METHOD FOR DEVELOPMENT OF STRAIN-SPECIFIC PCR PRIMERS BASED ON AFLP

Zhechko Dimitrov, Irina Gotova
LB-Bulgaricum Plc, R&D, 12-A Malashevska Str., Sofia 1202, Bulgaria
e-mail: zhechko.dimitrov@lbbulgaricum.bg

Key words: strain-specific primers, AFLP, L. bulgaricus

Introduction. One *L. bulgaricus* strain was selected with anti-inflammatory properties towards intestinal epithelium cells. The strain demonstrated satisfactory capability to survive in gastro-intestinal tract. Because of the probiotic properties are strain-specific, the use of reliable and discriminative molecular methods for characterization is very important.
The objective of this work is to present a method for development of strain-specific DNA markers, as well as AFLP genotyping for *L. bulgaricus* strains with satisfactory discriminative power and reproducibility.

Methods. AFLP genotyping was based on digestion of DNA with enzymes *Xho I* and *Taq I*, specially designed adapters, preselective and selective PCR primers (1, 2). The first stage of the process of strain-specific marker development was preparative AFLP for the *probiotic strain L. bulgaricus* with initial DNA quantity of 1 µg. A set of selective AFLP processes was performed using different combinations of selective nucleotides. The received AFLP fragments were used for preparation of AFLP library by the help of cloning kit (Promega) (3). The produced DNA arrays from AFLP fragments were hybridized with hydrolyzed DNA mix from 70 different *L. bulgaricus* strains. The combined DNAs were hydrolyzed by the same endonucleases used in AFLP – *Xho I* and *Taq I*. The received hydrolyzed DNA fragments were labeled with Cy5 dye (Amersham) as it was explained by the producer. The AFLP fragments which corresponded to those wells where the fluorescence signal was zero were selected for confirmation of their strain-specificity by hybridization on the individual DNA spots from many different *L. bulgaricus* strains. The strain-specificity of the selected AFLP fragments was confirmed by hybridization towards DNAs from large group of L. bulgaricus strains. After sequencing of the non hybridizing fragments, PCR primers with strain selectivity were developed.

Results. AFLP technique demonstrated high discriminative potential for typing of *L. bulgaricus* strains, as shown on Figure 1. AFLP derived ampliphicants were used successfully as a source of strain-specific hybridization probes towards one probiotic *L. bulgaricus* strain, and subsequently, based on their specific sequences - for design of strain-specific PCR primers. The specificity was confirmed among 70 *L. bulgaricus* strains. In order to study the strain-specificity of certain AFLP fragment it is necessary to prove the uniqueness of the fragment towards DNA from many strains of the same bacterial species. Searching such fragment we used DNAs from 70 different *L. bulgaricus* strains which were hydrolyzed with the same enzymes – *Taq I* и *Xho I* because other enzymes could break strain-specific sequences. If certain AFLP derived fragment from the targeted strain contains strain-specific sequence it will not hybridize with any DNA fragment derived from the other strains. Thus, in the corresponding well in the DNA arrays, containing AFLP fragments ,the fluorescence signal will be zero. The fragments demonstrating a lack of any hybridization signal were selected for further evaluation of their specificity. Following the upper procedure, five strain-specific fragments for the selected probiotic *L. bulgaricus* strain were found and the strain-specificity was confirmed by dot-blot hybridization. The lack of any hybridization with dot-blotted DNAs from other *L. bulgaricus* strains was clearly demonstrated. Simultaneously, the selected strain-specific fragment proved strong hybridization signal with DNA from *the* probiotic *L. bulgaricus* strain.

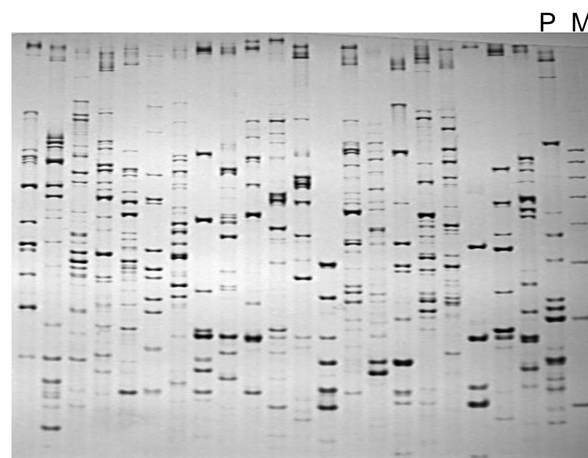

Fig.1 AFLP of different *L. bulgaricus* strains, silver staining. Line P – probiotic *L. bulgaricus* strain; line M – DNA marker 100 bp ladder.

Conclusions. The developed method for strain-specific DNA markers and PCR primers could be applied to every strain with beneficial properties. The developed specific DNA probes and PCR primers could be applied to evaluate the presence of certain strain with probiotic properties in complex microbial matrixes.

Acknowledgements. This work is supported by Bulgarian National Innovation Fund, project 6ИФ-02-33.

References.

1. Janssen P, Coopman R, Huys G, Swings J, Bleeker M, de Vos P, Zabeau M and Kersters K. (1996). *Microbiology* 142: 1881-1893.
2. Dimitrov Z., Minkova S., Michaylova M. (2008). *World J of Microbiology and Biotechnology*. 24:1305-1312
3. Dimitrov Z. (2012). *Biotechnol & Biotechnol eq.* 26:35-39