Blastobotrys (*Arxula*) *adeninivorans* - a promising alternative yeast for biotechnology and basic research

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/yea.3180

Abstract

Blastobotrys adeninivorans (syn. *Arxula adeninivorans*) is a non-conventional, non-pathogenic, imperfect, haploid, yeast, belonging to the Subphylum *Saccharomycotina*, which has to date received comparatively little attention from researchers. It possesses unusual properties such as thermo- and osmotolerance, and a broad substrate spectrum. Depending on the cultivation temperature *B.* (*A.*) *adeninivorans* exhibits different morphological forms and various post-translational modifications and protein expression properties which are strongly correlated with the morphology. The genome has been completely sequenced and in addition, there is a well-developed transformation/expression platform, which makes rapid, simple gene manipulations possible. This yeast species is a very good host for homologous and heterologous gene expression and is also a useful gene donor. *B.* (*A.*) *adeninivorans* is able to use a very wide range of substrates as carbon and/or nitrogen sources and is an interesting organism due to the presence of many metabolic pathways, for example, degradation of n-butanol, purines, and tannin. In addition, its unusual properties and robustness make it a useful bio-component for whole cell biosensors. There are currently a number of products on the market produced by *B.* (*A.*) *adeninivorans* and further investigation may contribute further innovative solutions for current challenges that exist in the biotechnology industry. Additionally it may become a useful alternative to existing commercial yeast strains and as a model organism in research. In this review we present information relevant to the exploitation of *B.* (*A.*) *adeninivorans* in research and industrial settings.

Keywords: *Arxula adeninivorans,* biosensor, recombinant proteins, dimorphism, yeast.

Introduction

Yeasts are eukaryotic microorganisms that play important roles in human life. The best known is *Saccharomyces cerevisiae*, which has been used since ancient times for the production of various foods and beverages, such as bread, beer and wine. Through the Yeast Genome Project (YGP), it was the first eukaryotic organism to have its genome sequenced which is recorded in the *Saccharomyces* genome database (http://www.yeastgenome.org/). Nevertheless, *S. cerevisiae* can have some limitations such as hyper glycosylation, poor secretion and/or incorrect folding of some heterologous proteins as well as relatively low robustness against osmotic and temperature stress in industrial applications. Thus other yeast systems have been sought that might not have these problems. Alternative yeast strains being employed in biotechnology includes *Blastobotrys adeninivorans* (*Arxula adeninivorans*), *Ogataea polymorpha (Hansenula polymorpha), Komagataella pastoris* (*Pichia pastoris*), *Kluyveromyces lactis* and *Yarrowia lipolytica.* This article describes the special properties of *B.* (*A.*) *adeninivorans* that makes it a first-rate system for commercial applications and an increasingly important model system for basic research.

History and phylogeny of *B. adeninivorans*

B. adeninivorans is a relatively recently discovered yeast species. The first report of this new organism was published by Middelhoven *et al.* in 1984. The yeast was isolated from soil and named *Trichosporon adeninovorans* (Middelhoven *et al.*, 1984). Six years later Gienow *et al.* (1990) described the second strain, LS3 (PAR-4), isolated from wood hydrolysates in Siberia. Further strains have been isolated from chopped maize herbage in the Netherlands and from humus-rich soil in South Africa (Van der Walt *et al.*, 1990). All of these wild type isolates were found to possess unusual properties such as nitrate assimilation and xerotolerance and the ability to use adenine, guanine, butylamine, soluble starch, melibiose, uric acid,

pentylamine, putrescine, propylamine and hexylamine as a sole source of carbon, nitrogen and energy (Middelhoven *et al.*, 1984). The species was given the name *Arxula adeninivorans* (Middelhoven *et al.*, 1991) and subsequently renamed *B. adeninivorans* (Kurtzman and Fell, 1998).

Another yeast species initially belonging to the *Blastobotrys* genus was subsequently discovered and is now named *Blastobotrys terrestris*. *B. adeninivorans* and *B. terrestris* exhibit some differences, for example, the ability to assimilate melibiose, D-glucosamine and galactitol (Kurtzman and Fell, 1998). Both were classified into *Trichomonascus* clade, which contains anamorphic genera such as *Blastobotrys*, *Candida* and *Sympodiomyces*, but also the ascosporic genera *Trichomonascus* and *Stephanoascus* (Kurtzman *et al.*, 2007). It was demonstrated, based on its complete genome sequence data (Kunze *et al.*, 2014), that *B.* (*A.*) *adeninivorans* is phylogenetically distant from the most characterised yeast model system, *S. cerevisiae*.

To date, more than 170 articles related to *A. adeninivorans* have been published. Although this yeast is fully characterised and its genome is completely sequenced, it is still not well known. The purpose of this paper is to provide information on this interesting and useful organism.

An apparently ordinary yeast with some extraordinary properties

A. adeninivorans is temperature tolerant and able to grow at up to 48 °C without previous adaptation. Depending on the cultivation temperature, LS3 wild types strain exhibit three morphological forms. Up to 42 °C, the cells reproduce by budding; at 42 °C, the cells form pseudomycelia and above 42 °C they become mycelial (Wartmann *et al.*, 1995, 2000 – Fig. 1). The occurrence of such dimorphism is quite common in the yeasts; however the factors, which cause the changes in the phenotype, are usually different in different yeast species. For

instance, in *Y. lipolytica*, the morphology depends principally on the pH, however carbon and nitrogen sources and temperature can also have an effect on the phenotype (Ruiz-Herrera *et al*., 2002). It was observed that under low temperature conditions this yeast grows mainly as mycelia, whereas under high temperature conditions, they grow as budding cells (Medoff *et al*., 1987; Swoboda *et al*., 1994), which is in contrast to *A. adeninivorans* (<42 °C - budding cells, 42°C –pseudomycelia, >42°C mycelial). The dimorphism of *A. adeninivorans* is reversible with the key factor being temperature e.g. when the temperature of a mycelial culture is reduced <42 °C the mycelia forms buds to produce new yeast cells. The morphology of *A. adeninivorans* is thus quite easy to control; however the exact mechanism underlying this dimorphism is not yet known.

Budding cells of the LS3 strain and mycelia of the mutant strain *A. adeninivorans* 135 (growing as mycelia at 30 °C) and cultivated under the same conditions, exhibit various biochemical and secretory properties. Except for the differences in dry cell weight (dcw), total RNA and intracellular protein content, the biggest difference between the two strains occurs in the total amount of secreted proteins. The mass of extracellular proteins in mycelial cultures is two-fold higher than in the budding cells. Hence, as reported by Wartmann *et al*. (2000), the two different morphological forms exhibit differences in their biochemical and secretory properties. However it is not yet known if the amount of protein produced by the different morphological forms varies for different proteins (Böer *et al.*, 2007).

The levels of post-translational modifications, specifically glycosylation or phosphorylation in *A. adeninivorans* are not constant but the mechanisms leading to these post-translational modifications are not yet understood. O-glycosylation occurs preferentially in budding cells, which indicates that the type of glycosylation is linked to the morphology. The extent of glycosylation and phosphorylation regulation, however, seems to depend only on the temperature of cultivation (Wartman *et al.*, 2002). Hyper-glycosylation is also known in *S.*

cerevisiae and its hyper-glycosylation machinery is able to create N-linked protein glycosylation terminated by mannose attached via the α-1,3 bond. This mannose type N-glycosylation induces an allergic response in humans (Gellissen *et al.*, 2005) and study of glycosylation in other yeasts may provide useful information on this response. The type of N-glycosylation in *A. adeninivorans* is still not well known. Böer *et al*. (2007) performed a comparative assessment experiment in *A. adeninivorans,* and *O. polymorpha* and *S. cerevisiae* (which are commercially available as expression systems) as hosts for the production of the human interleukin-6 (IL-6). They showed that only *A. adeninivorans* synthesizes the protein with the correct post-translational processing and it was also the most productive of the three yeasts. Analysis has shown that in case of *O. polymorpha* and *S. cerevisiae* N-terminal truncations of proteins occurred (Böer *et al.*, 2007). In 2015, Kumari *et al*. compared the secretion of lipase (YlLip11p) in two different hosts *A. adeninivorans* and *Y. lipolytica*. They observed differences in productivity, the pattern of glycosylation and thermostability of the enzyme. *A. adeninivorans* exhibited 1.6-fold higher productivity than the other strains and with a higher level of glycosylation and probably therefore, a higher thermostability and lower specific activity than that achieved by *O. polymorpha* enzyme (Kumari *et al*., 2015). These studies illustrate the value of using non-model systems in yeast research because these results indicate that they might have real value in improving the quality of various industrial products.

