasionas on the structure and functions of benthic macrofauna. Tartu 2000. 160 p.
64. **Georg Martin.** Phytobenthic communities of the Gulf of Riga and the inner sea the West-Estonian archipelago. Tartu, 2000. 139 p.
65. **Silvia Sepp.** Morphological and genetical variation of *Alchemilla L.* in Estonia. Tartu, 2000. 124 p.
66. **Jaan Liira.** On the determinants of structure and diversity in herbaceous plant communities. Tartu, 2000. 96 p.
67. **Priit Zingel.** The role of planktonic ciliates in lake ecosystems. Tartu 2001. 111 p.
68. **Tiit Teder.** Direct and indirect effects in Host-parasitoid interactions: ecological and evolutionary consequences. Tartu 2001. 122 p.
69. **Hannes Kollist.** Leaf apoplastic ascorbate as ozone scavenger and its transport across the plasma membrane. Tartu 2001. 80 p.
70. **Reet Marits.** Role of two-component regulator system PehR-PehS and extracellular protease PrtW in virulence of *Erwinia Carotovora* subsp. *Carotovora*. Tartu 2001. 112 p.
71. **Vallo Tilgar.** Effect of calcium supplementation on reproductive performance of the pied flycatcher *Ficedula hypoleuca* and the great tit *Parus major,* breeding in Nothern temperate forests. Tartu, 2002. 126 p.
72. **Rita Hõrak.** Regulation of transposition of transposon Tn*4652* in *Pseudomonas putida*. Tartu, 2002. 108 p.
73. **Liina Eek-Piirsoo.** The effect of fertilization, mowing and additional illumination on the structure of a species-rich grassland community. Tartu, 2002. 74 p.
74. **Krõõt Aasamaa.** Shoot hydraulic conductance and stomatal conductance of six temperate deciduous tree species. Tartu, 2002. 110 p.
75. **Nele Ingerpuu.** Bryophyte diversity and vascular plants. Tartu, 2002. 112 p.
76. **Neeme Tõnisson.** Mutation detection by primer extension on oligonucleotide microarrays. Tartu, 2002. 124 p.
77. **Margus Pensa.** Variation in needle retention of Scots pine in relation to leaf morphology, nitrogen conservation and tree age. Tartu, 2003. 110 p.
78. **Asko Lõhmus.** Habitat preferences and quality for birds of prey: from principles to applications. Tartu, 2003. 168 p.
79. **Viljar Jaks.** p53 — a switch in cellular circuit. Tartu, 2003. 160 p.
80. **Jaana Männik.** Characterization and genetic studies of four ATP-binding cassette (ABC) transporters. Tartu, 2003. 140 p.
81. **Marek Sammul.** Competition and coexistence of clonal plants in relation to productivity. Tartu, 2003. 159 p

82. **Ivar Ilves.** Virus-cell interactions in the replication cycle of bovine papillomavirus type 1. Tartu, 2003. 89 p.
83. **Andres Männik.** Design and characterization of a novel vector system based on the stable replicator of bovine papillomavirus type 1. Tartu, 2003. 109 p.
84. **Ivika Ostonen.** Fine root structure, dynamics and proportion in net primary production of Norway spruce forest ecosystem in relation to site conditions. Tartu, 2003. 158 p.
85. **Gudrun Veldre.** Somatic status of 12–15-year-old Tartu schoolchildren. Tartu, 2003. 199 p.
86. **Ülo Väli.** The greater spotted eagle *Aquila clanga* and the lesser spotted eagle *A. pomarina*: taxonomy, phylogeography and ecology. Tartu, 2004. 159 p.
87. **Aare Abroi.** The determinants for the native activities of the bovine papillomavirus type 1 E2 protein are separable. Tartu, 2004. 135 p.
88. **Tiina Kahre.** Cystic fibrosis in Estonia. Tartu, 2004. 116 p.
89. **Helen Orav-Kotta.** Habitat choice and feeding activity of benthic suspension feeders and mesograzers in the northern Baltic Sea. Tartu, 2004. 117 p.
90. **Maarja Öpik.** Diversity of arbuscular mycorrhizal fungi in the roots of perennial plants and their effect on plant performance. Tartu, 2004. 175 p.
91. **Kadri Tali.** Species structure of *Neotinea ustulata*. Tartu, 2004. 109 p.
92. **Kristiina Tambets.** Towards the understanding of post-glacial spread of human mitochondrial DNA haplogroups in Europe and beyond: a phylogeographic approach. Tartu, 2004. 163 p.
93. **Arvi Jõers.** Regulation of p53-dependent transcription. Tartu, 2004. 103 p.
94. **Lilian Kadaja.** Studies on modulation of the activity of tumor suppressor protein p53. Tartu, 2004. 103 p.
