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„Outer Membrane Vesicles in *Bordetella petrii*“

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1 INTRODUCTION

1.1 Outer Membrane vesicles (OMVs) produced by Gram-negative bacteria

OMVs are defined according to Beveridge (1999) and Kuehn & Kesty (2005) as small spherical structures or outer membrane blebs that are produced by gram-negative bacteria. OMVs contain amongst others biologically active proteins, DNA or RNA (Kulp & Kuehn, 2010; Dorward et al., 1989, Velimirov & Hagemann, 2011). Their diameter ranges between 20 – 250 nm, depending on the strain (Beveridge,1999) and studies by electron microscopy show that a bilayer membrane surrounds an electron dense body. Since the investigation of Perez-Cruz et al. (2013) a second type of OMV bearing a double bilayer was described, which was produced by the gram-negative bacterium *Shewanella vesiculosa*. A similar observation, confirming that this OMV type could also be produced by other bacterial species such as *Ahrensia kielensis* and *Pseudoalteromonas marina* was documented by Hagemann et al. (2013). Furthermore the literature distinguishes between OMVs according to their occurrence in the environment. OMVs can be detected in the planktonic environment (p-OMVs), which implies that after being released from the bacterial strain, the OMVs may drift away to fulfil a variety of biologically relevant functions (see below). The other possibility is that once released, the OMVs remain in close vicinity of the specific bacterial consortium, which forms biofilms (b-OMVs) and contributes to the construction of biofilms (Schooling & Beveridge, 2006, Schooling et al., 2009) by increasing the stability of the biofilm. Also the DNA contained in b-OMVs was found to be greater than for p-OMVs (Schooling et al., 2009).

OMVs are thought to be released only by gram negative bacteria, but recently extracellular vesicles have been described also for gram positive bacteria (Lee et al., 2009; Schrempf et al., 2011). It was suggested that extracellular vesicles of gram positive bacteria could fulfil similar roles as OMVs of gram negative bacteria (Schrempf et al., 2011).

1.2 Significance and function of OMVs

OMVs are released from the bacteria by budding but the mechanisms of OMV formation are poorly understood even though a number of molecular models on OMV

formation were proposed in the last 30 years (Wensink & Witholt, 1981; Zhou et al., 1998; Hayashi et al., 2002; Salkinoja-Salonen & Nurmiaho, 1978; Kadurugamuwa & Beveridge, 1995; Sabra et al., 2003; Mashburn-Warren & Whiteley, Kuehn & Kesty, 2005, 2006; Tashiro et al., 2011; Schwechheimer et al., 2013).

Because none of the so far formulated hypothesis on OMV formation is fully accepted only one example of the most recently proposed processes (Schwechheimer et al. 2013) is illustrated in Figure 1.

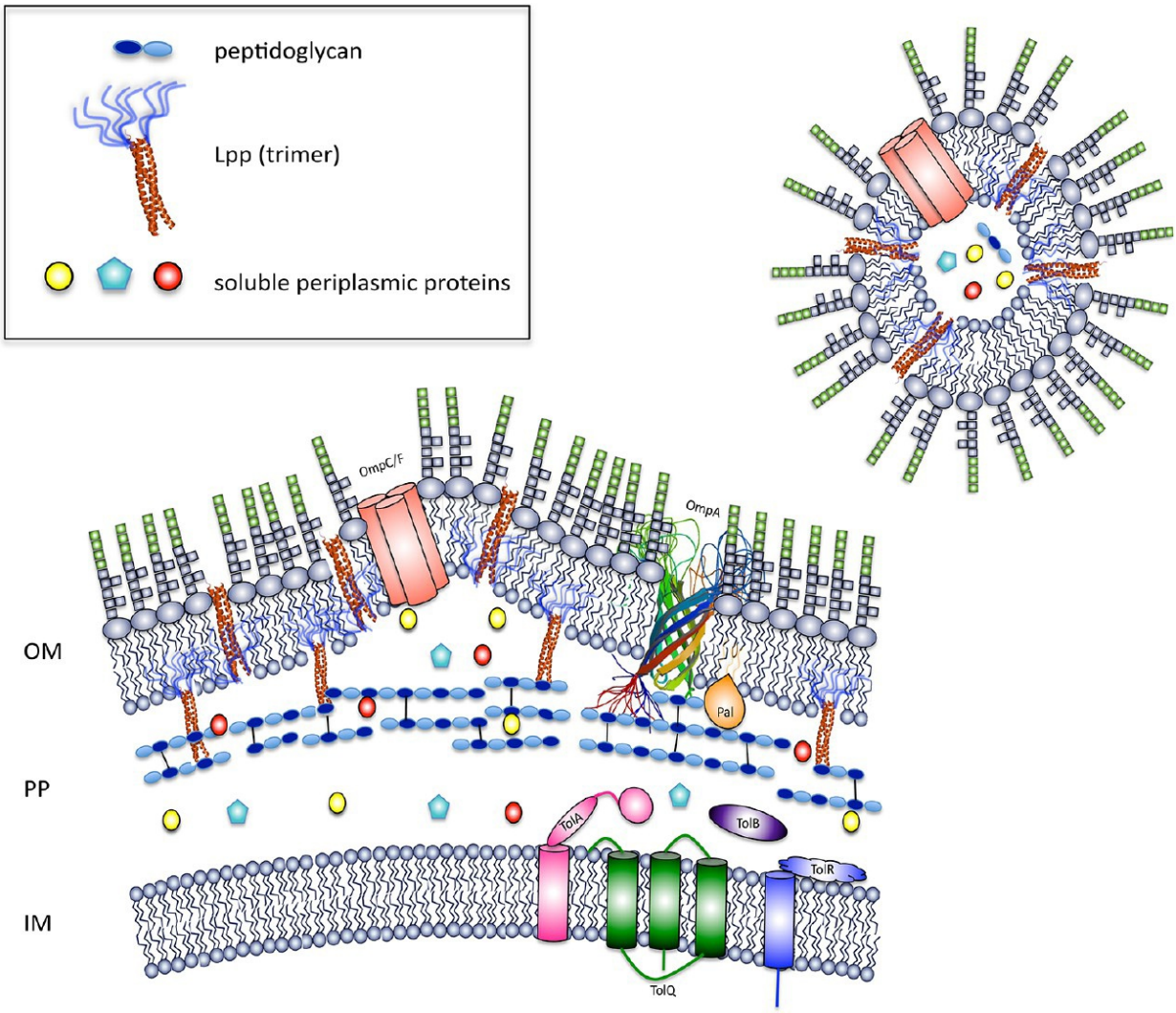

Fig. 1: OMV production model. Overview of Gram-negative envelope architecture in the context of OMV production, Schwechheimer et al., 2013.

As for the role of OMVs and their ecological significance a number of findings can be mentioned which are essential for the survival and functioning of procaryotes in general.

1.2.1 Interspecific competition

Within any bacterial consortium, the interspecies interactions shape the community structure. A cell specific fitness enhancement is therefore seen as a constant process necessary for a bacterial species to resist in competitive interactions. This is assumed to have led to the evolution of a large number of characteristics such as the production and secretion of antimicrobial factors to eliminate competing microorganisms.

OMVs were shown to disseminate such antimicrobial factors like the murein hydrolase (Li et al., 1996), and were called “predatory membrane vesicles” by Beveridge et al. (1997). The reason for the choice of this term is that the effect of the dissemination of murein hydrolase via OMVs is not only the reduction of competition within a polymicrobial environment but that the lytic bacterium is provided with nutrients liberated from lysed bacteria.

Other important biological roles of OMVs are interspecies communication and protein delivery such as bacterial toxins to eukaryotic cells.

1.2.2 Interspecies communication

Interspecies communication is a crucial mechanism in bacteria to monitor their population density and co-ordinate their group activities in response to cell density (Parsek & Greenberg, 2000). Bacteria are known to use chemical signals for communication and thereby modulate the expression of specific genes as a response, which is collectively termed Quorum sensing (QS). An important fraction of these QS molecules have hydrophobic character, which constrains their dissemination. Mashburn & Whiteley (2005) showed that such hydrophobic QS molecules are associated with OMVs, which serve as successful transport and protection vehicles.

1.2.3 Protein delivery

Many pathogenic bacteria produce OMVs containing toxins and these OMVs deliver the toxins to eukaryotic target cells. A study by Wai et al. (2003) showed that the E. coli cytotoxin cytolysin A (ClyA) was eightfold more potent when delivered by OMVs to eukaryotic target cells than when delivered in the form in which it is present in the bacterial periplasm. It seems therefore that ClyA is assembled into an active oligomeric form before being enclosed into the membrane vesicle.

A further essential role of OMVs is the trafficking of proteins between bacterial cells of the same species (Ciofu et al., 2000). *Pseudomonas aeruginosa* was shown to transfer the antibiotic resistance protein ß-lactamase between bacterial cells. This allows bacteria within the population to share the antibiotic resistance protein and it is not necessary that the gene encoding for the ß-lactamase protein is present within all cells of a *P.aeruginosa* population. There is a high probability that other gram-negative bacteria might use the same mechanism and a high production of OMVs with the specific protein by a few bacteria could be sufficient to protect a large portion of recipient bacteria from the respective antibiotics

1.2.4 Transfer of DNA by outer membrane vesicles

One specific attribute of OMV is that they induce the budding of new particles within the host cell.

The in-vitro induced OMV contained DNA with higher molecular weight than all other known particles, up to 370kbp (Chiura et al., 2011).

Once they have infected the host cells, these particles trigger the production of new OMV (Chiura et al., 2011). The electron-density of experimentally induced OMVs is significantly higher than that of other MVs or particles, which was assumend to be attributable to the high content of encapsulated DNA (Chiura et al., 2011).

The fact that OMVs contain significant amounts of DNA led to the hypothesis that genetic information may be transferred via OMVs between bacterial species. The first report, which documented the potential of gene transfer via OMVs was presented by Kahn & Goodgal (1982) and subsequent studies confirmed these observations (Kolling & Matthews, 1999, Yaron et al., 2000, Renelli et al., 2004). Experimental evidence, showing that the transfer of DNA via OMVs results in the restoration of genetic deficiencies in bacteria was provided by Chiura et al. (2011) and Velimirov & Hagemann (2011).

Thus it is obvious that beside the four well known mechanisms, which drive horizontal gene gtransfer (HGT), namely

1) transformation, i.e. the uptake of free DNA from the environment
2) conjugation which is the transfer of DNA from a bacterial donor cell to a recipient cell by the expression of a sex pilus
3) transduction as the phage mediated passage of bacterial genes and
4) Gene transfer agents (GTA) which is an bacteriophage –like vehicle of genetic exchange discovered in Rhodobacter capsulatus (Marrs, 1974, Solioz & Marrs, 1977, Lang & Beatty, 2000, Biers et al., 2008),

one is confronted with a fifth mechanism of HGT which is the formation by budding of DNA bearing outer membrane vesicles

These OMVs are mostly produced by cells in their stationary phase as is suggested by experimental observations (Chiura et al., 2011, Hagemann et al., 2013).

1.3 The bacterium *Bordetella petrii*

Bordetella petrii is a gram-negative bacterium, belonging to the genus Bordetella, which comprises 9 species. Nearly 107 years ago, Jules Bordet together with Octave Gengou isolated a pure culture of what would later be named after him, “the Bordetella” genus, and identified it as the cause behind whooping cough. All species, except *B. petrii*, are obligatorily associated with host organisms (Wintzingerode et al., 2001). *B. petrii* could be isolated from different habitants including humans (Fry et al., 2005).

Phylogenetically placed at the root of this genus is *Bordetella petrii*, the first environmental isolate of it, which was derived from an anaerobic, dechlorinating bioreactor culture enriched from river sediment and is, itself, capable of anaerobic growth. *B. petrii* takes a rather unique, evolutionary relevant position within the genus since it is in possession of several orthologous genes to virulence factors associated with the pathogenic Bordetella species, as well as common features of environmental bacteria. Therefore *B. petrii* can be seen as an attractive model organism for further experimentation and hypothesis testing.

Classification of *Bordetella petrii*	
Domain	Bacteria
Phylum	Proteobacteria
Class	Betaproteobacteria
Order	Burkholderiales
Family	Alcaligenaceae
Genus	*Bordetella*
Species	*Bordetella petrii*

Table 1: Systematic position of *Bordetella petrii*.

1.4 Aims of the investigation

Although there is a large number of studies dealing with OMVs which cover an impressive array of topics, some issues were poorly treated despite their relevance. It is remarkable that there is only one investigation dealing with sequence analysis of the OMV associated DNA (Hagemann et al., 2013). Furthermore, the majority of the investigated species with respect to DNA transfer were pathogenic species. Only in two bacterial species , both marine strains, the DNA sequencing of randomly chosen clones out of an established shotgun library were published. The studied species (Hagemann et al., 2013) belonged two the Alphaproteobacteria (*Ahrensia kielensis*) and the Gammaproteobacteria (*Pseudoalteromonas marina*). By integrating *Bordetella petrii* which is the only species of the genus Bordetella being derived from a natural aquatic environment into a similar survey, the information of sequence analysis may be extended to the Betaproteobacteria which were not investigated until now.

The major aims of the present study are therefore the following

- Obtain the establishment of a B. petrii liquid culture to reach the stationary phase and harvest OMVs
- Achieve an efficient DNA extraction from a cultured B. petrii population
- Establish a shotgun library from the DNA of the OMVs
- Sequence randomly chosen clones for the analysis of the OMV derived DNA
- Attempt to characterize the harvested OMVs by Transmission Electron Microscopy and if possible, present snap shots of *B. petrii* before or in the process of OMV production

Because the genome of *B. petrii* is completely sequenced and the species does not carry plasmids it should be possible to allocate the sequences to specific loci on the DNA. The final aim is to investigate whether there are pattern of DNA-packaging during OMV formation or whether we observe a random process.

2 MATERIAL AND METHODS

2.1 Strain DSM 12804

The strain DSM 12804 was originally isolated from a bioreactor culture in Germany enriched from river sediment (Wintzingerode et al., 2001). The genome of *Bordetella petrii* is completely sequenced.

2.2 Laboratory work

2.2.1 Cultivation of *Bordetella petrii*

The strain was obtained as freeze dried culture from the DSMZ (German collection of microorganisms and cell cultures) and cultivated in CASO nutrient medium.

Procedure and materials

- The lyophilized pellet was rehydrated for 30 minutes in 0.5 ml medium
- The mixture was transferred to a test tube with 5 ml medium
- 10 µl were stroked out on an agar plate and incubated overnight
- 5 ml medium were inoculated with a single colony
- Incubation overnight at 27.5° C and shaking (preculture)
- 250 ml medium were inoculated with 1.5 ml of the preculture and incubated until the appropriate optical density for harvesting the OMVs was reached

CASO-medium (1000 ml): 15 g peptone from casein, 5 g peptone from soymeal, 5 g NaCl; pH value adjusted to 7.3; autoclaving; for solid medium 15 g agar were added prior to autoclaving.

2.2.2 Monitoring the optical density (OD)

To establish a growth curve the optical density was determined by spectrophotometry using a “Biophotometer plus” from Eppendorf. The optical density is measured by letting visible light pass through the bacteria suspension, which scatters it depending on the cell concentration of the sample.

Procedure and materials

- 1 ml culture as probe (200 µl bacterial probe was diluted with 800 µl medium)
- 1 ml medium as blank

2.2.3 Epifluorescence microscopy

In this work epifluorescence microscopy was used for the visualization of bacteria and OMVs. Samples of bacterial cultures and of OMV suspensions were stained either with Acridine Orange or with SYBR gold and examined under a Leica DMRB epifluorescence microscope.

2.2.3.1 Staining with Acridine Orange

Procedure and materials

- 10 µl sample were mixed with 990 µl distilled water
- 500 µl dilution were put onto a 0.2 µm pore sized filter and 1 drop Acridine Orange was added
- 500 µl dilution (the rest) were added
- Filtration after one minute of incubation
- Drying in the dark

Acridine Orange solution: 0.3g Acridine Orange, 100 ml A. dest.

2.2.3.2 Staining with *SYBR®gold*

Procedure and materials

- 10 µl sample were mixed with 990 µl distilled water
- Filtration through a 0.02 µm pore sized filter
- Staining for 30 minutes in 60 µl SYBR Gold solution in the dark
- Filtration of 1000 µl distilled water
- Drying in the dark

SYBR Gold solution: 10 µl SYBR®GOLD nucleic acid gel stain (10000x in DMSO, Invitrogen), 390 µl A. dest.;

2.2.3.3 Removing of polysaccharides

In order to prepare bacteria and OMVs for fluorescence microscopy it was necessary to remove the polysaccharides, which appeared as a background of “white clouds”, by treatment with NaCl.

Procedure and materials

- 10 µl bacterial culture or OMV suspension were mixed with 300 µl 0.5M NaCl

• Centrifugation for 10 minutes at full speed
• Supernatant was discarded
• 100 µl distilled water was added
• 10 µl were diluted with 990 µl distilled water

2.2.4 Yield of OMVs

To acquire OMVs from bacterial cultures successive centrifugation and filtration steps were performed. Finally the OMVs were pelleted by ultracentrifugation using a Beckman XL-70 ultracentrifuge with a 55.2 Ti rotor.

Procedure and materials

• Transfer of the bacterial suspension into 50 ml centrifugation tubes
• Centrifugation for 40 minutes at 7500 g and 4°C
• The supernatant was filtered through - 0.45 µm pore sized filter, - 0.22 µm pore sized filter and at last a sterile 0.22 µl pore sized filter
• The sterile filtrate was transferred into quick seal tubes
• The ultracentrifugation was performed for 4 hours at 25.000 rpm and 4°C
• The pellet was dissolved in 100 µl TBT

1x TBT buffer (1000 ml): 5,84 g NaCl, 12,1 g Tris, 0,94 g $MgCl_2$; pH value adjusted to 7,6; autoclaving;

2.2.5 DNA extraction from OMVs

DNA extraction was done by a method established to extract genomic DNA from bacteria (Pitcher et al., 1989). In this method GES reagent is used for cell lyses and the DNA is precipitated by isopropanol.

Procedure and materials

• 100 µl OMV-suspension (in TBT) was mixed with 500 µl freshly prepared GES reagent
• Incubation for 5 minutes at room temperature and for 5 minutes on ice
• Adding of 250 µl ice cold 7.5M ammonium acetate, mixing
• Incubation for 10 minutes on ice

• Adding of 0.54 volume of ice cold isopropanol, mixing
• Incubation for 10 minutes on ice
• Centrifugation for 5 minutes (full speed)
• Discarding the supernatant
• Washing the pellet with 70% ethanol and then with ethanol absolute – both ice cold
• Dissolving the pellet in 10 µl 1x TE at room temperature (overnight)

7,5M ammonium acetate (100 ml): 57,81 g; sterile filtration;
GES reagent (100 ml): 60 g guanidium thiocynate, dissolved in 20 ml 0.5M EDTA and 20 ml distilled water at 65 °C; when cooled down to room temperature 5 ml 10% N-lauroyl-sarkosyl-solution were added and the volume filled up with distilled water; sterile filtration;

2.2.6 Agarose gel electrophoresis

By agarose gel electrophoresis the negatively charged nucleic acid fragments can be separated according their sizes due to their various migration speeds within an electric field. The smaller nucleic acid molecules move faster than the larger ones through an agarose gel in direction to the positive pole. To make the DNA fragments visible under UV light Midori Green was added either to the DNA probe or to the agarose gel.

Procedure and materials

• 1 g agarose was melt in 100 ml 1x TAE buffer
• After cooled down, 3 µl Midori Green were added
• The gel was poured into a gel rack with comb
• After polymerisation the gel was placed in a tank containing 1x TAE and loaded with the samples and the size markers

1x TAE (1000 ml) 4,84 g Tris, 1,14 ml acetic acid, 2 ml 0,5M EDTA;

2.2.7 Construction of a shot-gun library

In order to get sequence information about the “genome” of the OMVs a shot-gun library was constructed. At first the high molecular weight DNA was fragmented randomly by sonication, the DNA fragments “blunted” using the DNATerminator®

End Repair Kit from Lucigen® Corporation and separated by gel electrophoresis. The fragments of desired sizes were eluted from the gel using the illustra GFX™ PCR DNA and Gel Band Purification Kit from GE Healthcare and cloned using the CLONESMART® HCKan Chemically Competent Blunt Cloning Kit (SOLOs) from Lucigen® Corporation.

2.2.7.1 Sonication of DNA fragments

Procedure and material

• 5 µl OMV DNA were added to 195 µl distilled water, mixed and split into 2 reaction tubes (100 µl each)
• Sonication was done by a Branson Sonifier 450: the first sample for 7 seconds at 50 W, the second sample for 10 seconds at 50 W
• During this procedure the tubes were placed on crush ice
• The samples were pooled and precipitated with Ethanol absolute (+ 20 µl 2.5M NaAc + 440µl EtOH)
• Incubation for 1 hour at -20° C
• Centrifugation for 5 minutes at full speed and 4°C
• Washing with 70% EtOH and EtOH absolute
• Drying at room temperature

2.2.7.2 End repair for blunt end cloning

Procedure and material

• The pellet from the sonication step (2.2.7.1) was dissolved in 38 µl distilled water
• 10µl 5x DNA Terminator End Repair Buffer and 2 µl DNA Terminator End Repair Enzyme were added
• Incubation for 30 minutes at room temperature
• Enzyme inactivation by incubating for 15 minutes at 70° C
• Adding of 50 µl distilled water
• DNA precipitation (+ 10 µl 3M NaAc + 200 µl EtOH absolute) for 24 hours at 20°C

The kit used in this step was the „DNATerminator® End Repair Kit“ provided by Lucigen® Corporation.

2.2.7.3 Recovery of DNA fragments desired sizes

Procedure and material

• The pellet from the end repair step (2.2.7.2) was dissolved in 16 µl distilled water and 4 µl 5x DNA loading dye was added
• The probe was separated in a 1% agarose gel
• The desired DNA fraction was localized under UV light and cut out from the gel using a sterile scalpel
• The gel piece was weighed
• For each 10 mg 10 µl capture buffer type 3 were added
• To melt the gel slice an incubation at 60 °C for 15-30 minutes followed
• A GFX MicroSpin column was placed into a Collection tube
• The capture buffer mix was transferred onto the GFX MicroSpin column placed in the Collection tube
• Incubation for 1 minute at room temperature
• Centrifugation for 30 seconds at 16000 g
• The flow through was discarded and the GFX MicroSpin column was placed into the Collection tube again
• 500 µl Wash buffer type 1 were added
• Centrifugation for 30 seconds at maximum speed
• The GFX MicroSpin column was placed in a fresh 1,5 ml tube
• 10 µl A. dest. were transferred to the centre of the GFX MicroSpin column
• Incubation for 1 minute at room temperature
• Centrifugation for 1 minute at full speed
• The flow through contained the DNA

The kit used in this step was the „illustra GFX™ PCR DNA and Gel Band Purification Kit“ from GE Healthcare.

2.2.7.4 Cloning

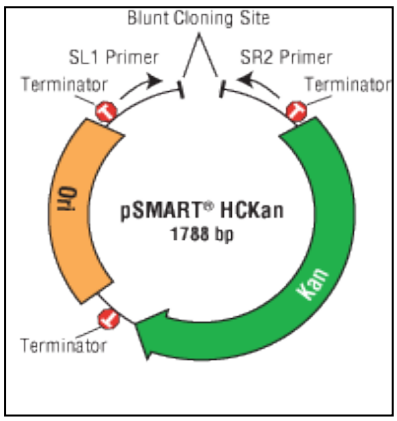

Fig. 2: pSMART® HCKan

The blunt end DNA fragments were ligated into the vector pSMART HCKan (Fig. x), that is component of the "CLONESMART® HCKan Chemically Competent Blunt Cloning Kit (SOLOs)" from Lucigen® Corporation. This high copy plasmid is 1788 bp long and the insert can be separated from the vector by the restriction enzyme EcoRI, which has two - the cloning site flanking - recognition sites within the polylinker. After transformation into the also kit provided competent E.cloni cells, plasmid containing colonies can be selected by the antibiotic Kanamycin.

2.2.7.4.1 Ligation

Procedure and material

- 6.5 µl DNA solution containing blunt end fragments
- Adding of 2.5 µl 4x CloneSmart Vector Premix (vector, ATP, buffer)
- Adding 1 µl CloneSmart DNA Ligase (2u / µl)
- Incubation for 2 hours at room temperature
- Incubation for 15 minutes at 70°C to stop the reaction

2.2.7.4.2 Transformation

Procedure and material

- 40 µl *E. cloni* cells where thawed completely on ice
- Adding of 1 µl ligation reaction
- Incubation on ice for 30 minutes
- Incubation for 45 seconds at 42 °C in a water bath (heat shock)
- Incubation on ice for 2 minutes
- Adding of 960 µl Recovery Medium
- Incubation for 1 hour at 37 °C and 250 rpm

• 100 µl transformed cells were plated on LB kan plates
• Incubation over night at 37 °C in the dark

LB /Kan (1000 ml): 10 g peptone, 5 g yeast extract, 10 g NaCl; pH value adjusted to 7.2-7.6; medium autoclaved; when cooled down 3 ml kanamycin solution (10mg/ml) were added; for solid medium: 15 g agar added prior to autoclaving;

The Recovery Medium is a component of the kit.

2.2.8 Clone analyses

2.2.8.1 Small scale DNA preparation by "Boiling prep"

Procedure and material

• 1.5 ml medium (LB/kan) were inoculated with a single colony taken from the transformation plate
• Shaking overnight at 37° C and 250 rpm (in the dark)
• The overnight culture was transferred into a 1.5 ml reaction tube
• Centrifugation 30 seconds and maximum speed
• The supernatant was removed completely
• The pellet was resuspended in 350 µl STET solution and 25 µl lysozyme solution were added
• Shaking extensively for 3 seconds
• Incubating for exactly 40 seconds in a boiling water bath
• Centrifugation for 10 minutes at maximum speed
• Removing the pellet with a sterile tooth pick
• The remaining supernatant was mixed well with 300 µl PCI
• Centrifugation for 5 minutes at maximum speed
• Transferring 250 µl from the upper phase into fresh tubes
• DNA precipitation by adding of 27 µl 2.5M NaAc (pH=5.2) and 280 µl isopropanol
• Incubation for 5 minutes at room temperature
• Washing with 100 µl ETOH absolute
• Drying at room temperature
• The pellet was dissolved in 10 µl distilled water

STET-solution (100 ml): 2 ml 5M NaCl, 1 ml 1M Tris/HCL (pH:8), 0.2 ml 500 mM EDTA, 5 ml Tritonx100.