Another property of *A. adeninivorans* is its halotolerance. Cells are able to grow on media containing up to 20% NaCl with only slight effects on transcription levels, secretion and growth being observed up to 10% NaCl (Tag *et al*., 1998; Yang *et al*., 2000). Osmotolerance is a very desirable feature both for fermentation as well as in bioremediation and biosensors.

Because of the importance of fatty acids in the biofuel, chemistry and cosmetic markets, their production in yeasts has been studied intensively. The medium-chain fatty acids (6 - 14

carbons) are the most valuable and occur in *S. cerevisiae* primarily as lipids. These fatty acids are absent in oleaginous yeasts such as *Y. lipolytica*. Nevertheless, yeasts remain a viable alternative to chemical synthesis because of their environmentally friendly production. It is known that, in general, low temperatures increase the fatty acid concentration in yeasts. Olstorpe *et al.* (2013) first described the temperature dependent fatty acid profile of C10 to C24 chain length fatty acids in *A. adeninivorans* (strains CBS 8244 and CBS 7377). They found that C16:0 is the main fatty acid present in wild-type strains (19.5 - 36.8% total fatty acids). C17:1 fatty acids were synthesized at up to 30.6% of total fatty acids and mono-unsaturated fatty acids accumulated to between 38.3% - 52.3%. Interestingly, the C18:1 chain demonstrated different profiles in different *A. adeninivorans* wild type strains. The first strain, *A. adeninivorans* CBS 8244, did not contain C18:1 (n-5), but C18:1 (n-7) was present at 7.4% at 15 °C and 9% at 30 °C incubation temperatures, while in another strain of *A. adeninivorans*, CBS 7377, C18:1 (n-5) was present at 4.5% at 15 °C and 12.6% at 30 °C. C18:1 (n-7) was only just detectable and the total fatty acids at low temperatures were lower compared to the total at higher temperatures. In both strains, the general trend of the profile of the fatty acids was similar, except for C16:1 (n-7), C18:1 (n-5) and C18:3 (n-3), where the trend was opposite. These strains, however, differed in the total fatty acid concentration, which is consistent with the notion of temperature dependent fatty acid production in yeast. Additionally the double bond index (DBI) increases with increased cultivation temperature. However the C16:C18 ratio is similar at 15 and 30 °C (Olstorpe *et al*., 2014). Hence, there is no predictable fatty acid composition in these yeast strains.

Froissard *et al*. (2015) analysed 16 strains belonging to the *Saccharomycotina*. They showed that *A. adeninivorans* (wild type strain CBS 8244) contains 50.12 ± 0.43 µg fatty acid methyl ester (FEME) mg^{-1} dcw after 24 h cultivation in YP medium at 28 °C. This strain however, is devoid of C18:3 fatty acids. The authors also found that the relative amount of fatty acids in

Saccharomycotina and *A. adeninivorans* is similar to the profile of fatty acids in *Y. lipolytica* with mainly C16:0, C18:0, C18:1 (n-9) and C18:2 (n-6) molecules present. *A. adeninivorans* LS3, which, although the most characterised *A. adeninivorans* wild type strain, has not yet been tested for its fatty acid profile.

Genome architecture and reproduction

In 2014, the *A. adeninivorans* LS3 genome was completely sequenced. The mitochondrial genome has a size of 31,662 bp and encodes 24 tRNAs and 15 proteins. The nuclear genome has a size of 11.8 Mb. The nuclear genome comprises four chromosomes, Arad1A (1,659,397 nt), Arad1B (2,016,785 nt), Arad1C (3,827,910 nt) and Arad1D (4,300,524 nt) with regional centromeres. There are 6,116 protein encoding genes, 33 pseudogenes and 914 introns present. A single rDNA cluster which plays an important role in molecular tools of *A. adeninivorans*, is located approximately 75 kb upstream of the Arad1D chromosome's right subtelomere (Kunze *et al.*, 2014; Rösel *et al*., 1996; Pich and Kunze, 1992). Although *A. adeninivorans* is phylogenetically distant from *S. cerevisiae*, it nevertheless follows the bacterial sparing rules and reads Leu CUN and Arg CGN codons, as in baker's yeast (Kunze *et al.*, 2014). In most cases, this is an advantage for heterologous gene expression. Based on the information obtained from whole genome sequences analysis of *A. adeninivorans*, a microarray for gene expression analysis containing 56,312 specific oligonucleotides was constructed (Fig. 2). This is an additional approach to investigate the changes in transcription profile that are dependent on environment conditions.

Sexual reproduction in *A. adeninivorans* has not been reported, thus it is considered to be a haploid asexual yeast. However, complete sequencing of the genome by Kunze *et al*. (2014) revealed the present of the *MAT* locus on chromosome Arad1D. The authors discussed the possibilities that *A. adeninivorans* really is an asexual organism, or alternatively, it is still

able to reproduce sexually but the opposite mating strain has not yet been discovered. It may also be that the ploidy of a strain is relevant and that haploid organisms adapt and evolve faster than diploid organisms and, therefore have become the dominant form (Orr *et al*., 1994; Gerstein *et al*., 2011). There is also the possibility that during the adaptation process of *A. adeninivorans* to harsh environments, this yeast became a haploid organism and lost the ability to reproduce sexually. Regardless of which factors led to the haploidy of *A. adeninivorans*, this property permits easy genetic manipulation (e.g. simple UV or nitrosoguanidine mutagenesis, followed by mutant selection), rapid selection of strains with desired properties, higher stability of the phenotype, rapid growth and the ability to disperse in a short period of time. On the other hand, asexual reproduction reduces molecular diversity. Samsonova *et al*. (1996) investigated the possibility of experimentally developing a parasexual cycle in *A. adeninivorans*. This experiment succeeded and most of the fusion hybrids obtained were relatively stable heterozygotes. Spontaneous mitotic segregation, which results in the misdistribution of the chromosomes and crossing-over or gene conversion, was found to be at a frequency of 0.1-1%.

Molecular tools for *A. adeninivorans*

The first successful transformation of *A. adeninivorans* was performed in 1998 by Rösel and Kunze. Homologous recombination of linearized cassettes containing a dominant selective marker were constructed and integrated into 25S rDNA of *A. adeninivorans* by gap repair. This strategy was based on previous studies on the characterization of the 25S rDNA from *A. adeninivorans* LS3. This rDNA sequence is similar to corresponding sequences from *Candida albicans* (91.7% similar), *S. cerevisiae* (90.5% similar) and *Schizosaccharomyces pombe* (83.8% similar). In most cases, 1-3 copies of the cassettes were detected in transformed strains. The transformants were stable and resistant to up to 2000-5000 µg mL^{-1}

of hygromycin B. In contrast, transformation with circular plasmids was not successful. Transformation did not appear to cause changes in the morphology of the cells (Rösel and Kunze, 1998).

In 2004a, Terentiev *et al*. developed an *A. adeninivorans* transformation/expression platform called Xplor1. It involved a hybrid plasmid, based on *E. coli* and *A. adeninivorans* fragments. The vector contained the conserved *A. adeninivorans* 25S rDNA derived sequences allowing specific insertion into the host genome and the *E. coli hph* gene, which confers resistance. The *TEF1* promoter sequence from *A. adeninivorans*, described by Rösel and Kunze (1998), was chosen for the expression control, while *PHO5* terminator from *S. cerevisiae* was selected for transcription termination. These improved the efficiency of integration of expression cassette in heterologous systems and were demonstrated to function in *S. cerevisiae*, *O. polymorpha*, *Pichia pastoris*, *Debaryomyces hansenii*, and *Debaryomyces polymorphus*. The study established the versatility of the Xplor1 platform and demonstrated activity of the *A. adeninivorans TEF1* promoter in heterologous yeast species (Terentiev *et al*., 2004a).

