Prof. James du Preez

Department of *Microbial, Biochemical and Food Biotechnology*

Overview

One of the highlights of the year for the Department of Microbial, Biochemical and Food Biotechnology was the inauguration of the BioPAD Platform for Metagenomics in March 2007, which forms part of a network of specialist platforms for research in South Africa. BioPAD is a Biotechnology Regional Innovation Centre established by the national Department of Science and Technology (DST).

This biotechnology research contract, worth R13.7 million and which includes equipment, student bursaries and postdoctoral fellowships awarded by the BioPAD Board of Trustees, is one of the biggest research contracts awarded to the UFS in recent years. The platform is managed by Dr Esta van Heerden, who also leads the research together with her two colleagues, Prof. Derek Litthauer and Dr Lizelle Piater. The steering committee includes representatives from BioPAD and the UFS. The focus of the research is the screening of mostly unculturable extremophiles and their (meta)genomes, since these are constantly exposed to ever-changing micro-environments that necessitate their perception of and adaptation to such conditions in order to survive.

Successful micro-organisms, probably better described as specialist communities, are believed to have evolved a variety of mechanisms, specifically genes and gene products, in this regard. Bioprospecting of these extreme biodiversities through the construction of metagenomic libraries presents infinite possibilities of unearthing extraordinary and unique micro-organisms and genes or gene products (especially extremozymes) that, ultimately, may contribute significantly to industrial processes, improved products, bioremediation of contaminated sites and, ultimately, the economy of the country. This research will also contribute to our understanding of the significance of our own extreme ecosystems. It is further anticipated that the research project will contribute to the preservation of biological and genetic material that is unique to South Africa.

The research infrastructure of the department for molecular biology was further improved as a result of Prof. Koos Albertyn's successful application for R760 000 from the National Research Foundation's (NRF) National Equipment Programme towards the purchase of a DNA sequencer, namely an ABI PRISM 313OXL-16 Capillary Genetic Analyser. The department also acquired a Molecular Dynamics GenePix4000B microarray slide scanner and a Bio-Rad Pharos Plus two-dimensional proteomics gel scanner, funded by an NRF National Equipment grant awarded to Prof. Hugh Patterton in 2007. In addition, an Applied Biosystems QTRAP 4000 hybrid ion-trap mass spectrometer fitted with an Agilent 1200 Nanoflow High Performance Liquid Chromatography system was acquired with partial funding from the Strategic Cluster initiative of the university. These items, housed in the departmental Facility for Genomics and Proteomics, provides a platform for modern, high-throughput biological research.

Another highlight in October was the start of construction of a Bioinformatics laboratory that will include a 30-workstation lecturing facility for bioinformatics and related teaching, adjacent to the Biotechnology building. This is the final result of a grant to Prof. Patterton for the establishment of a bioinformatics node at the UFS as a faculty facility that will link with the National Bioinformatics Network. The aim of the university's node is to perform internationally competitive research in bioinformatics, contribute to capacity building in bioinformatics by the training of students at under- and postgraduate levels, and to host training workshops for students and staff. The bioinformatics node will also be involved in providing computational support to projects that include bioinformatics components.

Planning has also started for upgrading the Biotechnology building, which will make provision for the increased demand for laboratory space by adding a wing that will include new undergraduate laboratories so that the existing undergraduate laboratories may be converted to research laboratories to accommodate the increase in research activities in the department.

The department has 11 NRF-rated academics, including four B-rated scientists. In the 2007 *Evaluation and Rating Facts and Figures* publication of the NRF, the university is listed in second place under the top performing higher education institutions in terms of the number of rated researchers (11) in the specialist field of Microbiology and Plant Pathology. Eight of these 11 rated scientists are members of the department.