Lysozym-solution (1 ml): 10 mg Lysozym.

PCI (50 ml): 25 ml phenol were mixed with 24 ml chloroform and 1ml isopropanol.

2.2.8.2 Restriction analyses

Procedure and material

• 6 µl distilled water, 1 µl plasmid DNA, 1 µl 10x buffer, 1 µl 10x RNAse and 1 µl restrictionenzyme EcoRI were mixed together
• Incubation for 2 hours at 37° C
• Adding of 3 µl 6x loading dye
• Agarosegel electrophoresis

2.2.8.3 Sequencing of selected clones

From those bacterial clones, which were selected for sequence analyses, overnight cultures were made and the recombinant plasmid DNAs isolated by the "QIAprep Spin Miniprep Kit" from Qiagen, resulting in small amounts highly purified DNA suitable for sequencing.

2.2.8.3.1 Small scale DNA preparation by "Spin preparation"

Procedure and material

• 2 ml overnight culture were centrifuged for 30 seconds at 13000 rpm
• Supernatant was removed completely (important!)
• Pellet was dissolved in 250 µl buffer P1
• 250 µl P2 buffer were added and the tube inverted 4-6 times for mixing
• 350 µl N3 buffer were added and the tube inverted for 4-6 times for mixing
• Centrifugation for 10 minutes at 13000 rpm
• Transferring the supernatant onto a spin column
• Centrifugation for 30 seconds at 13000 rpm
• The flow through was discarded

• 500 µl PB were added
• Centrifugation for 30 seconds at 13000 rpm
• The flow through was discarded
• 750 µl PE were added
• Centrifugation for 30 seconds at 13000 rpm
• The flow through was discarded
• Centrifugation for 1 minute at 13000 rpm
• Transferring the column to a fresh collection tube
• Adding of 50 µl distilled water
• Centrifugation for 30 seconds at 13 000 rpm to elute the DNA from the spin column

2.2.8.3.2 Sequencing

Sequencing was done by 4base-lab (Germany) using the primers SL1 (5´-CAG TCC AGT TAC GCT GGA GTC–3') and SR2 (5`-GGT CAG GTA TGA TTT AAA TGG TCA GT–3') that were components of the "CLONESMART® HCKan Chemically Competent Blunt Cloning Kit (SOLOs)" from Lucigen® Corporation.

2.2.9 Electrone microscopy

For this work the preparations were analysed in a transmission electron microscope (TEM, which are used for the investigation of the inner structures of cell thin sections. The preparation were kindly carried out by Ingrid Hassl and Regina Wegscheider. The examination under a Zeiss EM 902 TEM was done by Ao.Univ.Prof. Adolf Ellinger.

2.3 In silico analysis

To analyse the sequences the programs nucleotide blast and blastx were used (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.4 Used chemicals and instruments

Chemicals:	Producers:
A	
Acridine Orange	MERCK GmbH
Agar-Agar	Carl Roth GmbH&CoKG
Agarose	Promega Corporation
Ammonium Sulfate	Carl Roth GmbH&CoKG
Anodisc 25 filter (poresize 0.02µm)	Whatman
C	
Chloroform	Carl Roth GmbH&CoKG
Citifluor	Christiane Gröpel Elektronenmikroskopie
Cyclopore Tracketched Membrane (0.2 µm)	Whatman
D	
DNA Terminator End repair Kit	Lucigen Corporation
DNA Ligase (2u/µl)	Lucigen Corporation CloneSmart HCKan
E	
Ethanol	Carl Roth GmbH&CoKG
ECORI (10u/µl)	Roche
ECORI buffer H	Roche
F	
Formaldehyd	MERCK GmbH
G	
Gene Ruler 1kB Ladder 0,5µg/µl	Fermentas
Gene Ruler High Range DNA Ladder 0,1µg/µl	Fermentas
I	
Isoamylalkohol	Carl Roth GmbH&CoKG
Isopropanol	Carl Roth GmbH&CoKG
K	
Kanamycin (30µg/µl)	Carl Roth GmbH&CoKG
L	
Loading Dye 6X	Fermentas
Lysozyme	Carl Roth GmbH&CoKG
M	
Magnesium Sulfate	Carl Roth GmbH&CoKG
Midori Green	Gene Express
N	
Natrium Chlorid	Carl Roth GmbH&CoKG
Natrium Citrat	Carl Roth GmbH&CoKG

N-Laurylsarcosine	Sigma-Aldrich Handels GmbH
P	
Paraffin oil	Carl Roth GmbH&CoKG
PCI	Carl Roth GmbH&CoKG
Peptone from Casein	Carl Roth GmbH&CoKG
Peptone from Soymeal	Carl Roth GmbH&CoKG
Potassium dihydrogen phosphate	MERCK
Primer SL1 (3,2 pmol/µl)	Lucigen (Clone Smart HCKan)
Primer SR2 (3,2 pmol/µl)	Lucigen (Clone Smart HCKan)
Proteinase K (20 mg/ml)	Carl Roth GmbH&CoKG
pSMART®HCKan vector Kit	Lucigen (Clone Smart HCKan)
Q	
QIAprep Spin Mini Prep Kit	QIAgen GmbH
R	
Rotilabo syringe filter PVDF 0,22µm	Carl Roth GmbH&CoKG
Rotilabo syringe filter PVDF 0,22µm sterile	Carl Roth GmbH&CoKG
Rotilabo syringe filter PVDF 0,45µm	Carl Roth GmbH&CoKG
RNAse (DNAse free)	Roche
S	
SYBR®gold	Invitrogen GmbH
Sodium acetate	MERCK
T	
T4 DNA Ligase (Ligation Kit)	Promega
Trypton/Pepton	Carl Roth GmbH&CoKG
Y	
Yeast Extract	Carl Roth GmbH&CoKG

Instrument:	**Producer:**
Centrifuge	Sigma Laboatory centrifuge 3K30
Power Supply	Biorad
Certoclav	KELOMAT Sterilizer-Division
Dry heat sterilizer	Binder
Epifluorescence Microscope	Leica DMRB
Incubator	Binder
Micro centrifuge 5415D	Eppendorf AG
PCR workstation	Peqlab
Vakuum Pump	KNF Neuberger Type N035AN.18
Fine scale	Sartorius

Spectrophotometer	Eppendorf AG Bio Photometer Plus
Thermobloc	Thermobloc Thriller Peqlab
XL-70 Ultracentrifuge (55.2Ti Rotor)	Beckman Instruments, Inc.
Vertical Laminar Airflow Cabinet Faster, Biohazard BH-EN 2004	Szabo-Scandic
Waterbath	GFL

3 RESULTS

3.1 *Bordetella petrii* shows a rapid growth with typical growth phases

For this work the *Bordetella petrii* strain DSM 12804 was cultivated at 27.5° C in CASO nutrient medium. Growing in fluid medium, the bacteria showed – compared with other species - a fast growth with typical growth phases (Figure 3). The lag-phase lasted about 6 hours and was followed by the exponential phase. The optical density reached after 42.5 hours incubation its maximum and was within the following 24 hours reduced a little bit, thus reaching the 135 hours lasting stationary phase after about 66 hours of incubation. Not until 248 hours of incubation the death phase was reached.

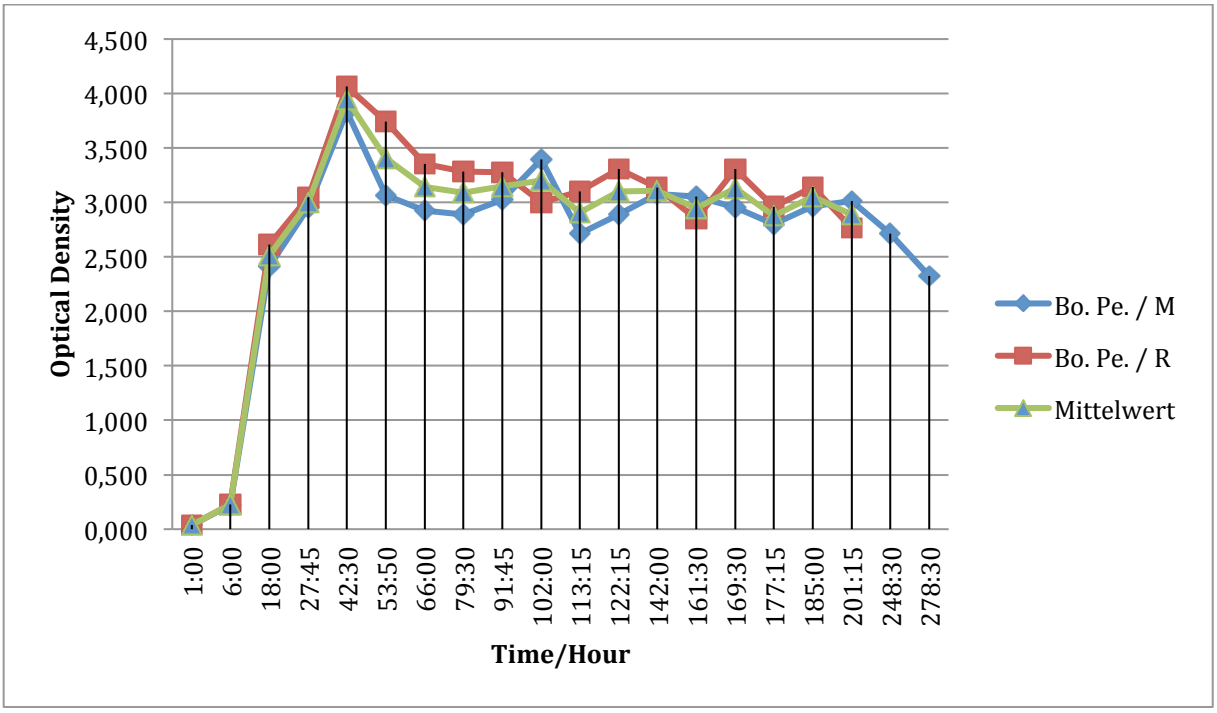

Fig. 3: The growth curve of *Bordetella petrii* shows the typical growth phases lag-, exponential-, stationary-, and death phase.

3.2 The morphological characteristics of *Bordetella petrii*

3.2.1 Epifluorescence microscopy

To analyze the morphotypes of *Bordetella petrii* the bacterial samples were either stained with acridinde orange or with SYBR gold. To remove the background of polysaccharides, that can be seen when nor further treatment was carried out (Figure 4A), the bacterial samples were pretreated with NaCl, resulting in bacterial preparations, which were background free (Figure 4B). As is shown in Figure 4B, *Bordetella petrii* are short or coccoid rods, varying in length between 1.0 and 2.8 and having a diameter of 0.4-0.7µm; sometimes the formation of chains can be observed (Figure 4B).

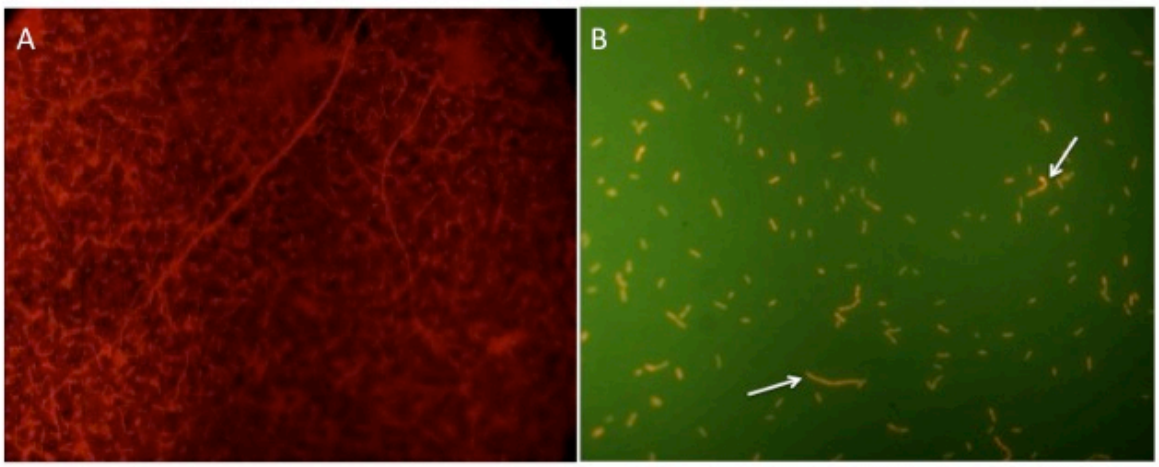

Fig. 4: Epiflourescence micrograph of a *Bordetella petrii* sample without NaCl treatment and stained with Acridine orange (A) and of a sample with NaCl treatment and stained with SYBR gold (B): rods, forming chains are indicated by white arrows.

3.2.2 Electron microscope visualisation

Inspection of *B. petrii* cells by Transmission electron microscope (TEM) towards 15 hours after initiation of the culture and before appearance of OMVs (Figure 5) showed a homogenous plasmic content of both length and cross sections with a dense filamentary network and occasionally electron dense central regions.

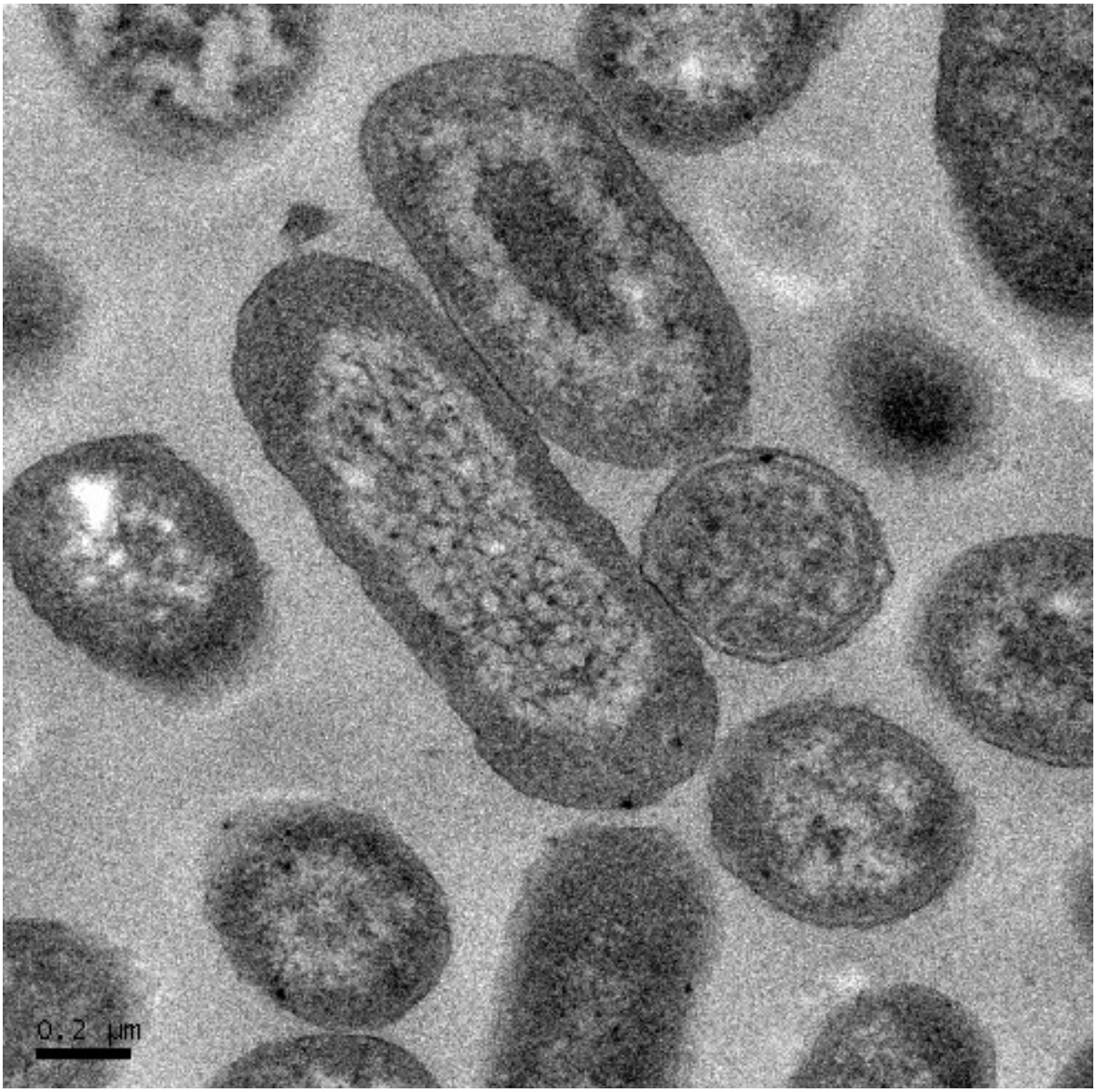

Fig. 5: Micrograph of a *Bordetella petrii* before vesiculasation

3.3 **Production of outer membrane vesicles (OMVs) by Bordetella *petrii***

3.3.1 Epifluorescence microscopy

In order find out when OMV production takes place in *B. petrii,* samples of the liquid culture were taken at 15, 18, 23, 24, 28, 36, 44, 49.5, 56 and 67.5 hours of incubation and stained with SYBR gold.

It could be observed that first OMVs appeared already after 18 hours. However at this time the culture showed a low OMV density, not sufficient for the harvest.

Appropriate OMV densities were found after 24 hours of incubation and this situation persisted up to 100 hours of incubation. The OMV harvests were performed after 56 hours of incubation. To eliminate the background all samples had again to be treated with NaCl.

All samples for AODC had again to be treated with 300 µl 0.5 M NaCl to allow density estimation (Figure 6 A,B).

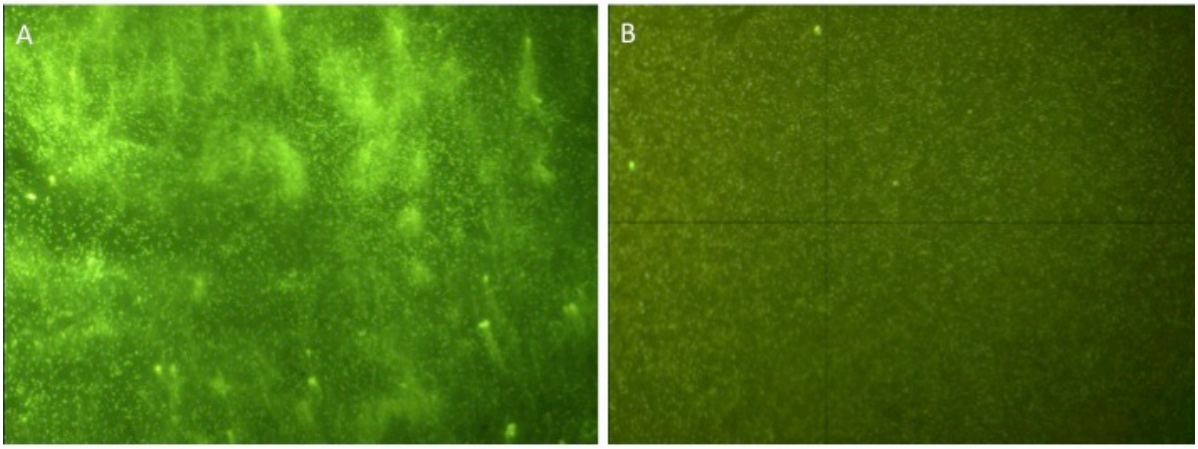

Fig. 6: Epiflourescence micrograph of an OMVs sample without NaCl treatment (A) and of an OMV sample with NaCl treatment, stained with SYBR gold.

3.3.2 TEM of OMVs produced by *B. petrii*

Obtained OMVs showed that their morphotype is a spherical vesicle with a diameter between 60 and 120 nm with a single double membrane (Figure 7).

Within all OMVs an electron dense content was visible, probably a protein / nucleic acid complex as described in earlier investigations (Kulp & Kuehn 2010, Beveridge 1999).

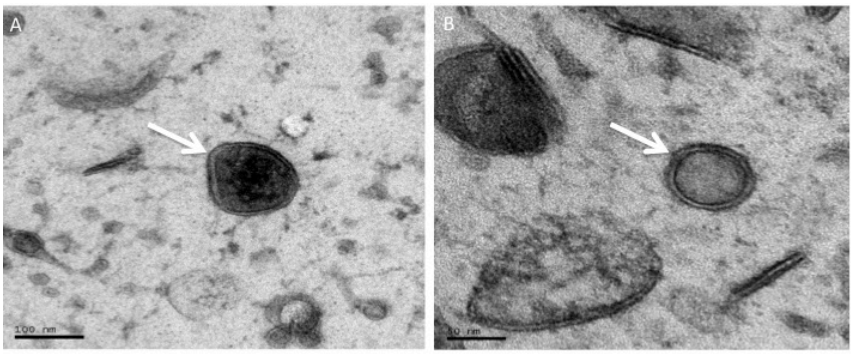

Fig. 7: TE micrograph of OMV samples.

3.4 **DNA extraction from OMVs of *Bordetella petrii***

OMV DNA was extracted from cultures incubated different times: 56 h, 47.5 h and 67.5 h and electrophoresed within a 1% agarosegel. As shown in Figure 8, the obtained DNA is high molecular weight. Considering that the migration of linear DNA fragments through an agarose is not linear but logarithmic, the molecular weight of the extracted DNA was estimated to be far above 40 kbp.

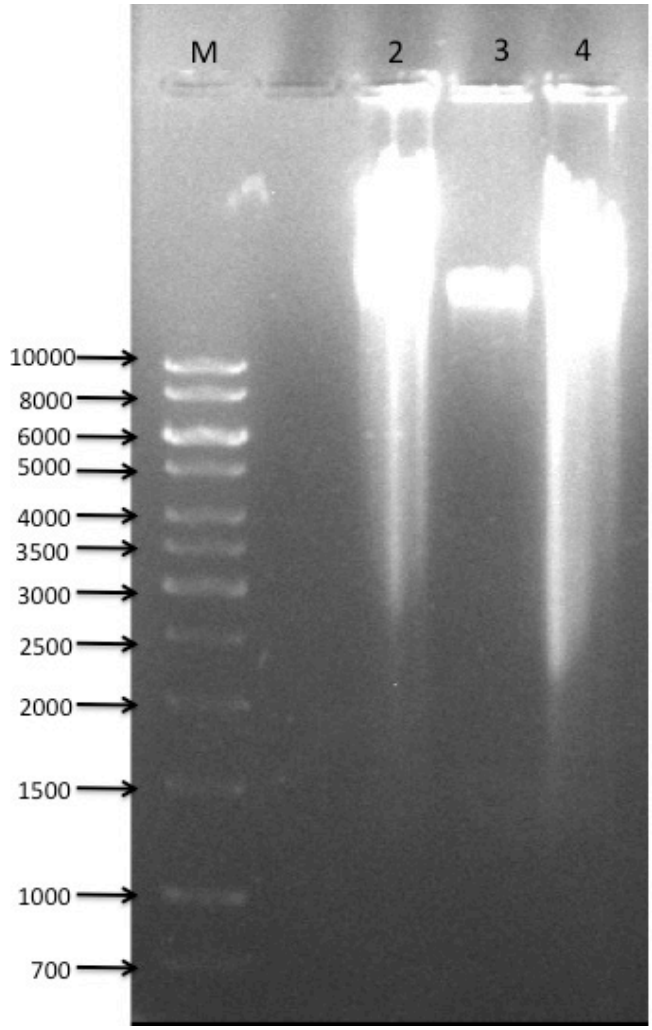

Fig. 8: 1 % Agarosegel electrophoresis of high molecular weight DNA, extracted from different old *B. petrii* cultures: 56 h (lane 2), 47.5 h (lane 3) and 67.5 h (lane 4) . For size estimation a 1kb ladder marker (M) was used, the fragment sizes are indicated at the left side.

3.5 Construction of a shotgun library from the "genome" of the OMVs from *B. petrii*

The shotgun library was constructed as described in chapter 2.2.7. The extracted DNA (DNA extraction separated in lane 1 of Figure 8) was sheared randomly by sonication, the ends of the fragments repaired for blunt-end cloning and purified as well as size fractionated by agarosegel electrophoresis (Figure 9 A, B). The gel slice containing fragments between 700bp and 3000bp was cut out and the DNA eluted and cloned using the CLONESMART® HCKan Chemically Competent Blunt Cloning Kit (SOLOs) from Lucigen® Corporation.

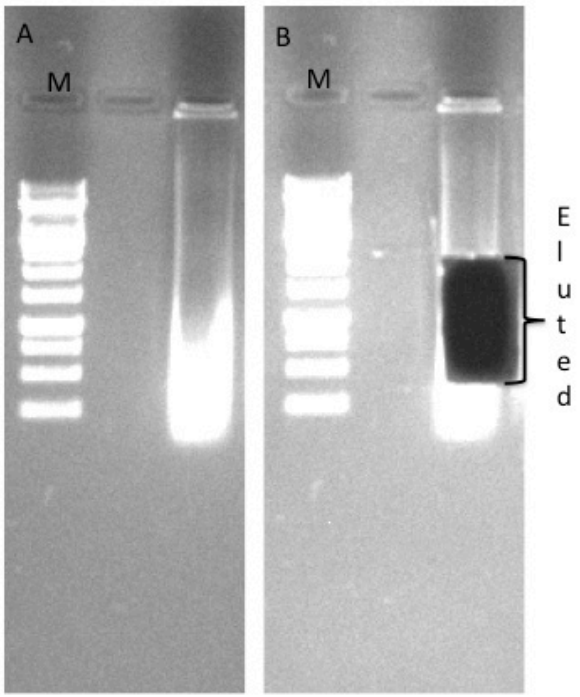

Fig. 9: 1 % Agarosegel preparitive electrophoresis gel with randomly shared OMV DNA before (A) and after (B) excision of the gel slice from which the DNA was eluted.

3.6 Selection of recombinant clones for sequence analysis

Plasmid DNA was extracted from 44 colonies by boiling prep and tested for the presence and size of the inserts by restriction enzyme treatment with EcoRI (Figure 19 A-D). All clones were prepared again using the QIAprep Spin Miniprep Kit to gain highly purified DNA for the sequencing reactions and their DNA concentrations were determined. Sequencing was carried out by the 4base lab in Germany from 43 clones (all with exception of clone 34). Together with the DNA the sequencing

primers SL1 and SR2, components of the used cloning kit, were shipped to the 4base lab.

