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RESEARCH PAPER

Biofilm formation on food contact surfaces and its inhibition

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ABSTRACT

Almost all moist surfaces are colonized by microbial biofilms. Biofilms implicated in cross-contamination of food products and various human infections such as dental cavities. In this investigation three bacterial samples were used for biofilm formation in 48 hrs in laboratory condition. Biofilms was determined by using spectrophotometric technique to overcome hazardous effect of biofilm on these biomaterials is suggested in this research. To subside the biofilms these parameters were used in the culture and after 24, 48, and 72 hrs. 0 day has been taken at 450 for *S. aureus* and at 600 for both *E. coli* and *Salmonella* spp. The result was found that this chemical parameter showed antimicrobial activity and was capable to inhibit the biofilms formation. The preservatives were more effective acetic acid for maggi bottle, benzoic acid for pickle bottle and sodium sulphite for both fizz bottle and coke cane.

Key Words : *Escherichia coli, Staphylococcus aureus, Salmonella* spp. Biofilm, Food contact surfaces

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Biofilms is a community (population) of micro-organisms that may include bacteria, fungi, yeasts and protozoa, attached to a solid surface. Biofilms are produced by micro-organisms and consist of a sticky structure of polysaccharides and other organic contaminants. This slime layer is anchored to a surface and provides a protective environment in which micro-organisms grow (Joseph *et al.*, 2001). Biofilms generally form on any surface that is exposed to non-sterile water or other liquids and are consequently found in many environmental, industrial, and medical environments. (Rao *et al.*, 2005) . Microbes form a biofilm in response to many factors, which may include cellular recognition of specific or non-specific attachment sites on a surface, nutritional cues, or in some cases, by exposure of plank tonic cells to sub-inhibitory concentrations of antibiotics (White and McDermott, 2001). When a cell switches to the biofilm mode of growth, it undergoes a phenotypic shift in behaviour in which large suites of genes are differentially regulated *Staphylococcus aureus; Escherichia coli* and *Salmonella* spp. are common pathogens responsible for community infection.

Formation of a biofilm begins with the attachment of free- floating micro-organisms to a surface (Donald, 2002). These first colonists adhere to the surface initially through weak, reversible adhesion via van der Waals

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forces (Jenkinson and Lappin-Scott, 2001). If the colonists are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion structures such as pili. Hydrophobicity also plays an important role in determining the ability of bacteria to form biofilms, as those with increased hydrophobicity have reduced repulsion between the extracellular matrix and the bacterium (Flemming, 1998). Some species are not able to attach to a surface on their own but are sometimes able to anchor themselves to the matrix or directly to earlier colonists. It is during this colonization that the cells are able to communicate via quorum sensing using products such as AHL. Some bacteria are unable to form biofilms as successfully due to their limited motility. Non motile bacteria cannot recognize the surface or aggregate together as easily as motile bacteria. Once colonization has begun, the biofilm grows through a combination of cell division and recruitment. Polysaccharide matrices typically enclose bacterial biofilms. In addition to the polysaccharides, these matrices may also contain material from the surrounding environment, including but not limited to minerals, soil particles, and blood components, such as erythrocytes and fibrin. The final stage of biofilm formation is known as dispersion, and is the stage in which the biofilm is established and may only change in shape and size.

RESEARCH METHODOLOGY

Biomaterials

Four biomaterials such as maggie sauce bottle, pickle bottle, Appy fizz bottle, coke cane bottle have been used in the present study.

Microorganisms and maintenance of culture :

Pure culture of *E.coli, S. aureus* and *Salmonella* spp. was obtained from the research laboratory of Microbiology and Fermentation Technology, SHIATS, Allahabad. The bacterial culture was maintained on nutrient broth (NB) medium at 4 ^{0}C. The slant was grown at 30 ^{0}C for 1 day.

Inoculums preparation :

Bacterial culture was inoculated onto nutrient agar medium in the slant, after 24 hrs incubation at 31 ^{0}C so these cultures were used to inoculation of Luria broth.

Screening of biomaterials :

For growth of biofilm four materials were screened. maggie bottle, pickle bottle, fizz bottle and coke cane bottle collected from market and cut in the form of 0.5cm square.

Preparation of cell broth and agar plate for bacterial colony count :

Cell broth and agar plates were prepared for bacterial counting.

Biofilm development and quantification :

The level of biofilm formation by *E.coli, S. aureus* and *Salmonella* spp. on maggi bottle, pickle bottle, fizz bottle and coke cane surfaces incubated in to vegetable broth and bacterial culture at 36 $\pm$ 1°C over 24, 48 and 72 hrs in the Eppendorff tube. After 24, 48, and 72hrs of incubation the coupons were withdrawn washed with d. water to remove none adhered cell. Again coupons were immersed in crystal violet (0.5%) for ½ hrs further in DMSO solution for 1 hrs till reading on spectrophotometer by Cuvette.

RESULTS AND REMONSTRATION

The growth of *E.coli* biofilms on biomaterials maggi bottle, pickle bottle, fizz bottle, coke cane increases from 24 hrs. The growth was the maximum at 48 hrs and eventually declined at 72 hrs. The results found are given in Table 1 to 15.

The growth of *S. aureus* biofilm on biomaterial increases from 24 hrs the growth is the maximum at 48 hrs and eventually declines at 72 hrs. The results are given in Table 6.

The growth of *Salmonella* spp. on biomaterials increases from 24 hrs, the growth was the maximum at 48 hrs and eventually declined at 72 hrs. When results are shown in Table 4.

