Editorial

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Deciphering Effective Application of Molecular Techniques

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Editorial

Advances in the field of molecular biology have allowed the development of a wide variety of approaches and techniques that allow assessment of practically every aspect related to the physiology of prokaryotes and eukaryotes. In the field of Food Science, molecular biology techniques are employed in the majority of the studies to:

- Create genotypic profiles of a set of individuals in order to cluster or differentiate them. The most common application is the genotypic profiling of microorganisms so that information regarding their biodiversity is gained.
- Identify a set of samples at a defined taxonomic level (genus, species, and serotype)
- Assess microbial transcriptomic response to defined stimuli.

In all cases, a wealth of literature is currently available that adequately highlights the key points that should be carefully considered for effective application of each approach. In addition, review or guideline articles have been published for each case. Failure to adhere to such recommendations compromises the outcome of the analysis and concomitantly creates confusion and misunderstandings. Therefore it is always useful to remind these key points, in which misconducting is often witnessed.

Genotypic profiling has become routine analysis in most microbiology laboratories. Effective genotyping requires the selection of the appropriate approach, careful standardization of the technical part, and proper interpretation of the results. In general, the methodology of choice should match the purpose of the study. More accurately, if exchange of data is desired, i.e. in the case of studies in which a conclusion of epidemiological nature should be reached, then a method that does not rely on PCR should be employed; in most of the cases this approach is Pulsed-Field Gel Electrophoresis (PFGE). However, the clonality of the microbial species under surveillance greatly affects the outcome of the analysis; therefore a combination with another technique, such as the sequence-based Multilocus Sequence Typing (MLST), may be necessary. When the purpose of the study is to depict the microbial consortium driving a process, without need for data exchange, at least at a genotypic profile level, then the desired taxonomic level aimed to be reached will define the method of choice. When the samples are unknown, a combination of culture-dependent (such as Random Amplified Polymorphic DNA, Restriction Fragment Length Polymorphism, repetitive element palindromic-PCR) and -independent (such as Denaturing Gradient Gel Electrophoresis, Single Strand Conformation Polymorphism) would be ideal. However, in the case of Food Microbiology, the dominant microbiota is culturable and therefore the former approach would be enough. When application of the culture-independent approach is necessary, it should be kept in mind that DNA, in which the vast majority of the studies rely on, may remain long after death of a microorganism and in that case the history rather than the current micro ecosystem composition will be depicted. Thus, the use of propidium monoazide-based approach as a means of differentiating dead from living cells should be selected but exercised with caution since standardization of additional factors is required [1]. Currently, the ideal approach would include the use of RNA instead of the aforementioned. Genotypic techniques, such as RAPD and RFLP are very effective both in terms of cost and quality of the result, taking accurate standardization for granted. Especially regarding the latter, due to the use of restriction enzymes, the inclusion of reference strains in the analysis is often advised. rep-PCR employing (GTG)5 as a primer has proved the ability to differentiate at subspecies level, therefore it seems a decent choice when the fate of a strain within a microbial consortium is the purpose of the study. In any case, the guidelines offered by van Belkum et al. [2] should be followed. Attention should also be given to the interpretation of the results. Guidelines for the interpretation of PFGE-derived analyses have been published [3] and the basic principles may be extended as well to the use of other genotypic profile-based techniques. Very often, interpretation of the results is based on cut-off similarity values that may range from 75% to 95%. However, it should be kept in mind that such values depict genetic relatedness as assessed by the approach applied, thus, very often; clustering based on such assumptions is not accurate.

Taxonomic level-specific PCR is applied to assign a sample to a taxonomic affiliation. The purpose of the application may include micro ecosystem composition assessment or fraud

detection. In addition, serotyping of foodborne pathogens through specific PCR is gaining interest and many protocols have been developed so far. The most important feature that such a protocol should offer is the ability to differentiate between closely related species. This capacity should always be verified before the actual analysis take place, since adjustment of the PCR conditions, either in terms of reagent concentration or thermocycling, may be necessary to obtain specificity and reproducibility.

The number of gene expression studies has increased over the recent years and is expected to increase further. To address the need for reliable and reproducible analysis, Bustin et al. [4] published the MIQE guidelines offering guidance for the standardization of every factor that may compromise the results obtained, from sample preparation to data analysis. These guidelines are strongly recommended to follow for all the right reasons mentioned with the addition of one more. Apart from the technical reasons, the outcome of the analysis is also affected by the occurrence of processes such as feed - back or -forward loops, negative or positive autoregulatory interactions etc. that, in most of the cases, are yet to be discovered. Therefore, data presentation should enable meta-analysis that could concomitantly assist their detection and overall improve our understanding of the physiological organization and traits of prokaryotes and eukaryotes.

Conflict of Interest

None

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