

## **A Biocompatible $\beta$ -Cyclodextrin Inclusion Complex Containing Natural Extracts: A Promising Antibiofilm Agent**

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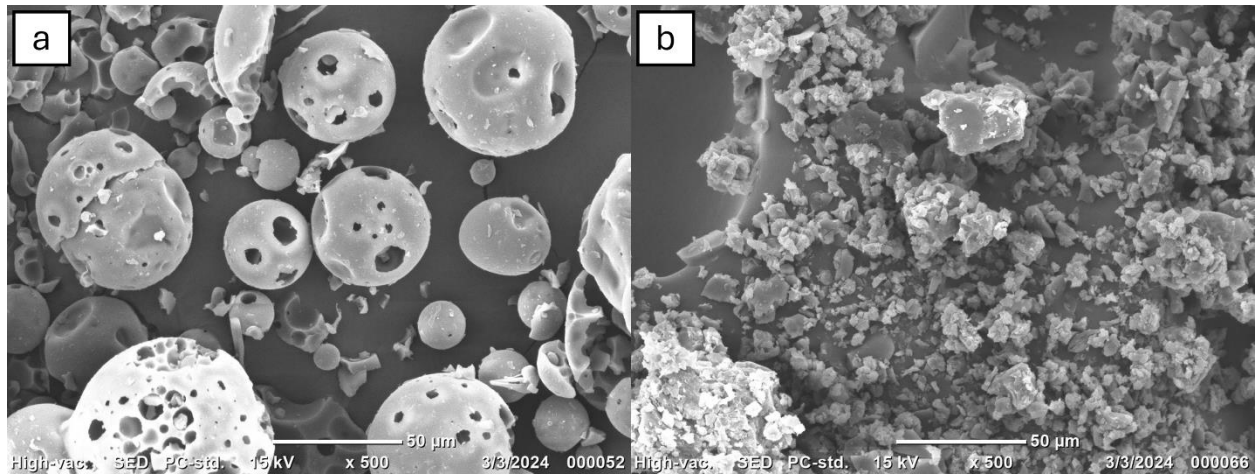
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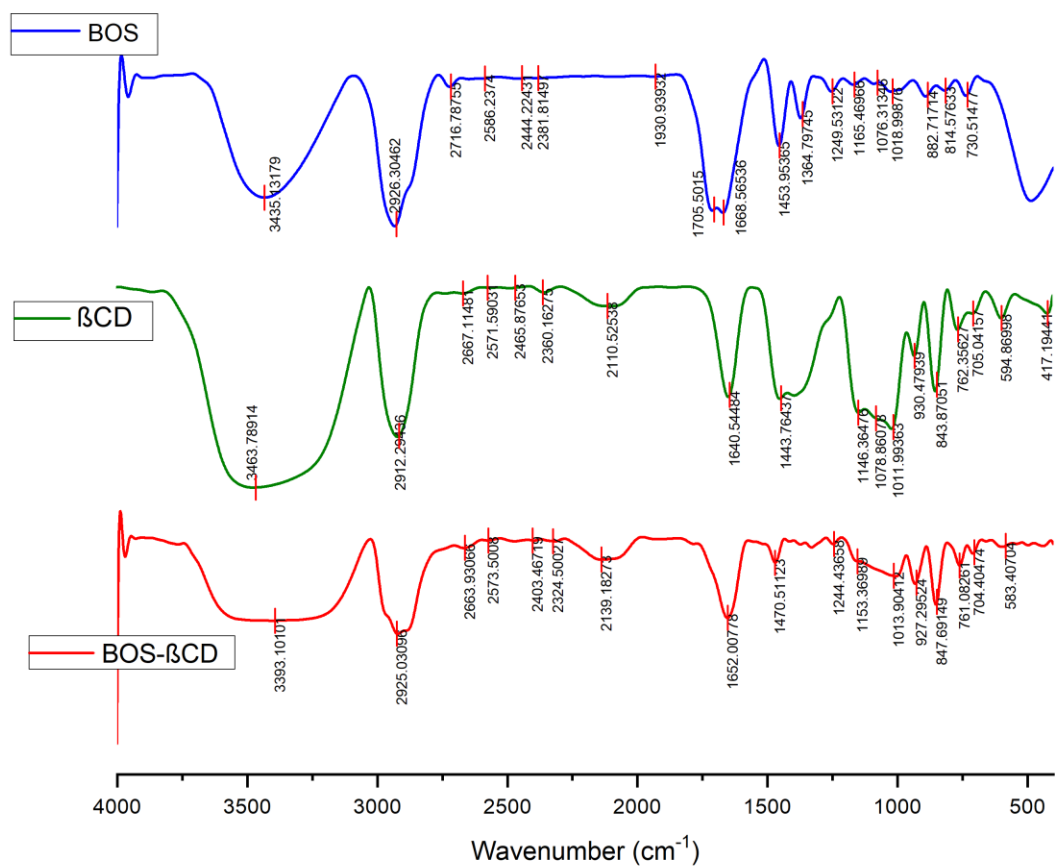
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### Supplementary Figure S1.

