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EXTENDED ABSTRACTS

Rapid Identification of E. coli Bacteriophages using Mass Spectrometry

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ABSTRACT

The current increasing interest within the application of mass spectrometry, especially MALDI-TOF MS, to identification of bacteria and fungi involves a requirement to utilise this technology for identification of other infectious agents like viruses. The aim of this study was to develop a rapid and reliable mass spectrometry-based proteomic method for identification of Escherichia coli phages. The approach was supported rapid in-solution tryptic digestion of suspensions of plaque-purified bacteriophage followed by mass spectral analysis. Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) and liquid chromatography – tandem mass spectrometry (LC-MS) were wont to analyse the tryptic digests. Processing of tandem mass spectrometry data and interpretation of results were achieved using Mascot software and therefore the Swiss-Prot database. Five bacteriophage species (Enterobacteria phages P2, T4, T5, T7 and Lambda) isolated in E. coli cultures were identified. The viral proteins were identified from a posh mixture of host bacterial proteins. additionally , employing a single ion monitoring method, a Lambda prophage derived protein was also identified.The data obtained demonstrate that LC-MS/MS are often used for accurate identification of E.coli- specific bacteriophages in both lytic and lysogenic cycles. Pepper (Capsicum annuum L.) is an economically important vegetable and spice crop. In our laboratory we've established a regeneration and transformation protocol for the sweet red pepper type 'Florinis' and for 2 pepper hybrids PO1 and C using hypocotyl explants. the speed of plant regeneration was found to depend upon the kinds of explants cultured and therefore the media used. In our regeneration protocol shoot bud initiation is simpler on MS media supplemented with IAA and BAP and shoot bud development is promoted with addition of GA 3. Rooted shoots are successfully established in soil. so as to realize the transformation of pepper we applied two different methods, using Agrobacterium and therefore the particle gun. Following the primary method fertile transgenic pepper plants were regenerated from hypocotyl explants that were co-cultivated with Agrobacterium tumefaciens strain LBA4404 harboring a plasmid that contains the gus reporter gene and therefore the nptII selection gene or a plasmid with the Cu/Zn SOD gene of tomato, that's expressed in chloroplasts. Transgenic pepper plants were developed, verified and characterized but the share of transformed plants obtained using Agrobacterium is quite small which is why we've applied as alternative the biolistics method. consistent with the tactic pepper hypocotyls as explants were bombarded by the hand gene gun of Bio-Rad. The plasmid that utilized in this transformation contains the gus reporter gene driven by the CaMV-35S promoter. The reporter gene facilitates the comparison of the 2 transformation methods, and indeed the amount of the kanamycin-resistant plants that were produced through the particle gun seem to be quite large.Pepper (Capsicum annuum L.) is a crucial crop plant grown worldwide for its use as a spice, vegetable or ornamental plant. Pepper is very vulnerable to fungal and viral pathogens, also on the environmental stresses, and these cause considerable damage to the crop. one among the solutions to the present problem is that the gene-splicing of sweet pepper for useful traits, which depends on an efficient and reliable transformation and regeneration protocol. While many members of the Solanaceae family are facile with reference to cell culture and regeneration, pepper (Capsicum annuum L.) is taken into account to be recalcitrant to regeneration. So far, the foremost successful method of regeneration involves direct organogenesis from cotyledons and hypocotyls and recently from young leaves. Pepper cultivars differ markedly in their regeneration requirements. the main problem during the in vitro regeneration process is shoot elongation. Regeneration is additionally severely limited thanks to the formation of ill-formed buds or shoot-like structures which either resist elongation or produce rosettes of distorted leaves that don't produce normal shoots. the foremost recent report for the regeneration of cayenne (Capsicum annuum) from cotyledon explants was developed by Husain et al.and may be a highly efficient three-stage protocol.

Keywords: Bacteriophage virus; Mass-spectrometry; Liquid chromatography; MALDI; LC-MS/MS; Lytic; Lysogenic; Enterobacteria; E.coli; Phage; Viruses

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