4.1 Final publishable summary report

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Project co-ordinator:	Prof. Dr. Helge B. Bode
Organisation:	Goethe Universität Frankfurt, Germany Institut für Molekulare Biowissenschaften Biozentrum/Campus Riedberg (N250, EG) Max-von-Laue-Str. 9 60438 Frankfurt am Main
Tel:	+49 69 798 29522
Fax:	+49 69 798 29527
E-mail:	h.bode@bio.uni-frankfurt.de

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1 Executive summary

GameXP has shown the great potential of *Xenorhabdus*/*Photorhabdus* bacteria as novel producers of bioactive natural products and thus has put these unexplored genera at the same level as well-known natural product producers like *Streptomyces*, *Bacillus* or Myxobacteria.The goal in the consortium of six partners from Thailand, Vietnam, the UK, and Germany, was to have as many natural products as possible specifically and probably unique from these bacteria in order to test them initially within GameXP but also to allow their testing in more detail and also against additional targets by pharmaceutical companies after the duration of GameXP.

The main activities and achievements within GameXP were:

- Isolation of bacteria from soil samples collected in Vietnam and Thailand
  - 1448 soil samples have been collected and from these
  - more than 200 *Xenorhabdus*/*Photorhabdus* strains have been isolated
  - this is probably one of the largest collection of *Xenorhabdus*/*Photorhabdus* strains in the world

- all isolated strains and several other strains obtained from lab and public strain collections were analysed chemically
  - more than 500 compounds have been identified in these strains using mass spectrometry, most of them unique to *Xenorhabdus*/*Photorhabdus*
  - a database has been built for *Xenorhabdus*/*Photorhabdus* specific compounds
  - 187 compounds have been isolated or have been synthesized after their structure was fully solved by mass spectrometry
  - several new compound classes and new derivatives of known compound classes have been identified

- the pure compounds were tested against several different target organisms including bacteria, fungi, protozoa, insects
  - several bioactive compounds have been identified with antibiotic activity
  - several active compounds against neglected tropical diseases have been identified

- more than 15 genomes of *Xenorhabdus*/*Photorhabdus* strains have been analysed for biosynthesis gene clusters involved in natural product biosynthesis
  - more than 20 of these gene clusters have been analysed in detail
  - molecular tools for accessing this chemical diversity have been established in order to increase the production of these compounds

- the scientific community has been pointed to GameXP
  - more than 24 scientific publications in peer reviewed journals have been written
  - a patent has been filed about the most abundant and novel compound class from *Xenorhabdus*/*Photorhabdus*
  - more than 70 additional dissemination activities (talks, posters, newspaper articles) have been done
  - pharmaceutical and chemical companies have been attracted to the results obtains during GameXP

2 Summary description of the project context and the main objectives

The proposed project (GAMEXP) combined European expertise in natural product chemistry, microbiology and molecular biology to isolate new small molecules for medicine.
75% of all compounds used to treat infectious diseases are either natural products, derived from natural products or are inspired from natural products (NP). This is even more astonishing since many more synthetic organic compounds are known than compounds derived from natural sources. However, it has been shown that natural products cover a different chemical space compared to synthetic compounds and often break the "rule of five" that refers to the chemical properties of medically used synthetic compounds that are indicative of their "drugability". The major sources for natural compounds currently in use are actinomycetes bacteria which have been investigated throughout within the last 60 years. Although new compounds with interesting biological activities can still be isolated, the rate of re-isolating already known compounds is high. To solve this problem, new sources for bioactive compounds have to be identified. This is of utmost importance since we are confronted with a plethora of emerging and re-emerging diseases all over the world (e.g. tuberculosis, plague, melioidosis, human ehrlichioses, tularaemia and trench fever) and because of the increasing resistance of several pathogenic bacteria (e.g. multi-resistant *Staphylococcus aureus* MRSA and *Mycobacterium* strains) even against the "last border" antibiotics (e.g. vancomycin-resistant enterococci). Here natural products seem to be a valuable resource as they have been developed in nature within a biological context that might already imply a high proportion of compounds with a biological activity against different targets. Although it is still not clear what the function of all NPs in the natural ecosystem is [6], it is clear for some of them that they function as chemical weapons that should kill and/or suppress competitors. With respect to this function it is not surprising that several "weapon-like" antibiotics have been identified from bacteria living in highly competitive environments like soil.
The great dilemma of anti-infective research is that almost all big pharmaceutical companies have stopped their research and development of new compounds as there is a profit problem in antibiotic R&D. Currently the interest in NPs seems to increase again which is also due to special programmes that support such research by pharmaceutical companies and because of our desperate need for these compounds. Without new anti-infectives we are rapidly approaching a situation prior to World War II where millions of people died of infectious diseases that are still curable at the moment but might not be in 5-10 years. As a result of this frightening development, pharmaceutical companies and biotech companies in the US are encouraged by tax cuts and long lasting patents when they develop new anti-infectives.