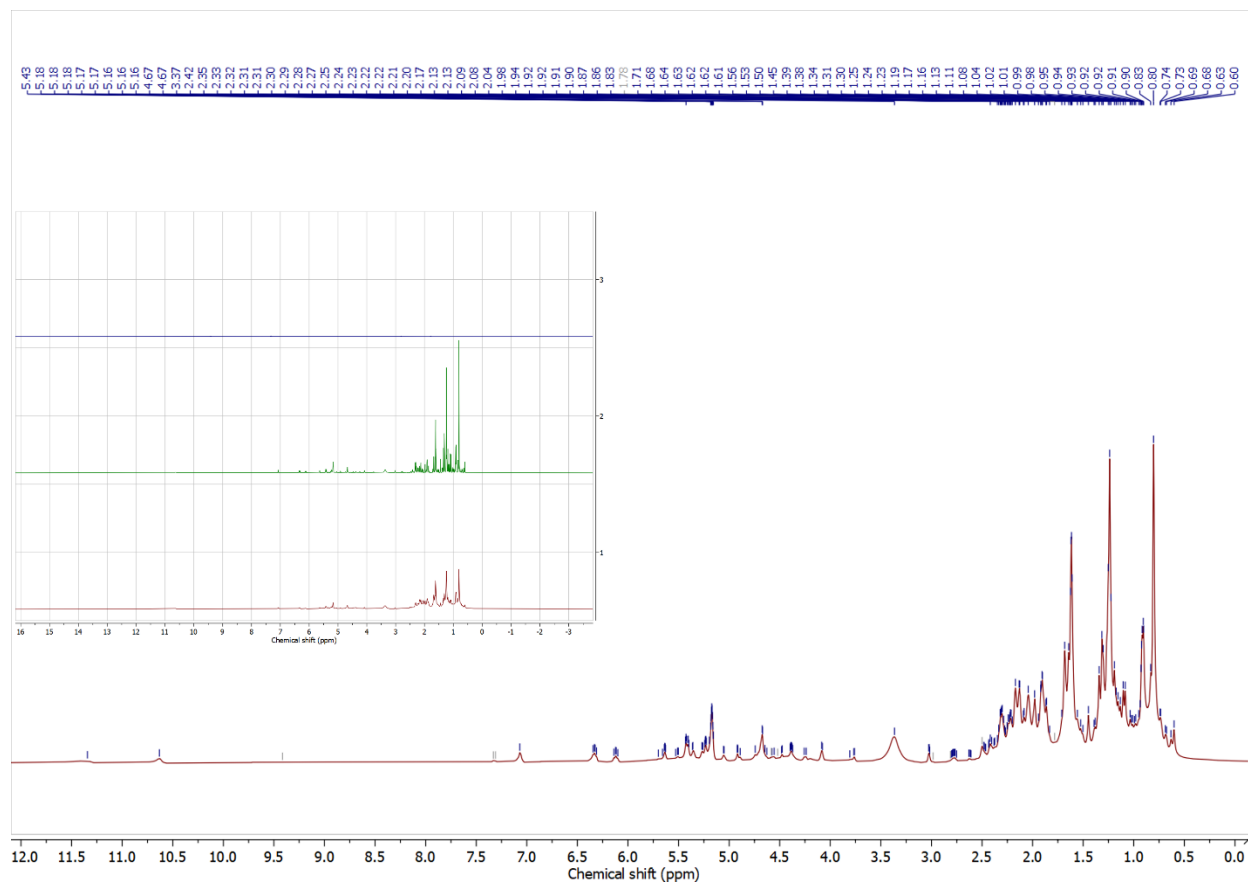


**Figure S1.** SEM images of (a)  $\beta$ CD and (b) BOS- $\beta$ CD inclusion complex. The  $\beta$ CD field demonstrated large ovoid crystals alongside rectangular particles, whereas the particles of BOS- $\beta$ CD inclusion complex were presented with significant reduction in size and substantial morphological alterations developing several aggregates as well. These observations suggest the potential complexation formation of BOS- $\beta$ CD inclusion complex, possibly involving an amorphous product interacting with other components in the established complex.