The Xplor®2 system has been developed from Xplor1 (Böer *et al*., 2009a – Fig. 3). Improvements to the original vector were made by a number of authors and have resulted in a versatile transformation/expression platform (Wartmann *et al*., 1998, 2003a, b; Steinborn *et al*. 2007a, b; Böer *et al*., 2009a). Xplor®2 enables heterologous gene expression with two different modules: a yeast rDNA integrative expression cassette (YRC), which targets genes into rDNA clusters, and a yeast integrative expression cassette (YIC), which targets genes randomly into genomic DNA. Both modules are included in the one vector and the presence of two different restriction sites enables simple and fast selection of the selected cassette. A multicloning site (MCS) can accommodate five modules. The antibiotic selection marker for yeast transformation has been replaced by auxotrophic selection markers, which overcomes

the problems associated with the presence of antibiotic resistant markers in organisms used in industrial biotechnology. Furthermore there are modules for constitutive and inducible gene expression (Böer *et al*., 2009a, b; Terentiev *et al*., 2004a; Wartmann *et al*., 2003a, b). Homologous recombination is an efficient transformation procedure that introduces linear DNA fragments into chromosomes. It was designed to reduce the size of the expression cassettes to a minimum for efficient transformation. Passaging of the transformants results in an increase in the stability of the constructs which is essential in yeast intended for industrial use.

The Xplor®2 transformation/expression platform with YRC and YIC cassettes was also trialled for homologous recombination in *Schwanniomyces occidentalis, O. polymorpha* and *S. cerevisiae*. Both systems resulted in successful transformations in these yeasts (Álvaro-Benito *et al*., 2013; Kumari *et al*., 2015).

The Xplor®2 system enables easy, rapid and efficient transformation to create stable transgenic strains for the production of industrially important proteins.

A. adeninivorans and Xplor®2 as a new system for recombinant protein production

Industry not only requires new molecules but it also requires them to be produced sustainably. It is also very important to reduce costs and maximize efficiency to convert a new system developed in the laboratory into a large-scale commercial production. Hence, for many purposes, there is still the need to search for alternative organisms, able to tolerate extreme environmental conditions such as high temperature fluctuations, media with high osmolarity and product accumulation to high levels.

A. adeninivorans contains useful enzyme encoding genes such as *glucoamylase* (*GAA* - Bui *et al*., 1996a), *tannase* (*ATAN1* - Böer *et al.*, 2009c), and *cutinase* (*ACUT1, ACUT2, ACUT3* - Bischoff *et al.*, 2015) genes which make this yeast a useful gene donor.

Homologous gene expression in A. adeninivorans

Many enzyme encoding genes and their regulation have been studied in *A. adeninivorans* and many of its enzymes have been biochemically characterized. Most of the secreted proteins are involved in the degradation of different chemical compounds. One of the potential markets for *A. adeninivorans* enzymes is the food and feed industries. The extracellular invertase, encoded by *AINV* gene, possesses high ß-fructosidase and low α-glucosidase activity (Böer *et al*., 2004). Invertases are used widely, especially in the confectionery industry and in the pharmaceutical industry for the production of probiotics. Another enzyme is the intracellular xylitol dehydrogenase, encoded by the *AXDH* gene (Böer *et al*., 2005a). This enzyme can be applied in the food industry for xylitol production for use as an alternative sweetener (Winkelhausen *et al*., 1998) and also for bioethanol production (Matsushika *et al*., 2008). In 2009c, Böer *et al*. characterised the *ATAN1* gene encoding tannin acyl hydrolase. This enzyme is involved in the hydrolysis of tannins, which are widely distributed in plants. Although tannases are produced by several microorganisms, they have limitations for industrial applications such as the amount produced, the intracellular localisation of enzyme and a limited substrate spectrum. In contrast, Atan1p has a wide substrate spectrum and 97% is secreted into the medium, which makes this enzyme easy and relatively inexpensive to purify. Currently a transgenic *A. adeninivorans* strain produces tannase with an activity of 1,642 U L^{-1} during growth in a shake flask and 51,900 U L^{-1} in fed-batch fermentation. Tannin acyl hydrolase is currently used in the food, feed, cosmetic and pharmaceutical industries and also in environmental bioremediation (Böer *et al*., 2009c, 2011; Kaiser *et al*., 2010).

Other enzymes with industrial importance produced by *A. adeninivorans* are the cutinases. These enzymes can be used for the degradation of natural and synthetic polymers as well as for selective esterification of fatty acids (Suzuki *et al*., 2005; Masaki *et al*., 2005). All three

cutinases have been purified and their biochemical properties determined. The activity of his-tagged cutinase 2 (Acut2p), produced by *A. adeninivorans* G1212/YRC102-ACUT2-6H cultivated under non-optimized fed-batch conditions reached 1064 U mL^{-1} (Bischoff *et al*., 2015). Although cutinases are classified as very unstable enzymes, PEG 200 has been shown to significantly stabilize these enzymes. Activity was observed for more than 24 h and the addition of PEG 200 to the reaction mixture also improved the enzyme activity by up to 200%. Other industrially significant enzymes obtained from *A. adeninivorans* are glucoamylase, lipase, transaldolase, and acid phosphatase (Bui *et al*., 1996b; Böer *et al*., 2005b; El Fiki et al., 2007; Kaur *et al*., 2007; Minocha *et al*., 2007).

Some metabolic pathways in *A. adeninivorans*, have also been investigated. An example is the purine degradation pathway comprising the enzymes adenine deaminase, xanthine oxidoreductase, urate oxidase, and guanine deaminase which have been shown to be useful in the production of food with reduced purine levels (Jankowska *et al*., 2013a, b; Trautwein-Schult *et al*., 2013, 2014). A second example is the tannin degradation pathway which has industrial importance. The exact mechanisms of this metabolic pathway are not yet elucidated. However early investigations have been undertaken (Sietmann *et al*., 2010). *A. adeninivorans* is able to use tannic acid and gallic acid (one of the hydrolysis product of tannins) as sole sources of carbon. Tannin acyl hydrolase, the enzyme involved in the first step of tannin degradation in *A. adeninivorans* has received particular attention because of its potential use in detannification of food, feed, beverages and cosmetics (Böer *et al*., 2009a, 2011).

Expression of heterologous genes in A. adeninivorans

A. adeninivorans is also suitable for commercial production of recombinant protein. In 2007 Böer *et al*. demonstrated the production of the correctly processed form of human interleukin-

6 in *A. adeninivorans*. The second human protein expressed in *A. adeninivorans* was human interferon α2a (IFNα2a). The concentration was 1 mg L^{-1}, which is five-fold higher than the concentration produced by *S. occidentalis* (Álvaro-Benito *et al*., 2013). Other heterologous proteins produced in *A. adeninivorans* include lipase 11 from *Y. lipolytica* (YlLip11p) (Kumari *et al*., 2015) and ß-galactosidase from *Kluyveromyces lactis*. This ß-galactosidase is able to perform selective hydrolysis of anomeric mixtures such as allyl α-D-gal and allyl ß-D-gal where only the allyl ß-D-gal anomer was hydrolysed by the enzyme with no cross reactivity to the α-anomer. This process can be used to replace the rather difficult synthetic process. The products obtained by the reaction carried out by this enzyme are used for the production of tensides and play a major role in medicine as precursor for the production of glycolipids and glycoproteins (Rauter *et al*., 2013). Additionally, the different glycosylation patterns seen in *A. adeninivorans*, *S. cerevisiae* and *O. polymorpha* offer the opportunity to trial different variants of a protein.

