Data Article

Data on quantitation of *Bacillus cereus sensu lato* biofilms by microtiter plate biofilm formation assay

Rener De Jesus, Gina Dedeles

**Abstract**

The microtiter plate biofilm formation assay is a method for the study of early biofilm formation on abiotic surfaces. It is a colorimetric technique that uses dyes, such as crystal violet, to stain attached biofilms and to quantify by using an absorbance microtiter plate reader. In this data, we evaluated the ability of 12 *Bacillus cereus sensu lato* isolated from soil and milk powder samples for their production of biofilms after a total of 48 hr incubation period in the 96-well microtiter plate. The biofilm production was induced by initially exposing them in diluted tryptic soy broth at its first 24 hr and then replacing with freshly prepared double strength broth for the next incubation period at 30°C. The optical densities of the bacterial growth in the wells were read at the absorbance wavelength of 630 nm while the stained biofilms that solubilized in absolute ethanol were read at 570 nm. The biofilm measurements were calculated and the degree of biofilm production of each isolate was classified according to biofilm formation categories adapted from previous researchers. Therefore, the assay concluded the negative impact of *B. cereus* group by ability to form biofilms on abiotic surfaces, such as food contact surfaces in...
1. Data

The microtiter plate (MTP), also known as 96-well plate, assay is a method for studying biofilms that are adhered on abiotic surfaces [1]. For quantitation of biofilms, the MTP assay is done using optically clear flat-bottom plates for measuring optical densities (ODs) by an absorbance MTP reader and the adhered biofilms are stained with crystal violet (CV).

In this method, the MTP assay was performed in three sets and in triplicates for each B. cereus s.l. isolate in every set. The raw data of conventional bacterial identification of 12 B. cereus s.l. isolates from soil and milk powder samples are available in Mendeley Data with DOI:10.17632/832g5m5yvb.1, https://data.mendeley.com/datasets/832g5m5yvb/1. All ODs datasets that were read at absorbance wavelengths of 570 nm and 630 nm are presented in Table 1 and Table 2, respectively. Specifically, in Table 1 presents ODs at 570 nm of attached B. cereus s.l. biofilms on well surfaces and were stained with 1% CV solution while Table 2...
shows ODs at 630 nm of bacterial growth in MTP wells after a total of 48 hr incubation period with TSB medium.

The specific biofilm formation (SBF) index from each set of assay was calculated and the mean SBF indices are shown in Table 3. Furthermore, Table 3 also shows the sample sources from which the *B. cereus* s.l. test strains were isolated and their assigned biofilm formation categories (BFCs) according to Ref. [2].
2. Experimental design, materials, and methods

2.1. Bacterial isolates

All *B. cereus s.l.* test organisms used in this study were isolated from soil samples and infant formula milk powders by the spread plate method in Ref. [3] using the *Bacillus cereus* selective agar® (Oxoid) supplemented with egg yolk emulsion and polymyxin B (Oxoid). A total of 12 *B. cereus s.l.* isolates were subjected to MTP biofilm formation assay. All isolates are kept in cryovials containing tryptic soy broth (Merck) with glycerol and stored at −80 °C for further analysis.

2.2. Biofilm formation of *B. cereus s.l.* isolates in 96-wells MTP

Following the described method in Ref. [2] with some modifications, the *B. cereus s.l.* isolates (*n* = 12) were grown overnight in 10 mL sterile TSB tubes at 30 °C in the incubator (Memmert GmbH + Co. KG). The polystyrene 96-well MTP (Corning®) were filled with 200 μL of TSB diluted with deionized water [1:20 (v:v)]. The wells were inoculated with 3 μL of the overnight *B. cereus s.l.* cultures and incubated at 30 °C without shaking for 24 hr. After incubation, the broth in MTP wells were removed by gentle pipetting, replenished with freshly prepared sterile double-strength TSB to support the biofilm formation and incubated again at 30 °C for 24 hr.

2.3. Quantification of biofilm production

The growth in MTP wells was read at wavelength of 630 nm using an absorbance MTP reader as shown in Table 2. The broth of each well was removed and the wells were gently washed once with 200 μL sterile PBS, pH 7.2 and then air-dried for 20 min. The attached biofilms in the wells were stained with 130 μL 1% CV (LabChem) solution for 5 min and washed thrice with 200 μL sterile distilled water. The stained attached biofilms in the wells were solubilized in 130 μL absolute ethanol (J.T Baker®) and the ODs were read at 570 nm, which are presented in Table 1.

