

EUROPEAN **CHITIN** SOCIETY

NEWSLETTER

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- Where to read and publish articles on chitin and chitosan
- Shigehiro Hirano
- Enzymatic conversion of chitin and chitosan to chito-oligosaccharides



December, 2004
No. 18

The European Chitin Society

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EDITORIAL

A new board was elected at the second board meeting in Poznan last September, as you will see from the minutes of the board meetings on page 9-10. The new board will continue to work according to the objectives of our society.

This edition of the Newsletter (No. 18) has been edited by the president in order to publish it in 2004, and our goal is to publish two issues each year.

You will find a list of the most recent forthcoming meetings on page 11. The next EUCHIS meeting will be a joint venture with the International Conference on Chitin and Chitosan to be arranged at Montpellier, France in September 2006 by Eric Guibal. You should also be planning for the EUCHIS conference in 2008, which will take place in Istanbul, Turkey.

We are working to up-grade the EUCHIS web-site, and this should be ready in January 2005. The internet is considered to be an important way of information exchange both with our members and others that are interested in chitin and chitosan.

Sincere thanks to the secretary George A.F. Roberts, assistant secretary Vincent G.H. Eijsink and treasurer Martin Graeve for their contributions since last September.

I wish you all a happy New Year 2005! We look forward to your contribution to the next EUCHIS Newsletter.

December 30., 2004.
Kjell M. Vårum

WHERE TO READ AND PUBLISH ARTICLES ON CHITIN AND CHITOSAN

Kjell M. Vårum

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Most of the EUCHIS members are involved in research on chitin and chitosan and rely on access to new results and/or publishing their own research. Where to find the information and how to publish results require substantial time and effort using different tools such as the internet and services provided by libraries. In relation to this, it could be helpful to know which scientific journals that actually publish most articles related to chitin and chitosan.

Using the **ISI Web of Science** database searching for:

CHITIN* or CHITOSAN*

for each of the years between 2000 and 2004, tables 1 to 5 were created containing the top ten journals which published the highest numbers of articles related to chitin and chitosan.

The list contains some interesting informations on the global research activities within our research field, and some comments are given below.

Table 1. Top 10 journals publishing articles related to chitin and chitosan in 2000.

Number of hits: **988**

Journal	% of hits
JOURNAL OF APPLIED POLYMER SCIENCE	3.0 %
JOURNAL OF BIOLOGICAL CHEMISTRY	2.5 %
CARBOHYDRATE POLYMERS	2.0 %
STP PHARMA SCIENCES	1.8 %
INTERNATIONAL JOURNAL OF PHARMACEUTICS	1.5 %
BIOSCIENCE BIOTECHNOLOGY AND BIOCHEMISTRY	1.3 %
JOURNAL OF MEMBRANE SCIENCE	1.3 %
MOLECULAR PLANT-MICROBE INTERACTIONS	1.3 %
ABSTRACTS OF PAPERS OF THE AMERICAN CHEMICAL SOCIETY	1.2 %
JOURNAL OF BIOSCIENCE AND BIOENGINEERING	1.2 %

Table 2. Top 10 journals publishing articles related to chitin and chitosan in 2001.

Number of hits: **1157**

Journal	% of hits
JOURNAL OF APPLIED POLYMER SCIENCE	3.8 %
ABSTRACTS OF PAPERS OF THE AMERICAN CHEMICAL SOCIETY	2.0 %
JOURNAL OF CONTROLLED RELEASE	1.9 %
CARBOHYDRATE POLYMERS	1.8 %
BIOMATERIALS	1.6 %
INTERNATIONAL JOURNAL OF PHARMACEUTICS	1.5 %
BIOSCIENCE BIOTECHNOLOGY AND BIOCHEMISTRY	1.4 %
JOURNAL OF BIOLOGICAL CHEMISTRY	1.2 %
PLANT PHYSIOLOGY	1.2 %
ADVANCED DRUG DELIVERY REVIEWS	1.1 %

Table 3. Top 10 journals publishing articles related to chitin and chitosan in 2002.
Number of hits: **1186**