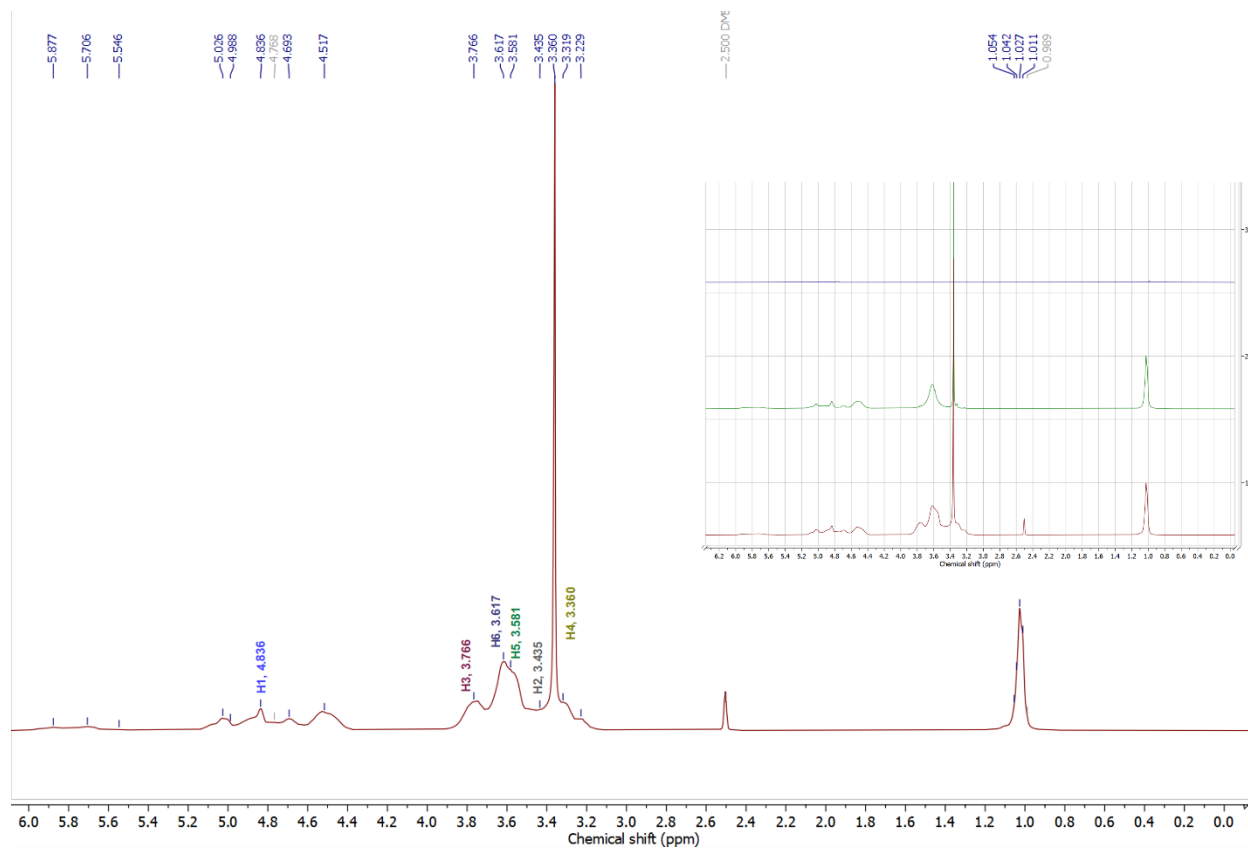
## Supplementary Figure S2.



