CURRENT STATE OF THE STUDY OF MICROBIAL LIPASES

K. Davranov and V. B. Khalameizer

This review gives a comparative analysis of information accumulated over the past 15 years on the isolation, purification, properties, and use of lipases of microbial origin.

The choice of lipases (E.C. 3.1.1.3 triacylglycerol acylhydrolases) as objects of study is due to the following considerations. In the first place, they are of interest from the point of view of general enzymology — as enzymes acting at an interphase surface. In the second place, interest in them is due to numerous practical problems and, above all, the employment of lipases as special "tools" for studying the composition of membranes and their use for concrete biotechnological processes connected with the treatment of lipid-containing raw material [1].

The natural substrates for lipases are triacylglycerols; however, other insoluble or partially soluble esters of glycerol and some esters of other alcohols are also capable of being hydrolyzed by this class of enzymes. Soluble esters are hydrolyzed by lipases extremely slowly since they do not accumulate at a phase separation surface [2].

Lipolytic enzymes are widely distributed in Nature. Sources of lipases are animal and vegetable tissues and microorganisms. The last-mentioned group of producing agents is attracting the intense attention of scientists through their advantages over other sources [3].

The synthesis of many hydrolases, including lipases, by the producing microorganisms can be regulated and directed by the choice of appropriate conditions of cultivation and, in particular, the composition of the nutrient medium. Moreover, many microbial enzymes are formed in response to the action of an inductor added to the nutrient medium, the activity of the induced enzyme rising many times during the growth of the microorganism in response to the addition of a specific substrate, while in a medium without the appropriate inductor the enzyme is formed in minimal amounts [4, 5].

Microorganisms possess the capacity for synthesizing extracellular enzymes the activity of which many times exceeds the level of activity of intracellular enzymes; i.e., a capacity for performing "supersynthesis." All this determines in full measure the promising nature of the microbiological synthesis of lipases.

The progress achieved in the study of microbial lipases has made possible the solution of a whole series of practical problems [6, 7] dictated by the tendencies in the development of the oils and fats industry and the necessity of obtaining products with predetermined properties. A no less important role in the stimulation of investigations of microbial lipases has been played by the demands of the medical and pharmaceutical industry and of public health — indeed, the etiology, prophylaxis, and treatment of many diseases are connected with the functioning of lipolytic enzymes [8]. The possible spheres of employment of these enzymes expanded after it became known that, in addition to their hydrolytic capacity, lipases possess the capacity for catalyzing such reactions as the synthesis of glycerides, the acylation and alkylation of lipids, esterification and transesterification reactions, ammonolysis, oximolysis, thioacyl and thioalkyl exchange, etc. [9, 10]. Many of these reactions are possible only in nonaqueous media, where there are changes in the characteristics of the enzymes — specificity and optimum conditions of functioning, catalytic parameters, and demands on the chemical structure of the substrate [11-14].

Results obtained in the last 10-15 years show that microbial lipases are acquiring deserved employment in fine organic synthesis, petrochemistry, the manufacture of pharmaceuticals, and the production of basically new types of surface-active agents [15-17].

Fig. 1. Change in the level of lipase activity of the fungus *Mucor miehei* during its cultivation on the addition of various components to the medium. 1) Minimal medium with glucose (basal level); 2) medium with the addition of cottonseed oil (1st threshold level); 3) medium with the addition of cottonseed oil and β-mercaptoethanol (2nd threshold level); 4) accumulation of biomass.

In addition to what has been said, the advances achieved in the development of effective methods for stabilizing and immobilizing microbial lipases have made realistic their use for the solution of concrete applied problems [17-19].

**CLASSIFICATION AND SPECIFICITY OF LIPASES**

According to the international nomenclature, lipases (E.C. 3.1.1.3 – triacylglycerol acylhydrolases) are hydrolases cleaving the ester bonds in a triglyceride molecule. In the classification of lipases the starting point is the fact that their substrates are triglycerides. On this basis, all lipases can be divided into three groups, depending on the positions and structures of the fatty acids (FAs) in the triglyceride molecule: 1) nonspecific lipases liberating any FAs from any position of a triglyceride (for example, lipases from *Candida rugosa* and *Oospora lactis*); 2) 1,3-specific lipases liberating FAs from position I or III of a triglyceride (lipases from *Rhizopus microsporus*, *Mucor miehei*, etc.); and 3) lipases liberating only a particular type of FA from any position of a triglyceride (for example, a lipase from *Geotrichum candidum* preferentially hydrolyzes cis-Δ9 unsaturated fatty acids). According to this classification, some lipases prove to be simultaneously in two groups (for example, the first and third) [20]. In order to resolve this contradiction, Jensen [21] proposed an improved classification of lipolytic enzymes on the basis of their specificity. He distinguished: 1) substrate specificity (specificity to mono-, di-, or triglycerides (MGs, DGs, or TGs); 2) positional specificity (specificity for a definite position of a triglyceride); 3) fatty-acid specificity (for a definite type of fatty acid); 4) stereospecificity; and 5) a combination of 1) and 4). This classification, based on the specificity of lipases, is the most popular, the most frequently used, and most convenient; however, it does not take into account the fact that the substrates of lipases are not only glycerides but also other esters.

Some authors propose to classify lipases according to their specificity with respect to the chemical structure and positions of the alcohol moiety and the fatty acid residue of esters [22]. Thus, it is possible to distinguish lipases specific for an alcohol residue with respect to: 1) chain length; 2) structure (i.e., primary or secondary alcohol or polyl); 3) the position of the ester bond if the alcohol is a polyl; 4) branchings (if an aromatic substituent is present); and 5) stereochemistry (i.e., sn-1 or sn-3 position of a glycerol residue). And lipases specific for a particular carboxylic residue, according to: 1) chain length; 2) unsaturation (configuration of the double bonds); 3) branching (closeness to the carboxy group and nature of the branched groups); and 4) stereochemistry (i.e. S- or R-isomers).

*Sic*. Ref. [21] does not refer to Jensen, who appears as the author of [22]. Other, similar, probable discrepancies in the numbering of the references have been detected and there are probably undetected ones as well — Translator.