2.4. Specific biofilm formation and biofilm formation category

The biofilm quantities produced by the *B. cereus s.l.* isolates were calculated using the formula provided by previous researchers in Ref. [4], which is SBF index = (AB–CW)/G. The SBF stands for specific biofilm formation while *AB* is the OD read at 570 nm then *CW* is the OD of stained control wells. The results showed that the isolates can be categorized as follows: *++*, weak biofilm producer; *++*, moderate biofilm producer; and *++++*, strong biofilm producer.

| Isolates | Sources | SBF index | mean SBF index ± SDb | CoV (%) | Biofilm Formation Categoryc |
|---|---|---|---|---|---|
| | | Set 1 | Set 2 | Set 3 | |
| B-01 | soil | 1.761 | 1.651 | 1.575 | 1.662 ± 0.094 | 5.6 | +++ |
| B-02 | soil | 0.739 | 0.707 | 0.830 | 0.759 ± 0.064 | 8.4 | ++ |
| B-04 | soil | 0.526 | 0.484 | 0.496 | 0.502 ± 0.022 | 4.3 | ++ |
| B-06 | soil | 2.441 | 2.228 | 2.428 | 2.366 ± 0.119 | 5.0 | +++ |
| B-07 | soil | 0.634 | 0.668 | 0.629 | 0.644 ± 0.021 | 3.3 | ++ |
| B-MS01 | milk powder | 0.676 | 0.729 | 0.793 | 0.733 ± 0.059 | 8.0 | ++ |
| B-03 | milk powder | 0.950 | 1.047 | 1.118 | 1.038 ± 0.084 | 8.1 | +++ |
| B-MS04 | milk powder | 0.850 | 0.959 | 0.866 | 0.893 ± 0.059 | 6.6 | ++ |
| B-05 | milk powder | 0.775 | 0.716 | 0.721 | 0.737 ± 0.033 | 4.4 | ++ |
| B-08 | milk powder | 0.691 | 0.695 | 0.730 | 0.705 ± 0.021 | 3.0 | ++ |
| B-09 | milk powder | 0.669 | 0.700 | 0.739 | 0.703 ± 0.035 | 5.0 | ++ |
| B-10 | milk powder | 2.534 | 2.528 | 2.696 | 2.586 ± 0.095 | 3.7 | +++ |

*++*, weak biofilm producer; *++*, moderate biofilm producer; and *++++*, strong biofilm producer.
b SD, standard deviation.
c CoV, coefficient of variation.
containing un-inoculated medium (to eliminate unspecific or abiotic OD values) that read only at 570 nm, and G is OD of cell growth in broth measured at 630 nm. The assay for control wells was performed in triplicates. The mean and standard deviation of the SBF index were calculated and the degree of biofilm production was classified in three categories as described in Ref. [2] and presented in Table 3: weak (SBF < 0.5), moderate (0.5–1.0), and strong (SBF > 1).

2.5. Statistical treatment

The precision and repeatability of mean ODs reading and SBF indices in the MTP assay were determined using the coefficient of variation (CoV) below 10% as shown in Tables 1–3

Acknowledgments

The authors would like to thank the Research Center for Natural and Applied Sciences, Thomas Aquinas Research Center, University of Santo Tomas in Manila, Philippines for allowing us to use the microtiter plate reader.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] B.M. Coffrey, G.G. Anderson, Biofilm Formation in the 96-well Microtiter Plate. Pseudomonas Methods and Protocols. Methods Mol. Biol. [Methods and Protocols], vol 1149, Humana Press, New York, NY, 2014, https://doi.org/10.1007/978-1-4939-0473-0_48.

[2] M. Martinez-Medina, P. Naves, J. Blanco, X. Aldeguer, J.E. Blanco, M. Blanco, C. Ponte, F. Soriano, A. Darfueille-Michaud, L.J. Garcia-Gil, Biofilm formation as a novel phenotypic feature of adherent-invasive Escherichia coli (AIEC), BMC Microbiol. 9 (2009) 202, https://doi.org/10.1186/1471-2180-9-202.

[3] U.S. Food and Drug Administration (USFDA), Bacteriological Analytical Manual Online (BAM Online): Chapter 14: Bacillus Cereus, 2012. Available from: https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam.

[4] C. Niu, E.S. Gilbert, Colorimetric method for identifying plant essential oil components that affect biofilm formation and structure, Appl. Environ. Microbiol. 70 (2004) 6951–6956, https://doi.org/10.1128/AEM.70.12.6951-6956.2004.