The department is represented on the editorial boards of several international journals. Prof. Bennie Viljoen serves on the editorial board of the journal *Food Microbiology* and Prof. James du Preez, Departmental Chairperson, is an associate editor of

Some of the foreign postgraduate students and postdoctoral fellows in the department during 2007 are front, from the left: Mss Kulsum Kondiah, Kenya, Kasweka Kakoma, Zambia, Mai Nguyen, Vietnam, Chikondi Mattaka, Malawi, Dr Khajamohiddin Syed, India, and Mr Simbarashe Mabwe Zimbabwe; back: Messrs Rudolf Schäfer, Germany, Joseph Katongole, Uganda, Mss Ji-Yun Lee, South Korea, Limpho Ramorobi, Nthabiseng Matsoha, both from Lesotho, Prof. James du Preez, Departmental Chairperson, Mr Olukayode Kuloyo, Ms Elizabeth Ojo, both from Nigeria, and Dr Antonio García- Moyano, Spain. Mr Schäfer is a chemical engineering undergraduate from the Mannheim University of Applied Science in Germany participating in a research programme to gain practical experience in fermentation biotechnology as part of his course requirements.

the *World Journal of Microbiology and Biotechnology* and serves on the editorial boards of *FEMS Yeast Research* and the e-journal *Biotechnology for Biofuels*.

The department was well represented at international congresses. Prof. Lodewyk Kock and six of his postgraduate students from the Lipid Biotechnology Group presented their research at the Asian Mycology Congress (AMC2007) and 10th International Marine and Freshwater Mycology Symposium held on the island of Penang in Malaysia. At this congress Mr Desmond Ncango, a Ph.D. student, was awarded first prize in the Biochemistry and Industrial Mycology section for his poster presentation and Mr Olihile Sebolai presented a lecture. These students were fully sponsored by the South African Fryer Oil Initiative (SAFOI), which is part of the Lipid Biotechnology Group. The 26th International Specialised Symposium on Yeasts (ISSY 26), held in Sorrento, Italy, was attended by Profs Kock, Du Preez (both members of the International Commission for Yeasts, which convened during the symposium), and Betty Lodolo (of the South African Breweries Limited (SAB) and also affiliate associate professor in the department) who presented a keynote lecture with Prof. Kock as co-author. The other members of the group were Profs Albertyn, Viljoen, Ms Chantal Smith, Ms Andri van Wyk and Ms Olga de Smidt who presented a paper from her Ph.D. thesis at this symposium. Prof. Kock also served on the International Scientific Committee of this symposium and acted as chair of one of the sessions. In addition, SAFOI partly sponsored ISSY 26.

Prof. Du Preez attended the 13th European Congress on Biotechnology held in Barcelona, Spain. Dr Van Heerden, Prof. Litthauer and Mr Dirk Opperman, a Ph.D. student, attended the 9th International Thermophiles Conference held in Bergen, Norway. Mr Opperman received a travel grant from the Oppenheimer Trust and the faculty contributed towards his conference registration. Participation in other congresses included the Deutsche Gesellschaft für Fettwissenschaft (DGF) Symposium in Hamburg, Germany attended by Dr Manjusha Joseph, Biocatalysis-2007, Moscow, St. Petersburg, Russia attended by Prof. Martie Smit, BIOTRANS 2007 in Oviedo, Spain attended by Prof. Smit and the World Veterinary Poultry Association Congress in Beijing, China where Prof. Rob Bragg presented seven lectures and chaired one of the sessions at the congress.

Other overseas visits included that of Prof. Litthauer and Dr Van Heerden to the Department of Biology at the University of Bergen to discuss collaboration on phage diversity in the environment. Dr Van Heerden, who is a member of the local organising committee for the international Extremophiles 2008 conference, also met with the international organising committee for the conference and visited the Department of Georadiochemistry at the Institute for Interdisciplinary Isotope Research in Leipzig, Germany to discuss collaborative projects. Prof. Smit visited the group of Dr Vlada Urlacher at the Institute for Technical Biochemistry in Stuttgart, Germany and attended a three-day workshop to determine priorities for a South Africa-UK Science Network in Catalysis in Cardiff, Wales.