Expert knowledge in nematode sampling and characterization of their symbiotic bacteria from Thailand and Vietnam was used to address the topic of bioprospecting *Xenorhabdus* and *Photorhabdus*. These drug producing bacteria live in symbiosis with entomopathogenic nematodes and are used by the nematodes to kill their insect hosts. Although these bacteria are proven producers of natural products, only a few compounds have been isolated and studied in-depth. The isolated natural products were used to build up a unique *Xenorhabdus*/*Photorhabdus*-specific compound library which was tested as drug leads for a variety of infectious diseases including anti-protozoal, anti-malaria, anthelminthic, and antibiotic activity. Additionally, insects were used as infection models in order to reduce the need for mammalian testing in preclinical analysis. The complete bioactivity data set for any given compound was collected as basis for future exploitation with pharmaceutical companies. Moreover, the most promising *Xenorhabdus*/*Photorhabdus* strains were used in

an extensive molecular biology program that would finally result in *E. coli* strains expressing the biosynthesis pathways responsible for the bioactivity of interest or in *Xenorhabdus*/*Photorhabdus* strains over-producing the desired compounds. Finally, the compound library with its connected bioactivity data was made accessible to pharmaceutical companies.

The ultimate goals of the project were therefore:

- The isolation of a diverse and large collection of *Xenorhabdus*/*Photorhabdus* strains from entomopathogenic nematodes.
- The isolation of new compounds from *Xenorhabdus/Photorhabdus* strains in order to build up a *Xenorhabdus/Photorhabdus*-specific compound library that would prove the great potential of *Xenorhabdus/Photorhabdus* for secondary metabolites.
- Multiple and parallel bioactivity testing against bacteria, fungi, protozoa, nematodes, insects and mammalian cytotoxicity of the compound library.
- 'Genome mining' of the five most promising strains including construction of cosmid/BAC libraries, RVA screening, genome sequencing and heterologous expression in *E. coli*
- In order to attract industrial partners after the duration of the project, all legal issues such as IPR, international agreements and access rights will be settled in advance of licensing the products to companies.

In order to achieve these goals, the project was subdivided into seven work packages (WP1 - WP7) which were inter-related as shown in Figure 1. WP1 – WP4 were the core work packages (connected by bold black arrows, Figure 1).