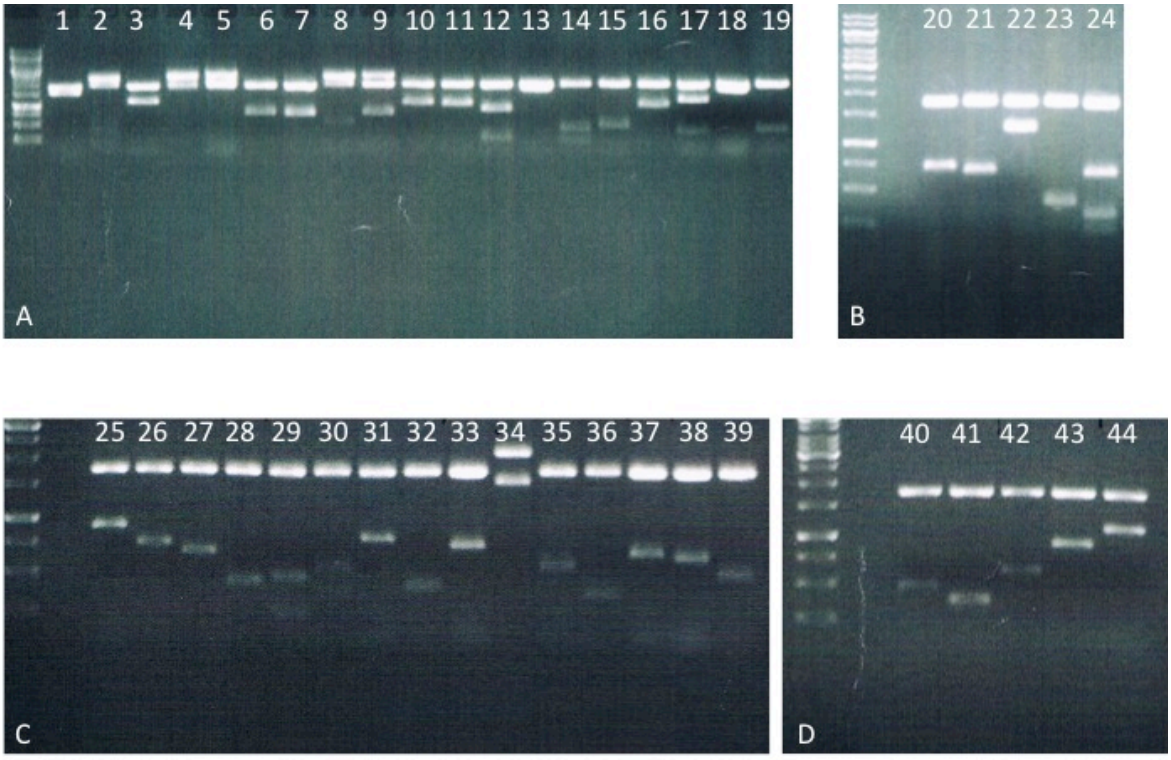

Fig. 10: Four (A-D) 1 % agarose gels with EcoRI digested DNA from 44 recombinant clones. For further analysis all clones (with exception of clone 34) were shipped to the 4base lab for sequencing.

3.7 DNA-sequence analyses of the selected clones

The raw data obtained from the 4base lab by email were processed: the vector sequences were deleted and sequences were joined together when an overlapping region was found in those clones that were sequenced from both directions. By blast search using the programs nucleotide blast and blastx the position of the sequence within the genome of *B. petrii* as well as the homologies to proteins were determined.

Clone TM1

Blastx search indicated a homology to the coding sequence of maleylacetate reductase and a further hypothetical protein.
E-Value: 0.0

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Query  1        GTTTCGCCCGGCGAAGTCGCGTATCTCGCGCCGCGCAAGAATTTTCGGATGTTGTGGCAG  60
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Sbjct  3962747  GTTTCGCCCGGCGAAGTCGCGTATCTCGCGCCGCGCAAGAATTTTCGGATGTTGTGGCAG  3962806

Query  61       CGCGGTGCACAGCAGCTGATTCTGAAGGTGCCCGACACCTTGCTGCGTTCCATTGTGGCC  120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3962807  CGCGGTGCACAGCAGCTGATTCTGAAGGTGCCCGACACCTTGCTGCGTTCCATTGTGGCC  3962866

Query  121      GAGGCGGGCGCGTGCCTGGACTTGCCGTCAACCATGAGATTGACGCCGGCAGCCGCCAGG  180
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Sbjct  3962867  GAGGCGGGCGCGTGCCTGGACTTGCCGTCAACCATGAGATTGACGCCGGCAGCCGCCAGG  3962926

Query  181      CAATGGCACTTGTTCATGCAATCGCTGCTCAACATCGGTACCGCGCCAAATGAGTTGCGG  240
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Sbjct  3962927  CAATGGCACTTGTTCATGCAATCGCTGCTCAACATCGGTACCGCGCCAAATGAGTTGCGG  3962986

Query  241      CTCGATGAAGGATGGGTTGACCATTTCGAGAAGAACATCGCGTTCTTCCTGCTGAGCCAG  300
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Query  301      AGGCCGTCGCAACAACGGCCGCGTGACGGAGTCGCCACTGCCTCGCCGCCAGTCCTTATT  360
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Sbjct  3963107  CGCTCTTCGTCCTGCACCGGTGGAACCTCGAGCGCCCTGGAGCGCCTGGAGGCGATGGAG  3963166

Query  421      CGTTACATCCGCAGGCACCTCTGTGCTCCGGTGGGCGTCATCGATCTCGCGCGGGCAGCG  480
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Sbjct  3963227  GGCGTGAGTGTTCGCACCCTCAACATCATTTGCCGGG  3963263
```

Clone TM2

Blastx search across the entire clone indicated a homology to the protein putative glycine betaine/choline/proline transport system.

e-Value: 1e-79

Locus: YP-001631920

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Sbjct  3472064  CGAGCGTGCCGCGGGCGATACGGCCGGACGGTTGCCGGCCAGCCGCATCGCCCCCGGCGC  3472123

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Sbjct  3472124  GCGGGCCCGGGGCGCGCGGCCTTGGCCGCGCACGGGCAAAACGCCGGACAGGCCGGCGGC  3472183

Query  120      gcgcgccatcacggcgcggcgcggcggcctgcttgccgcttacagcgcggcctggacgcg  179
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Sbjct  3472184  GCGCGCCATCACGGCGCGGCGCGGCGGCCTGCTTGCCGCTTACAGCGCGGCCTGGACGCG  3472243

Query  180      cgTCGCGACCTCGGCGGGCACCCACTTGGTCCAGACATCGGACTTGTTCTTCAGGAACCA  239
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Sbjct  3472244  CGTCGCGACCTCGGCGGGCACCCACTTGGTCCAGACATCGGACTTGTTCTTCAGGAACCA  3472303

Query  240      CTGGGCAACGGCGGACGAATCGTCGCCGGACTCTTCCATGTGCGCCAGGGTCTGGTCGAC  299
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Sbjct  3472304  CTGGGCAACGGCGGACGAATCGTCGCCGGACTCTTCCATGTGCGCCAGGGTCTGGTCGAC  3472363

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Sbjct  3472364  CTCGGGCAGCGGGATGCTGACTTTGGACAGGAACTCGGTCAGCTTGGGGGCCTGCTTCGA  3472423
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Sbjct  3472424  GAATTCGGTATTGACGGCGGTGAACACCGGGTTCTCGGGGTAGGCGCTGGGCTGCGGGTT  3472483

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Query  480      CTTGACCAGATCCAGCGCGCCCACCAGCGGGGTGGGATACCAATAG-AGAAC  530
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Sbjct  3472544  CTTGACCAGATCCAGCGCGCCCACCAGCGGGGTGGGATACCAATAGTAGAAC  3472595
```

Clone TM3

The blastx search across the entire clone indicated a homology of the DNA-sequence to the protein, amine oxidase, flavin-containing

E-value: 6e-175

Locus: YP-001632821

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Sbjct  4449136  GGGCAGGTCACCACGGCATAGTCGGTCTGCAGCACGCTCTCGCCCGCTGCGCCGTCGCGT  4449195

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Sbjct  4449196  TGGTAGACGATACGCGCACCGTCCGAGATGTTGTCAATGCGAATGGCAGTCCCGCCCAGC  4449255

Query  121      AACAAGCGTGGACCCAGCGCACGCGCGAAGGCTTCGCTGATGGCGTCCATGCCGCCTATG  180
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Sbjct  4449316  GGCTGCATCATTGTCGGCTGGTATTCGAGATGCTCGTCATACCAGAGCGGATACCAAAGG  4449375

Query  241      CGGCGATCGAGCAGTGTACGCAGTTCCAGCGAGTCGCGCAGGGTACCCGCGACTTCACCC  300
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Query  301      GCCGCGGGACTGACGGCATAGCCCGAGCGGCGCGACCCCGCGAAACTGCCGTCGACCCGC  360
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Sbjct  4449556  GAATTGAGCGCGCCCTGGCTGGCGCACTTGGCCAGCAACTCGGAGAGTAACCCGCGCGCG  4449615

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Sbjct  4449616  TCGCCTTGGAGTTGCCCCACCGTGTAGCGCCGGTCGCCCCCGCCCTCTTGAACCACGCAC  4449675

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Sbjct  4449736  CCCAGCAAAGCACGGTGGGTACTGGGGATGCGGGCTGCGCCCGCATTGAAGTACAGGTCA  4449795

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Sbjct  4449796  GAAGCAAAGCCGGCGTGCTGTACCGTGCCATCGTCGTAGCACAATGAGTCGCCGGCGCGT  4449855

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Sbjct  4450036  CCTCGTGGTACGAGAGGCGCACCCAGCGCCTCGGCCGCCGCCAACGCCGCCGCAGGCCCA  4450095

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Sbjct  4450096  ACCGCGGCGGCCAGTCCAACGATGAAAGCCCGTCTGCTCAGTTCCATCACGCCCCTTGAA  4450155

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Sbjct  4450156  AGCTCGGATGGGACCGGGAGCGCTAAGCAA  4450185
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Clone TM4

The blastx search across the entire clone indicated a homology of the DNA-sequence to the protein, Methionine biosynthesis protein MetWAmpG

E-value: 9e-165

Locus: YP-001629022

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Query  1        AGAGATTCTCGATGCCCACCGCGGCGGCCATCAGCCAGATGTTCTTGGGGCTGACGGCGA  60
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Sbjct  455458   AGAGATTCTCGATGCCCACCGCGGCGGCCATCAGCCAGATGTTCTTGGGGCTGACGGCGA  455399

Query  61       TGACCCAGTAGCCCAGGTTGGATATGGCCTGCAAGACGCCGAACGCCATCAATGAGCGAT  120
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Sbjct  455398   TGACCCAGTAGCCCAGGTTGGATATGGCCTGCAAGACGCCGAACGCCATCAATGAGCGAT  455339

Query  121      ACAGCCCCCAGCGCGCCATCAGCGAACCGCCGGCCAGCGCGCCGACGATAGTGGCGGCCA  180
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Query  301      CCAGCACGGTATAGGCGCCGCGCCGGCTGAAGAATTCGCGAAACGGCTCGACCACGGCCA  360
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Query  421      AGGCGCAGGCCAGCATCAGGCCGCCCATTAATACATAGGTGGCGCCCCAGCCCAGCCATT  480
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Query  601     CGAAGGCAATGTCCTGAGTAGCCGAGAAAAACGCCACCGCCACGGCCAGCA-GGTCAGCG  659
               ||||||||||||||||||||||||||||||||||||||||||||||||| || |||||
Sbjct  454858  CGAAGGCAATGTCCTGAGTAGCCGAGAAAAACGCCACCGCCACGGCCAGCAGGGCCAGCG  454799

Query  660     GCAACAGGGCCGAGGCCGGCGACAGCGTGCCCATGAGCATGATGGAAGC--GCAGCAGCA  717
               |||||||||||||||||||||||||||||||||||||||||||||||||    ||||||||
Sbjct  454798  GCAACAGGGCCGAGGCCGGCGACAGCGTGCCCATGAGCATGATGGAAGCCGCCAGCAGCA  454739

Query  718     CCTGCGTCA-CAGCAT-CAGCCGCGCCGGCGT-CGAGCACGCGCGGCACGTACCGGTCGA  774
               ||||||||| ||||||| |||||||||||||| |||||||   ||||||||||||||||||
Sbjct  454738  CCTGCGTCACCAGCATCCAGCCGCGCCGGCGTCCGAGCAACGGCGGCACGTACCGGTCGA  454679

Query  775     CCAGCGGCGCCCATAGGAACTTCAGCGTGTAGGCCGTTCCCACCAGGGTCAGGAAACCGA  834
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  454678  CCAGCGGCGCCCATAGGAACTTCAGCGTGTAGGCCGTTCCCACCAGGGTCAGGAAACCGA  454619

Query  835     TTTCTTCCAGCGATACGCCGTCGACCGTGGCCCACGCCTGCAGCGTGCCGCCCGTCAGCG  894
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  454618  TTTCTTCCAGCGATACGCCGTCGACCGTGGCCCACGCCTGCAGCGTGCCGCCCGTCAGCG  454559

Query  895     CCAGCGGCAGCCCGCTGGCAAACCCCAGCACCAGCAGGGGCGCGACCCGGCGGCTGAAAT  954
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  454558  CCAGCGGCAGCCCGCTGGCAAACCCCAGCACCAGCAGGGGCGCGACCCGGCGGCTGAAAT  454499

Query  955     AGACATGGGATGCGGCGGAAATAAGAAGCTCCTGGCAACAAAGGCGCCGCGGCGCCGGAC  1014
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  454498  AGACATGGGATGCGGCGGAAATAAGAAGCTCCTGGCAACAAAGGCGCCGCGGCGCCGGAC  454439

Query  1015    ACGGCATATTAACCAATGGCCGCCAGAAGCAGCTTCAATCGGCCGCGTAGCGGTACACCG  1074
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  454438  ACGGCATATTAACCAATGGCCGCCAGAAGCAGCTTCAATCGGCCGCGTAGCGGTACACCG  454379

Query  1075    CCAGCGTGCTGCGCCAGCCCGGCAACACGGTGATTTCGCGGCCTTCGTGGAAGGTGGCGC  1134
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  454378  CCAGCGTGCTGCGCCAGCCCGGCAACACGGTGATTTCGCGGCCTTCGTGGAAGGTGGCGC  454319

Query  1135    GTTCGAGGATGCGCAGCCCCAGATCCTGGGCCAGCCGCTCGAAATCGCGCAGCGTGCACA  1194
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  454318  GTTCGAGGATGCGCAGCCCCAGATCCTGGGCCAGCCGCTCGAAATCGCGCAGCGTGCACA  454259

Query  1195    GGTGGATGTTGGGCGTGTTGTACCACTGGTAGGGCATCTGCCCCGTCACGGGCATGCGCC  1254
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  454258  GGTGGATGTTGGGCGTGTTGTACCACTGGTAGGGCATCTGCCCCGTCACGGGCATGCGCC  454199

Query  1255    CGCGCAGGATCGACCAGCCGTGCGGCCAGTAGCCGAAATTGGGAAACGACACGATGCCGT  1314
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  454198  CGCGCAGGATCGACCAGCCGTGCGGCCAGTAGCCGAAATTGGGAAACGACACGATGCCGT  454139

Query  1315    AGCGCGCCACCCGCGCCATTTCGCGCAGAATGTGCTCGGTGCGGTGCATCGACTGCAGCG  1374
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  454138  AGCGCGCCACCCGCGCCATTTCGCGCAGAATGTGCTCGGTGCGGTGCATCGACTGCAGCG  454079

Query  1375    TCTGCG  1380
               ||||||
Sbjct  454078  TCTGCG  454073
```

Clone TM5

The blastx search across the entire clone indicated a homology of the DNA-sequence to the protein, A/G-specific adenine glycosylase.

E-value: 0.0

Locus: YP-001629100

```
Query  1       TGTACCCGGGGTCCGGGGCGGATGCAAGCTGGGGTTGTTCCGTCTGTTTTCTAGCGGCTT  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  542898  TGTACCCGGGGTCCGGGGCGGATGCAAGCTGGGGTTGTTCCGTCTGTTTTCTAGCGGCTT  542839

Query  61      CTCGCGGTTCCCTATCTTTCACCGTCCCGCCTCATCTGGGCAGGCGTGGAGCGCCCGTGC  120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  542838  CTCGCGGTTCCCTATCTTTCACCGTCCCGCCTCATCTGGGCAGGCGTGGAGCGCCCGTGC  542779
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Query  121     GTCGGGCTTAGTCCGGCCCCACGCCCGCCACGCCCAGGCGGCTAAAGAACTCAGAAACCA  180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  542778  GTCGGGCTTAGTCCGGCCCCACGCCCGCCACGCCCAGGCGGCTAAAGAACTCAGAAACCA  542719

Query  181     ATACAACCCAAACGCCAATGCGATAATCCCCGATTATGGATTTCGCCCCCCGCATCGTCG  240
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  542718  ATACAACCCAAACGCCAATGCGATAATCCCCGATTATGGATTTCGCCCCCCGCATCGTCG  542659

Query  241     CCTGGCAAGCCAGCCACGGCCGCCACGACCTGCCCTGGCAACGCACGCAAGACCCCTACC  300
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  542658  CCTGGCAAGCCAGCCACGGCCGCCACGACCTGCCCTGGCAACGCACGCAAGACCCCTACC  542599

Query  301     GGATCTGGCTGTCCGAGATCATGCTGCAGCAGACCCAGGTCGCCACCGTCATCCCGTACT  360
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  542598  GGATCTGGCTGTCCGAGATCATGCTGCAGCAGACCCAGGTCGCCACCGTCATCCCGTACT  542539

Query  361     ACCAGCGCTTCCTGGAACGCTTTCCCGACGTAAGCGCGCTGGCGGCCGCGCAGCAAGAAG  420
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  542538  ACCAGCGCTTCCTGGAACGCTTTCCCGACGTAAGCGCGCTGGCGGCCGCGCAGCAAGAAG  542479

Query  421     AAGTCATGCCCTACTGGGCCGGCCTGGGCTACTACGCGCGCGCCCGCAACCTGCACCGTT  480
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  542478  AAGTCATGCCCTACTGGGCCGGCCTGGGCTACTACGCGCGCGCCCGCAACCTGCACCGTT  542419

Query  481     GCGCGCAGGCCGTCATGAGCGAATGGGGCGGACGGTTTCCCGCCGCCGCGGAACAGATCG  540
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  542418  GCGCGCAGGCCGTCATGAGCGAATGGGGCGGACGGTTTCCCGCCGCCGCGGAACAGATCG  542359

Query  541     CCACCCTGCCCGGCATCGGCCGCTCAACCGCCGCCGCCATCGCGGCCTTCGCCTACGGCG  600
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  542358  CCACCCTGCCCGGCATCGGCCGCTCAACCGCCGCCGCCATCGCGGCCTTCGCCTACGGCG  542299

Query  601     AGCGCGCCCCCATTATGGATGGCAACGTCAAGCGT  635
               ||||||||||||||||||||||||||||||||||
Sbjct  542298  AGCGCGCCCCCATTATGGATGGCAACGTCAAGCGT  542264
```

Clone TM6

The blastx search across the entire clone indicated a homology of the DNA-sequence to the protein, fumarate reductase iron-sulfur.

E-value: 1e-118

Locus: YP-001629002

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Query  1       ACATCGCACGAGGCATAACAAACCCCGCAGCCAATGCACTCGATGCCCGCATCGGCCGCG  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  436057  ACATCGCACGAGGCATAACAAACCCCGCAGCCAATGCACTCGATGCCCGCATCGGCCGCG  435998

Query  61      CGCCGCCGCGGCGAAGACGGGTCGACGCGCGCCACCGGATCATGGCGGCCGGCAGGCCCG  120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  435997  CGCCGCCGCGGCGAAGACGGGTCGACGCGCGCCACCGGATCATGGCGGCCGGCAGGCCCG  435938

Query  121     GTGTAGCGGCCTTGCGCGCGCTGCCATTTGTCGAAGAACGGCGCCATGTCGGTGACCAGG  180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  435937  GTGTAGCGGCCTTGCGCGCGCTGCCATTTGTCGAAGAACGGCGCCATGTCGGTGACCAGG  435878

Query  181     TCTTTGACGATAGGCAGGTTGGACAAGGGCGCCAGCTCGATGCGGCCATTGCGCGCCACG  240
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  435877  TCTTTGACGATAGGCAGGTTGGACAAGGGCGCCAGCTCGATGCGGCCATTGCGCGCCACG  435818

Query  241     CGCGAGACATGAGTTCGGCAGGTCCAGCGCGCCACCCCATTGACCGTCATGGCGCACGAC  300
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  435817  CGCGAGACATGAGTTCGGCAGGTCCAGCGCGCCACCCCATTGACCGTCATGGCGCACGAC  435758

Query  301     CCGCACATGCCCACGCGGCACGCATAGCGGTACGACAGGGTGGAATCCAGGTGCCGCTGA  360
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  435757  CCGCACATGCCCACGCGGCACGCATAGCGGTACGACAGGGTGGAATCCAGGTGCCGCTGA  435698
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Query  361     ATGTGCGTGGCCACGTCCAGCACGGTCTGGTTATCGCGGCGCGGCACCTCGAACACCTGA  420
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  435697  ATGTGCGTGGCCACGTCCAGCACGGTCTGGTTATCGCGGCGCGGCACCTCGAACACCTGA  435638

Query  421     AAGCCGCCGTCGGCGCCGCCGCGCCACACACTGACCTGCAAAACGCCGGAAGTCGGATCT  480
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  435637  AAGCCGCCGTCGGCGCCGCCGCGCCACACACTGACCTGCAAAACGCCGGAAGTCGGATCT  435578

Query  481     TGTCGTGGCATGCCTGTTCAGCCCCCTACGCAATGCTCTTCATGGTGCCGCGCGCTTTGA  540
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  435577  TGTCGTGGCATGCCTGTTCAGCCCCCTACGCAATGCTCTTCATGGTGCCGCGCGCTTTGA  435518

Query  541     CAAAAGTAAAACTATTAATTATTTTCAGCAGGCCAAGTTTTTCTTATATATGCGCCATGT  600
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  435517  CAAAAGTAAAACTATTAATTATTTTCAGCAGGCCAAGTTTTTCTTATATATGCGCCATGT  435458

Query  601     CGTCGATCCGCCAATTCCGCACTGCGCTGGCCGCTGCCCGGCTGGGCAGCTTCGTGGCGG  660
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  435457  CGTCGATCCGCCAATTCCGCACTGCGCTGGCCGCTGCCCGGCTGGGCAGCTTCGTGGCGG  435398

Query  661     CCGGCCGCCACGTCGGACTGACCCAGGCCGCCGTCAGCCTGCAGATCAAGAAC  713
               |||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  435397  CCGGCCGCCACGTCGGACTGACCCAGGCCGCCGTCAGCCTGCAGATCAAGAAC  435345
```

Clone TM7

The blastx search across the entire clone indicated a homology of the DNA-sequence to the protein putative glycosyltransferase.