Profs Du Preez and Viljoen were invited to the University of Lund in Sweden

and the Norwegian University of Life Sciences in Norway, respectively, as the opponents in a Ph.D. defence. Prof. du Preez also presented a lecture on the role and regulation of alcohol dehydrogenases in *Saccharomyces cerevisiae* at the University of Lund. Prof. Patterton visited the Joliot-Curie Laboratory of the *Ecole Normale Superieure de Lyon* as invited professor from April to May and presented invited seminars at the Joliot-Curie Laboratory, CNRS in Lyon (with Dr Philippe Bouvet as his host), the Laboratoire de Biologie Moléculaire Eucaryote, CNRS in Toulouse (with Dr Michael Georgiev as his host), the Institut André Lwoff, CNRS in Paris (with Dr Ali Hamische as his host), and at the Université Joseph Fourier in Grenoble (with Dr Stefan Dimitrov as his host).

Members of the department participated in various collaborative research programmes. The Food Biotechnology group under the leadership of Prof. Viljoen was successful in obtaining a South Africa/Norway collaborative project based on a project entitled: *Prospecting probiotics and antimicrobials in indigenous fermented milks*. An amount of more than R2 million was allocated over a three-year period. The project on indigenous fermented foods in Southern Africa was expanded to include researchers from Zambia, Mozambique, Swaziland, Zimbabwe and Lesotho. Part of this work is sponsored by the Norwegian Council of Universities Committee for Development, Research and Education (NUFU). Prof. Viljoen is currently the external programme co-ordinator for the Institute of Food, Nutrition and Family Sciences at the University of Zimbabwe, and the School of Agricultural Sciences at the University of Zambia.

The department is also involved in the international National Earthquake Laboratory in South African Mines project (NELSAM) and has active contracts with GFZ (GeoForschungs Zentrum Potsdam) and the Oklahoma University in the United States of America (USA) for a five-year period to facilitate expertise transfer, student bursaries and equipment donations to the department.

Collaborative research projects are in place with the Laboratoire de Génétique des Microorganismes of the Institut National Agronomique, Paris-Grignon in France on the non-conventional yeast *Yarrowia lipolytica*, and with the Institute of Technical Biochemistry at the University of Stuttgart in Germany on the engineering of self-sufficient cytochrome P450 monooxygenases. Future collaboration with the department of Botany and Microbiology at the University of Lagos in Nigeria on the cloning of alkane degrading P450s from polluted environments is being investigated. Prof. Bragg has research agreements with Ewabo (Germany) and Mercordi (Belgium) for product testing. Other research collaboration includes the Obihiro University of Agriculture and Veterinary Medicine in Japan and the University College of Cork in Ireland.

Prof. Philippe Bouvet from the Laboratoire de Biologie Moléculaire de la Cellule at the Ecole Normale Supérieure de Lyon visited the department at the end of March as part of the CNRS/NRF collabo-

The "international" team that was the winner of the UFS section of the National Innovation Fund competition for students are, from the left: Ms Kasweka Kakoma, Zambia, Mr Lehlohonolo Mathengtheng, South Africa, Prof. Rob Bragg, mentor, and Ms Ji-Yun Lee, South Korea.

Some of the guests attending the launch of the BioPAD Platform for Metagenomics research contract are: Drs Siyabulela Ntutela, Deputy Director: Biotechnology at the Department of Science and Technology, Godfrey Netswera, Manager of Thuthuka and the Support Programme at the National Research Foundation (NRF), Esta van Heerden, Platform Manager and lecturer at the department, Mr Butana Mboniswa, Chief Executive Officer of BioPAD, and Mr Vuyisele Phehani, Portfolio Manager for BioPAD.