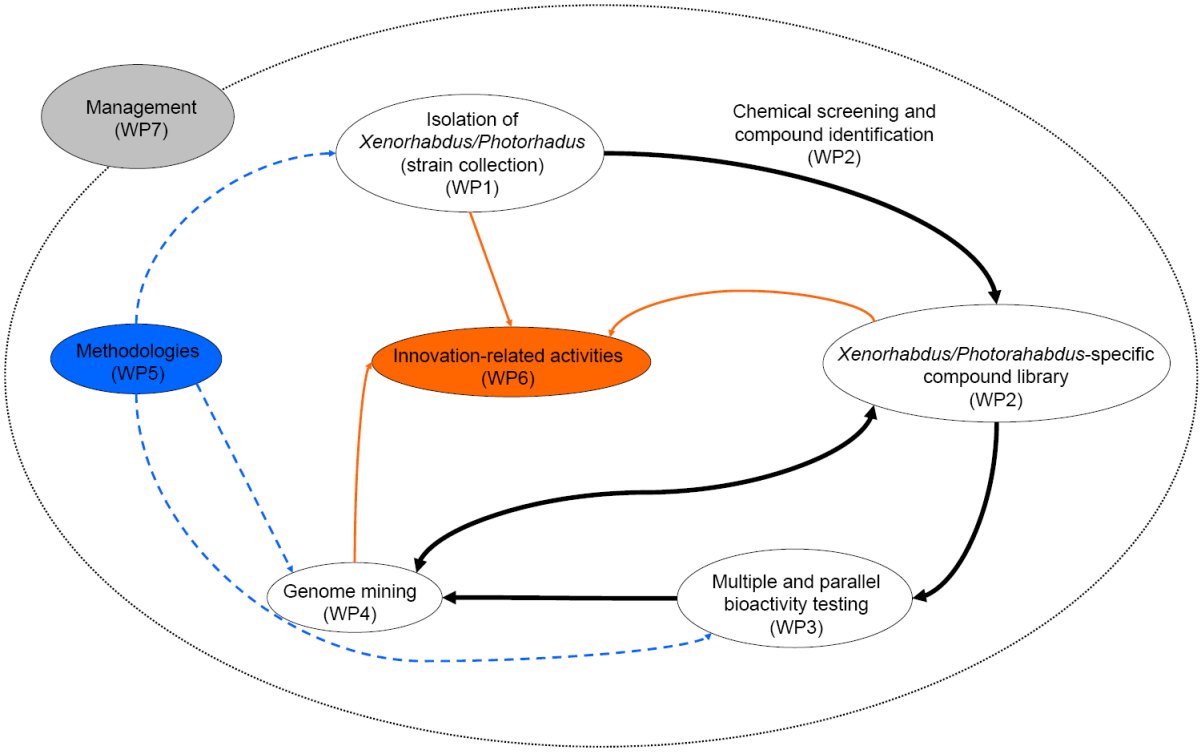

Figure 1. Illustration of a simplified workflow of the project which highlights the central roles of WP5 (Methodologies) as a harmonizing element to bring beneficiaries up to the same knowledge level and WP6 (Innovation-related activities) which focuses on dissemination and exploitation activities to be undertaken in the course of the project.

WP1 (Strain isolation/characterisation) was the basis for the whole project. A large and diverse strain collection of *Xenorhabdus/Photorhabdus* strains was obtained from different locations. The work in WP1 was carried out mostly by experts in nematology and microbiology from Thailand and Vietnam.

In **WP2** (Chemistry/compound library) all *Xenorhabdus/Photorhabdus* strains were analyzed using analytical methods and new compounds are isolated in a preparative scale from large scale cultivations or synthesized after their structure elucidation to build up a *Xenorhabdus/Photorhabdus*-specific compound library. This work was carried out by expert natural product chemists in Germany.

These compounds were analyzed in **WP3** (Bioactivity testing) against multiple different targets (bacteria, fungi, protozoa, nematodes, insects, cell cultures) in parallel. In order to rapidly identify interesting strains, crude extracts were also tested using the same assays. This work was carried out by biologists well familiar with these standard assays in the UK.

The aim of **WP4** (Genome mining) was to identify the biosynthesis gene clusters responsible for the production of the desired compounds allowing their future exploitation and enabling the heterologous production of these compounds in recombinant *E. coli*. This work was carried out by partners in the UK and Germany.

WP5 (Provision of harmonised set of methodologies) consisted of two sessions. The initial session was required to ensure that all beneficiaries use the same or a comparable methodology regarding especially the bioactivity testing. The consolidation session ensured that the used methods are still comparable but should also involve methods used within WP4 (genome mining).

WP6 (Innovation-related activities) included management of all GAMEXP related intellectual property right issues and coordination of dissemination activities, including the development of the overall dissemination strategy towards the scientific community and the broader public.

WP7 (Management) included the overall responsibility for managing the different work package activities, monitoring timely submission of deliverables and the overall project progress to fulfil the tasks and objectives set out in the work programme, collecting data and preparing interim reports.

3 Description of the main S & T results/foregrounds

The following describes selected examples of the results obtains during GameXP. As most of the results will be used in final publications that the GameXP consortium is currently writing or is sensitive and will be used for additional patents, the presented examples are taken from work already published as part of GameXP. A much more detailed description of the GameXP results can be found as part of the different deliverables as well as the periodic reports.

In **WP1 (Strain isolation and characterization)** more than 1400 soil samples have been collected in Thailand and Vietnam and more than 200 insect-pathogenic *Xenorhabdus*/*Photorhabdus* strains have been isolated from these soil samples using an insect baiting technique. All bacterial strains have been analysed using colony morphology as well as *recA* sequence as morphological marker. Additionally, the bacteria also several of the corresponding entomopathogenic nematode (EPN) partners have been collected and identified using morphological and molecular markers. As an example all *Xenorhabdus* strains from Thailand are shown in Figure 2.