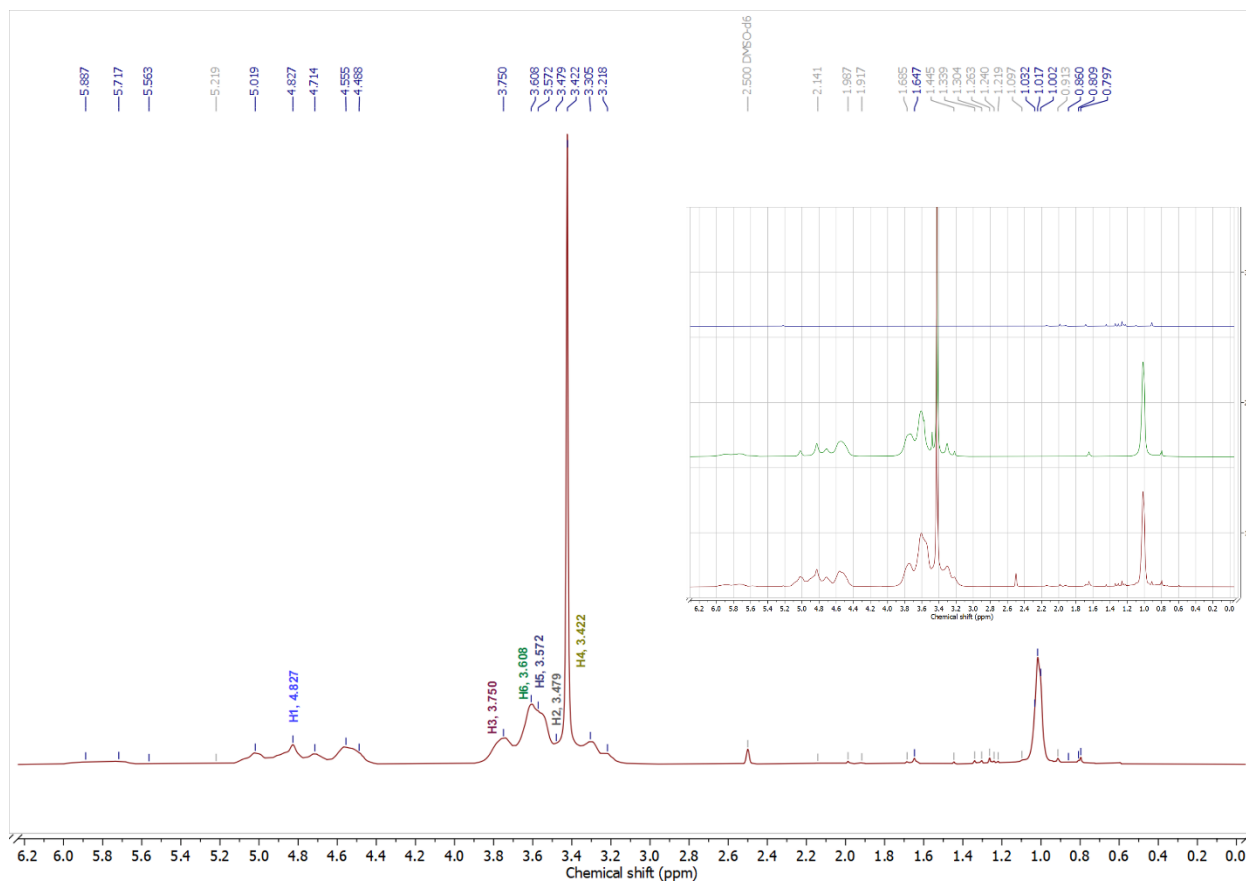
**Figure S2.** FTIR spectra for BOS,  $\beta$ CD, and BOS- $\beta$ CD inclusion complex.