It was found that biofilm growth on biomaterials like different coupons namely maggi bottle, fizz bottle, pickle bottle and coke cane. Three bacterial samples were used namely *E.coli, Staphylococcus aureus*, and *Salmonella* spp. It observed biofilm formation with different bacterial samples at different time interval 24 hrs, 48 hrs and 72 hrs. It was observed that maximum biofilms was formed after 48 hrs. by using spectrophotometer techniques. Further reading of three bacterial samples with three different preservatives

Table 1 : Absorbance (at 600 nm) taken for *E.coli* on biomaterials

Biomaterials	24 hrs	48 hrs	72 hrs
Maggi bottle	0.306	0.611	0.345
Pickle bottle	0.320	0.645	0.408
Fizz bottle	0.406	0.809	0.468
Coke cane	0.223	0.613	0.331

Table 2 : Absorbance (at 600 nm) taken for *E. coli* on maggi bottle coupons in presence of different preservatives

Preservatives	Maggi bottle coupons at 48 hrs
Control	0.615
Acetic acid	0.153
Benzoic acid	0.215
Sodium sulphite	0.246

Table 3 : *E.coli* biofilms on biomaterials on fizz bottle coupons in presence of different preservatives

Preservatives	Fizz coupons at 48 hrs
Control	0.806
Acetic acid	0.322
Benzoic acid	0.282
Sodium sulphite	0.201

Table 4 : Absorbance (at 600 nm) for *E. coli* on coke cane coupons in presence of different preservatives

Preservatives	Coke cane coupons at 48 hrs.
Control	0.611
Acetic acid	0.244
Benzoic acid	0.213
Sodium sulphite	0.152

Table 5 : Absorbance (at 600 nm) for *E.coli* on pickle bottle coupons in presence of different preservatives

Preservatives	Pickle bottle coupons at 48 hrs
Control	0.641
Acetic acid	0.224
Benzoic acid	0.160
Sodium sulphite	0.256

Table 6 : Absorbance (at 450 nm) taken for *S. aureus* on biomaterials

Biomaterials	24 hrs	48hrs	72 hrs
Maggi bottle	0.40	0.92	0.48
Fizz bottle	0.039	0.85	0.49
Coke cane	0.41	0.93	0.48
Pickle bottle	0.40	0.90	0.50

Table 7 : Absorbance at 450 nm for *S.aureus* on Maggi coupon in presence of different preservatives

Preservatives	Maggi bottle coupon at 48 hrs
Control	0.920
Acetic acid	0.230
Benzoic acid	0.322
Sodium sulphite	0.368

Table 8 : Absorbance (at 450 nm) for *S.aureus* on fizz bottle coupon in presence of different preservatives

Preservatives	Fizz bottle coupons at 48 hrs
Control	0.855
Acetic acid	0.342
Benzoic acid	0.322
Sodium sulphite	0.278

Table 9 : Absorbance at 450 nm for *S.aureus* on coke cane in presence of different preservatives

Preservatives	Coke cane at 48 hrs
Control	0.925
Acetic acid	0.323
Benzoic acid	0.277
Sodium sulphite	0.231

Table 10 : Absorbance at 450 nm for *S.aureus* on pickle bottle coupon in presence of different preservatives

Preservatives	Pickle bottle coupon at 48 hrs
Control	0.95
Acetic acid	0.332
Benzoic acid	0.237
Sodium sulphite	0.285

Table 11 : Absorbance (at 600nm) taken for *Salmonella* spp. on biomaterials

Biomaterials	24 hrs	48 hrs	72 hrs
Maggi bottle	0.400	0.467	0.354
Fizz bottle	0.230	0.741	0.554
Pickle bottle	0.180	0.611	0.315
Coke cane	0.090	0.470	0.215

Table 12: Absorbance (at 600 nm) for *Salmonella* spp. on maggi bottle coupon in presence of different preservatives

Preservatives	Maggi bottle coupon at 48 hrs
Control	0.460
Acetic acid	0.115
Benzoic acid	0.161
Sodium sulphite	0.184

Table 13: Absorbance (at 600 nm) for *Salmonella* spp. on fizz coupon in presence of different preservatives

Preservatives	Fizz bottle coupon at 48 hrs
Control	0.735
Acetic acid	0.294
Benzoic acid	0.220
Sodium sulphite	0.183

Table 14 : Absorbance (at 600 nm) for *Salmonella* spp. on coke cane coupon in presence of different preservatives

Preservatives	Coke cane bottle at 48 hrs
Control	0.860
Acetic acid	0.344
Benzoic acid	0.301
Sodium sulphite	0.215

Table 15: Absorbance (at 600 nm) for *Salmonella* spp. on pickle bottle coupon in presence of different preservatives

Preservatives	Pickle bottle coupon at 48 hrs
Control	0.855
Acetic acid	0.0.342
Benzoic acid	0.0213
Sodium sulphite	0.299

acetic acid, benzoic acid and sodium sulphite was also taken with respect to control. The result shows that different preservatives were effective in inhibition of bacterial biofilms on the different coupons. It was observed that for *E.coli,* the best preservative for biofilm inhibition was acetic acid for maggi bottle, benzoic acid for pickle bottle, and sodium sulphite for both fizz bottle and coke cane coupons. It was observed that for *S. aureus* the best preservative for biofilm inhibition was acetic acid for maggi bottle, benzoic acid for pickle bottle, and sodium sulphite for both fizz bottle and coke cane coupons. It is observed that for *Salmonella* spp. the best preservative for biofilm inhibition was acetic acid for Maggi bottle, benzoic acid for pickle bottle, and sodium sulphite for both fizz bottle and coke cane coupons. The best results was observed on *S. aureus* bacteria followed by *E.coli* and *Salmonella* spp. on certain coupons.

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