Direct detection of Burkholderia cepacia in susceptible pharmaceutical products using seminested PCR

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Abstract

Burkholderia cepaciahas recently received a considerable attention as one of the major risks in susceptible pharmaceutical products. This microorganism can easily propagate and cause vast and severe contamination, especially to the water supplies for pharmaceutical companies. Moreover, it proliferates within the products and can cause severe infections for humans. Therefore, fast and sensitive detection of these bacteria is of a great demand. The present study introduces improved application of a polymerase chain reaction assay with relatively high sensitivity and specificity for the direct detection ofB. cepaciafrom the aqueous pharmaceutical products. A semi-nested polymerase chain reaction approach using the primer set BCR1/BCR2 followed by BCR1/Mr yielding a 465 bp fragment of the recA gene was applied and tested using both crude lysate from isolated colonies and DNA directly extracted from artificially prepared and spiked reference syrup. The polymerase chain reaction assay showed no interference with other bacterial reference and environmental strains tested, includingStaphylococcus aureusATCC® 6538,Pseudomonas aeruginosaATCC® 9027,Escherichia coliATCC® 8739,Salmonella abonyNCTC® 6017,Bacillus subtilisATCC® 6633,Micrococcus luteus, Staphylococcus warneri, Pseudomonas fluorescens, Pseudomonas putida, andRalstonia pickettii Moreover, this semi-nested assay showed a detection limit of around 10 colony-forming units per sample and could detectB. cepaciastrains isolated from a municipal pre-treated potable water tank. Comparing the results for detection ofB. cepaciain 100 randomly collected commercial syrup preparations using both conventional standard method and polymerase chain reaction assay revealed thatB. cepaciawas detected in two samples using polymerase chain reaction assay while all samples showed negative results by conventional culturing and biochemical methods. These results highlight the advantage of using this polymerase chain reaction assay to detectB. cepaciain contaminated pharmaceutical products and even water for pharmaceutical purposes, without the need of culturing or pre-enrichment, where it may give false-negative results and may be misidentified when biochemically tested.

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