Journal	% of hits
JOURNAL OF APPLIED POLYMER SCIENCE	3.9 %
INTERNATIONAL JOURNAL OF PHARMACEUTICS	2.4 %
BIOMACROMOLECULES	2.3 %
CARBOHYDRATE POLYMERS	2.3 %
BIOMATERIALS	2.1 %
JOURNAL OF BIOLOGICAL CHEMISTRY	1.2 %
APPLIED AND ENVIRONMENTAL MICROBIOLOGY	1.1 %
ABSTRACTS OF PAPERS OF THE AMERICAN CHEMICAL SOCIETY	1.0 %
JOURNAL OF CONTROLLED RELEASE	1.0 %
JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY	0.9 %

Table 4. Top 10 journals publishing articles related to chitin and chitosan in 2003.
Number of hits: **1432**

Journal	% of hits
JOURNAL OF APPLIED POLYMER SCIENCE	5.0 %
BIOMATERIALS	2.5 %
BIOMACROMOLECULES	2.3 %
INTERNATIONAL JOURNAL OF PHARMACEUTICS	2.2 %
CARBOHYDRATE POLYMERS	2.0 %
MACROMOLECULAR BIOSCIENCE	1.7 %
JOURNAL OF BIOMEDICAL MATERIALS RESEARCH PART A	1.3 %
BIOSCIENCE BIOTECHNOLOGY AND BIOCHEMISTRY	1.1 %
CARBOHYDRATE RESEARCH	1.1 %
JOURNAL OF CONTROLLED RELEASE	1.1 %

Table 5. Top 10 journals publishing articles related to chitin and chitosan in 2004 (as of December 27th, 2004). Number of hits: **1502**

Journal	% of hits
JOURNAL OF APPLIED POLYMER SCIENCE	3.6 %
CARBOHYDRATE POLYMERS	2.3 %
BIOMATERIALS	2.1 %
BIOMACROMOLECULES	1.7 %
EUROPEAN JOURNAL OF PHARMACEUTICS AND BIOPHARMACEUTICS	1.5 %
INTERNATIONAL JOURNAL OF PHARMACEUTICS	1.4 %
JOURNAL OF CONTROLLED RELEASE	1.3 %
BIOSCIENCE BIOTECHNOLOGY AND BIOCHEMISTRY	1.1 %
PROCESS BIOCHEMISTRY	1.1 %
MICROBIOLOGY-SGM	1.0 %

The number one journal to publish articles related to chitin and chitosan is Journal of Applied Polymer Science, which publishes results on the practical application of polymer science. It should, however, be noted that articles related to chitin and chitosan are published in a very wide range of different journals, and that the top ten journals listed in tables 1-5 represent only between 17 and 22 % of the total number of articles published in the field. This reflects the fact that researchers in our field publish in a broad range of scientific journals, presumably reflecting the versatility of chitin and chitosan.

With respect to the quality of the journals in tables 1-5, their Impact factors was considered, although this parameter should be judged with caution. According to the Impact factors obtained by the **ISI Web of Science** database, the Journal of Applied Polymer Science has an Impact factor of 1.017 (as of 2003, which is the latest year where Impact factors were available). Considering the journals in Table 4 (2003), it should be mentioned that none of the journals has an impact factor below 1, and that four of the journals, i.e. Biomaterials, Biomacromolecules, Journal of Controlled Release and Microbiology have Impact factors of around 3, i.e. 2.903, 2.824, 3.298 and 3.044, respectively. The other journals all have Impact factors between 1 and 1.6. Journal of Biological Chemistry stands out with the highest Impact factor, i.e. between 7.36 and 6.696 in the years that this journal appears in the tables (2000, 2001 and 2002).

Did reading this article help you decide on which journal to publish your next article? Or where to look for new interesting articles? Perhaps not, but you may have learned something about other chitin/chitosan researchers way of publishing their results.

PROFESSOR SHIGEHIRO HIRANO

The death of Professor Shigehiro Hirano early in 2004 removed one of the leading figures of the world of chitin/chitosan chemistry. Professor Hirano was one of the first chemists to study these polymers in what might be termed the modern era of chitin research. He was an influential presence at the 1st International Chitin/Chitosan Conference in Boston in 1977 and has been a familiar figure at chitin conferences around the world ever since.