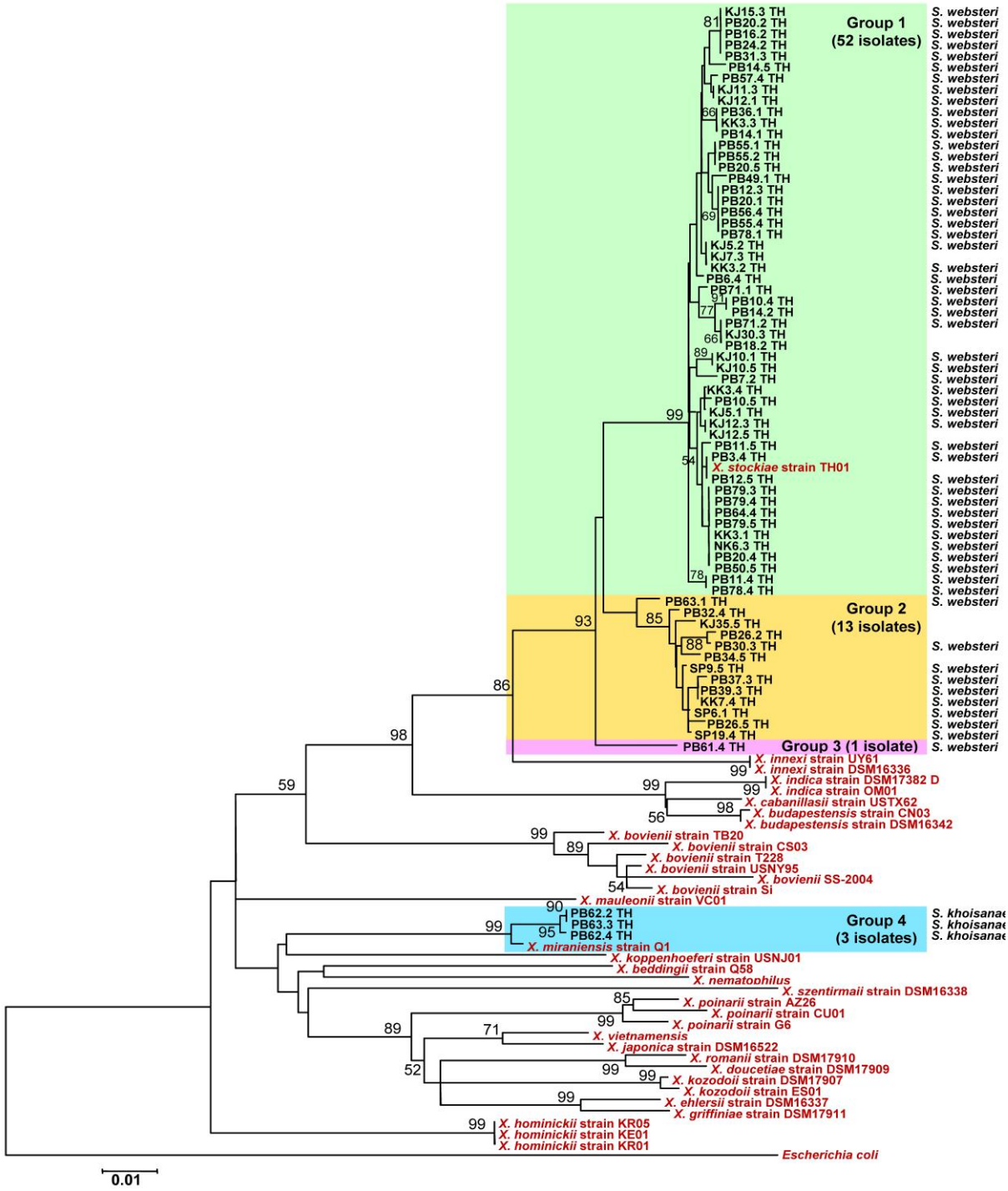

Figure 2. Maximum likelihood tree based on a 646 bp region of *recA* for 69 *Xenorhabdus* isolates from Thailand (codes ending with TH), together with *Xenorhabdus* sequences downloaded from the GenBank database (shown in red). Bootstrap values are based on 1,000 replicates. Numbers shown above branches are bootstrap percentages for clades supported above the 50% level. The bar indicates 1% sequence divergence. The EPN species from which they were isolated are shown.

In **WP2 (Chemistry/compound library)** all *Xenorhabdus*/*Photorhabdus* strains from Thailand and Vietnam as well as additional strains obtained from public strain collections (DSMZ, ATCC) as well as from other labs working with *Xenorhabdus*/*Photorhabdus*, were cultivated on a small scale and the cultures were extracted for subsequent analysis using HPLC coupled to mass spectrometry (HPLC/MS). Here the plan was originally to identify 50-100 compounds. However, *Xenorhabdus*/*Photorhabdus* were so potent producers of novel

chemical diversity, that more than 500 compounds were identified in these strains, most of them unique to *Xenorhabdus*/*Photorhabdus*. A MS-MS fragmentation-based database has been set up in order to identify these "known" compounds but also to identify even more novel compounds that can be analysed in the future.
In order to elucidate the structure of peptides among these compounds based on MS experiments, a new method was developed allowing the determination of the amino acid stereochemistry via a combination of labelling and detailed MS experiments (DOI: 10.1002/chem.201103479) as exemplified for the GameXPeptides found in *Photorhabdus luminescens*. Also as part of the GameXP search for novel compounds from *Xenorhabdus*/*Photorhabdus* a novel prodrug activation mechanism for nonribosomal peptides has been identified (DOI: 10.1038/nchembio.688).
A major goal of WP2 was also to build a *Xenorhabdus*/*Photorhabdus* specific compound library and at the end this library contains 187 *Xenorhabdus*/*Photorhabdus* compounds (these compounds obtained a unique HB# number). This large number of compounds have either been isolated from large scale cultivation of the respective strains or have been synthesized chemically after their structure has been solved by MS and labelling experiments as described above. For peptides, the synthesis is often superior to the isolation of the natural product, especially when the peptides are only produced in very small amounts by the original producer. Among the novel compounds also part of the compound library are the simple phenylethylamides showing an interesting biological activity (DOI: 10.1002/cbic.201100223) just to give one already published example.
The identification of all these compounds based on HPLC/MS also allowed the correlation between phylogeny (see Figure 2 as an example) and compounds present in the individual strains revealing the presence of several highly conserved natural products but also compounds being specific for a subclade of strains or present only in a few species.
Besides HPLC/ESI-MS also MALDI-MS was used for the identification of compounds, which turned out to be especially powerful for very large hydrophobic peptides and highly polar peptides containing several arginine moieties (DOI:10.1021/ac300372p).

