



During the first week of April the Southern African Society for Microbiology (SASM) held its 20th conference at Misty Hills conference centre, near Johannesburg. The conference was hosted by the Department of Biochemistry, Genetics and Microbiology, University of Pretoria with the theme “Microbe: Livelihoods, economy and environment”. The conference was attended by 261 delegates of which 126 were students. Apart from the 10 keynote presentations there was also 70 oral presentation of which more than 60% was presented by MSc and PhD students. More than 90 posters presentations were also delivered by students as the primary authors.

The generous support and participation of ISME at the conference was highly appreciated. Not only was the African ISME Ambassadors meeting held during the conference but members of the ISME’s executive board also used the opportunity to interact and inspire South African microbiologists. ISME sponsored a very successful poster session and Mark Bailey facilitated an interactive discussion session “Getting published”. Both Colin Murrell and Nicole Webster delivered keynote presentations.

The society was fortunate that ISME also sponsored two prizes for the best student presentations. This provided an ideal opportunity to introduce ISME to the wider community of South African microbiologists, working in the field Microbial ecology. Sarah Potgieter from the University of Pretoria received the prize for the best student oral presentation and Eduvan Bisschoff from the University of the Free State, for the best poster presentation related to microbial ecology.



Photo 1: Keynote presentation by Colin Murrell



Photo 2: Interactive session on how to get published facilitated by Mark Bailey



Photo 3: Certificate of appreciation handed over to Colin Murrell by the conference organiser, Fanus Venter



Photo 4: ISME sponsored poster session

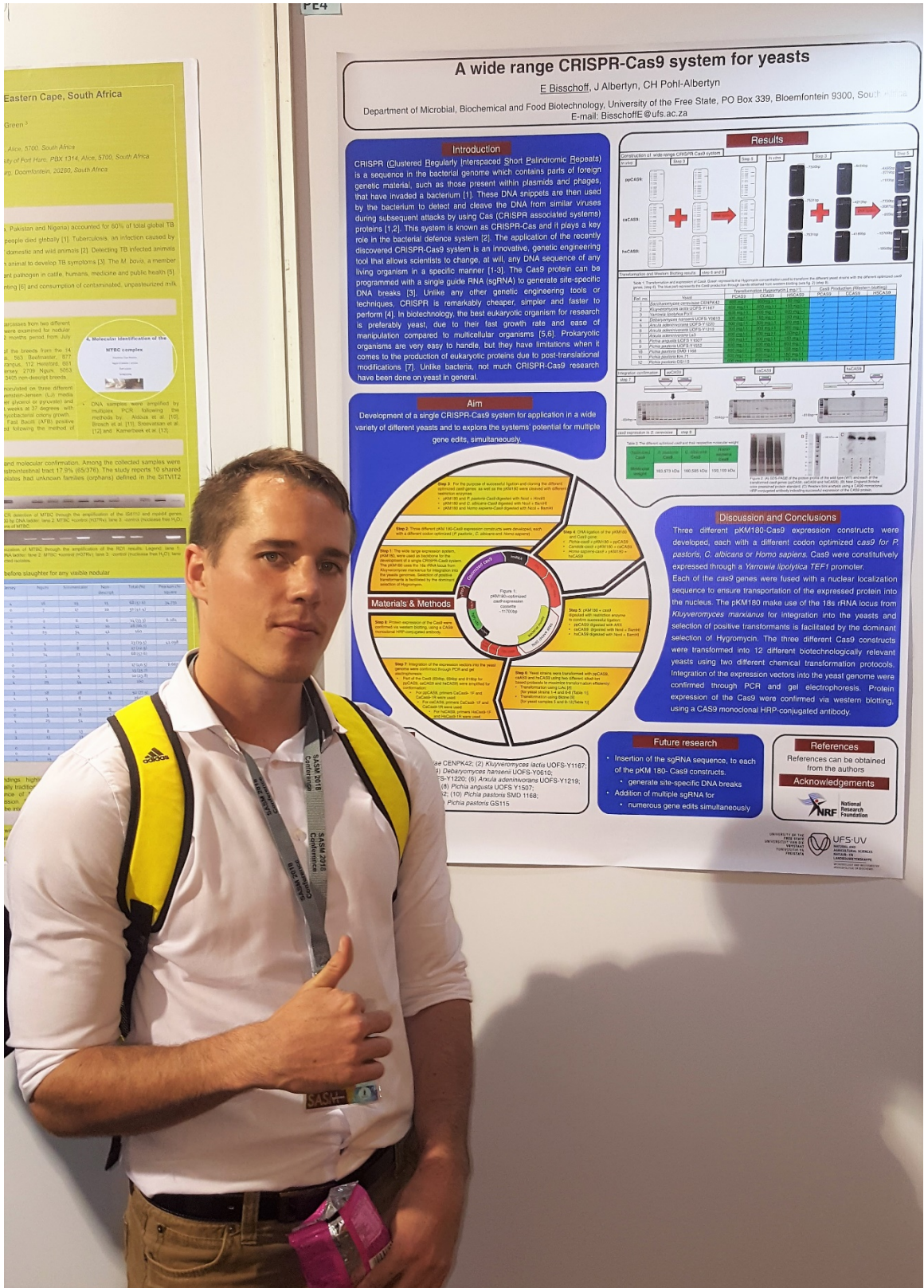


Photo 5: Winning poster presentation by Eduvan Bisschoff from the University of the Free State