His research covered a wide range but perhaps the most important was his development of the technique of homogeneous re-*N*-acetylation of chitosan without accompanying *O*-acetylation. This reaction has been applied in a number of areas including the preparation of chitin gels; the synthesis of F_A-homologous series of *N*-acetylchitosans of constant DP value, which have been important in numerous studies on structure-property relationships of chitosans; the synthesis of *N*-acylchitosans; and the preparation of amorphous chitin of high purity. It has proved to be a very versatile and valuable technique.

Professor Hirano had a great passion for chitin/chitosan research and was very generous in sharing his knowledge and enthusiasm. At a recent symposium to honour his memory many speakers told of how he had personally encouraged and helped them. To quote one speaker "He was my 'Guru' who freely taught me everything I wanted to know about chitin and chitosan". He was a major driving force in the establishment of first the Japanese Chitin & Chitosan Society and later of the Asia-Pacific Chitin/Chitosan Society, and justly deserves his status as the 'Grandfather and Godfather of S.E. Asian chitosan research'. He will be greatly missed, not only in Japan but within the community of chitin and chitosan researchers around the world.

George A.F. Roberts

Enzymatic conversion of chitin and chitosan to chito-oligosaccharides

Vincent Eijsink

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During the past years (e.g. at EUCHIS '04 and at previous EUCHIS conferences), there have been many reports on biological activities of both chitosan and chito-oligosaccharides (CHOS), e.g. in agriculture (plant protection), in medicine (bone repair, wound healing) and in the food industry (anti-microbial activity). It is likely that many of the known biological activities of CHOS mixtures in fact are due to one particular compound with a specific length and sequence. It is also likely that at least some of the biological activities described for chitosan are due to specific oligosaccharide breakdown products, which arise due to enzymatic activity. Because of limitations in producing individual CHOS species of known sequence (see below), the nature of the compounds that are causing the observed bioactivities is usually only partially known. Taken together, these possibilities and limitations create a need for the development of methods for controlled production, purification and characterization of CHOS.

Nature offers several enzymes for the hydrolysis of chitin and chitosan to CHOS, primarily chitinases (glycoside hydrolase families 18 & 19), chitosanases (glycoside hydrolase families 46 & 75 & 80, plus some members of families 5 & 8). Other potentially important enzymes for the production of bioactive CHOS are the chitin deacetylases (reviewed at EUCHIS '04 by Malgorzata Jaworska; abstract A4), which have a potential to be used for modification of CHOS. Glycoside hydrolases belonging to the same family are similar in terms of primary sequence, three-dimensional structure and catalytic mechanism. Nevertheless, family members may display important differences, e.g. in terms of substrate preferences (degree and position of acetylated/deacetylated units), and the type of action (exo-, endo-, processivity, directionality). Chitinases and chitosanases differ in terms of their preferences for acetylated (*N*-acetylglucosamine; GlcNAc) and deacetylated (Glucosamine; GlcN) sugars near the scissile bond. Cleavage by family 18 chitinases depends critically on the presence of an acetylated sugar in the so-called -1 subsite (that is, on the non-reducing site of the scissile glycoside bond), and these enzymes are known to cleave GlcNAc-GlcNAc and GlcNAc-GlcN sequences. Family 19 chitinases preferably cleave GlcNAc-GlcNAc and GlcN-GlcNAc sequences, whereas chitosanases prefer GlcN-GlcN and/or GlcN-GlcNAc and/or GlcNAc-GlcN. Thus, these enzymes show overlapping substrate specificities and all will degrade chitosans with some degree of acetylation.

