

Effect of substrate on biochemical expression of Bacterial Biofilms

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Introduction

Single or multi species bacteria are agglomerated together with the help of Extracellular Polymeric Substance (EPS) forming bacterial biofilms (BBs). BBs get attached to the biotic or abiotic surfaces. This surface based adhesion alters the biochemical expression of BBs. Under the optimum conditions, the biochemical expression of BBs can be maximized. The BBs can be used in various biotechnological applications like biofertilizers, drug discovery, biofuels etc. (Senaviratne, *et al.*, 2008). Thus, present study figure outs preferable surface for the BBs, which would enhance the functional properties of BBs.

Methodology

In this experiment, single species bacterial biofilms were formed in glass wool (G), metal fibers (M) and fungal filaments (F) immersed in a Low Cost Medium (LCM). Control (C) was maintained without surfaces or bacterial strain. There were three replicates for each treatment. Completely Randomized Design was followed as the experimental design. Attachment of bacterial biofilm with different surfaces was observed through the microscopy. Biochemical expressions of different biofilms were examined by analyzing the exudates using Fourier Transform Infrared (FTIR) spectroscopy. FTIR spectra were analyzed under six spectral windows *viz* finger print, polysaccharide, amide, fatty acid I, II, and mixed. Cluster analysis was performed under each window to see the similarity and the differences of the biochemical expressions of bacterial biofilms attached with different surfaces. Statistical analysis was done by using Minitab statistical package (version 16).

Results and Discussion

Morphologically, bacterial biofilm loosely attached with abiotic metal fibers where as it was tightly attached with the abiotic glass wool and biotic fungal filaments (figure 01).

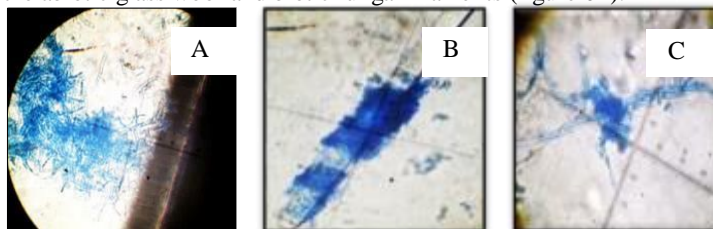


Figure 01. Bacterial biofilm attached to the metal surface (A), glass wool (B) and fungal filament (C).

Cluster analysis showed that, the biochemical expressions of bacterial biofilms totally depend on the surface of attachment. Dendrograms of amide window after a week of incubation and fatty acid I window after two weeks of incubation are given in figure 02 (A and B). Accordingly, the similarity of expressions of both amide and fatty acid compounds between treatments was 67%.

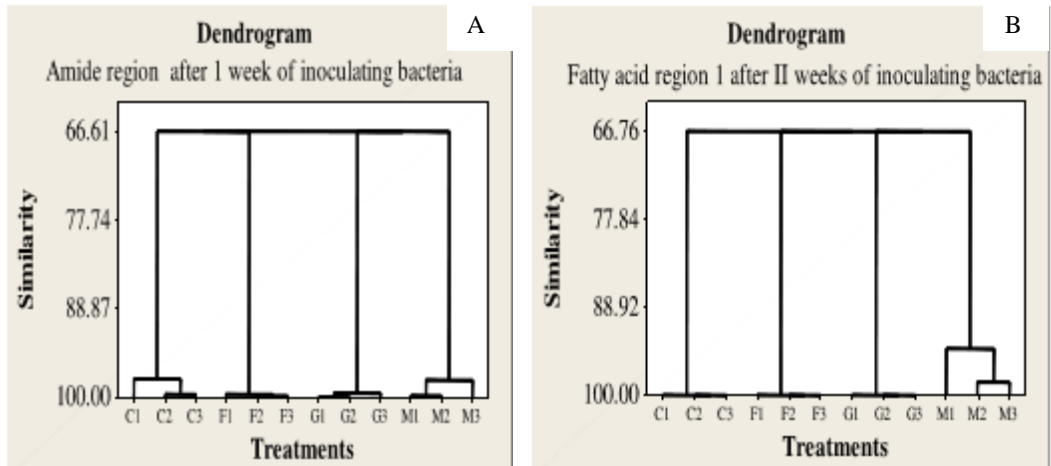


Figure 02. Dendrogram of amide window after one week of incubation (A), and fatty acid I window after two weeks of incubation (B)

When considering the changes of biochemical expression of treatments with the time, glass wool and metal fibers attached biofilms showed significantly high ($p < 0.05$) numbers of peaks in amide and fatty acid windows, respectively, after one week of incubation. During second week, metal fiber attached biofilm showed significantly higher ($p < 0.05$) peak numbers than other treatments.

Production of surface based unique compounds was a remarkable observation of this experiment. It depended on the incubation time. Fungal surface attached bacterial biofilm released a range of unique compounds (table 1), because direct contact of bacteria with fungus would make a biotic interaction altering the production of new compounds (Volker *et al.*, 2009), compared to the abiotic surface attached bacterial biofilms.

Table 01: Numbers of unique functional groups observed under four different treatments used in this study

	Control	Glass wool	Metal fiber	Fungal filament
Before attachment	4	1	5	5
After attachment	5	1	3	2
One week after attachment	-	1	-	2
Two weeks after attachment	1	5	1	9

Conclusions

It is concluded that morphologies of the attachment of bacterial biofilms are surface specific. Biochemical expression of bacterial biofilms is also affected by the provided surfaces. Bacterial biofilm-surface interaction produces unique chemical compounds specific to the surfaces. The fungal surface-attached bacterial biofilm produces a wider variety of compounds, possibly as a result of molecular interactions between the fungus and the bacterium.

References

Seneviratne, G., Zavahir, J., Bandara, W.M.M.S. and Weerasekara, M.L.M.A.W., 2008, Fungal-Bacterial biofilms: their development for novel biotechnological applications. *World Journal of Microbiology and Biotechnology* 24: 739-743.

Volker, S., Kirstin S., Hans-Wilhelm, N., Ekaterina S., Schmidt-Heck, W., Schuemann, J., Martin, K., Hertweck, C., Brakhage, A.A., 2009, Intimate bacterial–fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. *Proceedings of the National Academy of Sciences*, 106(34): 14558–14563.