E-value: 2e-102

Locus: YP-001629481

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Query  1       GTGTGTATGAATCCCTACGGCGGCAGACCTGTCGTGACTTCGAATGGCTGGTCGTGGATG  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  972460  GTGTGTATGAATCCCTACGGCGGCAGACCTGTCGTGACTTCGAATGGCTGGTCGTGGATG  972519

Query  61      ATGGTTCCACTGACCGCACCCACGAGGCCGTGCTCGCCTGGCAGGCGCAGGCCGATTTCC  120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  972520  ATGGTTCCACTGACCGCACCCACGAGGCCGTGCTCGCCTGGCAGGCGCAGGCCGATTTCC  972579

Query  121     CCATTCGCTACGTGTGGCAGAAAAACCGCCACAAGAAAACCGCATTCAATCGCGGCGTGC  180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  972580  CCATTCGCTACGTGTGGCAGAAAAACCGCCACAAGAAAACCGCATTCAATCGCGGCGTGC  972639

Query  181     GCGAGGCGCGCGGCGAGTTCGTGGTCACGCTGGATAGCGACGACGAAATTCCGCCGCGGG  240
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  972640  GCGAGGCGCGCGGCGAGTTCGTGGTCACGCTGGATAGCGACGACGAAATTCCGCCGCGGG  972699

Query  241     CGCTGCAGATCCTGCAAGAAGCCTGGGACGCTATTGCACCCGGGCAGCGCGAGGGTTATG  300
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  972700  CGCTGCAGATCCTGCAAGAAGCCTGGGACGCTATTGCACCCGGGCAGCGCGAGGGTTATG  972759

Query  301     TGGGCGTGACCGGCCTGTGCGCCCGCCCCGACGGTACCATCGTGGGCGACGCCTTTCCTC  360
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  972760  TGGGCGTGACCGGCCTGTGCGCCCGCCCCGACGGTACCATCGTGGGCGACGCCTTTCCTC  972819

Query  361     AGGATGTTTTCGATACGTCGGCGGTCGAGATGTATTTCCGCCATCGCATCAAGGGCGAGA  420
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  972820  AGGATGTTTTCGATACGTCGGCGGTCGAGATGTATTTCCGCCATCGCATCAAGGGCGAGA  972879

Query  421     AATTCGGCAGCATGCGCACCGACGTTTTGCGCCGCTTCCCGTTTCCCGAAGACGTGGAAG  480
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  972880  AATTCGGCAGCATGCGCACCGACGTTTTGCGCCGCTTCCCGTTTCCCGAAGACGTGGAAG  972939

Query  481     GCTTCGTGCCCGAAAGCCTGATCTGGTGGGCCATGGCGCGCGCCGGCTATCTGAACCGCT  540
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  972940  GCTTCGTGCCCGAAAGCCTGATCTGGTGGGCCATGGCGCGCGCCGGCTATCTGAACCGCT  972999

Query  541     GCATCAACCAGGTGGTGCGCATCTACCATCCCAGCCCCGATGGGCCTGAGCCGCGGCGCC  600
               |||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||
Sbjct  973000  GCATCAACCAGGTGGTGCGCATCTACCATCCCAGCCCCGAT-GGCCTGAGCCGCGGCGCC  973058
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Query  601     GTGTCGGTGCGCAACAATGCGCA-GGCCTGTACCTGCTGGCCTGGGACATCCTGGAACAC  659
               ||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct  973059  GTGTCGGTGCGCAACAATGCGCAGGGCCTGTACCTGCTGGCCTGGGACATCCTGGAACAC  973118

Query  660     CACATGGAACTGGTTCCGCTACCGCCC  686
               ||||||| ||||||||||||||| ||
Sbjct  973119  CACATGG-ACTGGTTCCGCTACCGGCC  973144
```

Clone TM8

The blastx search across the entire clone indicated a homology of the DNA-sequence to the protein transcriptional regulator, TetR-family

E-value: 2e-39

Locus: YP-001628968

```
Query  1       CGCCGGGCTGGTACCAGCGCGCCATCCAGTTCAGCATGGACAGCACCGCGCGCCCGGTGG  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  403341  CGCCGGGCTGGTACCAGCGCGCCATCCAGTTCAGCATGGACAGCACCGCGCGCCCGGTGG  403282

Query  61      ACGCGACGTCCATCGCCCGGAACTCGCCGCTGGCCATGCCATCGGCCAGAATGCCGCGCA  120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  403281  ACGCGACGTCCATCGCCCGGAACTCGCCGCTGGCCATGCCATCGGCCAGAATGCCGCGCA  403222

Query  121     GCATGCGCTCGTAGCCGTCGCGCAGACGGGCAGCGTCGTCGCGTTCGGGAATGGCCATGC  180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  403221  GCATGCGCTCGTAGCCGTCGCGCAGACGGGCAGCGTCGTCGCGTTCGGGAATGGCCATGC  403162

Query  181     CGGAATAGCCCACCAGCATGGTGACGAACTCCGGGTGGTGCTCTTCAAAATAGCGGGCGT  240
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  403161  CGGAATAGCCCACCAGCATGGTGACGAACTCCGGGTGGTGCTCTTCAAAATAGCGGGCGT  403102

Query  241     GGGCCAGCATGAACTGGCGCAAGCGCCCGGACGCCGTATCAGCCTGCGCCACCGCTTGCG  300
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  403101  GGGCCAGCATGAACTGGCGCAAGCGCCCGGACGCCGTATCAGCCTGCGCCACCGCTTGCG  403042

Query  301     ACACGGCGACGGTAAGGCCGCCCACCACTTCGAGAATGATGGCGTCGTAGATGTCTTGCT  360
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  403041  ACACGGCGACGGTAAGGCCGCCCACCACTTCGAGAATGATGGCGTCGTAGATGTCTTGCT  402982

Query  361     TGGTGGTGTAGTAATGGTAGATGGCCGCCTTGGACACCCCCAGGGCCGCGGCCAGGTCGG  420
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  402981  TGGTGGTGTAGTAATGGTAGATGGCCGCCTTGGACACCCCCAGGGCCGCGGCCAGGTCGG  402922

Query  421     CCACCGAACTGTTTTCGTAGCCGCTGCGGGCGAACAGGCGCGCCGCTTCTTCCAGGATGC  480
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  402921  CCACCGAACTGTTTTCGTAGCCGCTGCGGGCGAACAGGCGCGCCGCTTCTTCCAGGATGC  402862

Query  481     GT  482
               ||
Sbjct  402861  GT  402860
```

Clone TM9

The blastx search across the entire clone indicated a homology of the DNA-sequence to the conserved hypothetical protein Bpet 1311

E-value: 6e-80

Locus: YP-001629916

```
Query  1        CTACACGGTCAAAACGCATGTCCTTGGTTCCGCACCGGCCAAGCTTCCC-TCCCCGAGCC  59
                ||||||||||||||||||||||||||||||||||||||||||||| ||| ||||||||||
Sbjct  1374050  CTACACGGTCAAAACGCATGTCCTTGGTTCCGCACCGGCCAAGCTGCCCATCCCCGAGCC  1374109

Query  60       ACAGTGCCTGGAACTGGGCCAGCTCATCCAGCAGTTGGAGGAAATGCCGGCGCCCGATGG  119
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1374110  ACAGTGCCTGGAACTGGGCCAGCTCATCCAGCAGTTGGAGGAAATGCCGGCGCCCGATGG  1374169

Query  120      CTTCCGGCGTATCACCCACATGCTGGTCGATGCAGGCGCCCGCGACTTCACCTGGGTCGA  179
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1374170  CTTCCGGCGTATCACCCACATGCTGGTCGATGCAGGCGCCCGCGACTTCACCTGGGTCGA  1374229

Query  180      TCCGAAGCCGTCCAGGCTCATCGAAACACCCCCGGCGATCAGCTTCACGGTTTCGACCGC  239
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1374230  TCCGAAGCCGTCCAGGCTCATCGAAACACCCCCGGCGATCAGCTTCACGGTTTCGACCGC  1374289

Query  240      GAAGTTCGCCGGCCAGGTGACGATCCTCTACGATCGCGGCGGCGACACCTACGTGGTGGA  299
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1374290  GAAGTTCGCCGGCCAGGTGACGATCCTCTACGATCGCGGCGGCGACACCTACGTGGTGGA  1374349

Query  300      GCTGCATCGTGATGGTGAACTGGTCGATCGGCACGACGAGGTGTATTTCGACATGCTCGG  359
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1374350  GCTGCATCGTGATGGTGAACTGGTCGATCGGCACGACGAGGTGTATTTCGACATGCTCGG  1374409

Query  360      CGAAGTGCTGGAGCGCTTCATCGACGACGGGCGCTGGCGCCTGATCGACGTGAGCGTGAT  419
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1374410  CGAAGTGCTGGAGCGCTTCATCGACGACGGGCGCTGGCGCCTGATCGACGTGAGCGTGAT  1374469

Query  420      CGACACCAAGCCCGCCCGGCGACGCCAGGCAGTGCTTGTGTGACTTTCACGGCCAGCGGT  479
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1374470  CGACACCAAGCCCGCCCGGCGACGCCAGGCAGTGCTTGTGTGACTTTCACGGCCAGCGGT  1374529

Query  480      TCCTGGGTGGTGTATTCGCCCAACGAGTCGGCCGTCAGCGACAGTGCTGGCTTCTGGTCC  539
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1374530  TCCTGGGTGGTGTATTCGCCCAACGAGTCGGCCGTCAGCGACAGTGCTGGCTTCTGGTCC  1374589

Query  540      AACGAGTTCGGTGGGTCCCGCTCGACCAGGCGACGTGTTTCACGACCGATGAGGCCCGTG  599
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1374590  AACGAGTTCGGTGGGTCCCGCTCGACCAGGCGACGTGTTTCACGACCGATGAGGCCCGTG  1374649

Query  600      ACGCGGTGCTTCCGATATCGGTCGGCCACGACGCGCTGCTCGTTCCCCAGCTTGAGGCTC  659
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1374650  ACGCGGTGCTTCCGATATCGGTCGGCCACGACGCGCTGCTCGTTCCCCAGCTTGAGGCTC  1374709

Query  660      AACAGTTCTACGGCTGACCTCAAGACCGCC  689
                ||||||||||||||||||||||||||||||
Sbjct  1374710  AACAGTTCTACGGCTGACCTCAAGACCGCC  1374739
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Clone TM10

The blastx search across the entire clone indicated a homology of the DNA-sequence to the branched-chain amino acid transport system, permease component

E-value: 3e-94

Locus: YP-001632189

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Query  1        TTCGGCGGGTGGCGCCACGGCGCGCCGGCGGGCGCGCCATTGGGCCGGCAGGCCGGCCAG  60
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3790432  TTCGGCGGGTGGCGCCACGGCGCGCCGGCGGGCGCGCCATTGGGCCGGCAGGCCGGCCAG  3790373

Query  61       CCCGTCGGGCAGCAGCGCAACCAGCGCGATGATGGACAGGCCCAGCGGCAGGTGCCAGTG  120
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3790372  CCCGTCGGGCAGCAGCGCAACCAGCGCGATGATGGACAGGCCCAGCGGCAGGTGCCAGTG  3790313

Query  121      CTGGGCGTAGTCGCCGAAAATCGCCTGCGACTGGAACAGCTCCTGCAGCAGGATCAGCGC  180
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3790312  CTGGGCGTAGTCGCCGAAAATCGCCTGCGACTGGAACAGCTCCTGCAGCAGGATCAGCGC  3790253
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```
Query  181      GGCCGCGCCCAGCACGGCGCCGCCCAGCCGGCCCATGCCGCCCAGGATCACCATCAGCAG  240
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3790252  GGCCGCGCCCAGCACGGCGCCGCCCAGCCGGCCCATGCCGCCCAGGATCACCATCAGCAG  3790193

Query  241      CACCAGGCCGGATTGCTCCCAGGCCAGCAGTTCCGGCGTCACGAAGCCGTCTTTCAGCGC  300
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3790192  CACCAGGCCGGATTGCTCCCAGGCCAGCAGTTCCGGCGTCACGAAGCCGTCTTTCAGCGC  3790133

Query  301      GTACAGGAAGCCGGCCAGCCCGGCCAGCGCGGCGCCCACCACGTAGGCGGCCAGTTTGTA  360
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3790132  GTACAGGAAGCCGGCCAGCCCGGCCAGCGCGGCGCCCACCACGTAGGCGGCCAGTTTGTA  3790073

Query  361      CGGAAAGGTGGAATAGCCGGCCGCGCGCATGCGCTGCTCGTTGATGCGTATGCCCGCCAG  420
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3790072  CGGAAAGGTGGAATAGCCGGCCGCGCGCATGCGCTGCTCGTTGATGCGTATGCCCGCCAG  3790013

Query  421      CGCCGCGCCGAACGGCGAGCGCGCCAGCATGGCCAGGAAACCCCACGTCAAGGCCAGGCA  480
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3790012  CGCCGCGCCGAACGGCGAGCGCGCCAGCATGGCCAGGAAACCCCACGTCAAGGCCAGGCA  3789953

Query  481      GGCCAGCACGAACAGGTAGTAGACGCCGGGCTGGTCCAGGTCGAACGGCTGCCAGCCGCC  540
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3789952  GGCCAGCACGAACAGGTAGTAGACGCCGGGCTGGTCCAGGTCGAACGGCTGCCAGCCGCC  3789893

Query  541      CAGCGAGAGCTGCGGGCGAAAGTACAGGTAGATGCCGTCGCTGCCGCCGCCCAGGTCGGT  600
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3789892  CAGCGAGAGCTGCGGGCGAAAGTACAGGTAGATGCCGTCGCTGCCGCCGCCCAGGTCGGT  3789833

Query  601      GTCGTGGAACACGTAGTACGCCATCTGCGAGAACGCCAGCGTGACCATGATGAAATACAC  660
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3789832  GTCGTGGAACACGTAGTACGCCATCTGCGAGAACGCCAGCGTGACCATGATGAAATACAC  3789773

Query  661      GCCGCGCGTGCGCAGCGCCAGGGCGCCCGGTCACCAGCGCATACGCGGCCGCGCC  715
                |||||||||||||||||||||||| |||||||||||||||||||||||||||||
Sbjct  3789772  GCCGCGCGTGCGCAGCGCCAGGGCG-CCGGTCACCAGCGCATACGCGGCCGCGCC  3789719
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Clone TM11

The blastx search across the entire clone indicated a homology of the DNA-sequence to the dihydrolipoamide dehydrogenase.

E-value: 2e-98

Locus: YP- 001630123

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Query  1        GGTGTGGTGGTCGCCCTGGCCGTCGAACTGACCCGCGCTGGCCAACTCTAGCCAGGTCTC  60
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1564196  GGTGTGGTGGTCGCCCTGGCCGTCGAACTGACCCGCGCTGGCCAACTCTAGCCAGGTCTC  1564255

Query  61       GTAGTGCTCGGCTACCGTTTGGTACAGCAGCGTGCGTTCGGGGTGGCGTGGGTTGTAGAG  120
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1564256  GTAGTGCTCGGCTACCGTTTGGTACAGCAGCGTGCGTTCGGGGTGGCGTGGGTTGTAGAG  1564315

Query  121      CTTGGGCTTGGAAGTGGCTGACATGAATGCTGTACAAATGCACAGTATTCTGCGGCGATT  180
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1564316  CTTGGGCTTGGAAGTGGCTGACATGAATGCTGTACAAATGCACAGTATTCTGCGGCGATT  1564375

Query  181      GTTTGGGTACGCAAAGCACAAGGCCCGAACCGTCGCCAGTTCGGGCCTTGTCAGGGTAGC  240
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1564376  GTTTGGGTACGCAAAGCACAAGGCCCGAACCGTCGCCAGTTCGGGCCTTGTCAGGGTAGC  1564435

Query  241      TTGGCTGCGGACTTACTTGCGCGCAGGCGGCACATCCGTGCAGCTGCCATGCGCCACTTC  300
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1564436  TTGGCTGCGGACTTACTTGCGCGCAGGCGGCACATCCGTGCAGCTGCCATGCGCCACTTC  1564495

Query  301      CGCCGCCATGCCGATGCTCTCGCCCAGCGTCGGATGCGGGTGGATGGTCTTGCCGATGTC  360
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1564496  CGCCGCCATGCCGATGCTCTCGCCCAGCGTCGGATGCGGGTGGATGGTCTTGCCGATGTC  1564555
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Query  361      CACTGCGTCCGCACCCATCTCGATAGCCAGCGCGATCTCTCCGATCATGTCGCCCGCGTG  420
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1564556  CACTGCGTCCGCACCCATCTCGATAGCCAGCGCGATCTCTCCGATCATGTCGCCCGCGTG  1564615

Query  421      GGTGCCGACCATGCCACCGCCCAAGATCTTGCCGTGGCCCCGGCCAGCATGGCCGTCTCC  480
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1564616  GGTGCCGACCATGCCACCGCCCAAGATCTTGCCGTGGCCCCGGCCAGCATGGCCGTCTCC  1564675

Query  481      TGATCCTGCTTCGGGGCTGTCGTCGAACAGCAGCTTGGTGACGCCTTCATCGCGGCCGTT  540
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1564676  TGATCCTGCTTCGGGGCTGTCGTCGAACAGCAGCTTGGTGACGCCTTCATCGCGGCCGTT  1564735

Query  541      GGCAATGGCGCGGCCCGAGGCACTCCACGGGAACAGGCCCTTCTTGACCTTGATGCCCTG  600
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1564736  GGCAATGGCGCGGCCCGAGGCACTCCACGGGAACAGGCCCTTCTTGACCTTGATGCCCTG  1564795

Query  601      GGCCTTGGCCTGGTCTTCGGTGAGGCCGACCCACGCCACTTCGGGGTCGGTGTAGGCCAC  660
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1564796  GGCCTTGGCCTGGTCTTCGGTGAGGCCGACCCACGCCACTTCGGGGTCGGTGTAGGCCAC  1564855

Query  661      GCTGGGGATCACGCGGGCATTGAAGGCGGCGCTGGCCAGCTCCTTGTTGCCTTGCAGTTC  720
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1564856  GCTGGGGATCACGCGGGCATTGAAGGCGGCGCTGGCCAGCTCCTTGTTGCCTTGCAGTTC  1564915

Query  721      ACCGGCAATCACTTCCGCCGCCACATGCGCCTCGTGCACCGCCTTGTGCGCCAGCATGGG  780
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1564916  ACCGGCAATCACTTCCGCCGCCACATGCGCCTCGTGCACCGCCTTGTGCGCCAGCATGGG  1564975

Query  781      CTGACCGACGATGTCGCCGATGGCGAAGATGTGC-GCACGTTGGTGCGCATCTGGATGTC  839
                ||| |||||||||||||||||||||||||||| |||||||||||||||||||||||||||
Sbjct  1564976  CTGGCCGACGATGTCGCCGATGGCGAAGATGTGCGGCACGTTGGTGCGCATCTGGATGTC  1565035

Query  840      GACGTTGATGAAGCCGCGGTCTGTGACGGCCACGCCGGCCTTTTCAGCGGCAATCTTCTT  899
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1565036  GACGTTGATGAAGCCGCGGTCTGTGACGGCCACGCCGGCCTTTTCAGCGGCAATCTTCTT  1565095

Query  900      GCCGTTGGGCGTGCGGCCCACGGCCTGCAGCACGAGGTCG  939
                ||||||||||||||||||||||||||||||||||||||||
Sbjct  1565096  GCCGTTGGGCGTGCGGCCCACGGCCTGCAGCACGAGGTCG  1565135
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Clone TM12

The blastx search across the entire clone indicated a homology of the DNA-sequence to the conserved hypothetical protein Bpet 4301

E-value: 6e-111

Locus: YP-001632917

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Query  1        ATCGCAACCTGCTTCGACGACGGCCATGACCTGACCGCCATCCAGTTCGAGGGCTGCTGG  60
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1513703  ATCGCAACCTGCTTCGACGACGGCCATGACCTGACCGCCATCCAGTTCGAGGGCTGCTGG  1513762

Query  61       TATTGCAGCAAGCCAAGGCTGTTCGAGTTCGGCGGCAGCGGCTGCTACCTGAGCCGCGAG  120
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1513763  TATTGCAGCAAGCCAAGGCTGTTCGAGTTCGGCGGCAGCGGCTGCTACCTGAGCCGCGAG  1513822

Query  121      GTCCAGGTCTTCAGAACGTCCTCGCAGGCACTCCAACTCGGCGACCAACTGCGCAAAGCC  180
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1513823  GTCCAGGTCTTCAGAACGTCCTCGCAGGCACTCCAACTCGGCGACCAACTGCGCAAAGCC  1513882

Query  181      ATTAAGGCCAGCGACATCGAAGAGGCCTCGGCGTTGATCGCACTGGAGGCCGCCAATCTG  240
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1513883  ATTAAGGCCAGCGACATCGAAGAGGCCTCGGCGTTGATCGCACTGGAGGCCGCCAATCTG  1513942

Query  241      CTCGCGGGCATCAACGACGAGCATTTCCGCCTGAATGTGCGCCATCGCATCGCCGAACGG  300
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1513943  CTCGCGGGCATCAACGACGAGCATTTCCGCCTGAATGTGCGCCATCGCATCGCCGAACGG  1514002

Query  301      CTGGCCCAGACGCCAACGATCAGTGCCGACTGACGCATTTTCTCTTTTCAACCCACCGGG  360
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1514003  CTGGCCCAGACGCCAACGATCAGTGCCGACTGACGCATTTTCTCTTTTCAACCCACCGGG  1514062
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Query  361      TCCAATTCCCGGTGGCGGGGATTGGCTCCATTATTCAATCTGGAGAAATCCCATGTCCCA  420
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1514063  TCCAATTCCCGGTGGCGGGGATTGGCTCCATTATTCAATCTGGAGAAATCCCATGTCCCA  1514122

Query  421      AAATCCCAATCCCTTTGTCCGCGGCTACTGGAATCTGAAAATCGTCCGCACGCTGTCCAT  480
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1514123  AAATCCCAATCCCTTTGTCCGCGGCTACTGGAATCTGAAAATCGTCCGCACGCTGTCCAT  1514182

Query  481      CAGCTACGAGGACGGAAGCCCGCATGTGTGGCGAGCCATCCACGTGAGCCAACAGCACCT  540
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1514183  CAGCTACGAGGACGGAAGCCCGCATGTGTGGCGAGCCATCCACGTGAGCCAACAGCACCT  1514242

Query  541      CTCCGATGAGGAACTGGTTTCTTCCCCCTGCATCGTCACCAGCGAGTTCGCGGTGGTCAG  600
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1514243  CTCCGATGAGGAACTGGTTTCTTCCCCCTGCATCGTCACCAGCGAGTTCGCGGTGGTCAG  1514302

Query  601      GAATGGCACTGAACCTGTCAGCGCCGAACTGATGGCCGAATGCAATGCTGTTGAAAGGCG  660
                ||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||
Sbjct  1514303  GAATGGCACTGAACCTGTCAGCGCCGAACTGATGGCCGAATGCAATGCTGTTG-AAGGCG  1514361

Query  661      TCAGCGGCGAAAGGCGTAGTTGGTGCCGTGGTCTATGCCATTCATGGCGACGACTTCGAC  720
                |||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1514362  TCAGCGGCG-AAGGCGTAGTTGGTGCCGTGGTCTATGCCATTCATGGCGACGACTTCGAC  1514420

Query  721      GGGCCGCCCGATCCACGTC-GTGACACTTATTCGGCCGAAGCCGCGCGAGACGTGGTGCA  779
                 |||||||||||||||||| ||||||||||||||||||||||||||||||||||||||||
Sbjct  1514421  -GGCCGCCCGATCCACGTCGGTGACACTTATTCGGCCGAAGCCGCGCGAGACGTGGTGCA  1514479

Query  780      GCGGTTGAGTTTCGAGA-CGGCTATTACAG-CGATGCTGGGAAATCAGCAGCGCGCACAT  837
                |||||||||||||||| |||||||||||| ||||||||||||||||||||||||||||||
Sbjct  1514480  GCGGTTGAGTTTCGAGACCGGCTATTACAGCCGATGCTGGGAAATCAGCAGCGCGCACAT  1514539

Query  838      CAGCCGGAAAACCGGCCAGTACCTCGCCAACCTGGCAGACCTCGCCACGCCGGAGGCCTT  897
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1514540  CAGCCGGAAAACCGGCCAGTACCTCGCCAACCTGGCAGACCTCGCCACGCCGGAGGCCTT  1514599

Query  898      TCTGTTCATCGCCTTCCGGGTTCCGTACAGCCCGGCGATCGGCGTCAAGCTGATTTCCAC  957
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1514600  TCTGTTCATCGCCTTCCGGGTTCCGTACAGCCCGGCGATCGGCGTCAAGCTGATTTCCAC  1514659

Query  958      GCCCTGGACGGACCAGAACCTGGAGCATGCCGAGGGCATCGGCGCGGAGCAGCTTCGACA  1017
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1514660  GCCCTGGACGGACCAGAACCTGGAGCATGCCGAGGGCATCGGCGCGGAGCAGCTTCGACA  1514719

Query  1018     AGAGCACCGGAACAAGGGCATGCCGGACGACCTGGCGAACATCCTTGAACTGGCTGGCCA  1077
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1514720  AGAGCACCGGAACAAGGGCATGCCGGACGACCTGGCGAACATCCTTGAACTGGCTGGCCA  1514779

Query  1078     GGCCGATGTGCGCATTCTCATCCTCGACGCTGATGCGCCCGCGCTACTGGGCTTGCCGCT  1137
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1514780  GGCCGATGTGCGCATTCTCATCCTCGACGCTGATGCGCCCGCGCTACTGGGCTTGCCGCT  1514839

Query  1138     GGCCGAGTCCTAGCAGTCGCGCCACGCACCATTCTTCCTCTTTGTTCTCCCCCATGTCAG  1197
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1514840  GGCCGAGTCCTAGCAGTCGCGCCACGCACCATTCTTCCTCTTTGTTCTCCCCCATGTCAG  1514899

Query  1198     CCCGTCTCCCACCCGGAGCTGGGCTGATCCATTTTCATAGGAGCAACCTCATGTTCCCCG  1257
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1514900  CCCGTCTCCCACCCGGAGCTGGGCTGATCCATTTTCATAGGAGCAACCTCATGTTCCCCG  1514959

Query  1258     ACCTCATCTCGCATGCACCCGACTTCGAGCACCAGCTCGGAGCCTGCGTCAACGCCATGA  1317
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1514960  ACCTCATCTCGCATGCACCCGACTTCGAGCACCAGCTCGGAGCCTGCGTCAACGCCATGA  1515019

Query  1318     GCCAGGACGACGCCATCGGCCAGATCCTGGTATTCGAGCGCACGAGAGGCATGCTACACA  1377
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1515020  GCCAGGACGACGCCATCGGCCAGATCCTGGTATTCGAGCGCACGAGAGGCATGCTACACA  1515079

Query  1378     TTCGCCATATCGCCAGCGCCGACCTGGTGGACAGCGACATTGACGACTACGAA  1430
                | ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1515080  TGCGCCATATCGCCAGCGCCGACCTGGTGGACAGCGACATTGACGACTACGAA  1515132
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Clone TM13

The blastx search across the entire clone indicated a homology of the DNA-sequence to the hypothetical protein putative helicase.

E-value: 4e-41

Locus: YP-001632869

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Query  483      GGCTCTCGACTGGAGACCGTGAATGTCCGAAAGGGTTTGCCGACGCAATGGAGGCCAGAG  542
                |||  ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4514320  GGCGCTCGACTGGAGACCGTGAATGTCCGAAAGGGTTTGCCGACGCAATGGAGGCCAGAG  4514379

Query  543      GAGAACACCTCGTTGCCCTGCTGAACGAGAGTGGCATTCCTGAGCAAGTAAAGAATGAAA  602
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4514380  GAGAACACCTCGTTGCCCTGCTGAACGAGAGTGGCATTCCTGAGCAAGTAAAGAATGAAA  4514439

Query  603      TTCGATTTCTAATGGCCTGTATGCACAAAGGATGCGCCTGAAAATTGCGTTCAGTGGATA  662
                |||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||
Sbjct  4514440  TTCGATTTCTAATGGCCTGTATGCAC-AAGGATGCGCCTGAAAATTGCGTTCAGTGGATA  4514498

Query  663      ACCGGGCAGGTTGAGGGTCAGAAAATTCGTGACCTGCGAGCCGTCGGCTTCGCGCTTGGT  722
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4514499  ACCGGGCAGGTTGAGGGTCAGAAAATTCGTGACCTGCGAGCCGTCGGCTTCGCGCTTGGT  4514558

Query  723      GATGTTTCGCAGCAATGGCAG-AAGATTTGCTATCCCAGCTCGTTGCGAATCCGTCAAAT  781
                |||||||||||||||||||| ||||||||||||||||||||||||||||||||||||||
Sbjct  4514559  GATGTTTCGCAGCAATGGCAGAAAGATTTGCTATCCCAGCTCGTTGCGAATCCGTCAAAT  4514618

Query  782      GACGCGCTCAGTATTTTGGCCTATGCCATCTGGCGAGAGCAGCAGTTTGTTGAGAAATTC  841
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4514619  GACGCGCTCAGTATTTTGGCCTATGCCATCTGGCGAGAGCAGCAGTTTGTTGAGAAATTC  4514678

Query  842      AGCCTCGCCAACTTGCAGTCCATTCTGAATGCTCTGAACATCATGCTCAACATCAAGCAA  901
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4514679  AGCCTCGCCAACTTGCAGTCCATTCTGAATGCTCTGAACATCATGCTCAACATCAAGCAA  4514738

Query  902      TATCCGCCCCGGAAAGACGAGTGGACTGCTCGCAACTGGATTCGCGCCACGACAGAACCG  961
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4514739  TATCCGCCCCGGAAAGACGAGTGGACTGCTCGCAACTGGATTCGCGCCACGACAGAACCG  4514798

Query  962      CTTGAACTTCTGCTCGGCCTGCTTCGCACCCGTGCATCATCCACCCCCGAAATCAAGATT  1021
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4514799  CTTGAACTTCTGCTCGGCCTGCTTCGCACCCGTGCATCATCCACCCCCGAAATCAAGATT  4514858

Query  1022     CTCCTTCAGCCGCATCAAAAAATCACCAAGGAATTGGCCAAGAAAATCGAGCGAGTGACA  1081
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4514859  CTCCTTCAGCCGCATCAAAAAATCACCAAGGAATTGGCCAAGAAAATCGAGCGAGTGACA  4514918

Query  1082     GAAATTGTCACACTGTCCAACATCAAGCTCTTCTCCCGTGTGAAAATCAACATCCAGAAA  1141
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4514919  GAAATTGTCACACTGTCCAACATCAAGCTCTTCTCCCGTGTGAAAATCAACATCCAGAAA  4514978

Query  1142     CCCTCAGGCGACCGCACCCCCGATCTGCTCTACGCGCTGCGCCTGTACCTCACTGGTGAC  1201
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4514979  CCCTCAGGCGACCGCACCCCCGATCTGCTCTACGCGCTGCGCCTGTACCTCACTGGTGAC  4515038

Query  1202     GATGGTGCGAACGCTATTCACATATCTAGTGTTTCTGATGGAAATACTGATGAGACCATT  1261
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4515039  GATGGTGCGAACGCTATTCACATATCTAGTGTTTCTGATGGAAATACTGATGAGACCATT  4515098

Query  1262     TAATTTTGATAGATGATTTTCATATCTTGATCTTCATAGGCCGGGAAATTTCATTGAAAA  1321
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4515099  TAATTTTGATAGATGATTTTCATATCTTGATCTTCATAGGCCGGGAAATTTCATTGAAAA  4515158

Query  1322     TAGATATGGGTTATTTGGAGGCGTAAATTCGAAAACCATTTCTGGTTTGATAAGTAAATA  1381
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4515159  TAGATATGGGTTATTTGGAGGCGTAAATTCGAAAACCATTTCTGGTTTGATAAGTAAATA  4515218

Query  1382     GCCATGTAGCTTCCTCGATTTTCTGGCGCCTGTGACTTCGCAAGTCCAGATGTTCAGGCC  1441
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4515219  GCCATGTAGCTTCCTCGATTTTCTGGCGCCTGTGACTTCGCAAGTCCAGATGTTCAGGCC  4515278
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Query  1442     ACTTGGTTGCTTGCGATGCAATTGCAGTCGCTCGAAGCGCTTCTGCACCCACTGCCAATC  1501
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4515279  ACTTGGTTGCTTGCGATGCAATTGCAGTCGCTCGAAGCGCTTCTGCACCCACTGCCAATC  4515338

Query  1502     CTGCAGGTTCTCCTGTTTGGCCAGTTTGCCTACCTGCGGGTG-TCCTG  1548
                ||||||||||||||||||||||||||||||||||||||||||| |||||
Sbjct  4515339  CTGCAGGTTCTCCTGTTTGGCCAGTTTGCCTACCTGCGGGTGTTCCTG  4515386
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Clone TM14

The blastx search across the entire clone indicated a homology of the DNA-sequence to the two-component response regulator.