The outcome of an enzymatic degradation reaction with chitosan depends on several interrelated factors (see [1,2], EUCHIS '04 abstract A7 and posters 15 & 50), including:

1. The degree of acetylation of the chitosan. This will affect the average length and the sequences of the produced CHOS in a way that depends on the type of enzyme used.
2. The type of enzyme used. Several enzyme parameters affect the outcome of the reaction, such as (1) preferences for acetylated or deacetylated sugars in the various subsites, in particular in subsites -1 and +1 (where such preferences are most likely to be absolute); (2) the importance of binding energy for catalysis; the higher the number of subsites that need to be occupied for the formation of a productive enzyme-substrate complex, the longer the average length of the produced CHOS; (3) the enzyme's way of degrading the substrate (endo versus exo, processive versus non-processive – see [1] and poster 50 at EUCHIS '04).
3. The extent of the degradation reaction. As described in detail in ref [2], it may be beneficial to stop reactions at a rather well-defined points (degree of hydrolysis), since this may maximize the yield of certain oligosaccharides.

4. For example, it has been shown that processive enzyme initially mainly produces even-numbered CHOS, which are converted to odd-numbered CHOS later during the reaction [1].

The development of processes for the conversion of chitin and chitosan to bioactive CHOS not only depends on a versatile enzyme toolbox, but also on the availability of methods for purification and characterization of the products. Good analytical tools will allow further characterization of bioactive mixtures of CHOS and will ideally lead to purification and sequencing of specific bioactive oligosaccharides, which again would direct further development of production methods. For a long time, NMR has been the only reliable method for sequence determination of CHOS (e.g. [1]). Important recent work in this field has been done by Sven Bahrke and co-workers in the groups of Martin Peter and Jasna Peter-Katalinic, in collaboration with Primex (Iceland). In 2002, an effective mass spectrometry-based method for sequencing of CHOS was published [3], whereas recent work includes the purification of individual species of chito-oligosaccharides (with known sequence; several of the resulting oligosaccharide compounds showed highly promising effects in bone-repair; EUCHIS '04, poster 44).

During the past years enzymatic production of CHOS has become more common and successful. An increased understanding of how chitinases and chitosanases work, has improved the ability to design processes for the production of specific mixtures of CHOS (specific in length, and, to some extent, also in sequence), as illustrated by a recent paper by Pawel Sikorski et al. [2]. However, the efficient production of pure bioactive compounds is still quite far ahead, because structure-function relationships remain largely unknown, the biological activities themselves are not well-described, analytical tools remain limiting, and enzymatic production methods still need further improvement. Recent work has shown considerable progress and it seems likely that controlled production of “designer” CHOS becomes feasible in the future. These CHOS may result directly from enzymatic hydrolysis of chitosan followed by some purification steps. Alternatively, and more likely, bioactive compounds may emerge after chemical or enzymatic functionalization of chitosan-derived CHOS.

REFERENCES

- [1] Sørbotten A, Horn SJ, Eijsink VGH, Vårum KM, 2005. Degradation of chitosans with chitinase B from *Serratia marcescens*. Production of chito-oligosaccharides and insight into enzyme processivity. *FEBS journal* 272: 1-12.
- [2] Sikorski P, Stokke BT, Sørbotten A, Vårum KM, Horn SJ, Eijsink VGH, 2005. Development and application of a model for chitosan hydrolysis by a family 18 chitinase. *Biopolymers*, in press.
- [3] Bahrke S, Einarsson JM, Gislason J, Haebel S, Letzel MC, Peter-Katalinic J, Peter MG, 2002. Sequence analysis of chito-oligosaccharides by matrix-assisted laser desorption ionization postsources decay mass spectrometry. *Biomacromolecules* 3:696-704.

Minutes of the Board meeting of 1st September 2004

A meeting of the Board of the European Chitin Society was held at the IOR, Poznan, on the 1st September 2004. In the absence of the President, Professor Martin Peter, due to illness, the meeting was chaired by Professor Alain Domard.

The following members of the Board were present: Professor A Domard, Professor V G H Eijsink, Dr M Graeve, Dr M M Jaworska, Professor O Smidsrød, Dr L Szosland, Professor H Struszczyk, and Professor K M Vårum. Professor G Roberts was present as an observer.

1. Secretary's Report

As neither Secretary or Assistant Secretary was present no Secretary's Report was presented to the meeting.

