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Review

Understanding molecular identification and polyphasic taxonomic approaches for genetic relatedness and phylogenetic relationships of microorganisms

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ABSTRACT

The major proportion of earth's biological diversity is inhabited by microorganisms and they play a useful role in diversified environments. However, taxonomy of microorganisms is progressing at a snail's pace, thus less than 1% of the microbial population has been identified so far. The major problem associated with this is due to a lack of uniform, reliable, advanced, and common to all practices for microbial identification and systematic studies. However, recent advances have developed many useful techniques taking into account the house-keeping genes as well as targeting other gene catalogues (*16S rRNA*, *rpoA*, *rpoB*, *gyrA*, *gyrB* etc. in case of bacteria and 26S, 28S, β-tubulin gene in case of fungi). Some uncultivable approaches using much advanced techniques like flow cytometry and gel based techniques have also been used to decipher microbial diversity. However, all these techniques have their corresponding pros and cons. In this regard, a polyphasic taxonomic approach is advantageous because it exploits simultaneously both conventional as well as molecular identification techniques. In this review, certain aspects of the merits and limitations of different methods for molecular identification and systematics of microorganisms have been discussed. The major advantages of the polyphasic approach have also been described taking into account certain groups of bacteria as case studies to arrive at a consensus approach to microbial identification.

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Abbreviations: VBNC, viable but nut culturable; MLST, multi-locus sequence typing; MALDI-TOF, matrix assisted laser desorption ionisation-time of flight; MS, mass spectrometry; SMM, shotgun mass mapping; PCR, polymerase chain reaction; ERIC, enterobacterial repetitive intergenic consensus sequences; REP, repetitive element palindromic sequences; bp, base pair(s); HS, heat shock; ATP, adenosine triphosphate; ST, sequence type; SIP, stable isotope probing; IPP, intact protein profiling; LC, liquid chromatography; FAME, fatty acid methyl ester(s); nMDS, non-metric multidimensional scaling; ANOSIM, analysis of similarities; DFA, discriminant function analysis; FID, flame ionisation detection; MIS, microbial identification system; ATR, attenuated total reflection; FTIR, fourier transform infrared; DCIMS, direct chemical ionisation mass spectrometry; THM, thermal hydrolysis/methylation; TMAH, tetra methyl ammonium hydroxide; rDNA, ribosomal DNA; ITS, internal transcribed spacer; SSU, small subunit; LSU, large subunit; REVSEQ, reverse and complementary sequence; SPSS, statistical package for the social sciences; DGGE, denaturing gradient gel electrophoresis; TGGE, temperature gradient gel electrophoresis; CGGE, constant gradient gel electrophoresis; FC, flow cytometry; FACS, fluorescence activated cell sorting; TEFAP, Tag-encoded FLX-amplicon pyrosequencing; bTEFAP, bacterial tag encoded FLX titanium amplicon pyrosequencing; CBC, citrus bacterial canker; SDS, sodium dodecyl sulphate; PAGE, polyacrylamide gel electrophoresis; RPLP, restriction fragment length polymorphism; IVS, intervening sequences; TTC, triphenyltetrazolium chloride; ATCC, American type culture collection; RPHPLC, reverse phase high performance liquid chromatography; TOF-MS, time-of-flight mass spectra; IF, initiation factor; c553, cytochrome533; Trx, thioredoxin; VP, voges proskauer; ARDRA, amplified rDNA restriction analysis.

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1. Introduction

Microorganisms are omnipresent, extraordinarily diverse and perform specialized roles in the environment. They impart many harmful effects such as spoilage and health damages besides their beneficial role in the production of antimicrobials and bioactive compounds, bioremediation of toxic chemicals and involvement in food, beverages and pharmaceutical industries. Microbes have been present for over 3.8 billion years, however, their existence became obvious 300 years ago and yet still only <1% of total microbes are known. Taxonomic information of an unknown microbe is highly essential to establish its biodiversity, relationship among other organisms in the ecosystem and its functional aspects (Gevers et al., 2005). Thus, proper isolation and identification is mandatory before deducing the novel characteristic features of any microbial isolate. With the advent of genomics, the complexity of microbial world is largely understandable. In this regard, recent advancements in microbial systematics have led to a 'polyphasic taxonomic approach' which aims to generate all genotypic, phenotypic and phylogenetic information of a microbial taxon (Vandamme et al., 1996). The resulting biological data of the organisms find relevance and implications in biotechnological research such as production of bioactive molecules, microbial transformations, ecology and bioremediation using microbial isolate or consortium.

The prevalent conventional techniques are not sufficient to provide a complete draft for microbial taxonomy as these conventional techniques describe only shape, colour, size, staining properties, motility, host-range, pathogenicity and assimilation of carbon sources (Prakash et al., 2007). However, a comprehensive approach is required to furnish

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