**Figure S3.**  $^1\text{H}$  NMR spectrum and stacked H1 Sub-spectra of BOS EO.



**Figure S4.**  $^1\text{H}$  NMR spectrum and stacked H1 Sub-spectra of  $\beta\text{CD}$ .



**Figure S5.**  $^1\text{H}$  NMR spectrum and stacked H1 Sub-spectra of BOS- $\beta$ -CD.

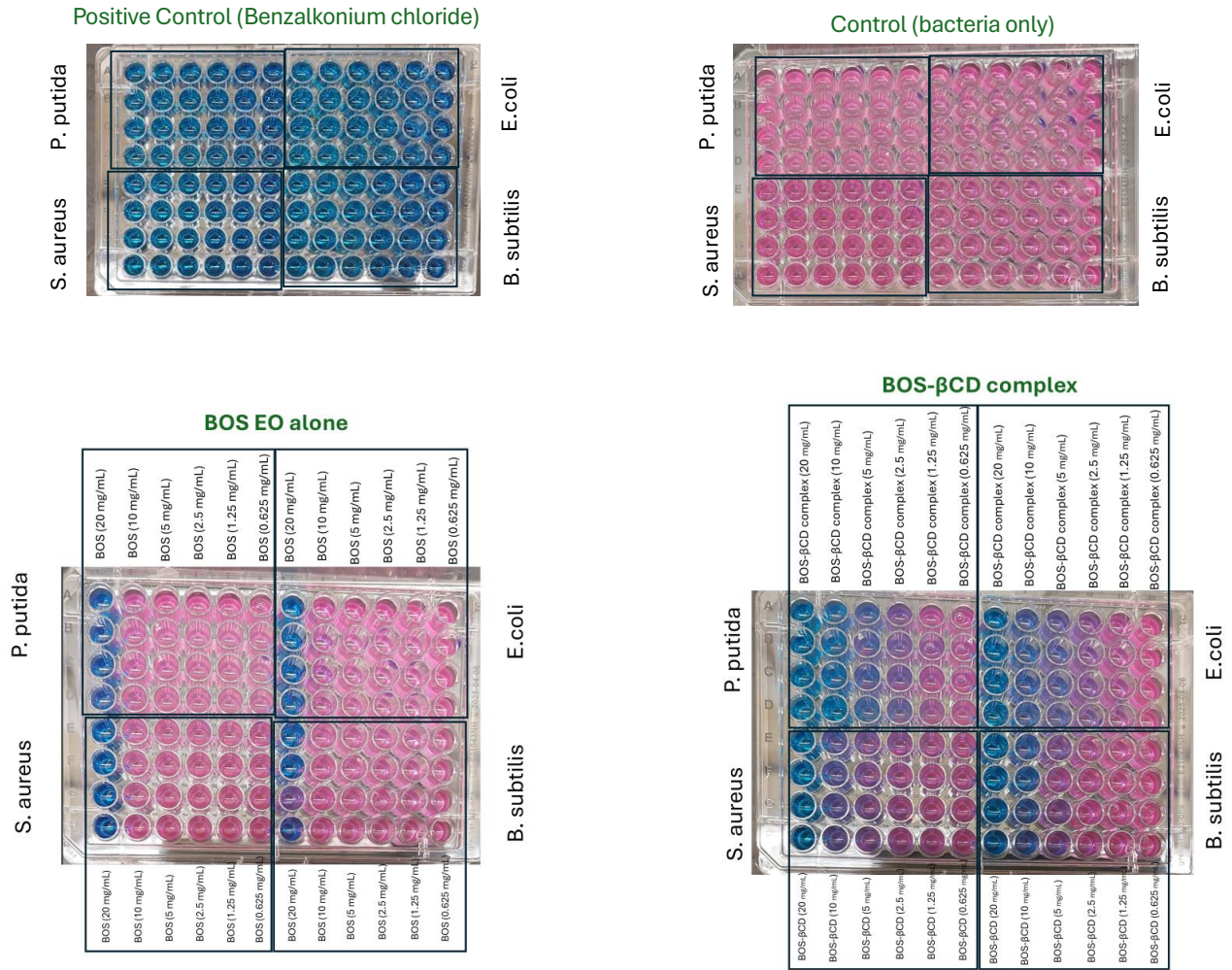
**Supplementary Table 1.** Chemical shifts ( $\delta$ ) for  $\beta$ CD and BOS- $\beta$ CD and differences in chemical shift ( $\Delta\delta$ )

Proton no.	$\beta$ CD ( $\delta$ /ppm)	BOS- $\beta$ CD ( $\delta$ /ppm)	$\Delta\delta$ BOS- $\beta$ CD/ $\beta$ CD ( $\Delta\delta$ /ppm)
H1	4.836	4.827	0.009
H2	3.435	3.479	-0.044
H3	3.766	3.750	0.016
H4	3.360	3.422	-0.062
H5	3.581	3.572	0.009
H6	3.617	3.608	0.009



Supplementary Figure S6.