rative exchange programme. Prof. Bouvet, who obtained his Ph.D. from Harvard University in the USA and completed postdoctoral training at the National Institute of Health, gave a seminar on the role of nucleolin in chromatin structure and gene expression and also met with several research groups in the department. Prof. Judy Narvhus (Norway), Dr Tony Mutukumira (Swaziland) and Dr Henry Gadaga (Lesotho) again visited the department in December as part of the Food Biotechnology programme. Prof. Bärbel Hahn-Hägerdal of the University of Lund in Sweden was appointed as Professor Extraordinary in the department. Prof. Edgar Da Silva from Paris, a current Professor Extraordinary in the department and former director of the United Nations Educational, Scientific, and Cultural Organisation's (UNESCO) Division of Life Sciences, visited the department in September and presented several lectures. Sadly, he passed away shortly afterwards while on a visit to India. This tragic loss was compounded by the death of Prof. Santosh Nigam, also a Professor Extraordinary in the department, in Berlin about a month earlier.

On national level the department collaborated on research with Onderstepoort Biological Products, ICA International Chemicals (Pty) Ltd., Mintek, Agave Distillers (Pty) Ltd., the NRF-DST Centre of Excellence in Catalysis hosted by the University of Cape Town (UCT), Stellenbosch University, the Tshwane University of Technology, the University of Fort Hare and Tiger Brands. Prof. Patterton collaborated with the National Bioinformatics Network on the development of bioinformatics in South Africa. Prof. Bragg of the Veterinary Biotechnology research group has an ongoing collaborative project with Prof. Ed Rybicki's group at UCT on the development of vaccines for the control of the Psittacine Beak and Feather disease virus. This virus is currently one of the major threats to the highly endangered and endemic Cape parrot. An experimental DNA vaccine has been successfully developed and testing in birds will start in January 2008.

Dr Arno Hugo undertook a project on Nguni meat quality in collaboration with researchers from the University of Fort Hare and the Nutrition and Food Science department of the Agricultural Research Council (ARC). In September, Dr Hugo presented the 32nd workshop on meat processing at the UFS. Also in September, Prof. Kock, Dr. Manjusha Joseph and students Ms Ntsoaki Leeuw and Ms Vernita Reid, all from the Lipid Biotechnology Group, presented a workshop for the North West Department of Health at their headquarters in Vryburg. Prof. Kock participated in a *Carte Blanche* television programme on food labelling in April. He also served on the NRF's assessment panel for Microbiology and Plant Pathology, while Prof. Viljoen served on the NRF's project panel for Microbiology and Plant Pathology.

Apart from international research collaboration, the department also has a significant number of foreign postgraduate students and postdoctoral fellows from various countries. At the 2007 graduation ceremonies, two Ph.D., 12 M.Sc. and 17 Honours degrees were conferred. One student who excelled was Ms Ané van Heerden, who received her M.Sc. with distinction and was awarded the Van der Walt Prize, the Sasol Prize, the S_2A_3 bronze medal and university academic colours at the faculty prize-giving ceremony. A team from the Veterinary Biotechnology Research group was the winner of the UFS section of the National Innovation Fund competition for students, with their business plan on the use of bacteriophages to combat specific bacterial infections in poultry. This team comprised Ms Kasweka Kakoma, Ms Ji-Yun Lee and Mr Lehlohonolo Mathengtheng. The team will now submit their project proposal to the national competition, which will be adjudicated early in 2008.

The department comprises three divisions, namely Microbiology and Biochemistry, Food Science and Consumer Science. Within these divisions there are various research groups. Although the overall research theme of the department is the broad field of microbial, biochemical and food biotechnology, the heterogeneous departmental composition causes some activities to fall outside this field. Activities of these research groups not reported above are indicated below.

In the **Food Science division**, Dr Hugo focuses his research on the manipulation of meat quality by nutrition. He presented his research on nutrition and meat quality of pork in South Africa at the 53rd International Congress of Meat Science and Technology in Beijing, China. His observations of a seasonal variation in fatty acid composition of pork attracted great interest from a large number of congress participants. Prof. Garry Osthoff is researching milk from wild animals in order to improve our understanding of milk as food in the broader animal kingdom. Not all compounds in milk are utilised for growth or energy. The oligosaccharides, for instance, have a prebiotic function, and are very important in some species. To research these molecules, Prof. Osthoff has entered into cooperation with a research group at the Obihiro University of Agriculture and Veterinary Medicine in Japan, where he spent some time during the year isolating and partially characterising oligosaccharides from elephant milk.