2. Treasurer's Report

The Treasurer presented a brief report of the accounts as of 31st December 2003. This showed a surplus slightly in excess of 10,000 Euro. Taking into account the income and expenditure since 1st January 2004 the current balance was approximately 12,350 Euro. This was considered a relatively healthy position although some costs would be incurred in setting up EUCHIS '06 and EUCHIS '08.

3. Membership

Membership was currently standing at 123 [69 active members; 10 active members (Eastern Europe); 6 donor active members; 14 associate members; 20 student members; 4 collective members.]

4. EUCHIS '08

The main business of the meeting was to decide on the venue for the EUCHIS '08 meeting. A presentation was given by Dr M Healy (Queen's University, Belfast) and Ms O Donnelly (NI Tourist Board) in support of the application to hold the conference in Belfast, Northern Ireland. This was followed by a presentation by Professor S Şenel (Hacettepe University, Ankara) in support of the application to hold the conference in Turkey.

After some discussion the Board decided that EUCHIS '08 should be held in Turkey and that Belfast should be offered the EUCHIS '10 conference.

5. Braconnot Prize

Only two nominations for the Braconnot Prize had been received by the Board. A jury of three Board members were asked to assess the applications and report back before the General Assembly Meeting.

6. Newsletter

There was some discussion over the relative merits of a 'paper' Newsletter and an electronic version. It was finally decided that the electronic version would be continued and Dr Vårum agreed to produce a hard copy version at low cost/no cost for those members who preferred the traditional type of Newsletter.

7. Honorary President

The Board decided unanimously to propose to the General Assembly that Professor Olav Smidsrød be elected to serve as Honorary President of the European Chitin Society.

December 20th, 2004

Signed: G A F Roberts, Secretary

Minutes of the Board meeting of 3rd September 2004

A meeting of the Board of the European Chitin Society was held at the IOR, Poznan on the 3rd September 2004 under the Chairmanship of Professor A Domard.

The following members of the Board were present: A Domard; V G H Eijsink; M Fenice; J Gislason; L F Gorovoj; M Graeve; M Jaworska; B Moerschbacher; G A F Roberts; S Senel; O Smidsrød; H Struszczyk; K M Vårum.

1. Election of officers

President: Professor K M Vårum was elected President and took over the Chair from Professor Domard.

The President made a short speech outlining his view of what are the important aims for the European Chitin Society in the short term. These are to:

Try to revitalise the Newsletter and produce 2 issues per year on a regular pattern, and containing information useful to members of the Society (Board members should be major sources of such information).

Upgrade the web-site which currently is not particularly user-friendly; possibly some professional assistance may be required. Also the server is in Germany and he would discuss its siting with Professor Peter.

Use EUCHIS more directly in the scientific area e.g. inter-laboratory trials for evaluation of chitosan characterisation techniques; classification/characterisation of chitin- and chitosan-degrading enzymes.

The remaining officers were then elected.

Vice-presidents: Professors A Domard and H Struszczyk

Treasurer: Dr M Graeve

Assistant Treasurer: Professor M Spindler-Barth

Secretary: Professor G Roberts

Assistant Secretary: Professor V G H Eijsink

2. AOB

The Treasurer suggested that it might be necessary to spend some of the Society's money as there was now an appreciable balance. Proposals for possible areas in which this might be done were running workshops (SS) and providing upfront funding for EUCHIS '06 (AD). The sum of 4,500 Euro was agreed.

It was decided that no Braconnot Prize was to be awarded this year. Two of the three jury members were concerned that neither candidate had supplied all of the required information and so they were not in a position to accurately assess them. It was decided that the rules for the prize would be published again in the Newsletter, stressing that they must be fully complied with. It was also decided that the two candidates could reapply for the prize next time.

It was proposed (VE) that the Society consider awarding a prize for the best poster by a student member, the prize being possibly free attendance at the next EUCHIS conference.

20th December 2004

Signed:

G A F Roberts, Secretary

Forthcoming Conferences

UK Society for Biomaterials: 4th Conference and Postgraduate Day

Nottingham University, Nottingham, UK

June 21-22, 2005

The programme will consist of a mix of keynote speakers and submitted presentations on the first day, and postgraduate presentations on the second day.