A wide range CRISPR-Cas9 system for yeasts

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Introduction

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a sequence in the bacterial genome which contains parts of foreign genetic material, such as those present within plasmids and phages, that have invaded a bacterium [1]. These DNA snippets are then used by the bacterium to detect and cleave the DNA from similar viruses during subsequent attacks by using Cas (CRISPR associated systems) proteins [1,2]. This system is known as CRISPR-Cas and it plays a key role in the bacterial defence system [2]. The application of the recently discovered CRISPR-Cas9 system is an innovative, genetic engineering tool that allows scientists to change, at will, any DNA sequence of any living organism in a specific manner [1-3]. The Cas9 protein can be programmed with a single guide RNA (sgRNA) to generate site-specific DNA breaks [3]. Unlike any other genetic engineering tools or techniques, CRISPR is remarkably cheaper, simpler and faster to perform [4]. In biotechnology, the best eukaryotic organism for research is preferably yeast, due to their fast growth rate and ease of manipulation compared to multicellular organisms [5,6]. Prokaryotic organisms are very easy to handle, but they have limitations when it comes to the production of eukaryotic proteins due to post-translational modifications [7]. Unlike bacteria, not much CRISPR-Cas9 research have been done on yeast in general.

Results

Construction of a wide range CRISPR-Cas9 system

Characterization and detection of Cas9

| Yeast Strain | hCas9 | mCas9 | yCas9 |
|---------------------------------------|-------|-------|-------|
| 1. Kluyveromyces fragilis UOF-S-1167 | + | + | + |
| 2. Kluyveromyces fragilis UOF-S-1167 | + | + | + |
| 3. Kluyveromyces fragilis UOF-S-1167 | + | + | + |
| 4. Kluyveromyces fragilis UOF-S-1167 | + | + | + |
| 5. Kluyveromyces fragilis UOF-S-1167 | + | + | + |
| 6. Kluyveromyces fragilis UOF-S-1167 | + | + | + |
| 7. Kluyveromyces fragilis UOF-S-1167 | + | + | + |
| 8. Kluyveromyces fragilis UOF-S-1167 | + | + | + |
| 9. Kluyveromyces fragilis UOF-S-1167 | + | + | + |
| 10. Kluyveromyces fragilis UOF-S-1167 | + | + | + |
| 11. Kluyveromyces fragilis UOF-S-1167 | + | + | + |
| 12. Kluyveromyces fragilis UOF-S-1167 | + | + | + |
| 13. Kluyveromyces fragilis UOF-S-1167 | + | + | + |
| 14. Kluyveromyces fragilis UOF-S-1167 | + | + | + |
| 15. Kluyveromyces fragilis UOF-S-1167 | + | + | + |
| 16. Kluyveromyces fragilis UOF-S-1167 | + | + | + |
| 17. Kluyveromyces fragilis UOF-S-1167 | + | + | + |
| 18. Kluyveromyces fragilis UOF-S-1167 | + | + | + |
| 19. Kluyveromyces fragilis UOF-S-1167 | + | + | + |
| 20. Kluyveromyces fragilis UOF-S-1167 | + | + | + |

Aim

Development of a single CRISPR-Cas9 system for application in a wide variety of different yeasts and to explore the systems' potential for multiple gene edits, simultaneously.

Materials & Methods

Step 1: The wide range CRISPR-Cas9 system was constructed by cloning the pKM180-Cas9 construct into the pKM180-sgRNA construct. The pKM180-Cas9 construct was cloned into the pKM180-sgRNA construct using the BamHI and XbaI restriction enzymes. The pKM180-sgRNA construct was cloned into the pKM180-Cas9 construct using the BamHI and XbaI restriction enzymes. The resulting pKM180-Cas9-sgRNA construct was transformed into the yeast strains.

Step 2: The yeast strains were transformed with the pKM180-Cas9-sgRNA construct. The transformation efficiency was determined by plating the transformed cells on a selective medium containing hygromycin. The resulting yeast strains were grown in a yeast extract peptone dextrose (YPD) medium.

Step 3: The yeast strains were grown in a yeast extract peptone dextrose (YPD) medium. The growth of the yeast strains was monitored by measuring the optical density (OD₆₀₀) of the yeast cultures. The yeast strains were harvested at the end of the growth period.

Step 4: The yeast strains were harvested and the DNA was extracted. The DNA was digested with the Cas9 protein to generate site-specific DNA breaks. The resulting DNA fragments were analyzed by gel electrophoresis.

Step 5: The DNA fragments were analyzed by gel electrophoresis. The resulting DNA fragments were visualized by staining with ethidium bromide. The DNA fragments were then sequenced to confirm the site-specific DNA breaks.

Discussion and Conclusions

Three different pKM180-Cas9 expression constructs were developed, each with a different codon optimized cas9 for *P. pastoris*, *G. albicans* or *Homo sapiens*. Cas9 was constitutively expressed through a *Neurospora crassa* TEF1 promoter. Each of the cas9 genes were fused with a nuclear localization sequence to ensure transportation of the expressed protein into the nucleus. The pKM180 make use of the 18S rRNA locus from *Kluyveromyces marxianus* for integration into the yeasts and selection of positive transformants is facilitated by the dominant selection of Hygromycin. The three different Cas9 constructs were transformed into 12 different biotechnologically relevant yeasts using two different chemical transformation protocols. Integration of the expression vectors into the yeast genome were confirmed through PCR and gel electrophoresis. Protein expression of the Cas9 were confirmed via western blotting, using a Cas9 monoclonal HRP-conjugated antibody.

Future research

- Insertion of the sgRNA sequence, to each of the pKM180-Cas9 constructs.
- Generate site-specific DNA breaks
- Addition of multiple sgRNA for numerous gene edits simultaneously

References

References can be obtained from the authors.

Acknowledgements

NRF
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Photo 6: Eduvan Bisschoff receiving his prize from Colin Murrell



Photo 7: Winning oral presentation by Sarah Potgieter from the University of Pretoria



Photo 8: Sarah Potgieter receiving her prize from Colin Murrell