**Some remarks how to write a good proposal**

One or two referees usually evaluate your proposal. These referees rate your previous experience, your knowledge in the respective field, and your personal impact. They will read a good application in detail, but put aside a bad application.

**What is a good application?**
You should know that a referee would not have more that 10-15 min time for each application. And it may happen, that the referee is **not** an expert on the special field of your application. That means that a paper longer than 10 written pages will have little chances for in-depth-discussions.
Insert headers, use actual (!) references, and insert graphics (a chemical paper without structures is like a soup without salt). Write as clear and understandable as possible. But do not explain trivial textbook facts and to not discuss minor detail, which you cannot know in advance anyway.
It must be obvious that you have written the application yourself: The referees do not want to evaluate your host, they want to grade *you*! Define milestones, and add a (realistic!) timetable

**A bad application:**
To long, to many details, no references or no actual references, proposal far away from your previous experiences, the importance of your theme is low or doesn't come out clearly. The worst thing is, if it comes out that the proposal was written by your future host!

It is understandable that you want to come as long as possible. Please remember, that only very few PhD grants are available (only ~ 10 per country and year!). There are, however, 30-40 short-term grants (2-12 months) available, and your chances will increase, if you apply for a shorter period.

If you are invited for an interview, please be **extremely well** prepared! You should be able to describe your project within 2-3 minutes, you should know about the special importance of the theme, the special techniques etc. Be able to explain all mentioned techniques *in detail*!! You should also know why you selected a special host (me in this case), and not somebody else.
Where is Germany, how many inhabitants, size, a few political and historical facts (e.g. who is president, chancellor, when was the re-unification...)

**This draft is giving just some ideas. Please modify, never take it as it is!!**

# Novel Sulfur (or Halogen or Phosphorus) containing Metabolites
# or
# Antibiotically Active (or cytotoxic or enzyme inhibitory) Metabolites

# from marine Micro-organisms (or *Bacteria/Fungi/Cyanobacteria etc.*)
# or
# Metabolites from endophytic microorganisms
# or
# Metabolite patterns in microbial life communities

**Application for a DAAD grant**
**by**
**(insert your name)**
**from**
**(your city/country)**

**date**

**Please do not forget to include your name, address and date and to delete the dummies!**

**Your Your application should contain the following information:**
**The CV must give answers on:**
**Name:** (write this one which you would put on a publication, please in CAPITALS)
**First name:**
**Born:**
**Sex:** (male/female); a photo will be very welcome
**married: ? / children: ?**
**Address:** (of your institute)
**e-mail:**

**Short CV (don't forget to mention your scientific experience in the filed of application)**

**2 recommendation letters of respected professors/senior scientists should be added (send the scanned letters) or sent directly to my e-mail address; you will need recommendations later anyway!**

## Summary

**The objective** of this research project in the group of Prof. Laatsch is to cultivate marine micro-organisms, to obtain and to separate their extracts, to isolate bioactive secondary metabolites and to elucidate their structure. My experience *((in the screening for xxx / in the isolation of plant metabolites / in xxxx ))* will help to isolate and to purify these metabolites. Techniques developed in the group of Prof. Laatsch will be used to speed up the procedures of isolation, dereplication and structure determination. I want to take part in some of these tasks and focus my interest on certain chemical groups of metabolites which are accessible by the so-called chemical screening. I suggest to look for *((….. elements, biological activity etc.))*, however, also other groups or elements are possible.

## Previous chemical studies on *((marine bacteria, marine fungi, the ecological interaction of … with …, and so on))*

Here you need to give a short (!) review of the current literature, but please don't copy this text! Please "decorate" the text with some chemical structures and give actual (!) references.

The vast resources of marine kingdom have received increasing attention from chemists and pharmacologists and played an important role in the explosive growth of biomedical science during the past two decades. The recent advent of sophisticated chromatographic (HPLC, gel filtration etc.), high field NMR & mass spectroscopic techniques coupled with force field calculations and x-ray crystallography have markedly enhanced the isolation and structure elucidation of more complex and diverse natural products and along with advanced bioassay methods has opened new possibilities in discovery of drugs and agrochemicals from natural sources.