For further details contact:

Angela Kuhn at uksb@nottingham.ac.uk

or

visit the website www.nottingham.ac.uk/schoolm3/research/Bioengineering/UKSB/

The 7th Asia-Pacific Chitin and Chitosan Symposium

Bexco, Busan, Korea

April 24-26, 2006

For further details contact:

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The Secretariat
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Seoul 135-957, Korea

Travel Awards for EUCHIS Conferences

The European Chitin Society will provide a limited number of travel awards for attendance at EUCHIS-meetings. Depending on the number of applicants selected and the funds available, awards may cover travel (economy return air, rail fare or car fuel costs), conference registration, and in some cases accomodation (economy class).

General rules:

Applicants must:

- Be a PhD student or junior scientist (i.e. medical resident or postdoctoral).
- Be an EUCHIS member in good standing (subscription paid).
- Make a contribution (oral or poster) to the conference.
- Have no other source of funds for this purpose, including supervisor's or University funding. If funds from elsewhere are subsequently obtained, EUCHIS should be informed immediately and the application for EUCHIS funding withdrawn, or the EUCHIS award declined/returned if already made, so that another applicant can be funded.

Application Procedure:

The deadline for applications is eight weeks before the deadline for early conference registration.

Please send an informal letter of application to:

Prof. Kjell M. Vårum
NOBIPOL, Department of Biotechnology
Norwegian University of Science and Technology
7491 TRONDHEIM, NORWAY
(E-mail: kvaarum@biotech.ntnu.no)

enclosing:

- Proof of status (usually a short statement from the supervisor or Head of Department), including the date or expected date of completion of PhD or medical qualification.
- Evidence of non-availability of other funds (usually part of the statement from the supervisor or Head of Department). Please state if other applications for funding are being made.
- Submitted abstract of the oral or poster contribution.
- Estimates of the costs of travel, accommodation and conference registration.
- Applicant's full address, phone and fax numbers, and e-mail address where available.

Awards

Unless all applicants can be funded, awards will be made by the Travel Awards Committee on a competitive basis. Applicants will be notified of the outcome by the Chair of the Committee, by letter, fax, or e-mail, no later than two weeks before the early registration deadline.

The award will be paid by cheque after submission of all relevant receipts including the original air or train tickets and the receipt for the registration fee. In exceptional cases, the award may alternatively be paid directly to a travel agent (or railway company, airline etc), and to the conference organizers, on submission of the original invoices.

EUCHIS Financial Report 2003

(per December 31, 2003) Account at Deutsche Bank, Bonn

POSITIVA

Balance per 31.12.2002	EUR 7.078,61		
members fees			
- collective members		EUR 900,00	
- active members		EUR 2.248,20	
- associate members		EUR 327,48	
- student members		EUR 280,00	
	EUR 7.078,61	EUR 3.755,68	
total			EUR 10.834,68

NEGATIVA

Bank charges		EUR -177,19	
Printing costs Newsletter		EUR -287,23	
Office expenses		EUR -260,00	
Internet charges		EUR -71,88	
total		EUR -802,30	EUR -802,30
Balance per December 31 2002			EUR 10.031,99

Bremen, 31.12.2002

Dr. Martin Graeve

EUCHIS Financial Report 2004

(per July 31, 2004) Account at Deutsche Bank, Bonn

POSITIVA

Balance per 31.12.2002	EUR 10.031,99		
members fees			
- collective members		EUR 540,00	
- active members		EUR 1.669,00	
- associate members		EUR 150,00	
- student members		EUR 160,00	
	EUR 10.031,99	EUR 2.519,00	
total			EUR 12.550,99

NEGATIVA

Bank charges		EUR -95,38	
Printing costs Newsletter			
Office expenses		EUR -	
Internet charges		EUR -41,94	
total		EUR -137,32	EUR -137,32
Balance per December 31 2002			EUR 12.413,67

Bremen, 31.07.2004

Dr. Martin Graeve