**BOS EO & BOS-βCD complex**  
**Microdilution assays for MICs determination -Alamar Blue**

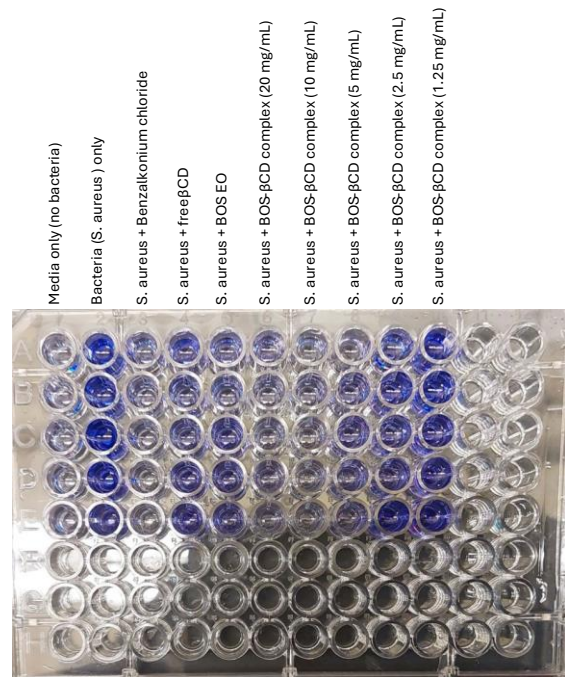


**Figure S6.** Microdilution assays for MICs determination of BOS EO & BOS-βCD complex (Alamar Blue).

Supplementary Figure S7I.

**Biofilm formation prevention-  
*S. aureus***

Well	1	2	3	4	5	6	7	8	9	10	11	12
A	0.297	5.268	0.441	2.924	1.054	0.339	0.370	0.563	1.001	0.911		
B	0.431	2.647	0.177	1.922	1.348	0.578	0.383	0.822	0.720	1.187		
C	0.434	5.609	0.207	1.853	0.482	0.309	0.387	0.436	0.532	0.901		
D	0.395	2.969	0.235	3.863	0.768	0.276	0.354	0.508	0.857	2.246		
E	0.482	2.877	0.089	1.781	0.890	0.264	0.269	0.499	0.461	2.702		



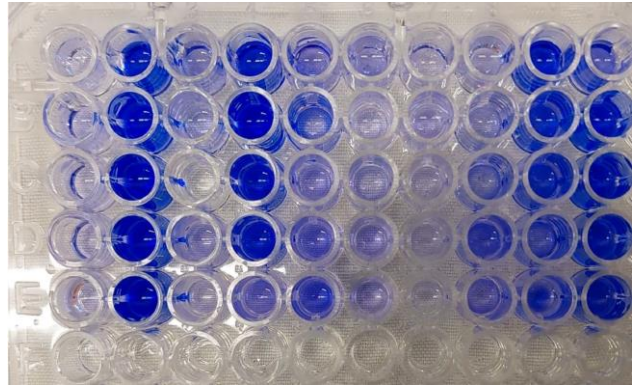
**Figure S7I.** Assay of Biofilm formation prevention against *S. aureus*.

Supplementary Figure S7II.

**Biofilm formation prevention-  
*P. Putida***

	1	2	3	4	5	6	7	8	9	10
A	0.254	2.170	0.529	1.954	0.750	1.143	0.203	0.376	1.643	1.081
B	0.358	3.577	0.374	3.848	0.193	0.298	0.489	0.308	1.581	2.819
C	0.311	3.967	0.060	2.309	0.566	0.159	0.345	2.791	1.570	2.638
D	0.158	3.897	0.468	2.099	0.831	0.501	0.293	1.169	0.585	2.224
E	0.274	4.784	0.253	0.793	1.231	0.425	0.220	0.632	1.851	2.177

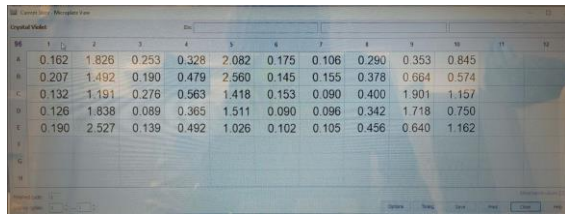
- Media only (no bacteria)
- Bacteria (*P. putida*) only
- P. putida* + Benzalkonium chloride
- P. putida* + free $\beta$ CD
- P. putida* + BOS EO
- P. putida* + BOS: $\beta$ CD complex (10 mg/mL)
- P. putida* + BOS: $\beta$ CD complex (5 mg/mL)
- P. putida* + BOS: $\beta$ CD complex (2.5 mg/mL)
- P. putida* + BOS: $\beta$ CD complex (1.25 mg/mL)
- P. putida* + BOS: $\beta$ CD complex (0.625 mg/mL)