The enzymatic synthesis of enantiomerically pure alcohols has been recently investigated. Because stereoisomers are often recognised as two different compounds by the biochemistry of living organisms, they frequently behave differently in cells. Ideally, only the stereoisomer with the desired activity should be included in therapeutics. Biological synthesis results in the production of only one stereoisomer whereas chemical synthesis always results in a racemic mixture. The production of stereo-selective alcohols has been demonstrated in *A. adeninivorans* strains. The expression of the *RrADH* gene encoding alcohol dehydrogenase from *Rhodococcus ruber* and the *BmGDH* gene encoding glucose dehydrogenase from *Bacillus megaterium* enabled the synthesis of 98% pure 1-(S)-phenylethanol with simultaneous regeneration of the cofactor (Rauter *et al*., 2014a). Permeabilization and immobilization of *A. adeninivorans* cells increased the stability and reusability of the construct and established a method that can be adapted for the use with other enzymes

(Rauter *et al*., 2014b). For example the construction of an *A. adeninivorans* strain to produce 1-(R)-phenylethanol resulted in the synthesis of a single isomer with no detectable by-products present (Rauter *et al*., 2015).

In 1992, Büttner *et al*. described the alcoholic fermentation in a number of *A. adeninivorans* wild type strains. The authors were investigating the direct conversion of starch to ethanol in aerobic and anaerobic cultivation conditions at different temperatures. The strains produced 9 – 17 g L^{-1} ethanol at 30 °C but much less at 45 °C (0.2 – 0.5 g $^{-1}$). In addition it was found that *A. adeninivorans* could use n-butanol as the sole source of carbon and energy for the organism (Kunze *et al.*, 2014). Furthermore it is possible to transfer new metabolic pathways into the yeast e.g. for n-butanol synthesis. In 2012 a patent was granted for a strain of *A. adeninivorans* that can synthesis n-butanol (Kunze *et al.*, 2012).

These examples indicate that *A. adeninivorans* is very useful host for the production of recombinant proteins and other chemicals.

A. adeninivorans as a biocomponent for biosensors

Rapidly growing pharmaceutical, chemical and cosmetic markets have contributed to the improvement of human health and the quality of life. However, an unintended side effect has been the increasing contamination of surface and ground water and the production of wastewater with difficult to detect and to remediate molecules, such as mammalian hormones. One consequence for example has been a strong influence on gender determination in fish leading to gender imbalance in some fish populations (Hunter *et al*., 1986). In 1998 Tag *et al.* reported the resistance of *A. adeninivorans* to high concentrations of sodium chloride in water (Tag *et al*., 1998) which, with the previously described properties, makes it possible to construct biosensors for use in brackish wastewater and other contaminated waters. In 2006, Hahn *et al.* developed the first *A. adeninivorans* cell-based

estrogen biosensor (A-YES). Recombinant strains containing the human estrogen receptor α (hERα) controlled by a strong *A. adeninivorans*-derived *TEF1* promoter and a phytase reporter gene from *Kliebsiella* sp. under the separate control of the *A. adeninivorans*-derived glucoamylase promoter (*GAA*), containing the estrogen-responsive element. Estrogen stimulated the production and export of phytase which was detected by an enzyme assay. It allowed the specific, sensitive and reproducible detection of estrogen in wastewater within 30 h without prior sample concentration (Hahn *et al.,* 2006). In the next iteration of the sensor (nAES), the detection time was reduced to between 7 and 25 h depending on the estrogen concentration (Kaiser *et al*., 2010). In 2011 the 'EstraMonitor' was developed as the first automated biosensor system. It contained immobilized transgenic *A. adeninivorans* cells which were reusable and allowed semi-online measurement (Pham Thi *et al*., 2012). The EstraMonitor version was further improved in 2013 allowing continuous and semi-online monitoring estrogenic compounds, in wastewater with NaCl concentrations as high as 5% (Pham Thi *et al*., 2013).

Some yeast species, such as *C. albicans* or *Paracoccidioides brasiliensis,* possess an estrogen binding protein (Ebp1p), which can be used to detect estrogen. Unfortunately they are pathogenic yeasts, which is a potential risk for human health. Chelikani *et al.* (2012) showed that *A. adeninivorans* has no detectable intrinsic response to estrogen compounds and Vijayan *et al.* (2015) presented the first transgenic *A. adeninivorans* strain to express the *EBP1* gene from *C. albicans*. A single-use electrode, with recombinant oestrogen binding protein for use with a portable potentiostat can determine estrogen concentrations in the range of observed environmental concentrations within 2 min.

Sensors based on transgenic *A. adeninivorans* strains for detection of other molecules such as omeprazole, lansoprazole (Pham Thi *et al*., 2015) and progesterone (Chamas *et al*., 2015a) have also been developed. Two bioassays for photometric and spectrophotometric detection

of glucocorticoids, A-YGS and A-YGFS, are operational but not yet commercialized (Pham Thi *et al*., 2016).

Furthermore, a HER-2 cancer cell detector utilizing surface plasmon resonance (SPR) has been constructed for the rapid diagnosis of a particularly aggressive type of breast cancer (Chamas *et al*., 2015b).

Commercial products

At present, there are ten commercially available products based on the yeast *A. adeninivorans*. The first protein produced by this non-traditional yeast was a recombinant tannase (Atan1p - Böer *et al*., 2011). Today it is also possible to order tannase and the three *A. adeninivorans* cutinases (Bischoff *et al*., 2015 - Acut1p, Acut2p, Acut3p) from ASA Spezialenzyme GmbH (Germany). 'Quo data' GmbH (Germany) has commercialized an A-YES kit for the detection of estrogenic activity, and 'new diagnostics' GmbH (Germany) offers diagnostic kits for dioxin (A-YDS Kit), estrogens in ultrapure and potable waters (A-YES®_aqua1.1), estrogens in saline waters (A-YES®_aqua^{+}1.1) androgens (A-YAS®) and progesterone (A-YPS_aqua). All these diagnostic kits use transgenic *A. adeninivorans* cells as the detection element.

Conclusion

Investigation of *A. adeninivorans* suggests major potential for this yeast in basic research and in industrial applications. It is a non-pathogen and widespread in some environments. The ability to grow in a wide range of environmental conditions is an advantage and the high level of secreted molecules enables exploitation of the organism for the production of biochemicals. The presence of many degradative pathways also makes *A. adeninivorans* useful in both bioremediation and in the food industry.

A well-developed transformation/expression system, the fully sequenced haploid genome makes it possible to design recombinant strains that are highly stable. Promising future applications include biofuel production, synthesis of enantiomerically pure biochemicals, and construction of new cell biosensors.

In comparison with current commercial yeast strains, the tools available for *A. adeninivorans* are in their infancy; however this non-conventional yeast is gathering increasing attention from researchers, which is leading to rapid progress in its application.

Acknowledgements

The researchers thank Dr. Twan Rutten and Dr. Adam S. Wilkins for their support. The research work was supported by grant from the Marie Curie Actions – Initial Training Networks (Grant No. FP7-PEOPLE-2013-ITN).

References

Álvaro-Benito M, Fernández-Lobato M, Baronian K, Kunze G. 2013. Assessment of *Schwanniomyces occidentalis* as a host for protein production using the wide-range Xplor®2 expression platform. *Appl Microbiol Biotechnol* **97**: 4443–4456.

Bischoff F, Litwinska K, Cordes A, Baronian K, Bode R, Schauer F, Kunze G. 2015. Three new cutinases from the yeast *Arxula adeninivorans* that are suitable for biotechnological applications. *Appl Environ Microbiol* **81**: 5497-5510.

Böer E, Wartmann T, Luther B, Manteuffel R, Bode R, Gellissen G, Kunze G. 2004. Characterization of the *AINV* gene and the encoded invertase from the dimorphic yeast *Arxula adeninivorans. Antonie van Leeuwenhoek* **86**: 121–134.