An analysis of phylogenetic distribution of these molecules from marine resources shows that their majority (95 %) is confined to four groups: macro algae, coelenterates, echinoderms and sponges. Despite these contributions it is evident that other important marine habitat organisms such as marine micro-organism have been largely untapped[1)]. Micro-organisms provide a number of potentially useful natural products agents; some of them are in clinical use since a long time. But rapid development of resistance is forcing chemistry to explore new sources of potent bioactive natural products: such a source can be found in marine bacteria.

A first review by Fenical et al.[2)] documents the rapid rate of discovery and pronounced structural diversity of marine derived bacteria secondary metabolic products. The tremendous chemical and biological diversity is most probably due to a wide variety of conditions in which marine organisms have evolved in order to survive: conditions which are more extreme and self limiting compared to their terrestrial counterparts, therefore it appears possible that marine bacteria are ideal targets and a potential source for discovery of totally new classes of drugs!

The first marine bacterial molecule was the highly brominated pentabromopseudilin, isolated by Burkholder et al. in 1966[3)]. In a series of papers beginning in 1972, the group of Okami has

discovered several structurally bioactive molecules starting with an benzanthraquinone antibiotic from *Cheinia purpurgena*[4]. Among the more interesting and unusual compounds isolated from marine bacteria are the istamycins[5] and aplasmomycin antibiotics[6]. High molecular weight antitumor agents have also been obtained from marine microorganisms. More recent work by Takahashi et al (1989) includes the isolation of a structurally novel monoterpene alkaloid altemycindin, an antitumor agent from a marine strain *Streptomyces sp.*[7]. Fenical et al. focused their attention on the deep sea bacteria which yielded a series of novel cytotoxic and antiviral macrolides[8]. The group of H. Laatsch is now working since more than 15 years on marine streptomycetes and has isolated a number of highly active metabolites (presently more than 100 antibiotics) from this source. (*see references on my homepage for details*)

Based upon the above data, it appears that marine bacteria[9] *(or fungi[10] etc.; select only one group)* are an ideal target for discovery of novel highly potential bioactive compounds.

** explain now that it may make sense to look selectively for certain chemical or biologically active groups (sulfur, halogen?, certain activities?). Another matter of interest are nitriles, isonitriles, or thiocyanates. Here you could screen by IR! Don't forget to explain the reason: In this case, it may be the simple detection method.
A second important topic is the ecological interaction (signalling, quorum sensing, chemical defense etc.). A very promising idea is also the insertion of genetic aspects: However, here you would need the help and cooperation of a local microbiologist.

Do not forget to insert some chemical structures of actual (!) metabolites. A chemical application without structures is like a soup without salt.

**Procedure**
Pre-Screening

The microbial isolates (obtained from culture collections or locally collected) will be cultured in multiple 1 L Erlenmeyer flasks, the fermentation medium could be of standard composition comprising starch, peptone, yeast, or glucose and malt extract, using synthetic sea water. It is also of interest, to investigate the influence of further nutrient compositions, as certain components may trigger the formation of new secondary metabolites. The fermentation will be allowed to proceed with shaking for 3-5 days at usually 28 °C, after which the entire fermentation broth will be extracted with organic solvents. On evaporation, the extracts will yield a crude mixture of microbial secondary metabolites and fats.

**(Bio)assay strategy**

*If you have own experience in the screening, please describe here the respective technique.*

Extracts from marine micro-organisms will be screened using chemical, physical and spectroscopic techniques (the so-called chemical screening) and various bioassays: These are antimicrobial tests, screening for phytotoxic and cytotoxic activity and industrial receptor assays.

The most effective test for antibacterial and antifungal activity is the agar diffusion method: A pure culture of rapidly growing aerobic bacteria such as *Streptococci, E. coli,* or other micro-organisms is plated evenly on special agar medium so as to produce an even layer. Several (6 - 8) filter paper discs, each containing a known amount of different crude extract, are placed on the plate and the plate is incubated overnight. An inhibition zone of growth around a disc indicates that the test organism is sensitive against the corresponding crude extract.