E-value: 1e-119

Locus: YP-001633578

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Query  4        CGATTTCAAGCCGATAGCCCAGGGCATACGAGGATGCCAGACGCACGCCGTTTTCGGGGC  63
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5219032  CGATTTCAAGCCGATAGCCCAGGGCATACGAGGATGCCAGACGCACGCCGTTTTCGGGGC  5218973

Query  64       GTAGTTGCAGCTTCTGGCGAATATTGGACAAATGCGTGTCCAGTGTGCGTGAGGTAGCGG  123
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5218972  GTAGTTGCAGCTTCTGGCGAATATTGGACAAATGCGTGTCCAGTGTGCGTGAGGTAGCGG  5218913

Query  124      GAATCTCGCGGCTCCATACGCTTTCAGCCAGCACGTCACGCGACAGCAGACGGCCTGCGT  183
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5218912  GAATCTCGCGGCTCCATACGCTTTCAGCCAGCACGTCACGCGACAGCAGACGGCCTGCGT  5218853

Query  184      TGCGAAAGAATAATTGCGCAAGTTCGAATTCTTTGGGTGCCAGCGCAATGGCCGTGCCGT  243
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5218852  TGCGAAAGAATAATTGCGCAAGTTCGAATTCTTTGGGTGCCAGCGCAATGGCCGTGCCGT  5218793

Query  244      TTAGCGCGATCGTGCGCGCGGTGGGATCGATGTCATACCCAGCCACCGAAAAAGGTGCAT  303
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5218792  TTAGCGCGATCGTGCGCGCGGTGGGATCGATGTCATACCCAGCCACCGAAAAAGGTGCAT  5218733

Query  304      GGTCGGGTTGCGCCGCCTGGCTGCGGCGTAGCAATGCCGCGATCCGCGCCAGCAATTCGG  363
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5218732  GGTCGGGTTGCGCCGCCTGGCTGCGGCGTAGCAATGCCGCGATCCGCGCCAGCAATTCGG  5218673

Query  364      CCGACCGCAACGGCTTTACGACATAATCGTCGGCGCCGGCATGCAGCCCCTGCACCAGAT  423
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5218672  CCGACCGCAACGGCTTTACGACATAATCGTCGGCGCCGGCATGCAGCCCCTGCACCAGAT  5218613

Query  424      CAATTTCCTGGCTGCGGCTGGTCAGGAAAATAATGGGAACCTGCATGCCGAGTGTGCTGC  483
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5218612  CAATTTCCTGGCTGCGGCTGGTCAGGAAAATAATGGGAACCTGCATGCCGAGTGTGCTGC  5218553

Query  484      GCAGGCGCCGCAGCACTTCGTCGCCATCCATGTCCGGCAATTGCCAATCGAGCATGATGA  543
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5218552  GCAGGCGCCGCAGCACTTCGTCGCCATCCATGTCCGGCAATTGCCAATCGAGCATGATGA  5218493

Query  544      GATCGAACGATTGGTTGCGCACGGCAGCCAGCAGATCGCGGCTGCGTTGATAGCTGTG  601
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5218492  GATCGAACGATTGGTTGCGCACGGCAGCCAGCAGATCGCGGCTGCGTTGATAGCTGTG  5218435
```

Clone TM15

The blastx search across the entire clone indicated a homology of the DNA-sequence to an unnamed protein product/hypothetical Protein Bpet 0369

E-value: 2e-04

Locus: YP-001628972

```
Query  2       TTCTTCAGACGCATTGGCCGCGCCGCCCTTGGGCGGCATGGCGGC-TTGCCCGTGAGAGC  60
               |||||||||||||||||||||||||||||||||||||||||||| | ||||||||||||||
Sbjct  407659  TTCTTCAGACGCATTGGCCGCGCCGCCCTTGGGCGGCATGGCGCCCTTGCCCGTGAGAGC  407718

Query  61      GACCTTCACCATGGCGTCGATGCCGGTCTTGATATAGGGCTCCCACGCGGCCTTGTCGCC  120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  407719  GACCTTCACCATGGCGTCGATGCCGGTCTTGATATAGGGCTCCCACGCGGCCTTGTCGCC  407778

Query  121     GAACTTCGGAGCGCCGGCTACGCCAGTGCCGTGGCAGGCGAAGCAGGTGGATTTGTAGAG  180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  407779  GAACTTCGGAGCGCCGGCTACGCCAGTGCCGTGGCAGGCGAAGCAGGTGGATTTGTAGAG  407838

Query  181     TTTTTCGCcggccgggttgacggcggcttcagcctttggggcggcggcgggcgcgggcgc  240
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  407839  TTTTTCGCCGGCCGGGTTGACGGCGGCTTCAGCCTTTGGGGCGGCGGCGGGCGCGGGCGC  407898

Query  241     ttcggccttgggcgcctcggccgcagccgcagcgggggccgcTGCCGGAGCCGGCGCGGC  300
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  407899  TTCGGCCTTGGGCGCCTCGGCCGCAGCCGCAGCGGGGGCCGCTGCCGGAGCCGGCGCGGC  407958

Query  301     TGCCGCTGCCTGCTTGGGAGCTTCGGCCTTGACGCCTTCGCCTTCCTGGGCGGGGGCGGC  360
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  407959  TGCCGCTGCCTGCTTGGGAGCTTCGGCCTTGACGCCTTCGCCTTCCTGGGCGGGGGCGGC  408018

Query  361     GGGTTCGTCGAACGAACCGCCCGACTGGTTGGCCATATAGACCACCGCGCGCGCCACTTC  420
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  408019  GGGTTCGTCGAACGAACCGCCCGACTGGTTGGCCATATAGACCACCGCGCGCGCCACTTC  408078

Query  421     GTAG  424
               ||||
Sbjct  408079  GTAG  408082
```

Clone TM16

The blastx search across the entire clone indicated a homology of the DNA-sequence to the Bactriophage-related transmembrane protein and hypothetical protein.

E-value: 2e-18 and 2e-41

Locus: YP-001629593 and YP-001629592

```
Query  1        ACACCTTCGGAGTCAGACACCTGTTCTGAGGCAGCCAGGCTCATCCTTCGAGTATCAGGC  60
               ||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  5024323  ACACCTTCGGAGTCAGACACCTGTTCTGAGGCAGCCAGGCTCATCCCTCGAGTATCAGGC  5024382

Query  61       ATCTGAATGCCTGGCCAACACGGGTCTCGCCAGGTGTCTGGCTCCGCAGGTGCCTGACAC  120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5024383  ATCTGAATGCCTGGCCAACACGGGTCTCGCCAGGTGTCTGGCTCCGCAGGTGCCTGACAC  5024442

Query  121      CGCACTATAGCGGCAGCCGGGGCACCCCACGGTGTCTGACACCTTCGGAGTCAGACACCT  180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5024443  CGCACTATAGCGGCAGCCGGGGCACCCCACGGTGTCTGACACCTTCGGAGTCAGACACCT  5024502

Query  181      GTTCTGAGGCAGCCAGGCTCATCCCTCGAGTATCAGGCATCTGAATGCCTGGCCAGCACG  240
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5024503  GTTCTGAGGCAGCCAGGCTCATCCCTCGAGTATCAGGCATCTGAATGCCTGGCCAGCACG  5024562

Query  241      GGTCTGGCCAGGTGTCTGGCTCCGCAGGTGTCTGGCTCCGCAGGTGCCTGACACCGCACC  300
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5024563  GGTCTGGCCAGGTGTCTGGCTCCGCAGGTGTCTGGCTCCGCAGGTGCCTGACACCGCACC  5024622
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Query  301      ATAGCGGCAGCCGTGGCACCCCACGGTGTCTGACACCTTCGG  342
                ||||||||||||||||||||||||||||||||||||||||||
Sbjct  5024623  ATAGCGGCAGCCGTGGCACCCCACGGTGTCTGACACCTTCGG  5024664
```

Clone TM17

The blastx search across the entire clone indicated a homology of the DNA-sequence to the hypothetical protein predicted by Glimmer and Criticahypothetical protein predicted by Glimmer/Critica.

E-value: 3e-47

Locus: YP-001629584

```
Query  1        GCCGAACGTGCTGGCGATTCCCAGCACGCAGCACGCGGCGCTGTATTCCAAGCGCCTGTC  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1063657  GCCGAACGTGCTGGCGATTCCCAGCACGCAGCACGCGGCGCTGTATTCCAAGCGCCTGTC  1063716

Query  61       GGACTCCGGCGGCCTGACGGTGGGCGAGTTCTTCGTCAAGCAGCACCCTGGCCTGCGCAT  120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1063717  GGACTCCGGCGGCCTGACGGTGGGCGAGTTCTTCGTCAAGCAGCACCCTGGCCTGCGCAT  1063776

Query  121      CGAGGAAGTGCCCGAGCTGTCCGACGCCGGCACCGGCGGCGTGTCGCTGGCGATCATGTA  180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1063777  CGAGGAAGTGCCCGAGCTGTCCGACGCCGGCACCGGCGGCGTGTCGCTGGCGATCATGTA  1063836

Query  181      CGAGCGCAGCGAAGAGAACCTGAGCATGGAAAACCCCATGCCGTTCAACCAGCTGGCCGC  240
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1063837  CGAGCGCAGCGAAGAGAACCTGAGCATGGAAAACCCCATGCCGTTCAACCAGCTGGCCGC  1063896

Query  241      TCAGGCGCGCAATCTCGAGCTGGTCGTGCCTTGCCTGGCGCGCACCGGCGGCGTCGCGGT  300
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1063897  TCAGGCGCGCAATCTCGAGCTGGTCGTGCCTTGCCTGGCGCGCACCGGCGGCGTCGCGGT  1063956

Query  301      GTACTACCCGCTGGCGCTCACCAAGGCGGAGGGCATCTGATATGGAAAAGCACGTCAAGA  360
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1063957  GTACTACCCGCTGGCGCTCACCAAGGCGGAGGGCATCTGATATGGAAAAGCACGTCAAGA  1064016

Query  361      ACCTGGCGGCTTTCGCCATCGTCACCGCCGCCGGCGAGGTCATCCCGCCCCTGCAGACCT  420
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1064017  ACCTGGCGGCTTTCGCCATCGTCACCGCCGCCGGCGAGGTCATCCCGCCCCTGCAGACCT  1064076

Query  421      CCAAGGCGCCCATCGACATCACCAAGCGCGACACCGCAGCGCTGATCAAGCGCGGGACGC  480
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1064077  CCAAGGCGCCCATCGACATCACCAAGCGCGACACCGCAGCGCTGATCAAGCGCGGGACGC  1064136

Query  481      TCGAGGTCGTGGGCGCCAAGGCTGGCAACAAGACCGCCGCCGGCGAGGGCGACGCGCCGC  540
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1064137  TCGAGGTCGTGGGCGCCAAGGCTGGCAACAAGACCGCCGCCGGCGAGGGCGACGCGCCGC  1064196

Query  541      TCGACCCGGCCACCATGAAGGCCGCGCAGCTCAAGGCCGAGCTGGACCAGCTGGGCGTCG  600
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1064197  TCGACCCGGCCACCATGAAGGCCGCGCAGCTCAAGGCCGAGCTGGACCAGCTGGGCGTCG  1064256

Query  601      AGTACGCCGGCAATGCCAGCACCGAGGTGCTGCGCGACCTGTACGTCGCCAAGCTGGCCG  660
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1064257  AGTACGCCGGCAATGCCAGCACCGAGGTGCTGCGCGACCTGTACGTCGCCAAGCTGGCCG  1064316

Query  661      AGCAGGCGGGGCAGTGACCATGGCCGCCACCGTCGAGCTACTGGACTTCCTGGCGCCGGC  720
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1064317  AGCAGGCGGGGCAGTGACCATGGCCGCCACCGTCGAGCTACTGGACTTCCTGGCGCCGGC  1064376

Query  721      GGTGGCGGGCATGTCGGCCGAGGACAAGCAGAAGGCCCTGGACATCGCCGCCGACTACCG  780
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1064377  GGTGGCGGGCATGTCGGCCGAGGACAAGCAGAAGGCCCTGGACATCGCCGCCGACTACCG  1064436

Query  781      GCCGGCGTGCCTGCCCGAGGCCAAGCAGGATGAGGCACAGGCCTGGTACGCCGCCTGGCT  840
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1064437  GCCGGCGTGCCTGCCCGAGGCCAAGCAGGATGAGGCACAGGCCTGGTACGCCGCCTGGCT  1064496
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Query  841      TCTTTATGGGCGGCTGCAACAGCAGGCCGCCCAGGACAGCGGCGGCGTGGCGCCCGCCGG  900
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1064497  TCTTTATGGGCGGCTGCAACAGCAGGCCGCCCAGGACAGCGGCGGCGTGGCGCCCGCCGG  1064556

Query  901      CGTGGTAAGCGAGAAAGAGGGTGATCTGTCGCGCACCTACGGGCGGGCGGCTGGCGCCGA  960
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1064557  CGTGGTAAGCGAGAAAGAGGGTGATCTGTCGCGCACCTACGGGCGGGCGGCTGGCGCCGA  1064616

Query  961      TGATC  965
                |||||
Sbjct  1064617  TGATC  1064621
```

Clone TM19

The blastx search across the entire clone indicated a homology of the DNA-sequence to the putative 3-oxoacyl-[acyl-carrier-protein] synthase I.

E-value: 2e-35

Locus: YP-001633054

```
Query  1        CTTCATCCAGCAAAATGGCCTCGCCGGCGTGCGGTATGAGCTCGTGTACGGGCCAGATCG  60
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4684884  CTTCATCCAGCAAAATGGCCTCGCCGGCGTGCGGTATGAGCTCGTGTACGGGCCAGATCG  4684825

Query  61       TCATGATGCCGCCAGAATCAGGCTGGTGTTGTTGCCGCCGAAGGCAAACGAATTGCTCAT  120
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4684824  TCATGATGCCGCCAGAATCAGGCTGGTGTTGTTGCCGCCGAAGGCAAACGAATTGCTCAT  4684765

Query  121      GGCAATGCGCCTGCGTCCCGCCGCGTACGCTTGCGTGCCATCGCAGAAGTCCAGCGGCGG  180
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4684764  GGCAATGCGCCTGCGTCCCGCCGCGTACGCTTGCGTGCCATCGCAGAAGTCCAGCGGCGG  4684705

Query  181      TAGCTCCGGGTCCGGAACGCCGTCCCAGGCATGCCGGGGCAAGGGGGCTCCAAGGCTTGT  240
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4684704  TAGCTCCGGGTCCGGAACGCCGTCCCAGGCATGCCGGGGCAAGGGGGCTCCAAGGCTTGT  4684645

Query  241      GCCATCGAGCGTCAGCCAGGCCAACGCAGCTTCAAGCGCGCCTGCCGTGCCAAGCGTGTG  300
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4684644  GCCATCGAGCGTCAGCCAGGCCAACGCAGCTTCAAGCGCGCCTGCCGTGCCAAGCGTGTG  4684585

Query  301      GCCGGTCAGGCCTTTGGTCGACGCGCAGGGAACGCCGGTCGGAAAGACGCTGTGCATGGC  360
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4684584  GCCGGTCAGGCCTTTGGTCGACGCGCAGGGAACGCCGGTCGGAAAGACGCTGTGCATGGC  4684525

Query  361      AT  362
                ||
Sbjct  4684524  AT  4684523
```

Clone TM20

The blastx search across the entire clone indicated a homology of the DNA-sequence to the putative acyl dehydratase.

E-value: 2e-77

Locus: YP-001629405

```
Query  2       TTGCTTTCGCCGCGCCACATGTCGAACTGCAGCGTTTCGCCCGGGTACACCGGCGACGAG  61
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  887570  TTGCTTTCGCCGCGCCACATGTCGAACTGCAGCGTTTCGCCCGGGTACACCGGCGACGAG  887629
```

```
Query  62      AAGCGCGCGTTCAGGCTGGCCAGCCGGCTGGCGTCGTAGCCGCACCAGGTCTTGACGATG  121
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  887630  AAGCGCGCGTTCAGGCTGGCCAGCCGGCTGGCGTCGTAGCCGCACCAGGTCTTGACGATG  887689

Query  122     GCGTGAGCCGCCATGCCATAGGTGCACAGGCCGTGCAGAATGGGCTGGGCATAGCCGGCC  181
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  887690  GCGTGAGCCGCCATGCCATAGGTGCACAGGCCGTGCAGAATGGGCTGGGCATAGCCGGCC  887749

Query  182     TTGCGGGCCACCTCGGGGTCGGCGTGCAGCGGGTTGCGGTCGGCGCTCAGGCGATACAGC  241
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  887750  TTGCGGGCCACCTCGGGGTCGGCGTGCAGCGGGTTGCGGTCGGCGCTCAGGCGATACAGC  887809

Query  242     AGCGCCGCGTTGGGCGCCACCGCCAGGGTGCAGCTTTGGTCGGGGGCCCGAGTGGGCGCG  301
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  887810  AGCGCCGCGTTGGGCGCCACCGCCAGGGTGCAGCTTTGGTCGGGGGCCCGAGTGGGCGCG  887869

Query  302     GCAGGCAAGGGCGGCGGGGCATCGTCGCCGCGGCCGAAGCCGCCGTCGCCCCGGCAGAAG  361
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  887870  GCAGGCAAGGGCGGCGGGGCATCGTCGCCGCGGCCGAAGCCGCCGTCGCCCCGGCAGAAG  887929

Query  362     GTGGTTTGCTGCAGAGTGGCCAGGCAGGCGCCGGCGCTGTCATGCAGCGTGCGCTCGGTG  421
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  887930  GTGGTTTGCTGCAGAGTGGCCAGGCAGGCGCCGGCGCTGTCATGCAGCGTGCGCTCGGTG  887989

Query  422     ATTACCAGCGCGCCCTTGTCGGCCCCTTTGTCGATGACATGCGTGACGCGGCTCTTGCCG  481
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  887990  ATTACCAGCGCGCCCTTGTCGGCCCCTTTGTCGATGACATGCGTGACGCGGCTCTTGCCG  888049

Query  482     ATGACGTTGCCGCTGGCGGGCAGCGGCGCGTGCAGGGCCAGGCGCTGCTCGCCGTGCACC  541
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  888050  ATGACGTTGCCGCTGGCGGGCAGCGGCGCGTGCAGGGCCAGGCGCTGCTCGCCGTGCACC  888109

Query  542     AGCCGCACCCAGTCGATGCCGGCGCGCGGATCGCTCATCCAGAAGCCGGGGTAGCCCAGC  601
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  888110  AGCCGCACCCAGTCGATGCCGGCGCGCGGATCGCTCATCCAGAAGCCGGGGTAGCCCAGC  888169

Query  602     ACGGTGGCTTGGGTTGGAAAGGCTTGCAGGCCCCGTTCGTATACGTAGCGCAGTTGCCCG  661
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  888170  ACGGTGGCTTGGGTTGGAAAGGCTTGCAGGCCCCGTTCGTATACGTAGCGCAGTTGCCCG  888229

Query  662     GCGTCGAGCGGGTCGTGCCCCAGGCCGATGCCCA-GGCGTACAGCATGGTGTC  713
               |||||||||||||||||||||||||||||||| ||||||||||||||||||
Sbjct  888230  GCGTCGAGCGGGTCGTGCCCCAGGCCGATGCCCAGGGCGTACAGCATGGTGTC  888282
```

Clone TM21

The blastx search across the entire clone indicated a homology of the DNA-sequence to the protein-L-isoaspartate O-methyltransferase.

E-value: 2e-50

Locus: YP- 001629092

```
Query  1       CGTCGGCCACGCCCAGCTTGGCCTGTTCGTAGCGTTGCCAGCGCGAA-AGACGTATAGCG  59
               |||||||||||||||||||||||||||||||||||||||||||||||  || |||||||||
Sbjct  529311  CGTCGGCCACGCCCAGCTTGGCCTGTTCGTAGCGTTGCCAGCGCGACCAGTCGTATAGCG  529370

Query  60      GCTGGGTCAGGACCAGGTCCCAGGCGCCGCGGGTGCCGTCGCGGCTGCGCGACGACATGT  119
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  529371  GCTGGGTCAGGACCAGGTCCCAGGCGCCGCGGGTGCCGTCGCGGCTGCGCGACGACATGT  529430

Query  120     TGCGGGTTGCGCGGGTTTCGTCGTAGGCGCCCAGGAGTTCGGCATTGACCGCCGGCAGCA  179
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  529431  TGCGGGTTGCGCGGGTTTCGTCGTAGGCGCCCAGGAGTTCGGCATTGACCGCCGGCAGCA  529490

Query  180     GGCTGGCCCGCGCCTGCGGCAATTGCTCGATGGACGCCCGGTATGAGGCGCGGGCCGCGG  239
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  529491  GGCTGGCCCGCGCCTGCGGCAATTGCTCGATGGACGCCCGGTATGAGGCGCGGGCCGCGG  529550
```

```
Query  240     CGTAGAGCGGGTCGCTGGCCAACGCCTGGCGCCAGATCTGCAGCAGGTCTTGGGCAGCGC  299
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  529551  CGTAGAGCGGGTCGCTGGCCAACGCCTGGCGCCAGATCTGCAGCAGGTCTTGGGCAGCGC  529610

Query  300     CGGCCGGCGCGCTCAGGCTTGCGGCAAGCGCCAGAACCAACGTGGCCGCAAGCGAACCGG  359
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  529611  CGGCCGGCGCGCTCAGGCTTGCGGCAAGCGCCAGAACCAACGTGGCCGCAAGCGAACCGG  529670

Query  360     CGCGCATGCGGAGGTGGCTAGAACTTGAACTGCGAAACCGTGGCCCCGCGCAGCGGCTTG  419
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  529671  CGCGCATGCGGAGGTGGCTAGAACTTGAACTGCGAAACCGTGGCCCCGCGCAGCGGCTTG  529730

Query  420     ACGACGGTTTCGAACAGGTTGACGGTCTCGAAGCTGGCGGCCGTGGTGCGCGTGATGCGA  479
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  529731  ACGACGGTTTCGAACAGGTTGACGGTCTCGAAGCTGGCGGCCGTGGTGCGCGTGATGCGA  529790

Query  480     CAGGCCGTCATGATGGGCGCCTGCCCCACGATGACCACCAGGCGGCCGCCGACGCGCAAC  539
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  529791  CAGGCCGTCATGATGGGCGCCTGCCCCACGATGACCACCAGGCGGCCGCCGACGCGCAAC  529850

Query  540     TGGTATTTCAGGGCGTCGGGCACCGCGGGCACCGAGCCGGTGACCAGGATGGCGTCGTAT  599
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  529851  TGGTATTTCAGGGCGTCGGGCACCGCGGGCACCGAGCCGGTGACCAGGATGGCGTCGTAT  529910

Query  600     TCGGTGGTGCCCCAGCCGTTGCGGCCGTCGCCGGTTTCGACCTTGACGTTGGTGACGTTG  659
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  529911  TCGGTGGTGCCCCAGCCGTTGCGGCCGTCGCCGGTTTCGACCTTGACGTTGGTGACGTTG  529970

Query  660     TTCATCTGCAGGTT  673
               ||||||||||||||
Sbjct  529971  TTCATCTGCAGGTT  529984
```

Clone TM22

The blastx search across the entire clone indicated a homology of the DNA-sequence to the B12-dependent methionine synthase, methionine synthase and 5-methyltetrahydrofolate--homocysteine methyltransferase

E-value: 0.0

Locus: YP-001629097 , WP-012247517 and CAP40826

```
Query  2       AATAAAAGCCCGACACGCTCGAGGCCGGATACATGGCATAGCTGTCGGTAAGCAGCATGC  61
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  539331  AATAAAAGCCCGACACGCTCGAGGCCGGATACATGGCATAGCTGTCGGTAAGCAGCATGC  539272