Böer E, Wartmann T, Schmidt S, Bode R, Gellissen G, Kunze G. 2005a. Characterisation of the *AXDH* gene and the encoded xylitol dehydrogenase from the dimorphic yeast *Arxula adeninivorans*. *Antonie van Leeuwenhoek* **87**: 233–243.

Böer E, Mock HP, Bode R, Gellissen G, Kunze G. 2005b. An extracellular lipase from the dimorphic yeast *Arxula adeninivorans*: molecular cloning of the *ALIP1* gene and characterization of the purified recombinant enzyme. *Yeast* **22**: 523–535.

Böer E, Steinborn G, Matros A, Mock HP, Gellissen G, Kunze G. 2007. Production of interleukin-6 in *Arxula adeninivorans, Hansenula polymorpha* and *Saccharomyces cerevisiae* by applying the wide-range yeast vector (CoMed™) system to simultaneous comparative assessment. *FEMS Yeast Res* **7**: 1181–1187.

Böer E, Bode R, Mock HP, Piontek M, Kunze G. 2009a. Atan1p - an extracellular tannase from the dimorphic yeast *Arxula adeninivorans*: molecular cloning of the *ATAN1* gene and characterization of the recombinant enzyme. *Yeast* **26**: 323-337.

Böer E, Piontek M, Kunze G. 2009b. Xplor®2—an optimized transformation/expression system for recombinant protein production in the yeast *Arxula adeninivorans*. *Appl Microbiol Biotechnol* **84**: 583–594.

Böer B, Schröter A, Bode R, Piontek M, Kunze G. 2009c. Characterization and expression analysis of a gene cluster for nitrate assimilation from the yeast *Arxula adeninivorans. Yeast* **26**: 83–93.

Böer E, Breuer FS, Weniger M, Denter S, Piontek M, Kunze G. 2011. Large-scale production of tannase using the yeast *Arxula adeninivorans*. *Appl Microbiol Biotechnol* **92**: 105-114.

Bui DM, Kunze I, Horstmann C, Schmidt T, Breunig KD, Kunze G. 1996a. Expression of the *Arxula adeninivorans* glucoamylase gene in *Kluyveromyces lactis*. *Appl Microbiol Biotechnol* **45**: 102–106.

Bui DM, Kunze I, Förster S, Wartmann T, Horstmann C, Manteuffel R, Kunze G 1996b. Cloning and expression of an *Arxula adeninivorans* glucoamylase gene in *Saccharomyces cerevisiae*. *Appl Microbiol Biotechnol* **44**: 610–619.

Büttner R, Bode R, Birnbaum D. 1992. Alcoholic fermentation of starch by *Arxula adeninivorans. Zbl Mikrobiol* **147**: 225-230.

Chamas A, Nieter A, Pham Thi MH, Giersberg M, Hettwer K, Uhlig S, Simon K, Baronian K, Kunze G. 2015a. Development of a recombinant *Arxula adeninivorans* cell bioassay for the detection of molecules with progesterone activity in wastewater. *Anal Bioanal Chem* **407**: 8109–8120.

Chamas A, Giersberg M, Friedrich K, Sonntag F, Kunze D, Uhlig S, Simon K, Baronian K, Kunze G. 2015b. Purification and immunodetection of the complete recombinant HER-2[neu] receptor produced in yeast. *Protein Expr Purif* **105**: 61–70.

Chelikani V, Downard AJ, Kunze G, Gooneratne R, Pasco N, Baronian KHR. 2012. Investigating yeast cell responses to oestrogen by electrochemical detection. *Electrochimica Acta* **73**: 136-140.

El Fiki E, El-Metabteb G, Bellebna C, Wartmann T, Bode R, Gellissen G, Kunze G. 2007. The *Arxula adeninivorans ATAL* gene encoding transaldolase - gene characterization and biotechnological exploitation. *Appl Microbiol Biotechnol* **74**: 1292-1299.

Froissard M, Canonge M, Pouteaux M, Cintrat B, Mohand-Oumoussa S, Guillouet SE, Chardot T, Jacques N, Casaregola SS. 2015. Lipids containing medium-chain fatty acids are specific to post-whole genome duplication *Saccharomycotina* yeasts. *BMC Evol Biol* **28**: 15-97.

Gellissen G, Strasser AWM, Suckow M. 2005. Production of recombinant proteins: novel microbial and eukaryotic expression systems. Key and criteria to the selection of an expression platform. WILEY-VCH Verlag GmbH & Co. KGaA,Weinheim.

Gerstein AC, Cleathero LA, Mandegar MA, Otto SP. 2011. Haploids adapt faster than diploids across a range of environments. *J Evol Biol* **24**: 531-540.

Gienow U, Kunze G, Schauer F, Bode R, Hofemeister J. 1990. The yeast genus *Trichosporon spec*. LS3; molecular characterization of genomic complexity. *Zbl Mikrobiol* **145**: 3-12.

Hahn T, Tag K, Riedel K, Uhlig S, Baronian K, Gellissen G, Kunze G. 2006. A novel estrogen sensor based on recombinant *Arxula adeninivorans* cells. *Biosens Bioelectron* **21**: 2078-2085.

Hunter GA, Solar II, Baker IJ, Domaldson EM. 1986. Feminization of coho salmon (*Oncorhynchus kisutch*) and chinook salmon (*Oncorhynchus tshawytscha*) by immersion of alevins in a solution of estradiol-17β. *Aquaculture* **53**: 295-302

Jankowska DA, Faulwasser K, Trautwein-Schult A, Cordes A, Hoferichter P, Klein C, Bode R, Baronian K, Kunze G. 2013a. *Arxula adeninivorans* recombinant adenine deaminase and its application in the production of food with low purine content. *J Mol Microbiol Biotechnol* **115**: 1134-1146.

Jankowska DA, Trautwein-Schult A, Cordes A, Hoferichter P, Klein C, Bode R, Baronian K, Kunze G. 2013b. *Arxula adeninivorans* xanthine oxidoreductase and its application in the production of food with low purine content. *J Mol Microbiol Biotechnol* **115**: 796—807.

Kaiser C, Uhlig S, Gerlach T, Körner M, Simon K, Kunath K, Florschütz K, Baronian K, Kunze G. 2010. Evaluation and validation of a novel *Arxula adeninivorans* estrogen screen (nAES) assay and its application in analysis of wastewater, seawater, brackish water and urine. *Sci Total Environ* **408**: 6017–6026.

Kaur P, Lingner A, Singh B, Böer E, Polajeva J, Steinborn G, Bode R, Gellissen G, Satyanarayana T, Kunze G. 2007. *APHO1* from the yeast *Arxula adeninivorans* encodes an acid phosphatase of broad substrate specificity. *Antonie van Leeuwenhoek* **91**: 45-55.

Kumari A, Baronian K, Kunze G, Gupta R. 2015. Extracellular expression of YlLip11 with a native signal peptide from *Yarrowia lipolytica* MSR80 in three different yeast hosts. *Protein Expr Purif* **110**: 138–144.

Kunze G, Gaillardin C, Czernicka M, *et al*. 2014. The complete genome of *Blastobotrys (Arxula) adeninivorans* LS3 - a yeast of biotechnological interest. *Biotechnol Biofuels* **7**: 66.

Kunze G, Hähnel U. 2012. Production of butanol by fermentation in *Arxula* sp., Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK) Patent no. EP 2508597.

Kurtzman CP, Fell JW. 1998. The yeasts - a taxonomic study: Edition 4. Elsevier.

Kurtzman CP, Robnett CJ. 2007. Multigene phylogenetic analysis of the *Trichomonascus, Wickerhamiella* and *Zygoascus* yeast clades, and the proposal of *Sugiyamaella gen.nov*. and 14 new species combinations. *FEMS Yeast Res* **7**: 141-151.

Masaki K, Kamini NR, Ikeda H, Iefuji H. 2005. Cutinase-like enzyme from the yeast *Cryptococcus* sp. strain S-2 hydrolyzes polylactic acid and other biodegradable plastics. *Appl Environ Microbiol* **71**: 7548–7550.