Other screening methods make use of the chemical screening where more or less selective spray reagents are used to visualize the spots in the tlc.

- *search for sulphur reagents in the literature*
- reagents for reducing metabolites (antioxidants), for peptides (e.g. the chlorine/tolidine reaction), for halogen compounds etc.
- *look for further techniques, like enzyme inhibitor tests etc.*

*Explain that a very efficient screening method is most important for a quick dereplication (= detection of known compounds). Give some ideas for the efficient combination of TLC and MS. (The dereplication can be done using our database AntiBase[11], see homepage, or by ESI/MS).*

**Upscaling, purification of active principles and structural elucidation**

*** please mentione that this chapter can only give a very general procedure. Point to your previous experience with other isolations or techniques on related fields.*

The most important aspect of this project will be isolation, purification, and structure elucidation. Strains with interesting activities will be scaled up by 20-50 L fermentation, the metabolites will be extracted, isolated and purified using normal phase chromatography, HPLC, countercurrent chromatography, size exclusion chromatography and solid phase extraction.

All fractions will be monitored by thin layer chromatography and 1H-NMR spectroscopy in order to isolate the major metabolites in the crude extracts. Bioactive fractions will be submitted to repeated silica gel chromatography and other techniques to obtain pure compounds.

The structure elucidation of secondary metabolites will be carried out by a combination of spectroscopic techniques and chemical conversions, particularly by 1 and 2D high field NMR spectroscopy (up to 600 MHz in Göttingen). Known types of compounds can be identified by means of databases developed in Göttingen for this purpose. Novel structures can be elucidated using different types of 2D-NMR experiments like COSY, HETCOR, COLOC, HMBC, HMQC and NOESY etc. X-ray crystallography also can be used for suitable cases.

Absolute stereochemistry will be determined based on the properties of derivatives (e.g. Mosher derivatives), by chromatography on chiral phases or by other techniques (e.g. exciton-chirality technique).

**Biosynthesis**

Secondary metabolites are usually synthesised biosynthetically from acetate, malonate, amino acids, sugars and other simple building blocks. Feedings experiments using labelled precursors can give insight into these biosynthetic pathways. After structure elucidation of new metabolites, it may makes sense to do some of these feeding experiments as well as studies for precursor-directed syntheses: In some cases it may be possible to obtain modified metabolites by feeding artificial precursors. If and when such experiments are done depends strongly on the previous results from structure elucidation.

**Scientific/social importance of this project**

why should your project be supported?

Do not forget to sign your proposal!

REFERENCES (this is only a selection! Please complete and control style and names, complete "et al.")


[1] *D. H. Attaway* and *R. Zaborsky*, Marine Biotechnology "Pharmaceutical and Bioactive Natural Products" vol. 1

[2] *W. Fenical*, Chemical Review **93** (1993) 1673 – 83; see also the many reviews of *D.J.Faulkner* and later *Blunt* and *Munroe* in Nat. Prod. Rep.

[3] *P. Burkholder, P.R. Pfister, R.M. Leitz*, Appl. Microbiol. **14** (1966), 649

[4] *Y.* Okami, Microb. Ecol. **12** (1986) 65 - 78

[5] *Y. Okami* et al, J. Antibiot. **29** (1986) 1019 - 1025

[6] *Y. Okami* et al., J. Antibiot. **32** (1979) 964 - 966

[7] *A. Takahashi* et al., J. Antibiot. **xx** (1989) 42

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[9] *H. Laatsch*, Marine Bacterial Metabolites, in: Frontiers in Marine Biotechnology (P. Proksch, W.E.G. Müller, eds.) p. 225-288, Horizon Bioscience, Norfolk, UK 2006. ISBN 1-904933-18-1

[10] *M. A. Biabani, H. Laatsch*: Advances in Chemical Studies on Low-Molecular Weight Metabolites of Marine Fungi, J. Prakt. Chemie **340** (1998) 589-607

[11] *H. Laatsch*, AntiBase, A Data Base for Rapid Structural Determination of Microbial Natural Products, and annual updates, Wiley-VCH, Weinheim, Germany 2007; see also http://www.gwdg.de/~ucoc/laatsch/