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Biotechnology Advances 24 (2006) 180–196
BIOTECHNOLOGY ADVANCES
www.elsevier.com/locate/biotechadv

Research review paper

Understanding *Candida rugosa* lipases: An overview

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Accepted 26 September 2005
Available online 8 November 2005

Abstract

Candida rugosa lipase (CRL) is one of the enzymes most frequently used in biotransformations. However, there are some irreproducibility problems inherent to this biocatalyst, attributed either to differences in lipase loading and isoenzymatic profile or to other medium-engineering effects (temperature, a_w, choice of solvent, etc.). In addition, some other properties (influence of substrate and reaction conditions on the lid movement, differences in the glycosylation degree, post-translational modifications) should not be ruled out. In the present paper the recent developments published in the CRL field are overviewed, focusing on: (a) comparison of structural and biochemical data among isoenzymes (Lip1–Lip5), and their influence in the biocatalytical performance; (b) developments in fermentation technology to achieve new crude *C. rugosa* lipases; (c) biocatalytical reactivity of each isoenzyme, and methods for characterising them in crude CRL; (d) state-of-the-art of new applications performed with recombinant CRLs, both in CRL-*second generation* (wild-type recombinant enzymes), as well as in CRL-*third generation*, (mutants of the wt-CRL).

Keywords: *Candida rugosa* lipases; Isoenzymes; Fermentation conditions; Recombinant *C. rugosa* lipases; Biocatalytical characterisation; Biocatalytical applications

Contents

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0734-9750/$ - see front matter
doi:10.1016/j.biotechadv.2005.09.003

1. Background. Historical "evolution" of *Candida rugosa* lipases

The potential applications of lipases in biotransformations–and in particular of *C. rugosa* lipases–are nowadays well established and fully documented with a plethora of examples, both in hydrolytical (aqueous media) or synthetical (organic media) approaches (Schmid and Verger, 1998; Bornscheuer and Kazlauskas, 1999; Drauz and Waldmann, 2002; Faber, 2004, and references therein). Furthermore, the rapid developments of molecular biology techniques, as well as the availability of more reliable high-throughput-screening methods, have enhanced the utility that enzymes offer for organic synthesis. Currently, biocatalysts of *second generation* (and even of third, fourth...) are being produced by adapting a wild-type enzyme to a desired application (for complete recent reviews in cloning and HTS fields see: Reetz, 2001; Goddard and Reymond, 2004; Otten and Quax, 2005; Schmidt and Bornscheuer, 2005, and references therein).

Within the hydrolase-based biocatalysis, lipases from *Candida rugosa* were firstly described as early as in the sixties, by isolating the yeast from natural soils due to its powerful lipase production capacity (Yamada and Machida, 1962; Yamada et al., 1963; Tomizuka et al., 1966). Later on, two isoenzymes–initially called LipA and LipB–were identified (Tomizuka et al., 1966; Shaw et al., 1989; Veeraragavan and Gibbs, 1989; Brahimi-Horn et al., 1990; Wu et al., 1990), purified (Rúa et al., 1993; Rúa and Ballesteros; López et al., 2000; Pernas et al., 2000; Xin et al., 2002), and genetically characterised (Longhi et al., 1992; Lotti et al., 1993). Nowadays it is well established that at least seven genes are involved in the *C. rugosa* lipase-producing machinery, being five of them (Lip1–Lip5) fully biochemically characterised (Lotti et al., 1994a,b; Brocca et al., 1995; Lotti and Alberghina, 1996). Nowadays the LipA/LipB nomenclature has been practically abandoned, and a new one based on numbers is often used: Lip1, Lip2, Lip3, up to Lip7.

Actually, all the commercial CRL samples are composed by a mixture of several isoenzymes in different proportions, and thus Lip1, Lip2 and Lip3 have been found in such commercial crude powders in variable proportions (Rúa et al., 1993; Linko and Wu, 1996; Diczfalusy et al., 1997; Ferrer et al., 2001; Pernas et al., 2001; López et al., 2004). The samples are stabilised with lactose, which plays an important role as water reservoir (Sánchez-Montero et al., 1991). Since modifications of the fermentation conditions lead to different lipase amounts and (iso)enzymatic profiles (Dalmau et al., 2000; Ferrer et al., 2001; Domínguez de María, 2002; Domínguez de María et al., 2005a, and references therein), it appears obvious why that isoenzymatic profile has been traditionally pointed out as one of the irreproducibility causes (among others) when working with crude *C. rugosa* lipases in slightly hydrated organic media (Kazlauskas et al., 1991; Chang et al., 1994; Lundell et al., 1998; Domínguez de Maria and Sinisterra, 1999; Domínguez de María et al., 2002, accepted for publication; Akoh et al., 2004; Alcántara et al., 2004; López et al., 2004). This is confirmed by the fact that each isoenzyme shows different biocatalytical properties (Rúa et al., 1993; Linko and Wu, 1996; Hernáiz et al., 1995; 1997; Moreno et al., 1997; Plou et al., 1997; Domínguez de María et al., 2002, accepted for publication; Alcántara et al., 2004; López et al., 2004). One could consider that a practical solution for the CRL system would be the cloning and external-hosting expression of the recombinant pure isolipases, hence only one active enzyme would be present. Unfortunately, *C. rugosa* yeast utilises a non-universal genetic code, in which codon CUG–normally read as leucine in the universal code–is expressed as serine (Kawaguchi et al., 1989). This drawback has led to many problems when attempts to obtain recombinant CRL enzymes were conducted. The bottleneck was recently circumvent, either by modifying the lipase genes by site-directed mutagenesis (Lip1, Lip2 and Lip4; Tang et al., 2000, 2001, 2003a,b; Lee et al., 2002; Chang et al., 2005) or by direct complete synthesis of the Lip1 gene (Mileto et al., 1998; Brocca et al., 1998, 1999). Moreover, the production of several recombinant CRL mutants has been reported (Brocca et al., 2000, 2003; Manetti et al., 2000; Tang et al., 2001; Schmitt et al., 2002; Secundo et al., 2004), and therefore new CRL of *second generation* (pure recombinant isoenzymes) and even of *third generation* (mutants produced from those recombinant isoenzymes) are also available nowadays (Schmitt et al., 2002; Vorlová et al., 2002; Secundo et al., 2004). Furthermore, a new application of the *C. rugosa* whole cells