Query  62      CGATATCGTCGCCATCCAGCACGCGGAACAGATCGCGCTTGACCACGTGTTCCGGGCAGG  121
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  539271  CGATATCGTCGCCATCCAGCACGCGGAACAGATCGCGCTTGACCACGTGTTCCGGGCAGG  539212

Query  122     CCGGATAGCCCGGCGCGGGCCGGATTCCCACGTATTTCTCGGCGATCATTTCGTCATTGG  181
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  539211  CCGGATAGCCCGGCGCGGGCCGGATTCCCACGTATTTCTCGGCGATCATTTCGTCATTGG  539152

Query  182     ACAGCGCCTCGTCGGCCGCGTAGCCCCACAGGTCCTGGCGCACGCGCGCGTGCAGGCATT  241
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  539151  ACAGCGCCTCGTCGGCCGCGTAGCCCCACAGGTCCTGGCGCACGCGCGCGTGCAGGCATT  539092

Query  242     CGGCGAAGGCTTCGGCCAGCCGGTCGGCCATGGCCTTGAGCATGATGCCGGAATAATCGT  301
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  539091  CGGCGAAGGCTTCGGCCAGCCGGTCGGCCATGGCCTTGAGCATGATGCCGGAATAATCGT  539032

Query  302     CCAGCGCGGCCTGGAACTCGGCCTCTTTCTTTTCGATGCCCAGCCCGGCGGTCACGGCGA  361
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  539031  CCAGCGCGGCCTGGAACTCGGCCTCTTTCTTTTCGATGCCCAGCCCGGCGGTCACGGCGA  538972
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Query  362     ACATGCCGATGTAGTCAGCCACCCCGCTCGACTTGGGCGCGATGAAATCGGCCAGGCACT  421
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  538971  ACATGCCGATGTAGTCAGCCACCCCGCTCGACTTGGGCGCGATGAAATCGGCCAGGCACT  538912

Query  422     TGTTGCTGACGCCTTCGCGCTTGACGCCCTGCTGGCGCAGGTTGCGATAAGTGAACAACA  481
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  538911  TGTTGCTGACGCCTTCGCGCTTGACGCCCTGCTGGCGCAGGTTGCGATAAGTGAACAACA  538852

Query  482     CCTCGCTGCGCGATTCGTCGGCGTAGACCTCGATGTCTTCGTCGTTGACGGTATTGGCCG  541
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  538851  CCTCGCTGCGCGATTCGTCGGCGTAGACCTCGATGTCTTCGTCGTTGACGGTATTGGCCG  538792

Query  542     GGTAGAACGCCACCGCGCCGTTGGCGGTCAGCCAGCGGCCCTCGATGATGCGCTTAAGCA  601
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  538791  GGTAGAACGCCACCGCGCCGTTGGCGGTCAGCCAGCGGCCCTCGATGATGCGCTTAAGCA  538732

Query  602     TGGCCTGCCCGTCGGCATATACCTTCTGGGCCTGCTCGCCCACCACTTTGTCTTCCAGAA  661
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  538731  TGGCCTGCCCGTCGGCATATACCTTCTGGGCCTGCTCGCCCACCACTTTGTCTTCCAGAA  538672

Query  662     TGGCGGGAAACTGCCCGAACAGGCTCCAGGTCTGGAAGAACGGCGTCCAGTCCAGATACC  721
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  538671  TGGCGGGAAACTGCCCGAACAGGCTCCAGGTCTGGAAGAACGGCGTCCAGTCCAGATACC  538612

Query  722     TGGCGATCTCGGCCAGGTCGTAGCTTTTGAACGACCGCCGACCAATGAACTTGGGGCGCG  781
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  538611  TGGCGATCTCGGCCAGGTCGTAGCTTTTGAACGACCGCCGACCAATGAACTTGGGGCGCG  538552

Query  782     GCGGCACATAGGCCGACCAATCGATCTGCGGgcgcgccgcgcgcgcCTCGGCCAGCGAAA  841
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  538551  GCGGCACATAGGCCGACCAATCGATCTGCGGGCGCGCCGCGCGCGCCTCGGCCAGCGAAA  538492

Query  842     CCAGCGGCGTCGCCTTGCGGTTGGCGTGGCGGCGCCGCACATCGGCGTATTCCTGCGCCA  901
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  538491  CCAGCGGCGTCGCCTTGCGGTTGGCGTGGCGGCGCCGCACATCGGCGTATTCCTGCGCCA  538432

Query  902     CTTCGTCGATGTAGGCCTGCGCCTGCTCGGACACCAGGTTGGCGGCCACGCCCACCGCGC  961
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  538431  CTTCGTCGATGTAGGCCTGCGCCTGCTCGGACACCAGGTTGGCGGCCACGCCCACCGCGC  538372

Query  962     GGCTCGCGTCTGGCGTGTAGATGACTGGCCCGTCGTAATTGGGCGCGATTTTCACGGCCG  1021
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  538371  GGCTCGCGTCTGGCGTGTAGATGACTGGCCCGTCGTAATTGGGCGCGATTTTCACGGCCG  538312

Query  1022    TATGCACCCGGCTGGTGGTGGCGCCGCCGATCATCAGCGGGATCTTGCGCTCGCGGAAGT  1081
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  538311  TATGCACCCGGCTGGTGGTGGCGCCGCCGATCATCAGCGGGATCTTGCGCTCGCGGAAGT  538252

Query  1082    ACGGGTCGCGCTGCATTTCCGAGGCCACATAGGCCATTTCTTCGAGGCTGGGCGTAATCA  1141
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  538251  ACGGGTCGCGCTGCATTTCCGAGGCCACATAGGCCATTTCTTCGAGGCTGGGCGTAATCA  538192

Query  1142    ACCCCGACAGGCCCACGATATCGGCCTGTTCTTCCTTGGCTTTTTCGAGAATCTGCGCGC  1201
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  538191  ACCCCGACAGGCCCACGATATCGGCCTGTTCTTCCTTGGCTTTTTCGAGAATCTGCGCGC  538132

Query  1202    ACGGCACCATGAC  1214
               |||||||||||||
Sbjct  538131  ACGGCACCATGAC  538119
```

Clone TM23

The blastx search across the entire clone indicated a homology of the DNA-sequence to the conjugal transfer protein.

E-value: 6e-70

Locus: YP-001632380

```
Query  1        GGGCATCGTTGCGAGTAATGCGGACACGGTCGCCCACAGACAGCTCTGCTCGGCTCAGGT  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3978078  GGGCATCGTTGCGAGTAATGCGGACACGGTCGCCCACAGACAGCTCTGCTCGGCTCAGGT  3978019

Query  61       GGTAGACCGATAACTTGGACATGGTTTTGGGGATGATTTCCATGGCTTGAGTAGGCGACT  120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3978018  GGTAGACCGATAACTTGGACATGGTTTTGGGGATGATTTCCATGGCTTGAGTAGGCGACT  3977959

Query  121      GGTCGGACTGGATCGATTCGACGGTAAGGCGGTCATGGCGTGTACTGCCGGTTACGCGAT  180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3977958  GGTCGGACTGGATCGATTCGACGGTAAGGCGGTCATGGCGTGTACTGCCGGTTACGCGAT  3977899

Query  181      ACATTTCGCCGCGTTTCAGCCCGCACTGATAGTCCCTTTCTGGAACCACGATGTCTCCCA  240
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3977898  ACATTTCGCCGCGTTTCAGCCCGCACTGATAGTCCCTTTCTGGAACCACGATGTCTCCCA  3977839

Query  241      CGGTGTAATACCGCGCTACGCTGCGTTCTGCACGAGTGGTGTCATGGCGAGTCAGGAGCT  300
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3977838  CGGTGTAATACCGCGCTACGCTGCGTTCTGCACGAGTGGTGTCATGGCGAGTCAGGAGCT  3977779

Query  301      GGCATCGGTGCCCCTTTCCTTCCAGACCGAGCAGGGCGTGGGTCCGTTCATTGATGGCTA  360
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3977778  GGCATCGGTGCCCCTTTCCTTCCAGACCGAGCAGGGCGTGGGTCCGTTCATTGATGGCTA  3977719

Query  361      GGCGAGACGCGTTGGTTCCAGTCACTACGATGG  393
               ||||||||||||||||||||||||||||||||
Sbjct  3977718  GGCGAGACGCGTTGGTTCCAGTCACTACGATGG  3977686
```

Clone TM24

The blastx search across the entire clone indicated a homology of the DNA-sequence to the following proteins:

1. hypothetical protein Bpet 0996

Evalue: 5e-129

Locus: YP-001629599

2. hypothetical protein

E-value: 6e-124

Locus: YP-001629600 and CAP41329

```
Query  1        GGTTGAGAAATCCGAACGGCTTGTCGCTGGACACCGTTATGACTTTGATCCCCACCCCGG  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1075455  GGTTGAGAAATCCGAACGGCTTGTCGCTGGACACCGTTATGACTTTGATCCCCACCCCGG  1075396

Query  61       CCGCCACTGGAATCCACCGATGCGGGTCATTCAGCAAGGCCTCTGCCGTGCTCGGGATGC  120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1075395  CCGCCACTGGAATCCACCGATGCGGGTCATTCAGCAAGGCCTCTGCCGTGCTCGGGATGC  1075336

Query  121      GGTTCATCCAGATGCGGACCTTGGCGTTGCCGGCATCCTGGACCACCACACGCGTCACAT  180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1075335  GGTTCATCCAGATGCGGACCTTGGCGTTGCCGGCATCCTGGACCACCACACGCGTCACAT  1075276

Query  181      CGAAAATCGGCTTGATGGCCGCGATGATCTCGGGCGTCGTGCCGTACCCGTTATTGACGG  240
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1075275  CGAAAATCGGCTTGATGGCCGCGATGATCTCGGGCGTCGTGCCGTACCCGTTATTGACGG  1075216

Query  241      CGATCTTCCAGTACAGCAGCTTGCGGTATTCGTAGTCGGGCAGCGTGGTCGAGCCCGCCA  300
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1075215  CGATCTTCCAGTACAGCAGCTTGCGGTATTCGTAGTCGGGCAGCGTGGTCGAGCCCGCCA  1075156
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Query  301      CCGTGCGCTCGTAGCGCCGGCGGATGCGCGCCCGAGAGAAGCCCTCGACACTGGGCTGGC  360
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1075155  CCGTGCGCTCGTAGCGCCGGCGGATGCGCGCCCGAGAGAAGCCCTCGACACTGGGCTGGC  1075096

Query  361      CCACGAATCCGAAAAAGGCCACGAATACGGCATTGTCCAGCACCCGCGACAGGCCTACGA  420
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1075095  CCACGAATCCGAAAAAGGCCACGAATACGGCATTGTCCAGCACCCGCGACAGGCCTACGA  1075036

Query  421      TCTCGCCGATGCCGTCGAGTTGCTTGCCTTCGGCAGCATCCAGCCAGCGCTTGTCGTATA  480
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1075035  TCTCGCCGATGCCGTCGAGTTGCTTGCCTTCGGCAGCATCCAGCCAGCGCTTGTCGTATA  1074976

Query  481      GGTCGCGCAGCGCCCCTTGCAGACCTTCGGCAGGCTTCAGCAACGCCTTCACCAGCGCCT  540
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1074975  GGTCGCGCAGCGCCCCTTGCAGACCTTCGGCAGGCTTCAGCAACGCCTTCACCAGCGCCT  1074916

Query  541      GCAAGCGTGGCTTGCCGTCGAATTGCGACAGCCAGTGATCCCAGGCAACCTGCCCATGGT  600
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1074915  GCAAGCGTGGCTTGCCGTCGAATTGCGACAGCCAGTGATCCCAGGCAACCTGCCCATGGT  1074856

Query  601      CTTGGTTCAAGTCCATCACGTCACCTCGACCCGGGTAGCATCAAACTTGGCAACCTCGAA  660
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1074855  CTTGGTTCAAGTCCATCACGTCACCTCGACCCGGGTAGCATCAAACTTGGCAACCTCGAA  1074796

Query  661      TTCCTGGATCGGGACGTTCTCGGCGCTGTAGTCGCCCTGGGTTGGAACGAAGCCCGGATC  720
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1074795  TTCCTGGATCGGGACGTTCTCGGCGCTGTAGTCGCCCTGGGTTGGAACGAAGCCCGGATC  1074736

Query  721      AGTGGAATGGGCAAAGACCAGATCCACCATGGCGATCCCCTGGGTCTGGTAGATCGCGCA  780
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1074735  AGTGGAATGGGCAAAGACCAGATCCACCATGGCGATCCCCTGGGTCTGGTAGATCGCGCA  1074676

Query  781      GAAGAAGCGCTGCTGCAGCACGTCCTGGCTGATCTGGTGGGCCTGGCCCGTGGCCAGGAT  840
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1074675  GAAGAAGCGCTGCTGCAGCACGTCCTGGCTGATCTGGTGGGCCTGGCCCGTGGCCAGGAT  1074616

Query  841      AGCGGCCGCCACTCGGGCGTAGCCATCAGTCGGGAATTCCTGCTCGCTTGCTGGCAGCAG  900
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1074615  AGCGGCCGCCACTCGGGCGTAGCCATCAGTCGGGAATTCCTGCTCGCTTGCTGGCAGCAG  1074556

Query  901      CGTCAGGCTGGCTTTGACCCAGACATAAACGGGCGCCGGCCGGTCGAAGCGGATCAGGTG  960
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1074555  CGTCAGGCTGGCTTTGACCCAGACATAAACGGGCGCCGGCCGGTCGAAGCGGATCAGGTG  1074496

Query  961      ATTGGCGCCCTCGTCGTCTTCGACCGACACCACCACACTGCCATACGTGTCGATCCCGCC  1020
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1074495  ATTGGCGCCCTCGTCGTCTTCGACCGACACCACCACACTGCCATACGTGTCGATCCCGCC  1074436

Query  1021     GGCCTTGGTCCGGAAGATAGCGTCCCCGATTTCATCGTCCAGGCCGCCATCGACGACCAC  1080
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1074435  GGCCTTGGTCCGGAAGATAGCGTCCCCGATTTCATCGTCCAGGCCGCCATCGACGACCAC  1074376

Query  1081     GTGCAGCGAGTGAGGCGGGCGGCCCGAGGCGTCCACCACATCGGAATCGTTCTGGTAGAC  1140
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1074375  GTGCAGCGAGTGAGGCGGGCGGCCCGAGGCGTCCACCACATCGGAATCGTTCTGGTAGAC  1074316

Query  1141     CTTGATCGCCGCCACGCCCACCACCTTGTCACGCACGTTCGGCGCGATGCT  1191
                ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1074315  CTTGATCGCCGCCACGCCCACCACCTTGTCACGCACGTTCGGCGCGATGCT  1074265
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Clone TM25

The blastx search across the entire clone indicated a homology of the DNA-sequence to the DNA (cytosine-5-)-methyltransferase, putative.

E-value: 5e-138

Locus: YP-001629550

```
Query  1        CTGTCAGAAACGCGCAGCACCACCGCCAAGTACCAAGCTCTCAACCGCCTGACGCTGCGC  60
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1039369  CTGTCAGAAACGCGCAGCACCACCGCCAAGTACCAAGCTCTCAACCGCCTGACGCTGCGC  1039310

Query  61       GGCGTCTGGCTGATGCTGGAGGCCTGGGCCGACGATCCGCCCGAGGTGATCCTGTTCGAG  120
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1039309  GGCGTCTGGCTGATGCTGGAGGCCTGGGCCGACGATCCGCCCGAGGTGATCCTGTTCGAG  1039250

Query  121      AACGTGCCGCGCATCGCCACCCGTGGCCGCCATCTGCTCGATCAGATCACCGGCATGCTG  180
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1039249  AACGTGCCGCGCATCGCCACCCGTGGCCGCCATCTGCTCGATCAGATCACCGGCATGCTG  1039190

Query  181      CGCCACTACGGCTACGTGGTCCGCGAAACCACGCATGACTGCGGCGAGCTGGGCGGCCTG  240
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1039189  CGCCACTACGGCTACGTGGTCCGCGAAACCACGCATGACTGCGGCGAGCTGGGCGGCCTG  1039130

Query  241      GCCCAGAGCCGGAGGCGGTTCCTGCTGATCGCCAGGCACGCCGAGAAAGTGCCGCCCTTC  300
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1039129  GCCCAGAGCCGGAGGCGGTTCCTGCTGATCGCCAGGCACGCCGAGAAAGTGCCGCCCTTC  1039070

Query  301      ATCTACGAGCCCCCCAAGCGGGCCCTGCGGTCGGTGGGCGAGGTGCTGAGCCGCTACGGC  360
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1039069  ATCTACGAGCCCCCCAAGCGGGCCCTGCGGTCGGTGGGCGAGGTGCTGAGCCGCTACGGC  1039010

Query  361      CGCCCGGGTGACCCGGCCATGGGCCCGATGCACCGCATCCCCAGCCTGAACTGGAAAACC  420
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1039009  CGCCCGGGTGACCCGGCCATGGGCCCGATGCACCGCATCCCCAGCCTGAACTGGAAAACC  1038950

Query  421      TGGGTGCGCCTGGCCTTCGTGGAAGCCGGCAAGGACTGGCGCAGCCTGAACCGGCTGGCC  480
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1038949  TGGGTGCGCCTGGCCTTCGTGGAAGCCGGCAAGGACTGGCGCAGCCTGAACCGGCTGGCC  1038890

Query  481      GTCGAAAACGGCCACCTGCGCGACTACCTAATCGTGCCCGAGGCCCACCATGGTTTCCTG  540
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1038889  GTCGAAAACGGCCACCTGCGCGACTACCTAATCGTGCCCGAGGCCCACCATGGTTTCCTG  1038830

Query  541      GGTGTGCAGCGCTGGGAGACGGCCAGCGGCACGATCTCCAGCCGCTGCGGCCCCA-CAAC  599
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||
Sbjct  1038829  GGTGTGCAGCGCTGGGAGACGGCCAGCGGCACGATCTCCAGCCGCTGCGGCCCCACCAAC  1038770

Query  600      GGCGCCTACAGCGTCGCCGACCCGCGCCAAGAGGTCTACTCGGCCGGCTACGGGGTCAAC  659
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1038769  GGCGCCTACAGCGTCGCCGACCCGCGCCAAGAGGTCTACTCGGCCGGCTACGGGGTCAAC  1038710

Query  660      GCATGGGAAGCGCCCACGGGTGCCGTGGCGGGCGAATCCCTGCCCAGCAACGGCCGCTTC  719
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1038709  GCATGGGAAGCGCCCACGGGTGCCGTGGCGGGCGAATCCCTGCCCAGCAACGGCCGCTTC  1038650

Query  720      TCGGTTGCGGACCCTCGCGCCGCGGCGGGCGCCAGCCAGTACCAGCAGTATGGCGTGCTG  779
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1038649  TCGGTTGCGGACCCTCGCGCCGCGGCGGGCGCCAGCCAGTACCAGCAGTATGGCGTGCTG  1038590

Query  780      CGCATGGATGACACGGCCGGCGCCGTCATCGGTGTGAAGAGTCCGGTTCAAGGCAGCTTC  839
                ||||||||||||||||||||||||||||||||||||||||||||||| ||| |||||||||
Sbjct  1038589  CGCATGGATGACACGGCCGGCGCCGTCATCGGTGTGAAGAGTCCGGGTCAGGGCAGCTTC  1038530

Query  840      AGCGTGGCCGACCCGCGCCACAGCGGCCCCGCCAAGCAT  878
                |||||||||||||||||||||||||||||||||||||||
Sbjct  1038529  AGCGTGGCCGACCCGCGCCACAGCGGCCCCGCCAAGCAT  1038491
```

Clone TM26

The blastx search across the entire clone indicated a homology of the DNA-sequence to the putative acyl dehydratase.

E-value: 9e-47

Locus: YP-001629405

```
Query  1       TTGCTTTCGCCGCGCCACATGTCGAACTGCAGCGTTTCGCCCGGGTA-ACCGGCGACGAG  59
               ||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  887570  TTGCTTTCGCCGCGCCACATGTCGAACTGCAGCGTTTCGCCCGGGTACACCGGCGACGAG  887629

Query  60      AAGCGCGCGTTCAGGCTGGCCAGCCGGCTGGCGTCGTAGCCGCACCAGGTCTTGACGATG  119
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  887630  AAGCGCGCGTTCAGGCTGGCCAGCCGGCTGGCGTCGTAGCCGCACCAGGTCTTGACGATG  887689

Query  120     GCGTGAGCCGCCATGCCATAGGTGCACAGGCCGTGCAGAATGGGCTGGGCATAGCCGGCC  179
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  887690  GCGTGAGCCGCCATGCCATAGGTGCACAGGCCGTGCAGAATGGGCTGGGCATAGCCGGCC  887749

Query  180     TTGCGGGCCACCTCGGGGTCGGCGTGCAGCGGGTTGCGGTCGGCGCTCAGGCGATACAGC  239
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  887750  TTGCGGGCCACCTCGGGGTCGGCGTGCAGCGGGTTGCGGTCGGCGCTCAGGCGATACAGC  887809

Query  240     AGCGCCGCGTTGGGCGCCACCGCCAGGGTGCAGCTTTGGTCGGGGGCCCGAGTGGGCGCG  299
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  887810  AGCGCCGCGTTGGGCGCCACCGCCAGGGTGCAGCTTTGGTCGGGGGCCCGAGTGGGCGCG  887869

Query  300     GCAGGCAAGGGCGGCGGGGCATCGTCGCCGCGGCCGAAGCCGCCGTCGCCCCGGCAGAAG  359
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  887870  GCAGGCAAGGGCGGCGGGGCATCGTCGCCGCGGCCGAAGCCGCCGTCGCCCCGGCAGAAG  887929

Query  360     GTGGTTTGCTGCAGAGTGGCCAGGCAGGCGCCGGCGCTGTCATGCAGCGTGCGCTCGGTG  419
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  887930  GTGGTTTGCTGCAGAGTGGCCAGGCAGGCGCCGGCGCTGTCATGCAGCGTGCGCTCGGTG  887989

Query  420     ATTACCAGCGCGCCCTTGTCGGCCCCTTTGTCGATGACATGCGTGACGCGGCTCTTGCCG  479
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  887990  ATTACCAGCGCGCCCTTGTCGGCCCCTTTGTCGATGACATGCGTGACGCGGCTCTTGCCG  888049

Query  480     ATGACGTTGCCGCTGGCGGGCAGCGGCGCGTGCAGGGCCAGGCGCTGCTCGCCGTGCACC  539
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  888050  ATGACGTTGCCGCTGGCGGGCAGCGGCGCGTGCAGGGCCAGGCGCTGCTCGCCGTGCACC  888109

Query  540     AGCCGCACCCAGTCGATGCCGGCGCGCGGATCGCTCATCCAGAAGCCGGGGTAGCCCAGC  599
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  888110  AGCCGCACCCAGTCGATGCCGGCGCGCGGATCGCTCATCCAGAAGCCGGGGTAGCCCAGC  888169

Query  600     ACGGTGGCTTGGGTTGGAAAGGCTTGCAGGCCCCCGTTCGTATACGTAGCGCAGTTGCCC  659
               |||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  888170  ACGGTGGCTTGGGTTGGAAAGGCTTGCAGG-CCCCGTTCGTATACGTAGCGCAGTTGCCC  888228

Query  660     GGCGTCGAGCGGGTCGTGCCCCAGGCCGATGCCCAGGGCGTACAGCATGGTGTC  713
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  888229  GGCGTCGAGCGGGTCGTGCCCCAGGCCGATGCCCAGGGCGTACAGCATGGTGTC  888282
```

Clone TM27

The blastx search across the entire clone indicated a homology of the DNA-sequence to the hypothetical protein Bpet 2300.

E-value: 3e-111

Locus: YP-001630911

```
Query  1        GTAGTATTGAAATGAACCTTCGCATGGTCGAAGCCGAGGCGAGTGAGGAGTCATCACCTT  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2389372  GTAGTATTGAAATGAACCTTCGCATGGTCGAAGCCGAGGCGAGTGAGGAGTCATCACCTT  2389431

Query  61       CAGTCAAGGCGCCGTCTGCTCCTATACCGTTGACTGCCGAGCAGGCGGATCTGCTGTATG  120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2389432  CAGTCAAGGCGCCGTCTGCTCCTATACCGTTGACTGCCGAGCAGGCGGATCTGCTGTATG  2389491

Query  121      AGGTTTTTGATTACAGCATATCCATTCGCGACATCGATAGGGCAATAAAATCCGCAAAAA  180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2389492  AGGTTTTTGATTACAGCATATCCATTCGCGACATCGATAGGGCAATAAAATCCGCAAAAA  2389551
```

```
Query  181      GGCTGCTGCTTGCCGGATATTATGTCCCGAAATTGATGCCAAAGATGGCACAGATTGCAT  240
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2389552  GGCTGCTGCTTGCCGGATATTATGTCCCGAAATTGATGCCAAAGATGGCACAGATTGCAT  2389611

Query  241      CGTTGAAATTCGACAATGAGTCGGCCAAAATAATAGCGATGCTGAGCCATTATGGCGATT  300
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2389612  CGTTGAAATTCGACAATGAGTCGGCCAAAATAATAGCGATGCTGAGCCATTATGGCGATT  2389671

Query  301      TGAATGGCATGATGGCGCTCCTGCAGGCTCGGTCGTATTCATCACAAGATATATCGAGAG  360
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2389672  TGAATGGCATGATGGCGCTCCTGCAGGCTCGGTCGTATTCATCACAAGATATATCGAGAG  2389731

Query  361      ACATTTTAGGGTTCATTACATTGCTGGCAAAGCTGATTTCACTTAAGCCGGATCGTGTAA  420
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2389732  ACATTTTAGGGTTCATTACATTGCTGGCAAAGCTGATTTCACTTAAGCCGGATCGTGTAA  2389791

Query  421      ATGATGCTATCTTCCTGATaaaaaaaTTTCCAGAACTGTTTGATGAAGATAAATTCGAAC  480
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2389792  ATGATGCTATCTTCCTGATAAAAAAATTTCCAGAACTGTTTGATGAAGATAAATTCGAAC  2389851

Query  481      GCATCCTTATCGGCATATTGATGTTGAATAGCAGGCGATCAATGGATCGTGTGCTTGGTT  540
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2389852  GCATCCTTATCGGCATATTGATGTTGAATAGCAGGCGATCAATGGATCGTGTGCTTGGTT  2389911

Query  541      TGCTTGCGGTCGAGCGTCCTCATTTGGCGCTTAGCGCCATAGAAGGTCTGCTCGACAGTG  600
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2389912  TGCTTGCGGTCGAGCGTCCTCATTTGGCGCTTAGCGCCATAGAAGGTCTGCTCGACAGTG  2389971

Query  601      GGGAAACGTCAGGCAACATTCGGATTGTTTATTCCCAGGCACTTTCTTAT  650
                ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2389972  GGGAAACGTCAGGCAACATTCGGATTGTTTATTCCCAGGCACTTTCTTAT  2390021
```

Clone TM28

The blastx search across the entire clone indicated a homology of the DNA-sequence to the putative secreted protein.

