Title Attachment strength and biofilm forming ability of *Bacillus cereus* on green-leafy vegetables: Cabbage and lettuce

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Abstract

The present study was designed to investigate the ability of six *Bacillus cereus* strains to attach and form biofilm on cabbage and lettuce surfaces. These six strains were; a reference strain DSMZ 345 and five biofilm-producing strains (aquatic strains; TUB8, TUB30, TUB31, TUB32 and TUB33) isolated from drinking-water distribution network. Hydrophobicity, biofilm formation ability, attachment strength (S_R) of spores and vegetative cells of the six *B. cereus* strains were also determined. Due to their high hydrophobicity, spores of all strains had high ability to attach polystyrene and did not affect by dilution of tryptone soy broth (TSB, 1:20 v/v) in the *in vitro* experiment. Significant ($p < 0.05$) enhancement *in vitro* biofilm formation by vegetative cells of *B. cereus* was recorded in the diluted TSB. The highest biofilm formation on cabbage and lettuce surfaces was obtained by spores and vegetative cells of all tested strains on the 4th hour of the incubation period. These populations were significantly ($p < 0.05$) increased by elongating incubation time from 4 h to 24 h except DSMZ 345 and TUB8. Biofilm formation behavior obtained by *B. cereus* spores and vegetative cells on the polystyrene surface was different compared with that recorded on produce surface. The S_R of both spores and vegetative cells of the studied strains to the lettuce surface was higher than that of the cabbage surface. The hydrophobicity, biofilm formation and S_R of spores and vegetative cells of the biofilm-producing strains were higher than that of the reference strain DSMZ 345. Scanning electron microscopy (SEM) exposed random distribution of cells either on the surface or cut edge, without clear obvious affinity for the surface structures. Increasing in the presence of large clusters of cells on leaf surfaces was demonstrated after 4 and 24 h.

In conclusion, use of aquatic environmental isolates is more useful for studying biofilm formation than the reference strain. Lettuce surface supported the attachment of *B. cereus* spores and vegetative cells compared with the cabbage surface. Further investigations are required to improve our knowledge of biofilm formation mechanisms by the human pathogenic microorganisms, especially by using the environmental and clinical isolates. To ensure safety level of green-leafy vegetables, biofilm formation after harvest should be considered as critical control point during handling of these vegetables.