95. **Jaak Truu.** Oil shale industry wastewater: impact on river microbial community and possibilities for bioremediation. Tartu, 2004. 128 p.
96. **Maire Peters.** Natural horizontal transfer of the *pheBA* operon. Tartu, 2004. 105 p.
97. **Ülo Maiväli.** Studies on the structure-function relationship of the bacterial ribosome. Tartu, 2004. 130 p.
98. **Merit Otsus.** Plant community regeneration and species diversity in dry calcareous grasslands. Tartu, 2004. 103 p.
99. **Mikk Heidemaa.** Systematic studies on sawflies of the genera *Dolerus, Empria,* and *Caliroa* (Hymenoptera: Tenthredinidae). Tartu, 2004. 167 p.
100. **Ilmar Tõnno.** The impact of nitrogen and phosphorus concentration and N/P ratio on cyanobacterial dominance and N_2 fixation in some Estonian lakes. Tartu, 2004. 111 p.
101. **Lauri Saks.** Immune function, parasites, and carotenoid-based ornaments in greenfinches. Tartu, 2004. 144 p.

102. **Siiri Rootsi.** Human Y-chromosomal variation in European populations. Tartu, 2004. 142 p.
103. **Eve Vedler.** Structure of the 2,4-dichloro-phenoxyacetic acid-degradative plasmid pEST4011. Tartu, 2005. 106 p.
104. **Andres Tover.** Regulation of transcription of the phenol degradation *pheBA* operon in *Pseudomonas putida*. Tartu, 2005. 126 p.
105. **Helen Udras.** Hexose kinases and glucose transport in the yeast *Hansenula polymorpha*. Tartu, 2005. 100 p.
106. **Ave Suija.** Lichens and lichenicolous fungi in Estonia: diversity, distribution patterns, taxonomy. Tartu, 2005. 162 p.
107. **Piret Lõhmus.** Forest lichens and their substrata in Estonia. Tartu, 2005. 162 p.
108. **Inga Lips.** Abiotic factors controlling the cyanobacterial bloom occurrence in the Gulf of Finland. Tartu, 2005. 156 p.
109. **Kaasik, Krista.** Circadian clock genes in mammalian clockwork, metabolism and behaviour. Tartu, 2005. 121 p.
110. **Juhan Javoiš.** The effects of experience on host acceptance in ovipositing moths. Tartu, 2005. 112 p.
111. **Tiina Sedman.** Characterization of the yeast *Saccharomyces cerevisiae* mitochondrial DNA helicase Hmi1. Tartu, 2005. 103 p.
112. **Ruth Aguraiuja.** Hawaiian endemic fern lineage *Diellia* (Aspleniaceae): distribution, population structure and ecology. Tartu, 2005. 112 p.
113. **Riho Teras.** Regulation of transcription from the fusion promoters generated by transposition of Tn*4652* into the upstream region of *pheBA* operon in *Pseudomonas putida*. Tartu, 2005. 106 p.
114. **Mait Metspalu.** Through the course of prehistory in india: tracing the mtDNA trail. Tartu, 2005. 138 p.
115. **Elin Lõhmussaar.** The comparative patterns of linkage disequilibrium in European populations and its implication for genetic association studies. Tartu, 2006. 124 p.
116. **Priit Kupper.** Hydraulic and environmental limitations to leaf water relations in trees with respect to canopy position. Tartu, 2006. 126 p.
117. **Heili Ilves.** Stress-induced transposition of Tn*4652* in *Pseudomonas Putida.* Tartu, 2006. 120 p.
118. **Silja Kuusk.** Biochemical properties of Hmi1p, a DNA helicase from *Saccharomyces cerevisiae* mitochondria. Tartu, 2006. 126 p.
119. **Kersti Püssa.** Forest edges on medium resolution landsat thematic mapper satellite images. Tartu, 2006. 90 p.
120. **Lea Tummeleht.** Physiological condition and immune function in great tits (*Parus major* l.): Sources of variation and trade-offs in relation to growth. Tartu, 2006. 94 p.
121. **Toomas Esperk.** Larval instar as a key element of insect growth schedules. Tartu, 2006. 186 p.

122. **Harri Valdmann.** Lynx (*Lynx lynx*) and wolf (*Canis lupus*) in the Baltic region: Diets, helminth parasites and genetic variation. Tartu, 2006. 102 p.
123. **Priit Jõers.** Studies of the mitochondrial helicase Hmi1p in *Candida albicans* and *Saccharomyces cerevisia*. Tartu, 2006. 113 p.
124. **Kersti Lilleväli.** Gata3 and Gata2 in inner ear development. Tartu, 2007. 123 p.