Matsushika A, Watanabe S, Kodaki T, Makino K, Sawayama S. 2008. Bioethanol production from xylose by recombinant *Saccharomyces cerevisiae* expressing xylose reductase, $NADP^+$-dependent xylitol dehydrogenase, and xylulokinase. *J Ferment Bioeng* **105**: 296-299.

Medoff G, Painter A, Kobayashi GS. 1987. Mycelial - to yeast-phase transitions of the dimorphic fungi *Blastomyces dermatitidis* and *Paracoccidioides brasiliensis*. *J Bacteriol* **169**: 4055-4060.

Middelhoven WJ, Hoogkamer-Te Niet MV, Kreger-Van Rij NJW. 1984. *Trichosporon adeninovorans sp. nov.*, a yeast species utilizing adenine, xanthine, uric acid, putrescine and primary n-alkylamines as the sole source of carbon, nitrogen and energy. *Antonie van Leeuwenhoek* **50**: 369–378.

Middelhoven WJ, de Jong IM, de Winter M. 1991. *Arxula adeninivorans*, a yeast assimilating many nitrogenous and aromatic compounds. *Antonie van Leeuwenhoek* **59**: 129–137.

Minocha N, Kaur P, Satyanarayana T, Kunze G. 2007. Acid phosphatase production by recombinant *Arxula adeninivorans*. *Appl Microbiol Biotechnol* **76**: 387–393.

Olstorpe M, Pickova J, Kiessling A, Passoth V. 2014. Strain- and temperature-dependent changes of fatty acid composition in *Wickerhamomyces anomalus* and *Blastobotrys adeninivorans. Biotechnol Appl Biochem* **61**: 45-50.

Orr HA, Otto SP. 1994. Does diploidy increase the rate of adaptation? *Genetics* **136**: 1475-1480.

Pham Thi MH, Giersberg M, Uhlig S, Hanke G, Simon K, Kunath K, Baronian K, Kunze G. 2012. EstraMonitor – A monitor for amperometric detection of estrogenic activity with *Arxula adeninivorans* yeast cells as the biocomponent. *Sens Actuators B* **161**: 137–145.

Pham Thi MH, Kunath K, Gehrmann L, Giersberg M, Tuerk J, Uhlig S, Hanke G, Simon K, Baronian K, Kunze G. 2013. Application of modified *Arxula adeninivorans* yeast cells in an online biosensor for the detection of estrogenic compounds in wastewater samples. *Sens Actuators B* **185**: 628–637.

Pham Thi MH, Giersberg M, Gehrmann L, Hettwer K, Tuerk J, Uhlig S, Hanke G, Weisswange P, Simon K, Baronian K, Kunze G. 2015. The determination of pharmaceuticals in wastewater using a recombinant *Arxula adeninivorans* whole cell biosensor. *Sens Actuators B* **211**: 439–448.

Pham Thi MH, Chamas A, Nieter A, Giersberg M, Rutten T, Gehrmann L, Hettwer K, Tuerk J, Uhlig S, Simon K, Baronian K, Kunze G. 2016. Determination of glucocorticoids using photometric (A-YGS) and spectrofluorometric (A-YGFS) bioassays based on modified *Arxula adeninivorans* cells: Applications in environmental analysis. *Sens Actuators B* **223**: 540–549.

Pich U, Kunze G. 1992. Genome organisation of mitochondrial DNA from the non-saccharomycete yeast *Arxula adeninivorans* LS3. *Curr Genet* **22**: 505-506.

Rauter M, Schwarz M, Becker K, Baronian K, Bode R, Kunze G, Vorbrodt HM. 2013. Synthesis of benzyl β-D-galactopyranoside by transgalactosylation using a β-galactosidase produced by the over expression of the *Kluyveromyces lactis LAC4* gene in *Arxula adeninivorans*. *J Mol Catalysis B: Enzymatic* **97**: 319–327.

Rauter M, Kasprzak J, Becker K, Baronian K, Bode R, Kunze G, Vorbrodt HM. 2914a. *ADH* from *Rhodococcus ruber* expressed in *Arxula adeninivorans* for the synthesis of 1-(S)-phenylethanol. *J Mol Catalysis B: Enzymatic* **104**: 8–16.

Rauter M, Kasprzak J, Denter S, Becker K, Baronian K, Bode R, Kunze G, Vorbrodt HM. 2014b. Reusability of ADH and GDH producing *Arxula adeninivorans* cells and cell extract for the production of 1-(S)-phenylethanol. *J Mol Catalysis B: Enzymatic* **108**: 72–76.

Rauter M, Prokoph A, Kasprzak J, Becker K, Baronian K, Bode R, Kunze G, Vorbrodt HM. 2015. Coexpression of *Lactobacillus brevis ADH* with *GDH* or *G6PDH* in *Arxula adeninivorans* for the synthesis of 1-(R)-phenylethanol. *Appl Microbiol Biotechnol* **99**: 4723-4733.

Rösel H, Kunze G. 1995. Cloning and characterisation of a *TEF* gene for elongation factor1a from the yeast *Arxula adeninivorans*. *Curr Genet* **28**: 360-366.

Rösel H, Kunze G. 1996. Identification of a group-I intron within the 25S rDNA from the yeast *Arxula adeninivorans. Yeast* **12**: 1201-1208.

Rösel H, Kunze G. 1998. Integrative transformation of the dimorphic yeast *Arxula adeninivorans* LS3 based on hygromycin B resistane. *Curr Genet* **33:** 157-163.

Ruiz-Herrera J, Sentandreu R. 2002. Different effectors of dimorphism in *Yarrowia lipolytica*. *Arch Microbiol* **178**: 477–483.

Samsonova I, Kunze G, Bode R, Böttcher F. 1996. A set of genetic markers for the chromosomes of the imperfect yeast *Arxula adeninivorans. Yeast* **12**: 1209-1217.

Sietmann R, Uebe R, Böer E, Bode R, Kunze G, Schauer F. 2010. Novel metabolic routes during the oxidation of hydroxylated aromatic acids by the yeast *Arxula adeninivorans*. *J Appl Microbiol.* **108**: 789-99

Smyth GK. 2005. Limma: linear models for microarry data. In: Gentleman R, Carey V, Dudoit SI, Huber W, editors. Bioinformatics and computational biology solutions using R and Bioconductor. New York: Springer; p. 397-420.

Smyth GK. 2004. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* **3**: 3.

Steinborn G, Gellissen G, Kunze G. 2007a. A novel vector element providing multicopy vector integration in *Arxula adeninivorans*. *FEMS Yeast Res* **7**: 1197–1205.

Steinborn G, Wartmann T, Gellissen G, Kunze G. 2007b. Construction of an *Arxula adeninivorans* host–vector system based on *trp1* complementation. *J Biotechnol* **127**: 392–401.

Swoboda RK, Bertram G, Delbrück S, Ernst JF, Gow NA, Gooday GW, Brown AJ. 1994. Fluctuations in glycolytic mRNA levels during morphogenesis in *Candida albicans* reflect underlying changes in growth and are not a response to cellular dimorphism. *Mol Microbiol* **13**: 663-672.

Suzuki K, Noguchi MT, Shinozaki Y, Koitabashi M, Sameshima-Yamashita Y, Yoshida S, Fujii T, Kitamoto HK. 2014. Purification, characterization, and cloning of the gene for a biodegradable plastic degrading enzyme from *Paraphoma*-related fungal strain B47-9. *Appl Microbiol Biotechnol* **98**: 4457–4465.

Tag K, Lehmann M, Chan C, Renneberg R, Riedel K, Kunze G. 1998. *Arxula adeninivorans* LS3 as suitable biosensor for measurements of biodegradable substances in salt water. *J Chem Technol Biotechnol* **73**: 385-388.

Terentiev Y, Pico AH, Böer E, Wartmann T, Klabunde J, Breuer U, Babel W, Suckow M, Gellissen G, Kunze G. 2004a. A wide range integrative yeast expression vector system based on *Arxula adeninivorans*-derived elements. *J Ind Microbiol Biotechnol* **31**: 223–228.