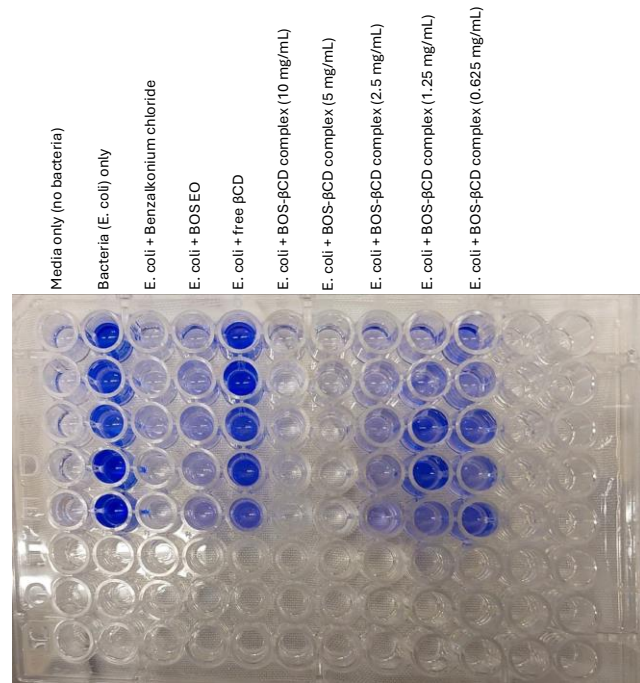
**Figure S7II.** Assay of Biofilm formation prevention against *P. putida*.

Supplementary Figure S7III.

**Biofilm formation prevention-  
*E. coli***



	1	2	3	4	5	6	7	8	9	10	11	12
A	0.162	1.826	0.253	0.328	2.082	0.175	0.106	0.290	0.353	0.845		
B	0.207	1.492	0.190	0.479	2.560	0.145	0.155	0.378	0.664	0.574		
C	0.132	1.191	0.276	0.563	1.418	0.153	0.090	0.400	1.901	1.157		
D	0.126	1.838	0.089	0.365	1.511	0.090	0.096	0.342	1.718	0.750		
E	0.190	2.527	0.139	0.492	1.026	0.102	0.105	0.456	0.640	1.162		

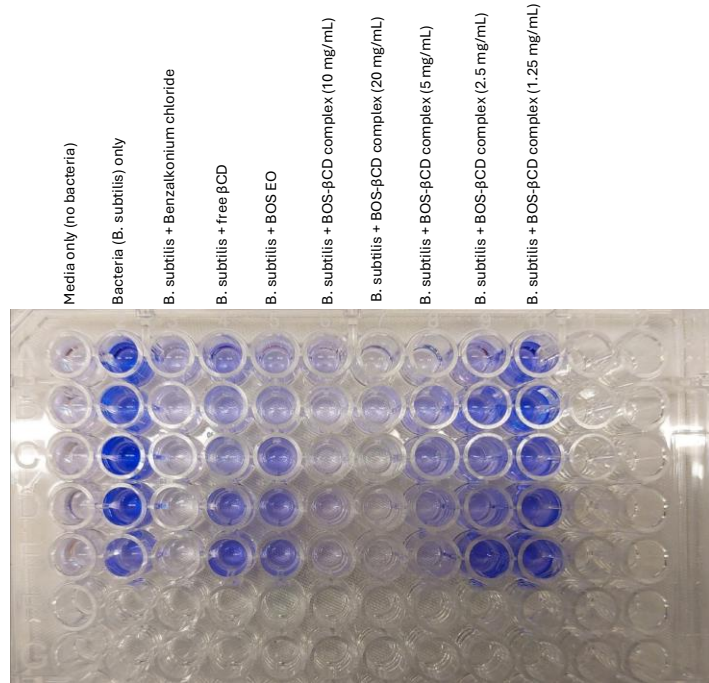


**Figure S7III.** Assay of Biofilm formation prevention against *E. coli*.

Supplementary Figure S7IV.