E-value: 9e-75

Locus: YP-001631732

```
Query  1        CGACATTGCTACCCGCATCATCGCCGACAAGCTCGCCGGCAAGCTGGGG--GTCGGTAGT  58
                ||||||||||||||||||||||||||||||||||||||||||||||||   |||||||||
Sbjct  3279679  CGACATTGCTACCCGCATCATCGCCGACAAGCTCGCCGGCAAGCTGGGCCAGTCGGTAGT  3279738

Query  59       GGTCGAGAACCGGGCCGGCGCGGCGGGCATCATCGGCACACAGCAGTTGGTGCGGGCCAA  118
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3279739  GGTCGAGAACCGGGCCGGCGCGGCGGGCATCATCGGCACACAGCAGTTGGTGCGGGCCAA  3279798

Query  119      CCCCGACGGCTACACGCTGATCATGGGCAACATAGGCCCCAATGCCATCAACTACAGCAT  178
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3279799  CCCCGACGGCTACACGCTGATCATGGGCAACATAGGCCCCAATGCCATCAACTACAGCAT  3279858

Query  179      GTACCGCGAGCTGCCGTACAAGGCAACGGACTTCGCCCCCATTACGCGGGTGATTTCAGT  238
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3279859  GTACCGCGAGCTGCCGTACAAGGCAACGGACTTCGCCCCCATTACGCGGGTGATTTCAGT  3279918

Query  239      GCCCAATGTGCTGGTCGTGAACGCGCAATCGCCGGCCCGCAGCGCCGGCGAGCTGATCGA  298
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3279919  GCCCAATGTGCTGGTCGTGAACGCGCAATCGCCGGCCCGCAGCGCCGGCGAGCTGATCGA  3279978

Query  299      GATACTGCGCAAAGATCCCGGCAAGTCTTCGTTCGGCTCGTCCGGCACCGGGCAGTCGCC  358
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3279979  GATACTGCGCAAAGATCCCGGCAAGTCTTCGTTCGGCTCGTCCGGCACCGGGCAGTCGCC  3280038

Query  359      GCACTTGTCCGGCGAGCTTTTCAAGCAGCGCGCGGGCGTGCAGGCCACGCA  409
                ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3280039  GCACTTGTCCGGCGAGCTTTTCAAGCAGCGCGCGGGCGTGCAGGCCACGCA  3280089
```

Clone TM29

The blastx search across the entire clone indicated a homology of the DNA-sequence to the transposase and ISRSO8-transposase orfA protein.

E-value:2e-58 and 2e-45

Locus: YP-001628796 and YP-001631623

```
Query  1        CGCCTTTGGATTCAGCGTGCACGGCCTTGATGTGCACCAGCAGAGCGTCGTTGCTGATGC  60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2201700  CGCCTTTGGATTCAGCGTGCACGGCCTTGATGTGCACCAGCAGAGCGTCGTTGCTGATGC  2201759

Query  61       GTCGGCGCGGTCGGTCAGTATCGACATCCCGCACCTTGCGCGCGTGGTAACCGCTGGGGC  120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2201760  GTCGGCGCGGTCGGTCAGTATCGACATCCCGCACCTTGCGCGCGTGGTAACCGCTGGGGC  2201819

Query  121      TGACACCCAGCACCTGGCAGCTCAGGGACACCGGCCATTGTCGGCTGTGAAGCTCGATCC  180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2201820  TGACACCCAGCACCTGGCAGCTCAGGGACACCGGCCATTGTCGGCTGTGAAGCTCGATCC  2201879

Query  181      AGGCGTACTTCATGCCGACACCTTCGCAAAGTACGCCGTGGCTTTTCCCAATATGTCGCG  240
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2201880  AGGCGTACTTCATGCCGACACCTTCGCAAAGTACGCCGTGGCTTTTCCCAATATGTCGCG  2201939

Query  241      CTCCATCTTCACGCGTGCCAACTCCGCGCGCAGCCGGGCAATCTCCATCTGTTCTGGAGA  300
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2201940  CTCCATCTTCACGCGTGCCAACTCCGCGCGCAGCCGGGCAATCTCCATCTGTTCTGGAGA  2201999

Query  301      AACCTGTTTGCCTGCGCCGTTCAGCTTCCCAGCGGCATCGGCCTTCACCCAGTTGTGCAG  360
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2202000  AACCTGTTTGCCTGCGCCGTTCAGCTTCCCAGCGGCATCGGCCTTCACCCAGTTGTGCAG  2202059

Query  361      CGTCTTCGGGCTGATGCCCAGTATCTTCGCCACCGCCGCCATGCTCTGGCCGCCGCGGAC  420
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2202060  CGTCTTCGGGCTGATGCCCAGTATCTTCGCCACCGCCGCCATGCTCTGGCCGCCGCGGAC  2202119

Query  421      CATGCGCACGGCTTCCAGCATGAATTCCTGCGTATATCGAGCTCTCGGATTACCCATCTT  480
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2202120  CATGCGCACGGCTTCCAGCATGAATTCCTGCGTATATCGAGCTCTCGGATTACCCATCTT  2202179

Query  481      TCCTCCCTCCTTGCGTGAGTTTTACACACTCAGCAAGGGATCCATTTTTTGCGGGCAAGC  540
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2202180  TCCTCCCTCCTTGCGTGAGTTTTACACACTCAGCAAGGGATCCATTTTTTGCGGGCAAGC  2202239

Query  541      TCAGACCGACCAGGACGTGGGCCAGCAGATGCTGGCGCAACTGTTCTCGAACCCCAGCGT  600
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2202240  TCAGACCGACCAGGACGTGGGCCAGCAGATGCTGGCGCAACTGTTCTCGAACCCCAGCGT  2202299

Query  601      GCTGTACCT-ACCGCGGGCATTATCGGCATCATGGGGCTGATTCCCAATATGCCGCATGT  659
               ||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2202300  GCTGTACCTGACCGCGGGCATTATCGGCATCATGGGGCTGATTCCCAATATGCCGCATGT  2202359

Query  660      G  660
               |
Sbjct  2202360  G  2202360
```

Clone TM31

The blastx search across the entire clone indicated a homology of the DNA-sequence to the autotransporter.

E-value: 1e-04

Locus: YP-001632594

```
Query  1        CCGAGGCCAGCGTAACGACCCACGGCGACAACATCCTGGGTGTCGTGG-GCAAAGCCtgg  59
                ||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct  4201763  CCGAGGCCAGCGTAACGACCCACGGCGACAACATCCTGGGTGTCGTGGCGCAAAGCCTGG  4201704

Query  60       gcggccagggcggcgacggaggcgatggcacggcgctggccggccagggcggtggcggcg  119
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4201703  GCGGCCAGGGCGGCGACGGAGGCGATGGCACGGCGCTGGCCGGCCAGGGCGGTGGCGGCG  4201644

Query  120      gattcggcggAAACGCCAGCGATGTCTATATCTCGGCGCAATCTGGCGCCGCCATTTCCA  179
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4201643  GATTCGGCGGAAACGCCAGCGATGTCTATATCTCGGCGCAATCTGGCGCCGCCATTTCCA  4201584

Query  180      CGACGGGCGATTTCGCGACCGGCATGTTGGCGCATTCCATcggcggcggcggcggcacgg  239
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4201583  CGACGGGCGATTTCGCGACCGGCATGTTGGCGCATTCCATCGGCGGCGGCGGCGGCACGG  4201524

Query  240      gcggcgatttcgtggccgtgctcggagggcagggcggtaacggcggcAACGGCGGTGATG  299
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4201523  GCGGCGATTTCGTGGCCGTGCTCGGAGGGCAGGGCGGTAACGGCGGCAACGGCGGTGATG  4201464

Query  300      CTGGCCAGGCCGCCATTTCCAGCCTGGCCACCCTGTCTACCACCGGCGATCATGCCTACG  359
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4201463  CTGGCCAGGCCGCCATTTCCAGCCTGGCCACCCTGTCTACCACCGGCGATCATGCCTACG  4201404

Query  360      GCATGCTGGCGCAATCCATTGCcggcagcggcggcgcgggcggcgtggatacttccgcgg  419
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4201403  GCATGCTGGCGCAATCCATTGCCGGCAGCGGCGGCGCGGGCGGCGTGGATACTTCCGCGG  4201344

Query  420      ccgtggcgctgggggggcgatggcgccggAGGCGGCGCCGCCAACCAGGTCAACATTACGA  479
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4201343  CCGTGGCGCTGGGGGGCGATGGCGCCGGAGGCGGCGCCGCCAACCAGGTCAACATTACGA  4201284

Query  480      ACTACGGAACGATCACCACGGGCGGGTACAGCGCGCATGGCATCGTCGCCCAGTCCATcg  539
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4201283  ACTACGGAACGATCACCACGGGCGGGTACAGCGCGCATGGCATCGTCGCCCAGTCCATCG  4201224

Query  540      gcggcggcggcggcgcggcgggcagcgccaacggcgcgctcagtgtgggcggcaacgccg  599
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4201223  GCGGCGGCGGCGGCGCGGCGGGCAGCGCCAACGGCGCGCTCAGTGTGGGCGGCAACGCCG  4201164

Query  600      ccggTGAAACGCCCTCTTCGGGCGGCAGTGTATGGATCAACAACCAGGCGGCGATCACCA  659
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4201163  CCGGTGAAACGCCCTCTTCGGGCGGCAGTGTATGGATCAACAACCAGGCGGCGATCACCA  4201104

Query  660      CCACGGGCGATGCCGCGGTGGGGATGCTGGCGCAATCGATcggcggcgggggcggcagcg  719
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4201103  CCACGGGCGATGCCGCGGTGGGGATGCTGGCGCAATCGATCGGCGGCGGGGGCGGCAGCG  4201044

Query  720      ggggcgatgccaacggcgtggtcaccgtgggcggctcgggcgCCAGCGCCGGC  772
                ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4201043  GGGGCGATGCCAACGGCGTGGTCACCGTGGGCGGCTCGGGCGCCAGCGCCGGC  4200991
```

Clone TM32

The blastx search across the entire clone indicated a homology of the DNA-sequence to the LPS(lipopolysaccharides) - export associated outer membrane protein.

E-value: 0.0

Locus: YP-001629363

```
Query  1       ATTTACGCTCTTTCTTGACCGGAAAGGTCAGGTACGGCGACGCCAGTAG-GGCACGTCTT  59
               ||||||||||||||||||||||||||||||||||||||||||||||| || ||||||||||
Sbjct  841015  ATTTACGCTCTTTCTTGACCGGAAAGGTCAGGTACGGCGACGCCAGCAGCGGCACGTCTT  840956

Query  60      TGAAGTACAGCACGCCGTTGCGCGCCACGCCCTCGTTTTCGTCGAAATCGAGGTCGACGC  119
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  840955  TGAAGTACAGCACGCCGTTGCGCGCCACGCCCTCGTTTTCGTCGAAATCGAGGTCGACGC  840896
```

```
Query  120     TGTCGGCCTTGATGTACCACGACGGCTTGGGACACGGGCAGCCGCTGTACTGCACGGTGG  179
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  840895  TGTCGGCCTTGATGTACCACGACGGCTTGGGACACGGGCAGCCGCTGTACTGCACGGTGG  840836

Query  180     TCAGCCGCATTTGCGAGCGGCTGAAGATCTGGGCCCGTTCGGCGCGGGCAAAGCCGCCGC  239
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  840835  TCAGCCGCATTTGCGAGCGGCTGAAGATCTGGGCCCGTTCGGCGCGGGCAAAGCCGCCGC  840776

Query  240     TGGCGCCGATCCAGAAATCGGGAGACTCGATTTCGCCGCTGTCGGTGTCGATGTTGAACT  299
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  840775  TGGCGCCGATCCAGAAATCGGGAGACTCGATTTCGCCGCTGTCGGTGTCGATGTTGAACT  840716

Query  300     GCGCCGACGGGCCGGTGACCAGCGTGCCGTCACGCATCAGGCGCGCGTTGCCCTGGATGT  359
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  840715  GCGCCGACGGGCCGGTGACCAGCGTGCCGTCACGCATCAGGCGCGCGTTGCCCTGGATGT  840656

Query  360     CGACCGCGCCCGTGTCGCTGGCGTAGTCGAT  390
               ||||||||||||||||||||||||||||||||
Sbjct  840655  CGACCGCGCCCGTGTCGCTGGCGTAGTCGAT  840625
```

Clone TM33

The blastx search across the entire clone indicated a homology of the DNA-sequence to the MFS permease.

E-value: 2e-40

Locus: YP-001631812

```
Query  1        CGACACGCCCGCGCTTTCGCGCAGCACTTGCGCAAAGGGCACCAGTGC-ACCTGCCCCAC  59
               ||||||||||||||||||||||||||||||||||||||||||||||| || ||||||||||||
Sbjct  3363903  CGACACGCCCGCGCTTTCGCGCAGCACTTGCGCAAAGGGCACCAGCGCCACCTGCCCCAC  3363962

Query  60       CGAACCGCCCGCGCTGGCCAGGCCCAGCGCCATGCTGCGCCGCTGCGGCGTGGCGGCGCG  119
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3363963  CGAACCGCCCGCGCTGGCCAGGCCCAGCGCCATGCTGCGCCGCTGCGGCGTGGCGGCGCG  3364022

Query  120      CCCCACCGCGGCCAGCACCACGCCGAAGCTGGTGCAGCTGATACCTATGCCCACCAGCAC  179
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3364023  CCCCACCGCGGCCAGCACCACGCCGAAGCTGGTGCAGCTGATACCTATGCCCACCAGCAC  3364082

Query  180      GCCCATGCCGATGACCAGCAATACCGGCGATTGCGCGGCCGTGGCCAGCGCCAGCCCCGC  239
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3364083  GCCCATGCCGATGACCAGCAATACCGGCGATTGCGCGGCCGTGGCCAGCGCCAGCCCCGC  3364142

Query  240      CGCGAAGGCCGCGGCGCCGAAGGCCACCACCGGCGCCGATCCGTAGCGGTCGGCCGCCGC  299
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3364143  CGCGAAGGCCGCGGCGCCGAAGGCCACCACCGGCGCCGATCCGTAGCGGTCGGCCGCCGC  3364202

Query  300      GCCGGCAAAGGGCTGCGCCGCGCCCCACACCAGGTTGTGCACGGCGATGGCGAATGCGAT  359
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3364203  GCCGGCAAAGGGCTGCGCCGCGCCCCACACCAGGTTGTGCACGGCGATGGCGAATGCGAT  3364262

Query  360      CTGCGTCACCGGCAGGCCGCGGTCGAACGAAAAGGGATTGATGAACAGCCCGAATGTCTG  419
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3364263  CTGCGTCACCGGCAGGCCGCGGTCGAACGAAAAGGGATTGATGAACAGCCCGAATGTCTG  3364322

Query  420      CCGCGCGCCCATCGCGGCACTCAGTATCAGCGCCGCCGCGATGACCACCCCGACGACAGG  479
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3364323  CCGCGCGCCCATCGCGGCACTCAGTATCAGCGCCGCCGCGATGACCACCCCGACGACAGG  3364382

Query  480      GCGCGCCAGCGGATGCGCGGGATTTGCGGTTTGCATGATGAGGCGACCT  528
               ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3364383  GCGCGCCAGCGGATGCGCGGGATTTGCGGTTTGCATGATGAGGCGACCT  3364431
```

Clone TM35

The blastx search across the entire clone indicated a homology of the DNA-sequence to putative chaperonin GroEL.

E-value: 5e-43

Locus: YP-001632546

```
Query  1        GTGCTGGCCCAGGCCATCGTGCAGGAAGGCCTGAAGTACGTTGCCGT-GGCTTCA-CCCC  58
                ||||||||||||||||||||||||||||||||||||||||||||||  ||||||| ||||
Sbjct  4146842  GTGCTGGCCCAGGCCATCGTGCAGGAAGGCCTGAAGTACGTTGCCGCCGGCTTCAACCCC  4146901

Query  59       ATCGACCTGAAGCGCGGCATCGACAAAGCCGTGTCCGCCGCCGTGGCTGAACTGGCCAAG  118
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4146902  ATCGACCTGAAGCGCGGCATCGACAAAGCCGTGTCCGCCGCCGTGGCTGAACTGGCCAAG  4146961

Query  119      CAATCCAAGCCGGTCACCACCAGCAAGGAAATCGCCCAGGTTGGCTCGATCTCGGCCAAC  178
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4146962  CAATCCAAGCCGGTCACCACCAGCAAGGAAATCGCCCAGGTTGGCTCGATCTCGGCCAAC  4147021

Query  179      AGCGACGAATCCATCGGCAAGATCATCGCCGACGCGATGGACAAGGTCGGCAAGGAAGGC  238
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4147022  AGCGACGAATCCATCGGCAAGATCATCGCCGACGCGATGGACAAGGTCGGCAAGGAAGGC  4147081

Query  239      GTCATCACCGTCGAAGACGGCAAGTCGCTGGACAACGAACTGGACGTGGTCGAAGGCATG  298
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4147082  GTCATCACCGTCGAAGACGGCAAGTCGCTGGACAACGAACTGGACGTGGTCGAAGGCATG  4147141

Query  299      CAGTTCGACCGCGGCTACCTGTCGCCCTACTTCATCAACAACCCCGACAAGCAAGTCGCC  358
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4147142  CAGTTCGACCGCGGCTACCTGTCGCCCTACTTCATCAACAACCCCGACAAGCAAGTCGCC  4147201

Query  359      GCGCTGGACGATCCGTACGTCCTGATCTTCGACAAGAAGATCAGCAACATCCGCGACCTG  418
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4147202  GCGCTGGACGATCCGTACGTCCTGATCTTCGACAAGAAGATCAGCAACATCCGCGACCTG  4147261

Query  419      CTGCCCGTGCTGGAACAAGTCGCCAAGTCGAGCCGTCCGCTGCTGATCATCGCTGAAGAC  478
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4147262  CTGCCCGTGCTGGAACAAGTCGCCAAGTCGAGCCGTCCGCTGCTGATCATCGCTGAAGAC  4147321

Query  479      GTCGAAGGCGAAGCGCTGGCCACCCTGGTGGTGAACAACATCCGTGGCATCCTGAAGACC  538
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4147322  GTCGAAGGCGAAGCGCTGGCCACCCTGGTGGTGAACAACATCCGTGGCATCCTGAAGACC  4147381

Query  539      ACCGCCGTCAAGGCGCCT  556
                ||||||||||||||||||
Sbjct  4147382  ACCGCCGTCAAGGCGCCT  4147399
```

Clone TM36

The blastx search across the entire clone indicated a homology of the DNA-sequence to the serine-type D-Ala-D-Ala carboxypeptidase.

E-value: 2e-29

Locus: YP-001633426

```
Query  1        GCGCATTGAACACCACGTAGGCGGTCATGATCTTGGTGAGCGAGGAAGGGTCGATCTGCA  60
                ||||||||||||||||||||||||||||||||||||||||||||||  || ||||||||||
Sbjct  5074391  GCGCATTGAACACCACGTAGGCGGTCATGATCTTGGTGAGCGAGGCCGGCTCGATCTGCA  5074450

Query  61       TGTCGGGATTGGCCGCCGCCAGCACCTGGCCGCTGCTGGCATCGATGGTGATCCAGGCGC  120
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5074451  TGTCGGGATTGGCCGCCGCCAGCACCTGGCCGCTGCTGGCATCGATGGTGATCCAGGCGC  5074510
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```
Query  121      GCGCCGCGATGGTGGGCGCGGGCACCGACGACAATTCCGCCACCGGCGTCGTGTCCGCCG  180
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5074511  GCGCCGCGATGGTGGGCGCGGGCACCGACGACAATTCCGCCACCGGCGTCGTGTCCGCCG  5074570

Query  181      CCGCGGGCGCGGTGCCGGCCGTCGGCGCTGCCGGCTGCTGCTGCGCCCAGGCAGGCGCGG  240
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5074571  CCGCGGGCGCGGTGCCGGCCGTCGGCGCTGCCGGCTGCTGCTGCGCCCAGGCAGGCGCGG  5074630

Query  241      ATGCCGCCATCAACGCCGACAACACGGCGCCGGCCAACGCGCGGCGGGAAAACGCAACAG  300
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5074631  ATGCCGCCATCAACGCCGACAACACGGCGCCGGCCAACGCGCGGCGGGAAAACGCAACAG  5074690

Query  301      AAGACGGAAGGGAACGATTGTTTTTCATCGGCAAGAGTAGTCCACGATCAAGAG  354
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5074691  AAGACGGAAGGGAACGATTGTTTTTCATCGGCAAGAGTAGTCCACGATCAAGAG  5074744
```

Clone TM37

The blastx search across the entire clone indicated a homology of the DNA-sequence to the hypothetical protein Bpet 2406 and hypothetical protein Bpet 2407.

E-value: 6e-68 and 1e-59

Locus: YP-001631017 and YP-001631018

```
Query  1        CATGTTCCGCGCCACCCGCATCGACCCCGATGATCCATGGGTGCGCTGGGCGGTGGAGTC  60
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2521381  CATGTTCCGCGCCACCCGCATCGACCCCGATGATCCATGGGTGCGCTGGGCGGTGGAGTC  2521322

Query  61       GCTGGAACGCACCAGCGGCAAGAAGGCGGCGATTCTGCCCAACCTGGGCGGTTCGCTGCC  120
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2521321  GCTGGAACGCACCAGCGGCAAGAAGGCGGCGATTCTGCCCAACCTGGGCGGTTCGCTGCC  2521262

Query  121      GAACGATATCTTTACGGAAGTGCTGGGCCTGCGCACCATATGGGTGCCGCACTCGTATCC  180
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2521261  GAACGATATCTTTACGGAAGTGCTGGGCCTGCGCACCATATGGGTGCCGCACTCGTATCC  2521202

Query  181      GGGCTGCTCGCAGCATGCGCCTAACGAACACCTGCCGCCGGAACTGCTGCGCGAAGGCCT  240
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2521201  GGGCTGCTCGCAGCATGCGCCTAACGAACACCTGCCGCCGGAACTGCTGCGCGAAGGCCT  2521142

Query  241      GACCCTGATGACCGGGCTGTATTGGGACCTGGGCGCGGGCGATACGCCGCCGCGCTGATG  300
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2521141  GACCCTGATGACCGGGCTGTATTGGGACCTGGGCGCGGGCGATACGCCGCCGCGCTGATG  2521082

Query  301      GCCCCGGAACCAGCCGGACGCGGCCGCTACAGATACGCCTGGTAGACCCGCGTGAGGCCC  360
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2521081  GCCCCGGAACCAGCCGGACGCGGCCGCTACAGATACGCCTGGTAGACCCGCGTGAGGCCC  2521022

Query  361      CAGCGCCTGACCAGCAGTACTTCGTAGGGGTGCTTGCGGCCCTGGTATTCGGGGCCGTCC  420
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2521021  CAGCGCCTGACCAGCAGTACTTCGTAGGGGTGCTTGCGGCCCTGGTATTCGGGGCCGTCC  2520962

Query  421      ATGCCGGGCGAGCCCAGCGGCATGCCGGGTACGGCCAGCCCGATGGCGGCCGGCCGTTCG  480
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2520961  ATGCCGGGCGAGCCCAGCGGCATGCCGGGTACGGCCAGCCCGATGGCGGCCGGCCGTTCG  2520902

Query  481      CGCAGCATGCGCTGGATGTCGGAGGCGGGCACGTGGCCTTCAAGGGCATAGCCATCAACC  540
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2520901  CGCAGCATGCGCTGGATGTCGGAGGCGGGCACGTGGCCTTCAAGGGCATAGCCATCAACC  2520842

Query  541      AGCCCGGTATGGCAAGAACCGTACTCAGCCGGTATGCCCAGCCGATTGCGCACTTCGTCT  600
                |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  2520841  AGCCCGGTATGGCAAGAACCGTACTCAGCCGGTATGCCCAGCCGATTGCGCACTTCGGCG  2520782

Query  601      ATGCTGGGCACTTCTTGCACCTCGACCCGGAAACCGTGATCGCGCAGGTGGGAG  654
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2520781  GTGCTGGGCACTTCTTGCACCTCGACCCGGAAACCGTGATCGCGCAGGTGGGAG  2520728
```

Clone TM39

The blastx search across the entire clone indicated a homology of the DNA-sequence to the bacteriophage protein.

E-value: 7e-76

Locus: YP-001629597

```
Query  1        CGACCTGGTGAAGCTGGAAAGCCGCAGCGTCCAGGGCTTCTACCGCGGGGACGGCGTGCG  60
                |||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  1072174  CGACCTGGTGAAGCTGGAAAGCCGCAGCGTCCAGGGCTTCTACCGCGCGGACGGCGTGCG  1072233

Query  61       TCACGTGGGTGACAGCGACGGCGGCGACTGGCAGACCCAGTTGGACCTGGTAGACCGCTA  120
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1072234  TCACGTGGGTGACAGCGACGGCGGCGACTGGCAGACCCAGTTGGACCTGGTAGACCGCTA  1072293

Query  121      CGCAGCCCGCAAGAAGGCCCGACCATGACCGATCCTGTAACCGACCTGCGCCGCCTCATC  180
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1072294  CGCAGCCCGCAAGAAGGCCCGACCATGACCGATCCTGTAACCGACCTGCGCCGCCTCATC  1072353

Query  181      GCCGCCGAGCTGGCCGAGGTCCACACCACGCTGCCGGGCGTCGTCGTGGCCTACGACGGC  240
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1072354  GCCGCCGAGCTGGCCGAGGTCCACACCACGCTGCCGGGCGTCGTCGTGGCCTACGACGGC  1072413

Query  241      AAGATGGCCGTGGTCCGACCGGCATTGCCCAAGCAACTGGCCAACGGCCAAGTCCTGCCG  300
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1072414  AAGATGGCCGTGGTCCGACCGGCATTGCCCAAGCAACTGGCCAACGGCCAAGTCCTGCCG  1072473

Query  301      GCGCCGCAGATCGTCAGCGTGCCGGTGTGCTGGCCGGTGGGCGACGTGGCCGGCGCGCTG  360
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1072474  GCGCCGCAGATCGTCAGCGTGCCGGTGTGCTGGCCGGTGGGCGACGTGGCCGGCGCGCTG  1072533

Query  361      GCGCTGATCAGCGTTCCGCTGAAGGCCGGCGATCCTGTGGTGCTGCACTTTTCTGAGCGT  420
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1072534  GCGCTGATCAGCGTTCCGCTGAAGGCCGGCGATCCTGTGGTGCTGCACTTTTCTGAGCGT  1072593

Query  421      GCCCTGGAATCCTGGCTGGCCGGCAGCGACGAGCCCCCCGACGATCCCCGGCAGTTCGAC  480
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1072594  GCCCTGGAATCCTGGCTGGCCGGCAGCGACGAGCCCCCCGACGATCCCCGGCAGTTCGAC  1072653

Query  481      CTGACCGACTGCTTCGCCG  499
                |||||||||||||||||||
Sbjct  1072654  CTGACCGACTGCTTCGCCG  1072672
```

Clone TM40

The blastx search across the entire clone indicated a homology of the DNA-sequence to the hypothetical protein Bpet 0935.