125. **Kai Rünk.** Comparative ecology of three fern species: *Dryopteris carthusiana* (Vill.) H.P. Fuchs, *D. expansa* (C. Presl) Fraser-Jenkins & Jermy and *D. dilatata* (Hoffm.) A. Gray (Dryopteridaceae). Tartu, 2007. 143 p.
126. **Aveliina Helm.** Formation and persistence of dry grassland diversity: role of human history and landscape structure. Tartu, 2007. 89 p.
127. **Leho Tedersoo.** Ectomycorrhizal fungi: diversity and community structure in Estonia, Seychelles and Australia. Tartu, 2007. 233 p.
128. **Marko Mägi.** The habitat-related variation of reproductive performance of great tits in a deciduous-coniferous forest mosaic: looking for causes and consequences. Tartu, 2007. 135 p.
129. **Valeria Lulla.** Replication strategies and applications of Semliki Forest virus. Tartu, 2007. 109 p.
130. **Ülle Reier**. Estonian threatened vascular plant species: causes of rarity and conservation. Tartu, 2007. 79 p.
131. **Inga Jüriado**. Diversity of lichen species in Estonia: influence of regional and local factors. Tartu, 2007. 171 p.
132. **Tatjana Krama.** Mobbing behaviour in birds: costs and reciprocity based cooperation. Tartu, 2007.
133. **Signe Saumaa.** The role of DNA mismatch repair and oxidative DNA damage defense systems in avoidance of stationary phase mutations in *Pseudomonas putida.* Tartu, 2007. 172 p.
134. **Reedik Mägi**. The linkage disequilibrium and the selection of genetic markers for association studies in european populations. Tartu, 2007. 96 p.
135. **Priit Kilgas.** Blood parameters as indicators of physiological condition and skeletal development in great tits (*Parus major*): natural variation and application in the reproductive ecology of birds. Tartu, 2007. 129 p.
136. **Anu Albert**. The role of water salinity in structuring eastern Baltic coastal fish communities. Tartu, 2007. 95 p.
137. **Kärt Padari.** Protein transduction mechanisms of transportans. Tartu, 2008. 128 p.
138. **Siiri-Lii Sandre.** Selective forces on larval colouration in a moth. Tartu, 2008. 125 p.
139. **Ülle Jõgar.** Conservation and restoration of semi-natural floodplain meadows and their rare plant species. Tartu, 2008. 99 p.
140. **Lauri Laanisto.** Macroecological approach in vegetation science: generality of ecological relationships at the global scale. Tartu, 2008. 133 p.
141. **Reidar Andreson**. Methods and software for predicting PCR failure rate in large genomes. Tartu, 2008. 105 p.

142. **Birgot Paavel.** Bio-optical properties of turbid lakes. Tartu, 2008. 175 p.
143. **Kaire Torn.** Distribution and ecology of charophytes in the Baltic Sea. Tartu, 2008, 98 p.
144. **Vladimir Vimberg.** Peptide mediated macrolide resistance. Tartu, 2008, 190 p.
145. **Daima Örd.** Studies on the stress-inducible pseudokinase TRB3, a novel inhibitor of transcription factor ATF4. Tartu, 2008, 108 p.
146. **Lauri Saag.** Taxonomic and ecologic problems in the genus *Lepraria* (*Stereocaulaceae*, lichenised *Ascomycota*). Tartu, 2008, 175 p.
147. **Ulvi Karu.** Antioxidant protection, carotenoids and coccidians in greenfinches – assessment of the costs of immune activation and mechanisms of parasite resistance in a passerine with carotenoid-based ornaments. Tartu, 2008, 124 p.
148. **Jaanus Remm.** Tree-cavities in forests: density, characteristics and occupancy by animals. Tartu, 2008, 128 p.
149. **Epp Moks.** Tapeworm parasites *Echinococcus multilocularis* and *E. granulosus* in Estonia: phylogenetic relationships and occurrence in wild carnivores and ungulates. Tartu, 2008, 82 p.
150. **Eve Eensalu.** Acclimation of stomatal structure and function in tree canopy: effect of light and CO_2 concentration. Tartu, 2008, 108 p.
151. **Janne Pullat**. Design, functionlization and application of an *in situ* synthesized oligonucleotide microarray. Tartu, 2008, 108 p.
152. **Marta Putrinš.** Responses of *Pseudomonas putida* to phenol-induced metabolic and stress signals. Tartu, 2008, 142 p.
153. **Marina Semtšenko.** Plant root behaviour: responses to neighbours and physical obstructions. Tartu, 2008, 106 p.
154. **Marge Starast.** Influence of cultivation techniques on productivity and fruit quality of some *Vaccinium* and *Rubus* taxa. Tartu, 2008, 154 p.
155. **Age Tats.** Sequence motifs influencing the efficiency of translation. Tartu, 2009, 104 p.