The **Lipid Biotechnology Group** led by Prof. Kock and Dr Carlien Pohl in collaboration with Prof. Pieter van Wyk (of the Centre for Confocal and Electron Microscopy at the UFS) and Prof. Lodolo (from SAB) concentrated on the uncovering of novel antifungals and antiflocculants with antimitochondrion activity. This finds application in the brewing industry where the control of flocculation is important in beer clarification and in the termination of fermentation. This research is also of special medical importance since fungal infections annually contribute towards many deaths worldwide, including in South Africa. It was uncovered that inexpensive acetylsalicylic acid acts as a potent antimitochondrial antifungal in a large number of yeasts with potential for human application. This research resulted in seven articles published and accepted in ISI-accredited scientific journals and three students successfully completing their M.Sc. studies (all with distinctions). This work was also published as a review in one of the foremost specialised yeast journals, FEMS Yeast Research (2007;7:1207-1217). Dr Pohl is currently also investigating the effect of unusual non-methylene interrupted fatty acids, specifically sciadonic acid, on the opportunistic yeast pathogens *Candida albicans* and *Candida dubliniensis*. Sciadonic acid is an arachidonic acid homologue, but unlike arachidonic acid it cannot be metabolised to form prostaglandins – important virulence factors of these yeasts. This research will provide a better understanding of lipid metabolism in these yeasts and may also lead to the development of sciadonic acid as a novel antifungal agent against pathogenic yeasts.

The **Biocatalysis research group** of Prof. Smit participates in the DST-NRF Centre of Excellence in Catalysis, hosted by UCT and the High Throughput Biology Research Niche Area of the Advanced Biomolecular Research Cluster of the UFS. The main focus is on the terminal hydroxylation of alkanes and alkylben-

zenes by cytochrome P450 monooxygenases, but benzylic hydroxylation of alkyl phenols by vanillyl alcohol oxidases is also receiving attention. Previous work mainly involved the cloning of yeast P450s into the yeast *Yarrowia lipolytica* followed by whole cell biotransformations in shake flasks.

During 2007 the group ventured into a number of new activities that included P450 enzyme assays with cell free extracts and microsomal fractions; expression of P450s in *E. coli*; cloning of P450s into *Pseudomonas* strains; cloning and expression of bacterial P450s as well as enzyme modelling. Molecular biology projects are done in collaboration with Prof. Albertyn (**Molecular Biology research group**). During 2007 the group also commissioned an InFors Sixfors multireactor which was acquired during 2006 with funding from the UFS, the DST-NRF Centre of Excellence in Catalysis and the NRF. This multireactor system makes it possible to simultaneously run six small-scale bioreactor experiments with online monitoring of pH and oxygen and control of pH, stirring and substrate feed. A second postdoctoral fellow, Dr Rama Krishna Gudiminchi, joined the group in August.

In the **Fermentation Biotechnology research group,** led by Profs du Preez and Stephanus Kilian, work on the production and application of prebiotics continued. Previous work of Prof. Kilian, focusing on the prebiotic sugar neokestose, was extended to determine its potential to stimulate the growth of a known probiotic yeast strain and a number of other yeasts with potential probiotic properties. The resistance of neokestose to intestinal conditions and enzymes and the comparison of the properties of neokestose in this regard to inulin, a commercial prebiotic, is also under investigation. The research project of Prof. Du Preez together with Dr Carlien Pohl-Albertyn and Mr Piet Botes on the fermentation of Agave juice extract was continued. This research was undertaken in collaboration with Agave Distillers (Pty) Ltd., the only company in the world outside Mexico that produces Agave spirit (known in Mexico as tequila). A number of *Saccharomyces cerevisiae* yeast strains were evaluated in terms of their temperature-ethanol tolerance profiles and fermentation performance. A strain of *Kluyveromyces marxianus* was isolated from the juice and shows promise for use in the process to improve the efficiency of the fermentation. The collaborative project of Prof. Albertyn (**Molecular Biology research group**) and Prof. Du Preez on the regulation and physiological role of the alcohol dehydrogenase (ADH) isozymes in *S. cerevisiae* has turned up some interesting results. The effect of multiple *adh* deletions in strains of *S. cerevisiae* on its phenotype is under further investigation, using aerobic glucose-limited continuous cultures.