In **WP3 (Bioactivity testing)** the pure compounds were tested against bacteria, fungi, nematodes, protozoa including species causing tropical neglected diseases, insects and insect cells and other eukaryotic cell lines. Here especially antibiotically active compounds have been identified of which some were new or new derivatives of known compound classes (like the xenorhabdins and the xenocoumacins). Even more important, several compounds showed an activity and or selectivity against neglected tropical diseases as tested by the Swiss Tropical and Public Health Institute (Swiss TPH). Among them structurally novel peptide classes but also several low molecular weight compounds. The activity of a few compound classes was in fact so good that further tests are planned in order to evaluate these compounds also in vivo.
When using insect cells as target, microscopic high content analysis was performed as shown for two compounds (as an example, see Figure 3). Here, differences could be observed even no cytotoxicity could be obtained in some cases.

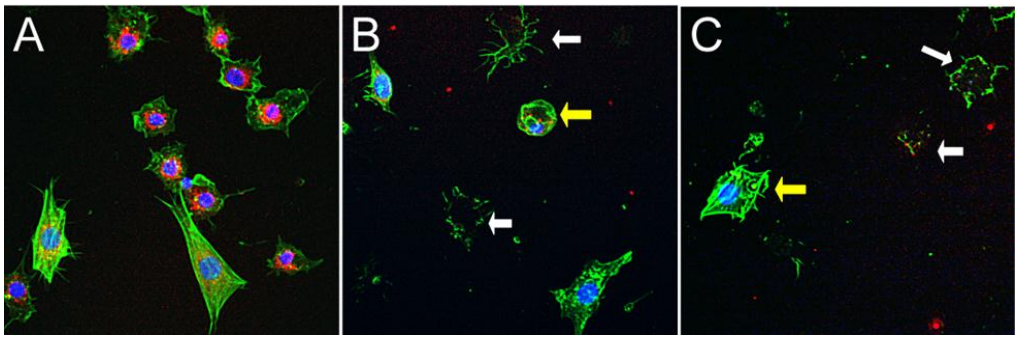

Figure 3. Hemocyte morphology subsequent to treatment with A. 2% DMSO control B. HB41 and C. HB36. White arrows indicate cytoskeletal corpses with absent nucleus, and yellow modification of the cytoskeletal structure. (Blue – nucleus, Green – F-actin cytoskeleton, Red- viable mitochondria.)

In **WP4 (Genome mining)** 15 strains of *Xenorhabdus*/*Photorhabdus* were analysed for the presence of biosynthesis gene clusters involved in the production of natural products. Thus, the genomes of several *Xenorhabdus*/*Photorhabdus* strains selected based on the production of several different compound classes or the production of conserved and/or bioactive compound classes have been sequenced. Although most genomes were not closed, from the contig data the biosynthesis gene clusters could be identified. Here, we could identify the biosynthesis gene clusters of the most promising bioactive compound class (against tropical neglected diseases) were identified in several different genomes finally resulting in the submission of a patent in order to protect these genes, the corresponding proteins as well as the compounds derived thereof. Additionally, more than 200 other biosynthesis gene clusters have been identified. Several of these have been assigned to a biological activity in the Rapid Virulence Annotation (RVA) approach.

15 biosynthesis gene clusters have been studied in either of the following ways:

- genes being part of the biosynthesis gene were disrupted or deleted in the original producer, resulting in the production of modified derivatives. This led to the identification of several novel biosynthesis mechanisms, which will also be studied in detail in the future.
- biosynthesis gene clusters were expressed heterologously in E. coli, resulting in the production of derivatives of the natural products and/or in higher yields
- the native promoter was exchanged against strong constitutive or inducible promoters in the native producer resulting in the activation of previously “silent” biosynthesis gene clusters and thus also in the production of the corresponding natural products for the first time (DOI:10.1016/j.jbiotec.2011.10.002).
- the same approach was also very efficient in increasing the yields of already known compounds

In order to achieve all these goals methods for promoter exchange approach or the hetereologous expression have been developed and/or optimized for *Xenorhabdus*/*Photorhabdus.*

For *Photorhabdus asymbiotica*, which is not only entomopathogenic but can also infect mammals and thus is also an emerging human pathogen, the transcriptome was analysed revealing the presence of several virulence islands activated in human serum or at 37°C, the temperature of the human body. Here RNA seq methodologies were performed which gave a huge but very robust data set that will also be analyzed in the future. A first result was that

also some natural product biosynthesis gene clusters were differentially regulated pointing to the corresponding compounds as virulence factors. Subsequently, compounds were identified, that inhibit the human immune system and thus might play an important part in the pathogenicity of *P. asymbiotica*.