Trautwein-Schult A, Jankowska D. A, Cordes A, Hoferichter P, Klein C, Matros A, Mock HP, Baronian K, Bode R, Kunze G. 2013. *Arxula adeninivorans* recombinant urate oxidase and its application in the production of food with low uric acid content. *J Mol Microbiol Biotechnol* **23**: 418–430.

Trautwein-Schult A, Jankowska D.A, Cordes A, Hoferichter P, Klein C, Matros A, Mock HP, Baronian K, Bode R, Kunze G. 2014. *Arxula adeninivorans* recombinant guanine deaminase and its application in the production of food with low purine content. *J Mol Microbiol Biotechnol* **24**: 67–81.

Van der Walt JP, Smith MT, Yamada Y. 1990. *Arxula* gen. nov. (*Candidaceae*), a new anamorphic, arthroconidial yeast genus. *Antonie Van Leeuwenhoek* **57**: 59–61.

Vijayan V, Giersberg M, Chamas A, Mehrotra M, Chelikani V, Kunze G, Baronian K. 2015. Use of recombinant oestrogen binding protein for the electrochemical detection of oestrogen. *Biosens Bioelectron* **66**: 379–384.

Wartmann T, Krüger A, Adler K, Duc B, Kunze I, Kunze K. 1995. Temperature-dependent dimorphism of the yeast *Arxula adeninivorans* LS3. *Antonie van Leeuwenhoek* **68**: 215–223.

Wartmann T, Rösel H, Kunze I, Bode R, Kunze G. 1998. *AILV1* gene from the yeast *Arxula adeninivorans* LS3 – a new selective transformation marker. *Yeast* **14**: 1017-1025.

Wartmann T, Erdman J, Kunze I, Kunze K. 2000. Morphology - related effects on gene expression and protein accumulation of the yeast *Arxula adeninivorans* LS3. *Arch Microbiol* **173**: 253-261.

Wartman T, Stephan U, Bube I, Böer E, Melzer M, Manteuffel R, Stoltenburg R, Guengerich L, Gellissen G, Kunze G. 2002. Post-translational modifications of the *AFET3* gene

product – a component of the iron transport system in budding cells and mycelia of the yeast *Arxula adeninivorans*. *Yeast* **19**: 849-862.

Wartmann T, Bellebna C, Böer E, Bartelsen O, Gellissen G, Kunze G. 2003a. The constitutive *AHSB4* promoter - a novel component of the *Arxula adeninivorans*-based expression platform. *Appl Microbiol Biotechnol* **62**: 528-535.

Wartmann T, Stoltenburg R, Böer E, Sieber H, Bartelsen O, Gellissen G, Kunze G. 2003b. The *ALEU2* gene—a new component for an *Arxula adeninivorans*-based expression platform. *FEMS Yeast Res* **3**: 223–232.

Winkelhausen E, Kuzmanova S. 1998. Microbial conversion of **D**-xylose to xylitol *J Ferment Bioeng* **86**: 1-14.

Yang X, Wartmann T, Stoltenburg R, Kunze G. 2000. Halotolerance of the yeast *Arxula adeninivorans* LS3. *Antonie van Leeuwenhoek* **77**: 303-311.

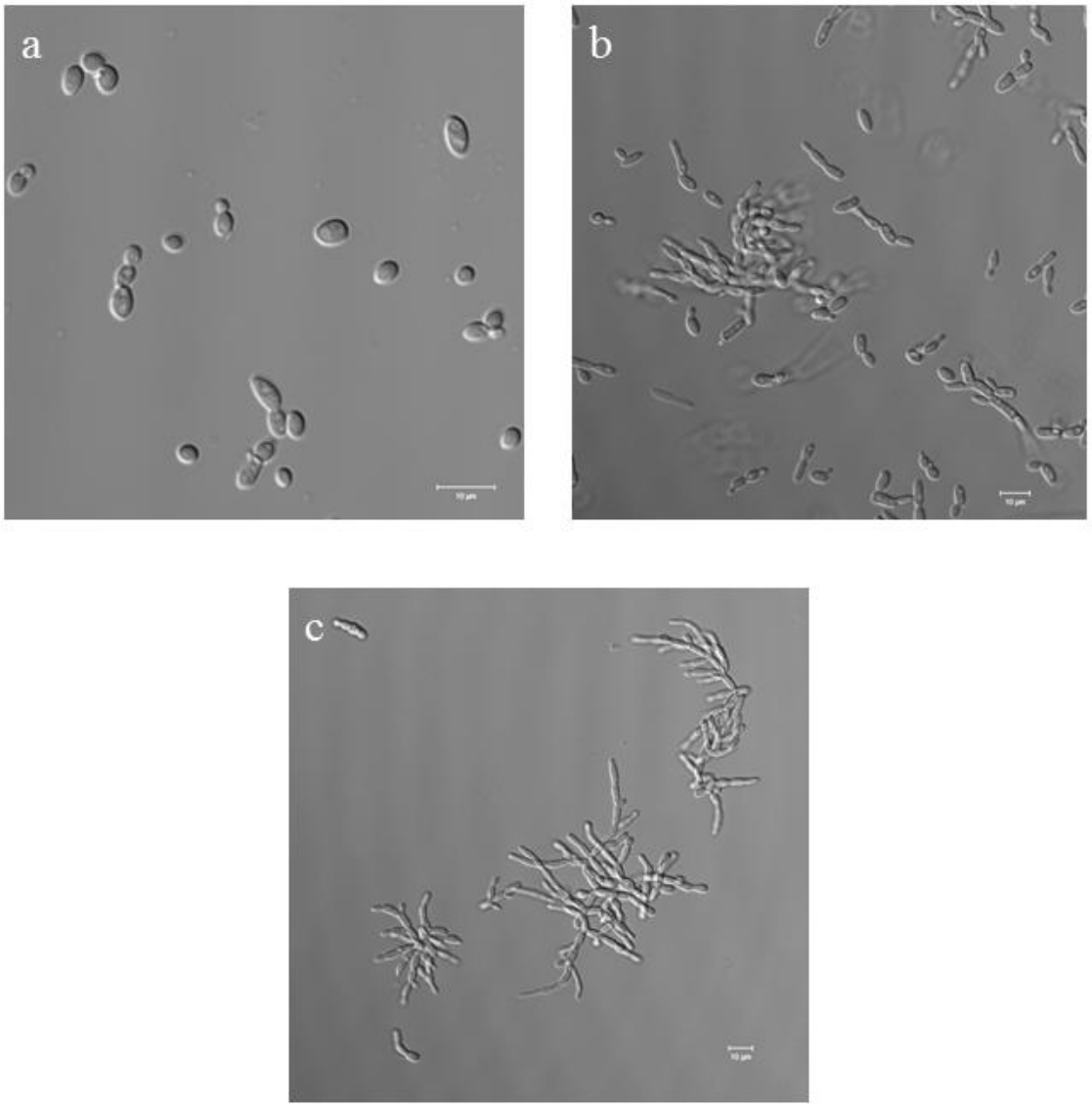

Fig. 1. Temperature dependent dimorphism of *A. adeninivorans* LS3.

Cell morphology of *A. adeninivorans* LS3: budding cells (a), pseudomycelia (b) and mycelia (c). The yeast cells were cultured in YEPD medium for 18 h at 30 °C, 42 °C and 45 °C respectively. Bright field images were obtained using CLSM (Zeiss LSM 780, with laser 633 nm).

