3.3.3.1 Randomly Amplified Polymorphic DNA

The RAPD technique (Williams et al. 1990), is characterized by the fact it uses just one

primer, which has the special characteristics of being particularly short (approximately

ten nucleotides) and having an arbitrary sequence. The RAPDs-PCR reaction is also

characterized by the low hybridization temperature used (37°C). Thus, the pairings

between the oligonucleotide and the DNA are determined by the short and arbitrary

sequence this has, and favoured by the low temperature used, setting off the amplifi-

cation of diverse fragments of DNA distributed all the way along the genome. The

result is a pattern of amplified products of different molecular weight that can be char-

acteristic of the species or of the different strains or isolates within the same species

(Bruns et al. 1991; Paffetti et al. 1995).

The main advantage of the method is that one does not need previous informa-

tion about the sequence to design the primer. Moreover, the technique enables one

to analyse the variability along the whole genome, thus revealing more polymor-

phism than other techniques that analyse specific regions. However, owing to the low

hybridization temperature used (37°C) the amplification profiles obtained are unsta-

ble and difficult to reproduce and it is necessary to carry out several repetitions for

each sample, starting off with different DNA extractions. Only the bands present in

all the repetitions will be taken into account. This fact together with the need to

combine the amplification results with several oligonucleotides to obtain a good res-

olution power means that the technique is not apt for routine application at an

industrial level. Consequently, the technique has not been used much for the char-

acterization of strains.

The efficiency of the technique to differentiate at strain level has been demon-

strated by analysing reference strains belonging to different species (Quesada and

Cenis 1995; Baleiras Couto et al. 1996; Romano et al. 1996; Tornai-Lehoczki

and Dlauchy 2000; Pérez et al. 2001a; Cadez et al. 2002). Recent applications of the

technique to S. cerevisiae and other yeast species associated with different foods and

beverages are shown in Table 3.3.

3.3.3.2 PCR of Repetitive Regions of the Genome (Microsatellites and

Minisatellites)

There are repeated regions in the genome that represent potential targets for molec-

ular identification at strain level, as they show great variability. These areas are the

microsatellites and the minisatellites that constitute motifs of very varied length,

repeated in tandem abundantly and at random along the genome. The microsatel-

lites are usually less than 10 bp in length, while the minisatellites are between 10- and

100-bp long. The variability found in these regions can be shown by means of PCR

amplification using specific oligonucleotides, such as (GTG)5

, (GAG)5

, (GACA)4 or

M13. The ability of these oligonucleotides to reveal polymorphism among strains of

S. cerevisiae was demonstrated by Lieckfeldt et al. (1993) using hybridization tech-

niques. The same authors were the first to use these sequences as primers in a PCR

reaction, showing the usefulness of this technique for characterization at strain level.

The technique was used by other authors later for the study of reference strains

(Baleiras Couto et al. 1996; González Techera et al. 2001; Hennequin et al. 2001;

Pérez et al. 2001a, b; Marinangeli et al. 2004) and recent applications are shown in

Table 3.3. The amplified products obtained are approximately 700 and 3,500 bp in

size; therefore, they can be visualized in agarose gels. Visualization of the amplified

products obtained is usually carried out in acrylamide gels, although it can also be

done in automatic sequencers. This means that the technique is not very useful for

routine application, in spite of its high resolution and its high reproducibility. The

resolution power of this technique is comparable to d elements and restriction analy-

sis of mtDNA.

3.3.3.3 Amplification of d Sequences

d sequences are elements measuring 0.3 kb that flank the retrotransposons Ty1

(Cameron et al. 1979). Around 100 d copies are present in the yeast genome as part of

the retrotransposons Ty1 or as isolated elements. However, these d sequences are con-

centrated in genomic regions adjacent to the transfer RNA genes (Eigel and Feldmann

1982). The number and the localization of these elements demonstrate certain

intraspecific variability that Ness et al. (1993) took advantage of to develop specific

primers (d1 and d2

) that are useful to differentiate strains of S. cerevisiae. These authors

showed that the d elements are stable enough for this technique to be used as an iden-

tification method of S. cerevisiae strains at an industrial level, as demonstrated by

other authors later (Table 3.3). Some of these studies show the great variability this

technique reveals between isolates of the S. cerevisiae species compared with other

highly resolving techniques, such as restriction analysis of the mtDNA and elec-

trophoresis of chromosomes (Pramateftaki et al. 2000; Fernández-Espinar et al. 2001).

Recently, Legras and Karst (2003) optimized the technique by designing two new

primers (d12 and d21) that are located very near to d1 and d2

. The use of d12 and d21

or of d12 with d2 reveals greater polymorphism, which is reflected by the appearance

of a greater number of bands. Consequently, the new primers are able to differenti-

ate more strains: 53 commercial strains were differentiated unequivocally (Legras

and Karst 2003). Shuller et al. (2004) confirmed it later, showing that the combina-

tion of d2 and d12 identified twice as many strains as the set of primers designed by

Ness et al. (1993).

An important drawback of this technique is the influence that the concentration

of DNA can have on the profile obtained, as shown by Fernández-Espinar et al.

(2001) and commented on by Shuller et al. (2004). Although this problem is avoided

by standardizing the concentration of DNA, the comparison of results between lab-

oratories is complicated. Another problem of this technique is the appearance of

“ghost” bands due to the low annealing temperature (42°C) used during the ampli-

fication reaction. Recently, Ciani et al. (2004) used an annealing temperature of

55°C to characterize wine strains of S. cerevisiae. In this way, the amplification pro-

files obtained are much stabler, although fewer bands are obtained.

3.3.4 Amplified Fragment Length Polymorphism

Although this technique is fundamentally based on PCR amplification, we will con-

sider it in a different section, owing to its complex methodology, which implies the

use of other methodologies, as we will see later.

Amplified fragment length polymorphism (AFLP) is a technique that involves

the restriction of genomic DNA followed by the binding of adapters to the frag-

ments obtained and their selective amplification by PCR. The adapter sequence and

the restriction sites are used as the primers’ target for PCR amplification. The frag-

ments are separated in DNA sequencing gels and visualized by auto-X-ray or in

automatic sequencing (Vos et al. 1995). Figure 3.10 outlines this technique.

As in the case of RAPDs, previous information about the sequence is not needed

to design the primer, it is easily reproduced and it offers a great deal of information.

AFLP is a useful technique to discriminate between yeasts at strain level, as shown

by de Barros Lopes et al. (1999); however, it has the drawback of being a very labo-

rious technique, since it requires automatic sequencers, which are very sophisticated

for use in industry, and also the data are difficult to interpret. Although the tech-

nique has been very widely used to study bacteria, plants and animals, in the case of

yeasts, there are few works in this respect (de Barros Lopes et al. 1999; Azumi and

Goto-Yamamoto 2001; Boekhout et al. 2001; Theelen et al. 2001; Borst et al. 2003;

Dassanayake and Samaranayake 2003; Trilles et al. 2003).