In order to ensure that all partners use the same methods and know all methods used within GameXP, two method manuals (Deliverable D5.1 and D5.2) were written as part of **WP5 (Provision of harmonized set of methodologies)**. D5.1 was dedicated to the isolation of nematodes and bacteria from soil samples and D5.2 was dedicated to all aspects of genome mining used during GameXP. Due to the fact that novel tools for genome mining (promoter exchange approach, efficient heterologous expression), D5.2 was updated during GameXP.

As part of **WP6 (Innovation-related activities)** a website for GameXP was established (http://www.gamexp.eu), allowing also the establishment of the communication structures and its use as data repository for reports and publications. As part of WP6, all IPR issues were solved regarding strains and nematodes or natural products derived thereof. Additionally, SWOT analyses and PUDK analysis were an integral part of WP6.

The **management** of GameXP **(WP7)** was performed by the company EURICE, specialized in EC-project management. This included the organisation of different project meetings as well as the preparation and submission of project reports.

4 Description of the potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and the exploitation of results

"*The time has come to close the book of infectious diseases*" stated by the U.S. General Surgeon William H. Stewart in 1969 has been proven fatally wrong. This statement was given when antibiotic research and discovery had its zenith and new classes of potent drugs entered the market, resulting in the disappearance of many formerly deadly infectious diseases – at least in the industrialized nations.

Infectious diseases, however, have made a stunning comeback. Syphilis, gonorrhea, and tuberculosis are all re-emerging with vengeance and the advent of AIDS, hepatitis C, and severe acute respiratory syndrome (SARS) has been a humbling experience. However, the less publicised trend of bacterial resistance may pose an even greater threat. Statistics from WHO hint as to what the future may hold: *Vibrio cholerae* is 100% resistant to both tetracycline and chloramphenicol in Tanzania and Rwanda; in some regions, penicillin-resistant *Streptococcus pneumoniae* makes up 70% of strains; worldwide, multidrug-resistant *Mycobacterium tuberculosis* accounts for 1 to 22% of all new cases. In the USA, methicillin-resistant *Staphylococcus aureus* now accounts for nearly 60% of hospital-acquired staphylococcal infections, and 20% of nosocomial infections in US hospitals are reported to be multidrug resistant. Although the numbers are slightly better in Europe, unfortunately Europe is catching up fast.

Even without multidrug resistant strains, nosocomial infections are increasingly important as it has been shown that nearly $5 billion are added to US health costs every year as a result of infections that patients get while they are hospitalized for other health problems.[1] Furthermore, it is reported that that nearly 2 million patients annually acquire infections while being treated for other illness or injury in hospital, and nearly 88,000 die as a direct or indirect result of this infection (4th Decennial Conference on Nosocomial and Health-care associated Infections, Atlanta USA). This means, that approximately 1 in 10 hospitalized patients will acquire an infection after admission (Emerging infectious diseases, Vol. 10, No. 4, April 2004). The extra costs arise largely due to the extra days that the patient has to spend in hospital. In general the number of days that a patient has to spend in the hospital varies depending on the type of infection he or she gets: an estimated 1 to 4 days for urinary tract infections, 7 to 8 days for infection at the site of surgery procedure, 7 to 21 days for bloodstream infections and 7 to 30 days for pneumonia. The costs vary from anywhere like $600 for urinary tract infections to $5.000 or more for pneumonia. Prolonged bloodstream infections can top $50,000. Other sources reported, that the annual economic costs amount to $6,7 billion per year in the US and approximately US $1,7 billion in the UK indicating that nosocomial infections are in fact in a global problem similarly as infections in general are a global problem.

Even with the adoption of drugs with novel mechanisms of action, we do not have to ask the question *if* resistance will develop but only *when* it will occur. This is simply the consequence of the enormous speed at which microorganisms exchange and mutate their genes. It is predictable that the winner in this game will always be the microbe, simply because of its huge numbers, short generation times, and mutation rates.