Departmental delegates at the 26th International Specialised Symposium on Yeasts (ISSY 26) held in Sorrento, Italy are, from the left: Ms Olga de Smidt, Ph.D. student, Prof. Bennie Viljoen, Ms Chantal Smith, researcher, Profs Bettie Lodolo, Affiliate Associate Professor, Koos Albertyn, Lodewyk Kock and James du Preez, Departmental Chairperson, with Mount Vesuvius in the background.

Large numbers of new genes have become available due to the progress of various genome projects. This necessitates the development of highly efficient expression systems for the production of heterologous proteins, for both industrial and academic purposes. The **Molecular Biology research group**, led by Prof. Albertyn, is currently analysing the expression potential of three yeast hosts in collaboration with Prof. Gotthard Kunze of the Institut für Pflanzengenetik und Kulturpflanzenforschung in Germany. In this study a xylanase is expressed using an integration plasmid based on the conserved 26S rDNA locus which allows a single plasmid to be transformed in all three yeast hosts. These will subsequently be compared to the expression levels using host-specific plasmids. These include plasmids developed in a previous study expressing epoxide hydrolase genes in *Y. lipolytica*. Analysis of the expressed protein will be performed to determine expression levels (on both mRNA and protein level) and glycosylation levels. Furthermore, this research group is in the process of developing a series of plasmid-based vectors to allow the transformation of and heterologous expression in various yeasts.

The Epigenomics and DNA Function research group of Prof. Patterton continued work on the role of chromatin structure on the regulation of gene expression on a genome-wide scale. Work showing that the linker histone H1 was absolutely required for compaction of chromatin in the stationary phase was completed. The surprising finding was made that H1 did not act as a general transcriptional repressor in the semi-quiescent state, despite its central structural position in the nucleosome. Instead, strong evidence was found for H1 being displaced by transcriptional activity. These findings suggested that in lower eukaryotes, such as *Saccharomyces cerevisiae*, the primary role of compaction of chromatin in the stationary phase may not be for gene shutdown, but may be related to an inhibition of homologous recombination, thus maintaining the genome integrity over extended periods of time during semi-quiescence.

Staff

Division Microbiology and Biochemistry:

Professors: Profs James du Preez, Rob Bragg, Stephanus Kilian, Lodewyk Kock, Derek Litthauer, Martie Smit, Bennie Viljoen

Associate Professors: Profs Koos Albertyn, Hugh Patterton

Senior Lecturers: Drs Esta van Heerden, André van Tonder

Lecturers: Drs Lizelle Piater, Carlien Pohl-Albertyn

Researcher: Ms Laurinda Steyn

Professors Extraordinary: Profs Edgar DaSilva, Bärbel Hahn-Hägerdal, Santosh Nigam

Affiliate Associate Professors: Profs Mary DeFlaun, Bettie Lodolo

Secretary: Ms Millie Cohen

First Professional Officer: Mr Piet Botes

Senior Professional Officer: Dr Manjusha Joseph

Professional Officers: Mss Mapula Maropola, Suzanne Brandt, Andri van Wyk, Anita de Necker, Yvette Makaum, Nel-Marie Agenbag, Dalene van den Berg

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Division Food Science:

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Lecturers: Dr Maryna de Wit, Ms Carina Bothma,

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Research outputs

Research articles

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