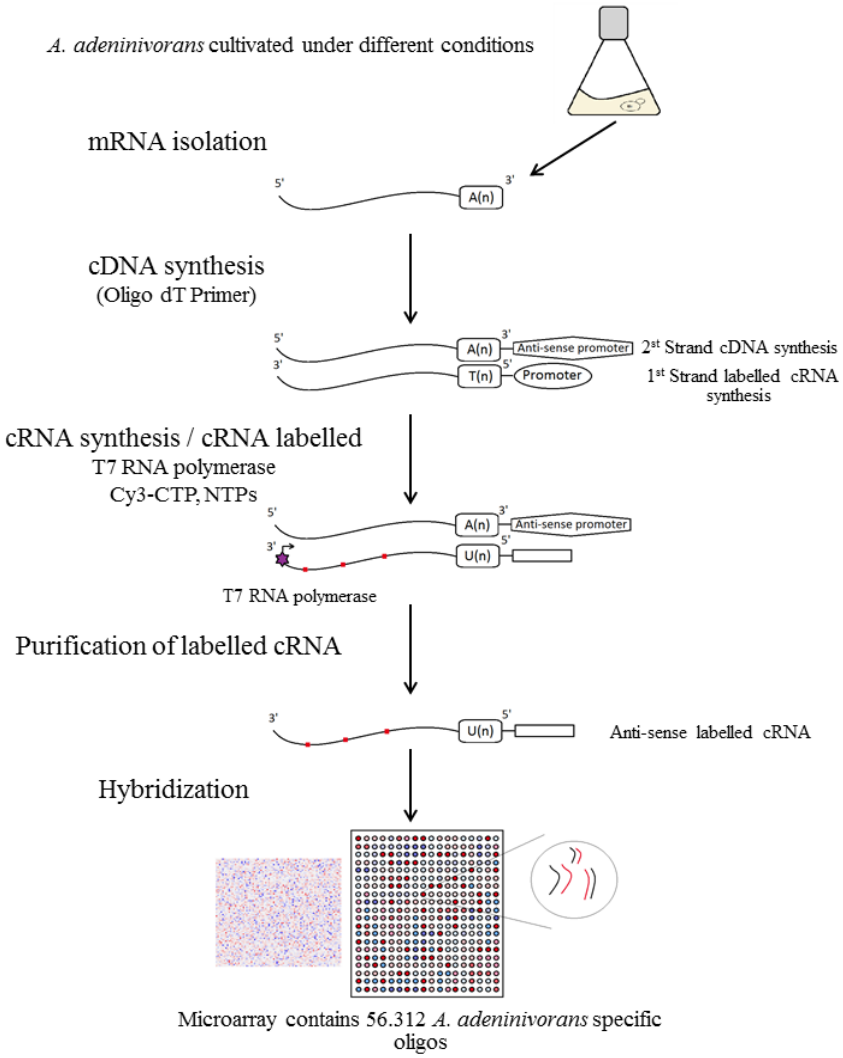

Fig. 2. Microarray design and hybridization for gene expression analyses in *A. adeninivorans LS3*.

Based on 6025 annotated chromosomal sequences and 36 putative mitochondrial genes, oligos were designed using Agilent Technologies eArray software (https://earray.chem.agilent.com; design number 035454). Depending on the length of the genes up to 10 60-mers per gene were created resulting in a total of 56312 *A. adeninivorans* specific oligos. The microarray was produced by Agilent Technologies in 8x60k format.

A. adeninivorans LS3 cells cultured under different conditions were harvested and total RNA was isolated. Probe labeling and microarray hybridization (duplicates) were executed according to the manufacturer's instructions (Agilent Technologies "One-Color Microarray-Based Gene Expression Analysis"; v6.5).

Analysis of microarray data was performed with the R package limma (Smyth, 2005). Raw expression values were background corrected using "normexp" and normalized between arrays using "quantile". Differentially expressed genes were detected by fitting a linear model to log2-transformed data by an empirical Bayes method (Smyth, 2004). The Bonferroni method was used to correct for multiple testing.

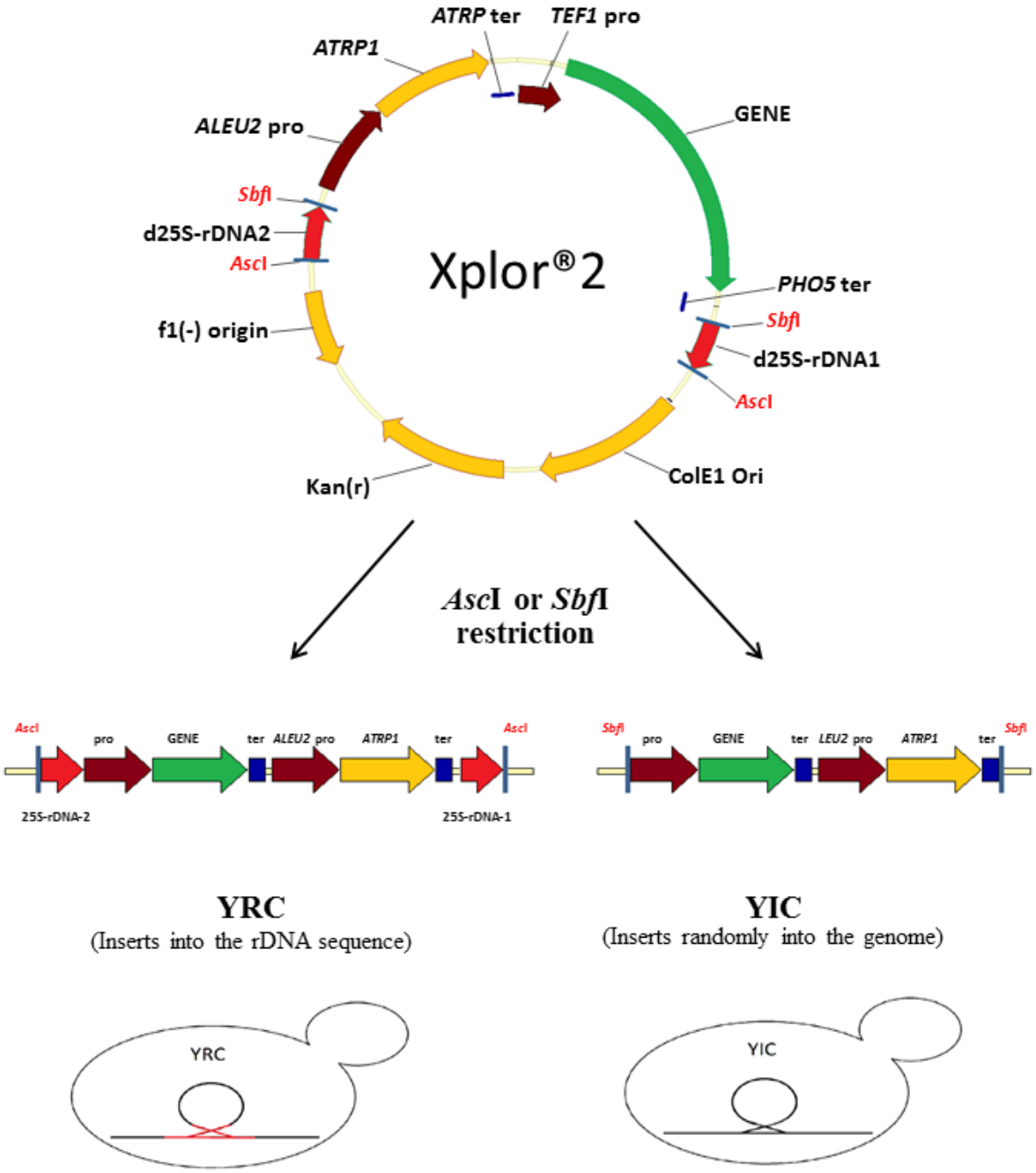

Fig. 3. Principle of the transformation procedure of *A. adeninivorans* based on the Xplor®2 transformation/expression platform.
The system is based on a bacterial vector backbone, with yeast selection markers and expression modules inserted between two 25S rDNA segments. After the construction of the plasmids in *E. coli*, all bacterial sequences are removed by *Asc*I and *Sbf*I restriction. The choice of the restriction enzyme determined whether the rDNA fragment flanks the expression cassette (*Asc*I) or not (*Sbf*I), so that the gene of interest can be integrated in the yeast genome via homologous recombination in the rDNA as Yeast rDNA Integrative Cassette (YRC) or via non-homologous recombination as Yeast Integrative Cassette (YIC).