The best way to combat antimicrobial resistance or at least delay it, is by reducing or, preferably, eliminating the misuse of antibiotics in medicine and agriculture. Moreover, we also have to find new drugs with new mode of actions and therefore new sources of these

[1] 4th Decennial Conference of Nosocomial and Health-care associated infections, Atlanta, USA, 2000.

drugs have to be identified as it is the goal of the current call HEALTH 2.3.4-3 in which this proposal is submitted.
A number of genome sequencing projects within the last 5 years have revealed several bacterial species that seem to be capable to produce secondary metabolites. Instead the isolation of new species of well-known natural product producers (e.g. actinomycetes) from extreme or not-yet explored environments (e.g. deep sea), one way might be to bioprospect bacterial species that seem to be good NP producers (e.g. *Burkholderia*, *Xenorhabdus*, *Photorhabdus*).

Improving health in general is important in its own right. But is also an important part of the solution to address a number of key challenges facing Europe such as population ageing, security threats or labour shortage. Obviously, infectious diseases play a major role within all these challenges and therefore the question is how bioprospecting projects such as GAMEXP might be involved in their solution?
Possible answers to this can be the following long-term benefits which might arise directly or indirectly from this project:

1. New anti-infective compounds: In an ideal case a compound found within this project is developed into a pharmaceutical product within the next 10 years by a pharmaceutical company to treat one or several diseases.
2. The methods applied within this project (activation of silent biosynthesis gene clusters to increase the chemical diversity) are used for the identification of anti-infective compounds from other organisms.
3. The identification of several biologically active compounds from *Xenorhabdus*/*Photorhabdus* will stimulate other research groups or even pharmaceutical companies to explore this huge resource and thereby might lead to the identification of desperately needed cures.

As all current EU problems with infectious diseases are mirrored in countries all over the world, bioprospecting in general will also benefit global health issues, e.g. in Thailand and Vietnam and other developing countries with important tropical diseases (e.g. melioidosis, malaria).
Here it is noteworthy that one of us (Dr. Aunchalee Thanwisai who obtained her PhD within GameXP) has decided to start her independent career in the field of *Xenorhabdus*/*Photorhabdus* in Thailand.

With the aim of expediting the development of new diagnostics, drugs, vaccines and tools for transmission control for effective prevention, treatment and control, the GAMEXP consortium will be established to address several infectious diseases including selected neglected diseases. The proposed work will establish bacteria of the genera *Xenorhabdus* and *Photorhabdus* as new source for natural products with potential biological activity. Compounds should be isolated resulting in a library *Xenorhabdus*/*Photorhabdus*-specific compounds which are then analyzed in bioactivity tests against a plethora of infectious diseases including several emerging and neglected diseases. This would include assays against multi-resistant Gram-negative and –positive bacteria (*Vibrio*, *Pseudomonas*, *Staphylococcus*, *Mycobacterium*) as well as assay against emerging human bacterial infections (e.g. *Burkholderia*). Moreover, assays will be conducted against opportunistic pathogenic fungi or yeasts (e.g. *Candida* involved in AIDS), *Acantamoeba* as model-protozoa to detect compounds useful to treat trypanosomiasis or leishmaniasis, *C. elegans* as model

for anthelminthic compounds to treat river blindness and filiariasis. Additionally, cytotoxicity against mammalian cell cultures and insects will be determined as model organisms for higher organism.
The compounds with the most promising bioactivity data will be protected. Furthermore, in order to truly establish *Xenorhabdus*/*Photorhabdus* as new source of natural products genome mining will be performed that will result also in the finding of the biosynthesis gene clusters responsible for the production of the compounds of interest. This is a prerequisite for biotechnological exploitation of these bacteria.
The unique approach of combining rapid chemical identification, genome mining and massively parallel bioactivity testing in non-animal models will vastly increase the current speed of drug discovery and development. The rigorous patent strategy will also attract partners at an early stage in order to take-over novel bioactives for drug development and deployment.

Regarding general dissemination activities 24 publications in peer-reviewed scientific journals have already been published (Table 1), three more are already accepted or in press.

Table 1. List of all published scientific and peer-reviewed publications obtained during GameXP.