E-value: 1e-96

Locus: YP-001629538

```
Query  1        CGCCCTGGCCAAATGGCCCGATGTACCCGCCGTGTACGGCTGGCTGTTG-TGGACGAACG  59
                |||||||||||||||||||||||||||||||||||||||||||||||| | ||||||||||
Sbjct  1028666  CGCCCTGGCCAAATGGCCCGATGTACCCGCCGTGTACGGCTGGCTGTCGCTGGACGAACG  1028607

Query  60       CGGGCGCTGGCGCCTGCATCCTGTGGGCGACGCCACCGGCGGCGGGCCTGGCGAATCCAT  119
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1028606  CGGGCGCTGGCGCCTGCATCCTGTGGGCGACGCCACCGGCGGCGGGCCTGGCGAATCCAT  1028547
```

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Query  120      CGAGAACACCCAGATCCTGGACTTCATCGGCCGCAATTACGATCATGACGACGCAGGCCG  179
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1028546  CGAGAACACCCAGATCCTGGACTTCATCGGCCGCAATTACGATCATGACGACGCAGGCCG  1028487

Query  180      CTGGTTTTTCCAGAACGGCCCGCAGCGCGTGTACGTGCGCCTGGATGCCGCGCCGTTCCT  239
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1028486  CTGGTTTTTCCAGAACGGCCCGCAGCGCGTGTACGTGCGCCTGGATGCCGCGCCGTTCCT  1028427

Query  240      GCTGCGCCGCGCCGACGACGGCGTCGGCCTGGTCACGCATACCGGCCGGACGGTGCGGGC  299
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1028426  GCTGCGCCGCGCCGACGACGGCGTCGGCCTGGTCACGCATACCGGCCGGACGGTGCGGGC  1028367

Query  300      GGTGACAGCCTGGCTGCTGGACGACACGGGGCGCCTGTACGCGCAGACCGATGCGGGGCC  359
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1028366  GGTGACAGCCTGGCTGCTGGACGACACGGGGCGCCTGTACGCGCAGACCGATGCGGGGCC  1028307

Query  360      AGCCATGGTCGAAGGCCGCGAACTGCCCGCGCTGCTCGACTCGTTGCGCGATGTGCAGGG  419
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1028306  AGCCATGGTCGAAGGCCGCGAACTGCCCGCGCTGCTCGACTCGTTGCGCGATGTGCAGGG  1028247

Query  420      CCGGCCGCTGGCCGATGCGCTGGAATCGGCCGCCGCGGGCATATC  464
                |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1028246  CCGGCCGCTGGCCGATGCGCTGGAATCGGCCGCCGCGGGCATATC  1028202
```

Clone TM41

The blastx search across the entire clone indicated a homology of the DNA-sequence to the TonB-dependent outer membrane receptor.

E-value: 1e-17

Locus: YP-001633406

```
Query  1        CTCGGCATATTCCGCGCGCAGGTCGACGCGATTGCTGCGCAACTTCACGTATGGCGGATC  60
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5056566  CTCGGCATATTCCGCGCGCAGGTCGACGCGATTGCTGCGCAACTTCACGTATGGCGGATC  5056625

Query  61       TTCCTCTCCGTGCTCATGATCGTGATCGTGGTCATGGTCGTGATCATGGCCGCCGCAATG  120
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5056626  TTCCTCTCCGTGCTCATGATCGTGATCGTGGTCATGGTCGTGATCATGGCCGCCGCAATG  5056685

Query  121      CAGGTGCGTGCCGTGGGGGTGGCACCCTTCGTATTCGTGGTTGTGCCCAGGCAGCCCGTA  180
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5056686  CAGGTGCGTGCCGTGGGGGTGGCACCCTTCGTATTCGTGGTTGTGCCCAGGCAGCCCGTA  5056745

Query  181      TTTGCTTTCCAGGTAGGTATAGGCCACGCCCACATAGCCGCGCGGCGTGATCCACGACAG  240
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5056746  TTTGCTTTCCAGGTAGGTATAGGCCACGCCCACATAGCCGCGCGGCGTGATCCACGACAG  5056805

Query  241      GCCCACCGTGCCTTGCGACGACCTGCTGTACGAGCCTTCCAGTTCGCCGCCTGGCCAGTC  300
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5056806  GCCCACCGTGCCTTGCGACGACCTGCTGTACGAGCCTTCCAGTTCGCCGCCTGGCCAGTC  5056865

Query  301      GGGCACGTTGTAGTCGTCCGACCGGCGCTTCAGGCCCTCGACCCGCACGGCGAATTcgcc  360
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5056866  GGGCACGTTGTAGTCGTCCGACCGGCGCTTCAGGCCCTCGACCCGCACGGCGAATTCGCC  5056925

Query  361      cgtgcccgccgtaatgcccacggcgcccgcgcgctcgccggtgccggtggcgccgcgcAC  420
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5056926  CGTGCCCGCCGTAATGCCCACGGCGCCCGCGCGCTCGCCGGTGCCGGTGGCGCCGCGCAC  5056985

Query  421      CTCGGCTTCGGCTTCCACGCCCTTTTCCGGCACCGCCGTGGGAATCTTGCGGACCAGCA-  479
                |||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||
Sbjct  5056986  CTCGGCTTCGGCTTCCACGCCCTTTTCCGGCACCGCCGTGGGAATCTTGCGGTCCAGCAC  5057045

Query  480      ATTGACCACGC  490
                ||||||||||| 
Sbjct  5057046  ATTGACCACGC  5057056
```

Clone TM42

The blastx search across the entire clone indicated a homology of the DNA-sequence to the hypothetical protein Bpet 2847.

E-value: 6e-34

Locus: YP-001631457

```
Query  1        CGGTGCGATCGGGCGATACGCTGTTTCGCATTGCACGCCGCAACGTGGTGCCGGACGCCA  60
                |||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  2991472  CGGTGCGATCGGGCGATACGCTGTTTCGCATTGCACGCCGCAACGCGGTGCCGGACGCCA  2991413

Query  61       GCGTGTACCAGATGCTGGTGGCGCTGTGGCGCGCCAACCCGCAGGCGTTCATCCAGAACA  120
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2991412  GCGTGTACCAGATGCTGGTGGCGCTGTGGCGCGCCAACCCGCAGGCGTTCATCCAGAACA  2991353

Query  121      ACATGAACCTCGTGCGCGCCGGCGAGACGCTGTCCATCCCGGACGCGGCCACCGTGCGTG  180
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2991352  ACATGAACCTCGTGCGCGCCGGCGAGACGCTGTCCATCCCGGACGCGGCCACCGTGCGTG  2991293

Query  181      CCATCGATCCCGCTGAGGCCCGACGCATTTTCGCCGAGCAGGCCGAGGCCTTTGCACGCT  240
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2991292  CCATCGATCCCGCTGAGGCCCGACGCATTTTCGCCGAGCAGGCCGAGGCCTTTGCACGCT  2991233

Query  241      ATCGCGGCCGCGCGGGCGCCGATGTCGCCGGCGGCTCGGCCATCAATGCGGGGCGCGGAG  300
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2991232  ATCGCGGCCGCGCGGGCGCCGATGTCGCCGGCGGCTCGGCCATCAATGCGGGGCGCGGAG  2991173

Query  301      CCGCGTCGGGTCAGGTGGGCCAGGCGCCCGCGCAGCAGGCCGCCACGGGATCGACGCCCC  360
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2991172  CCGCGTCGGGTCAGGTGGGCCAGGCGCCCGCGCAGCAGGCCGCCACGGGATCGACGCCCC  2991113

Query  361      AAGACCGCCTGCGCCTGAGCGCGGCGACGCCCGACGAGGCGCAATCCGATGCGCGCACCT  420
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2991112  AAGACCGCCTGCGCCTGAGCGCGGCGACGCCCGACGAGGCGCAATCCGATGCGCGCACCT  2991053

Query  421      CCAGCGAGCACGCCATGCGTGACGCCCAGCAACGCGTCAATGCGCTGCAAGGCAACGTCG  480
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2991052  CCAGCGAGCACGCCATGCGTGACGCCCAGCAACGCGTCAATGCGCTGCAAGGCAACGTCG  2990993

Query  481      ATGCCCTGAACCGCGCCGCGGACGGGCAGGGGGCCGCGGCCGATGGCGGTTCCGAGGGTG  540
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2990992  ATGCCCTGAACCGCGCCGCGGACGGGCAGGGGGCCGCGGCCGATGGCGGTTCCGAGGGTG  2990933

Query  541      CGGCGGGCAGCGGGGCCGCCGGCGCAGCCGGGACTCCCGGTGCGGCAGGCG  591
                ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2990932  CGGCGGGCAGCGGGGCCGCCGGCGCAGCCGGGACTCCCGGTGCGGCAGGCG  2990882
```

Clone TM43

The blastx search across the entire clone indicated a homology of the DNA-sequence to the protein, peptide chain release factor 3.

E-value: 7e-68

Locus: YP-001628691

```
Query  1       CATTTCGGTGGCCTCGTCGGTGATGCAGATGGAATACCGCGAATGGGTCGTCAACCTGCT  60
               |||||||||||||||||||||||||||||||||||||||||| || ||| ||||||||||
Sbjct  102181  CATTTCGGTGGCCTCGTCGGTGATGCAGATGGAATACCGCGACTGCGTCATCAACCTGCT  102122
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Query  61      CGATACCCCGGGCCACCAGGACTTCTCCGAAGACACGTATCGCGTGCTTACCGCCGTGGA  120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  102121  CGATACCCCGGGCCACCAGGACTTCTCCGAAGACACGTATCGCGTGCTTACCGCCGTGGA  102062

Query  121     CGCCGCGCTGATGGTCATCGACGCGGCCAACGGCGTCGAGCCGCAGACCATCCGCCTGTT  180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  102061  CGCCGCGCTGATGGTCATCGACGCGGCCAACGGCGTCGAGCCGCAGACCATCCGCCTGTT  102002

Query  181     GCAGGTCTGCCGCGCGCGCAACACGCCCATCATCACGTTCATCAACAAGATGGACCGCGA  240
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  102001  GCAGGTCTGCCGCGCGCGCAACACGCCCATCATCACGTTCATCAACAAGATGGACCGCGA  101942

Query  241     AGTGCGCGAACCGCTCGACCTGTTGTCCGAGATCGAAGGCCACCTGGGCATGGACGCGGT  300
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  101941  AGTGCGCGAACCGCTCGACCTGTTGTCCGAGATCGAAGGCCACCTGGGCATGGACGCGGT  101882

Query  301     GCCGTTCTCATGGCCCGTGGGCATGGGCAAATCGTTCGGCGGCGTGTTGGACATCCGCCG  360
               |||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct  101881  GCCGTTCTCATGGCCCGTGGGCATGGGCAAATCGTTCGGCGGCGTGTTCGACATCCGCCG  101822

Query  361     CGACCGCATGCGCGTGTTCCGCCCGGGGCAGGAACGCCGCTCCGAGGACGACGACATCAT  420
               |||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  101821  CGACCGCATGCGCGTGTTCCGCCCGGGGCAGGAACGCCGCTCCGACGACGACGACATCAT  101762

Query  421     CGACGGCCTGGACAATCCCGAAATCGCCAGCCGCTTCGGCTCGGCCTTCGAGCAGGCCAA  480
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  101761  CGACGGCCTGGACAATCCCGAAATCGCCAGCCGCTTCGGCTCGGCCTTCGAGCAGGCCAA  101702

Query  481     CGGCGAAATCGAGCTCATCCAGGAGGCCGCCCCGGCCTTCGACCGCGAGGCCTTCCTGGC  540
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  101701  CGGCGAAATCGAGCTCATCCAGGAGGCCGCCCCGGCCTTCGACCGCGAGGCCTTCCTGGC  101642

Query  541     CGGCCGGCAGACGCCGGTGTTCTTCGGCTCGGCCATCAACAACTTCGGCGTGCAGGAAGT  600
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  101641  CGGCCGGCAGACGCCGGTGTTCTTCGGCTCGGCCATCAACAACTTCGGCGTGCAGGAAGT  101582

Query  601     GCTGGACGCCCTGGTGGAACAGGCGCCGCCCCCGGGCCCGCGCCAGGCGCT  651
               ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  101581  GCTGGACGCCCTGGTGGAACAGGCGCCGCCCCCGGGCCCGCGCCAGGCGCT  101531
```

Clone	Blast Result	E value	Position / B. petrii
TM1	1) maleylacetate reductase 2) hypothetical protein predicted by Glimmer/Critica	0	3962747-3963263
TM2	1) glycine betaine/choline/proline transport system substrate-binding protein 2) glycine/betaine ABC transporter substrate-binding protein 3) putative glycine betaine / choline/proline transport system substrate-binding protein	2e - 76	3472064-3472595
TM3	1) Amine oxidase 2) Amine oxidase - flavin containing	6e - 175	4449136-4450185
TM4	1) Muropeptide transporter 2) AmpG protein	3e - 178	454073-455458
TM5	1) A/G-specific adenine glycosylase 2) Adenine glycosylase	5e - 93	407659-408082
TM6	1) Fumarate reductase iron-sulfur protein 2) Fumarate reductase	1e - 113	435345-436057
TM7	Glycosyltransferase	8e - 149	972460-973146
TM8	TetR family transcriptional regulator	2e - 110	402860-403341
TM9	1) Hypothetical protein Bpet1311 2) GTPase 3) Conserved hypothetical protein	3e - 77	1374050-1374739
TM10	1) Branched-chain amino acid transport system, permease 2) ABC transporter permease	3e - 94	3789719-3790432
TM11	Dihydrolipoamide dehydrogenase	2e - 98	1564196-1565135
TM12	1) Hypothetical protein Bpet1047 2) Hypothetical protein 3) Conserved hypothetical protein	1e - 40	1513703-1515132
TM13	1)Hypothetical protein Bpet4253 2) DNA-binding protein	8e - 132	4514320-4515386
TM14	1) Two-component response regulator 2) XRE family transcriptional regulator	5e - 122	5218435-5219036
TM15	1) Hypothetical protein Bpet0369 2) Cytochrome C 3) Unnamed protein product	9e - 44	407659-408082
TM16	1) Hypothetical protein Bpet0989 2) Hypothetical protein predicted by Glimmer/Critica	3e - 40	1068273-1068818
TM17	1) Hypothetical protein Bpet0981 2) Hypothetical protein Bpet0982 3) Hypothetical protein Bpet0983	5e - 70	1063657-1064621
TM19	3-oxoacyl-ACP synthase	7e - 41	4684523-4684884
TM20	1) Acyl dehydratase 2) 3-alpha,7-alpha,12-alpha-trihydroxy-5-beta-cholest-24-enoyl-CoA hydratase	1e - 145	887570-888282
TM21	Protein-L-isoaspartate O-methyltransferase	2e - 50	529311-529984
TM22	1) B12-dependent methionine synthase 2) Methionine synthase 3) 5-methyltetrahydrofolate--homocysteine methyltransferase	0	538119-539331
TM23	Conjugal transfer protein	2e - 82	3977686-3978078
TM24	Hypothetical protein Bpet0996	6e - 129	1074265-1075455
	Hypothetical protein Bpet0997	6e - 124	
TM25	DNA (cytosine-5-)-methyltransferase	7e - 153	1038491-1039369

TM26	1) Acyl dehydratase 2) 3-alpha,7-alpha,12-alpha-trihydroxy-5-beta-cholest-24-enoyl-CoA hydratase	1e - 115	887570-888282
TM27	Hypothetical protein Bpet2300	1e - 120	2389372-2390021
TM28	1) Hypothetical protein Bpet3122 2) ABC transporter substrate-binding protein	6e - 77	3279679-3280089
TM29	Transposase	1e - 59	2201700-2202360
TM31	Autotransporter	3e - 37	4200991-4201763
TM32	LPS-export associated outer membrane porin	2e - 71	840625-841015
TM33	MFS permease	2e - 40	3363903-3364623
TM35	Molecular chaperone GroEL	4e - 109	4146842-4147399
TM36	1) Serine-type D-Ala-D-Ala carboxypeptidase 2) Cytochrome C550	2e - 29	5074391-5074744
TM37	Hypothetical protein Bpet2406	6e - 68	2520728-2521381
	Hypothetical protein Bpet2407	1e - 59	
TM39	Bacteriophage protein	8e - 76	1072174-1072672
TM40	Hypothetical protein Bpet0935	1e - 91	1028202-1028666
TM41	TonB-dependent outer membrane receptor	2e - 74	5056566-5057056
TM42	1) Hypothetical protein Bpet2847 2) Peptidoglycan-binding protein LysM	4e - 85	2990882-2991472
TM43	Peptide chain release factor 3	1e - 141	101317-102181

Table 2: Summary of blast results.

4 DISCUSSION

B. petrii produced large amounts of OMVs and high pressure freezing (HPF) preparation of the pelleted material followed by TEM analysis showed that the vesicle type was clearly a spherical bleb with a single bilayer membrane and an electron dense luminal content. These findings on the structure of *B.petrii* derived OMVs are in contrast to the OMVs produced by *A. kielensis* and *P. marina* which were able to produce two types of OMVs (Hagemann et al., 2013), namely the classical form as originally described by Beveridge (1999) and reviewed by Kuehn & Kesty (2008) as well as the type with a double bilayer, first recorded by Perez-Cruz et al., (2013) in *Shewanella vesiculosa* $M7^T$. It seems therefore that the OMVs which are derived from marine bacteria, namely *S. vesiculosa*, *A. kielensis* and *P. marina* are able to produce both types of OMV while *B.petrii* which was isolated from a fresh water environment and which belongs to a genus harbouring mainly pathogens produces only one type of OMVs. However, this hypothesis needs further verification thus requesting more information on structural features of OMVs from various limnic, marine and pathogenic bacterial species.

The successful staining of harvested OMVs with SYBR Gold (Figure 6) indicated that they contained nucleic acids but as no DAPI staining was conducted it is unknown how much of the OMV associated nucleic acids are DNAs.

The extracted DNA from *B. petrii* derived OMVs was high molecular weight. This is well in agreement with data of DNA from *A. kielensis* and *P. marina* (Hagemann et al., 2013) as well as with the upper range of so far published data (Dorward et al., 1989), ranging from 3.3 to 36 kbp.

Despite the large amount of published information on OMVs which was published in the last 30 years, there is only one investigation dealing with the quality of the OMV associated DNA by sequencing randomly chosen clones from a shotgun library (Hagemann et al., 2013).

In the present study 27352 bp of the MV genome were determined, providing the opportunity to compare the available information with new sequence data from an other bacterial species. It should be pointed out that the published data indicate that all sequences in *A. kielense* and *P. marina* were single copy and that all sequences, with one exception were similar to prokaryotic sequences. No inserted viral sequences were detected.

In the case of *B. petrii* a similar situation occurs. With one exception all analysed sequences are prokaryotic. However, the sequence of the clone TM39 can be translated into a bacteriophage protein (e-value: 8e – 76). As all OMVs contained (with the one mentioned bacteriophage sequence) only sequences from the donor bacterium *B. petrii* and all sequences were single copy sequences, the investigation confirms earlier findings that there is no specific OMV genome. Hence it is assumed that OMVs derived from *B.petrii* package DNA randomly.

The results of the sequence analysis in table 2 were also inspected to find out whether specific DNA sequences, able to express metabolically specific proteins, dominate the OMV associated DNA which would allow to detect a pattern in the DNA encapsulation processes and reject the hypothesis of the random packaging during vesiculation.

As expected, the majority of the sequences can be translated into hypothetical proteins (clones TM9, TM12, TM13, TM15, TM16, TM17, TM24, TM27,TM28, TM37,TM38, TM40,TM42) for which there is no evidence that they are expressed in vivo. Transporter and transporter related sequences were found in clones TM2, TM4, TM10, TM31 and tranferases for glycosyl and methyl were seen in TM7, TM22 and TM25 (Table 2). A conjugal transfer protein was found in clone TM23. Also two reductases were found in clones TM1 and TM6 and synthases in TM 19 and TM22. All other sequences belonged to single expressions and could therefore not be allocated to a dominant specific functional groups. An overview of the relative abundance of the most abundant sequences is given in Figure 11.

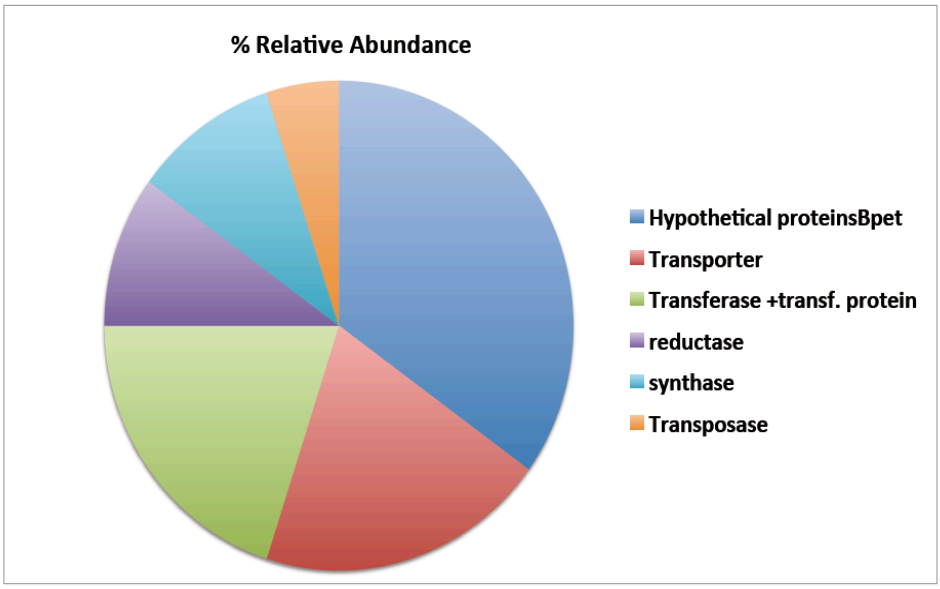

Figure 11: Relative abundance (%) of Blastx-results for qualitative different DNA-sequences in OMVs derived from *Bordetella petrii*. Sequences occuring only once were not integrated into the diagram.

With the exception of the hypothetical proteins where a functional allocation is impossible, the majority of the sequences code for membrane associated proteins such as transporter system proteins, TonB dependent receptor proteins and export associated outer membrabe proteins. Another group of sequences codes for proteins involved in anabolic processes, defense/ survival strategies and a transposase.

The completely sequenced genome of *B. petrii* has 5 287 950 bp (Gross et al.,2008). The obtained OMV associated DNA sequences seem to be positioned randomly all over the genome. A region with a denser occurrence of loci could be observed ranging between bp position 1,068 273, coding for a hypothetical protein (TM16) and bp position 1, 075 455 coding again for a hypothetical protein. With in the distance of 11 798 bp there are an other 3 hypothetical proteins, a DNA –methyltransferase and a bacteriophage protein.

Conclusion

The successful establishment of a shotgun library from the DNA of the *B. petrii* derived OMVs and its subsequent analysis led to the following conclusion:

The obtained results witness that the hypothesis of random DNA-packaging into OMVs is still a maintainable concept, nonetheless it seems that specific pattern can be recognized with respect to the position and the quality of the sequenced DNA-segments.

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Zusammenfassung

Gram-negative Bakterien geben während ihres Wachstums spezielle Vesikel, die als Outer Membrabe Vesicles (OMVs) oder Membran-Vesicle-like Particles bezeichnet werden, ab. Diese Arbeit befasst sich mit den OMVs, welche vom Bakterium *Bordetella petrii* abgegeben werden, und zwar mit besonderem Fokus auf die Zeitspanne, in der die Partikel-Abgabe beginnt, auf die Morphologie der besagten Partikel sowie auf ihre DNA.

Derzeit ist bekannt, dass vor allem marine Bakterien, die Großteils noch nicht identifiziert worden sind, OMVs mit großen, linearen DNAs zwischen 20-500 kbp produzieren. Basierend auf diesem Wissen wurde das Bakterium *Bordetella petrii* zwecks OMV-DNA-Isolierung kultiviert und in verschiedenen Stadien des bakteriellen Wachstums die OMVs mittels Filtration und Ultrazentrifugation von den Bakterien getrennt, um die DNA aus den Vesikeln zu extrahieren, zu klonieren und zu sequenzieren. Dafür wurde die extrahierte, hoch-molekulare DNA mechanisch fragmentiert und eine Shotgun-Library hergestellt. 43 Klone dieser Sequenzbibliothek wurden sequenziert und die Sequenzdaten wurden computergestützt ausgewertet.

Abstract

Gram-negative bacteria spontaneously release certain vesicles during their growth that are described as Outer Membrane Vesicles (OMVs) or Membrane Vesicle like Particle. This study focuses on the OMVs released by *Bordetella petrii*, with added attention to the timeframe in which the particle release is initiated by the organism, to the morphology of said particles as well as their DNA and its functionality
Currently, it is known that marine bacterial strains – that are, for the most part unidentified – produce outer membrane vesicles containing a large, linear DNA (20 – 500kb). This knowledge inspired the cultivation of the bacterium *Bordetella petrii* for OMV isolation by utilizing filtration and ultracentrifugation at a variety of bacterial culture ages, while the DNA of the released particles was extracted and sequenced. For this, the extracted, high-molecular-weight DNA was mechanically fragmented and a shotgun library established. 43 clones of this library were sequenced and the sequences were computer-assisted analyzed.

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