N°	Title	Main author	Title of the periodical or the series	Year of publi-cation	Permanent identifiers
1	Insect-Associated Microorganisms as a Source for Novel Secondary Metabolites with Therapeutic Potential	Helge B. Bode	Insect Biotechnology	2011	DOI: 10.1007/978-90-481-9641-8_5
2	A robust phylogenetic framework for the bacterial genus Photorhabdus and its use in studying the evolution and maintenance of bioluminescence:A case for 16S, gyrB, and glnA	Scott M. Peat	Molecular Phylogenetic Evolution	2010	DOI:10.1016/j.ympev.2010.08.012
3	New plasmids and putative virulence factors from the draft genome of an Australian clinical isolate of Photorhabdus asymbiotica	Richard H. ffrench-Constant	FEMS microbiology letters	2010	DOI:10.1111/j.1574-6968.2010.02030.x
4	Synthesis of szentiamide, a depsipeptide from entomopathogenic Xenorhabdus szentirmaii with activity against Plasmodium falciparum	Helge B. Bode	Org Chem	2012	DOI:10.3762/bjoc.8.60
5	Determination of the absolute configuration of peptide natural products by using stable isotope labelling and mass spectrometry	Helge B. Bode	Chemistry – A European Journal	2012	DOI: 10.1002/chem.201103479
6	Drosophila embryos as model systems for monitoring bacterial infection in real time	Isabella Vlisidou	PLOS pathogens	2009	DOI:10.1371/journal.ppat.1000518
7	Host–Pathogen Interactions: Proline Gives Insect Pathogens the Green Light	Nicholas R. Waterfield	Current Biology	2010	DOI: 10.1016/j.cub.2009.11.018
8	A natural prodrug activation mechanism in nonribosomal peptide synthesis	Helge B. Bode	Nature Chemical Biology	2011	DOI: 10.1038/nchembio.688
9	Cytotoxic fatty acid amides from Xenorhabdus	Helge B. Bode	chembiochem	2011	DOI: 10.1002/cbic.201100223
10	Structure elucidation and biosynthesis of lysine-rich cyclic peptides in Xenorhabdus nematophila	Helge B. Bode	Org. Biomol. Chem	2011	DOI: 10.1039/c1ob05097d
11	Diversity of Xenorhabdus and Photorhabdus spp. and Their Symbiotic Entomopathogenic Nematodes from Thailand	Narisara Chantratita	PLoS One	2012	DOI:10.1371/journal.pone.0043835
12	Comparative genomics of the emerging human pathogen Photorhabdus asymbiotica with the insect pathogen Photorhabdus luminescens	Richard H. ffrench-Constant	BMC genomics	2009	DOI:10.1186/1471-2164-10-302

12	Comparative genomics of the emerging human pathogen Photorhabdus asymbiotica with the insect pathogen Photorhabdus luminescens	Richard H. ffrench-Constant	BMC genomics	2009	DOI:10.1186/1471-2164-10-302
13	Insects: True Pioneers in Anti-Infective Therapy and What We Can Learn from Them	Helge B. Bode	Angew. Chem. Int. Ed.	2009	DOI: 10.1002/anie.200902152
14	Entomopathogenic bacteria as a source of secondary metabolites	Helge B. Bode	Current Opinion in Chemical Biology	2009	DOI:10.1016/j.cbpa.2009.02.037
15	Photorhabdus and a Host of Hosts	Nicholas R. Waterfield	Annual review of Microbiology	2009	DOI:10.1146/annurev.micro.091208.073507
16	Identification and isolation of insecticidal oxazoles from Pseudomonas spp	Helge B. Bode	Org Chem	2012	DOI:10.3762/bjoc.8.85
17	Neutral loss fragmentation pattern based screening for arginine-rich natural products in xenorhabdus and photorhabdus	Helge B. Bode	Analytical Chemistry	2012	DOI:10.1021/ac300372p
18	Triggering the production of the cryptic blue pigment indigoidine from Photorhabdus luminescens	Helge B. Bode	Journal of Biotechnology	2012	DOI:10.1016/j.jbiotec.2011.10.002
19	Photorhabdus adhesion modification protein (Pam) binds extracellular polysaccharide and alters bacterial attachment	Nicholas R. Waterfield	BMC microbiology	2010	DOI:10.1186/1471-2180-10-141
20	New plasmids and putative virulence factors from the draft genome of an Australian clinical isolate of Photorhabdus asymbiotica	Richard H. ffrench-Constant	FEMS Microbiol Lett.	2010	DOI:10.1111/j.1574-6968.2010.02030.x
21	The KdpD/KdpE two-component system of Photorhabdus asymbiotica promotes bacterial survival within M. sexta hemocytes	Nicholas R. Waterfield	Journal of Invertebrate Pathology	2010	DOI:10.1016/j.jip.2010.09.020
22	Influence of the Photorhabdus luminescens phosphomannose isomerase gene, manA, on mannose utilization, exopolysaccharide structure, and biofilm formation	Nicholas R. Waterfield	Applied and Environmental Microbiology	2011	DOI:10.1128/AEM.02326-10
23	Genome-wide analysis reveals loci encoding anti-macrophage factors in the human pathogen Burkholderia pseudomallei K96243	Richard H. ffrench-Constant	PLoS One	2010	DOI:10.1371/journal.pone.0015693
24	Pdl1 is a putative lipase that enhances Photorhabdus toxin complex secretion	Nicholas R. Waterfield	PloS Pathogens	2012	DOI:10.1371/journal.ppat.1002692

Additionally, one patent on the most abundant and novel class of compounds from *Xenorhabdus*/*Photorhabdus* showing activity against neglected tropical diseases has been filed. 78 additional dissemination activities (web releases, press releases, newspaper articles) were performed as part of GameXP among them 45 talks and presentations given as well as 14 posters presented by GameXP members at national and international conferences and meetings.