**Biofilm formation prevention-  
*B. subtilis***

Well	1	2	3	4	5	6	7	8	9	10
1	0.179	1.074	0.303	0.523	0.385	0.325	0.144	0.245	0.998	1.013
2	0.219	0.890	0.231	0.375	0.260	0.231	0.249	0.379	0.759	1.122
3	0.195	1.412	0.172	0.383	0.590	0.165	0.131	0.471	0.745	0.891
4	0.147	1.102	0.116	0.620	0.539	0.318	0.143	0.233	0.472	0.924
5	0.181	0.738	0.226	0.716	0.518	0.226	0.154	0.350	0.973	1.009

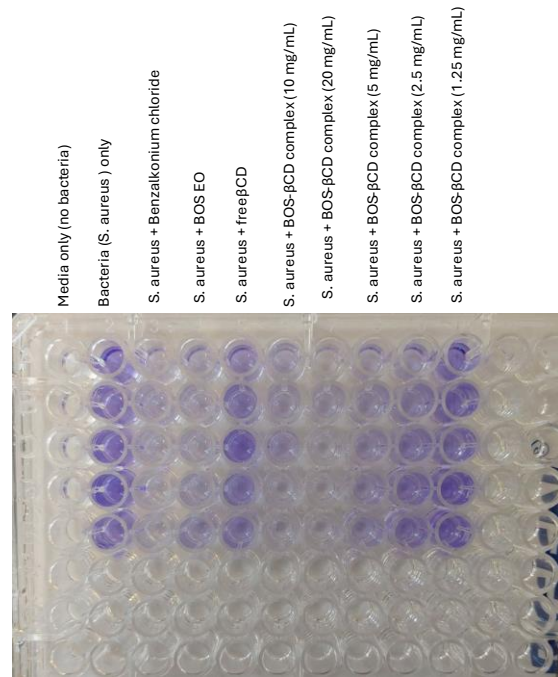


**Figure S7IV.** Assay of Biofilm formation prevention against *B. subtilis*.

Supplementary Figure S8I.

**Biofilm disruption -  
*S. aureus***

OD	1	2	3	4	5	6	7	8	9	10
A	0.070	0.825	0.172	0.278	0.525	0.344	0.147	0.255	0.319	0.933
B	0.089	0.876	0.309	0.237	0.590	0.267	0.237	0.338	0.426	0.933
C	0.094	1.003	0.169	0.242	0.881	0.355	0.143	0.344	0.537	0.706
D	0.071	1.167	0.073	0.234	0.606	0.163	0.156	0.342	0.638	1.181
E	0.087	0.830	0.152	0.366	0.518	0.199	0.155	0.369	0.660	1.038

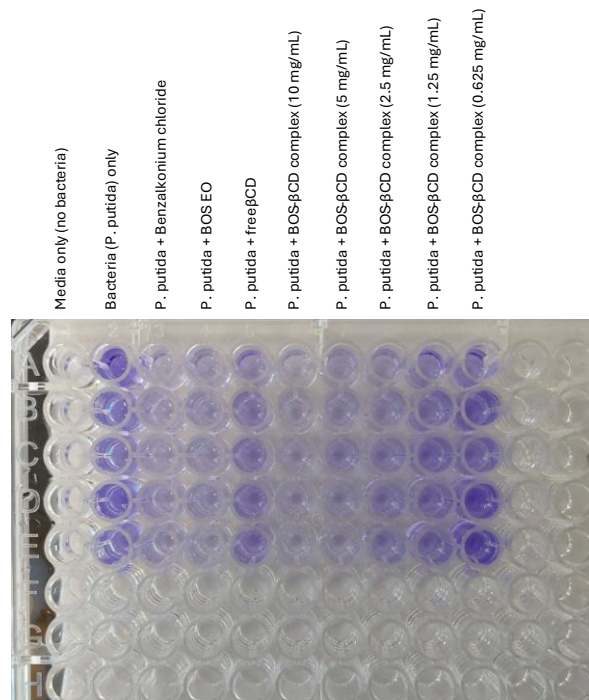


**Figure S8I.** Assay of established biofilm disruption against *S. aureus*.

Supplementary Figure S8II.

**Biofilm disruption -  
*P. Putida***

	1	2	3	4	5	6	7	8	9	10
A	0.079	1.605	0.234	0.430	0.490	0.238	0.387	0.371	0.537	0.786
B	0.077	1.030	0.254	0.426	0.558	0.269	0.358	0.357	0.622	1.194
C	0.042	0.870	0.278	0.355	0.579	0.272	0.442	0.478	0.856	0.928
D	0.082	1.396	0.269	0.386	0.639	0.247	0.334	0.504	0.719	1.689
E	0.095	0.970	0.301	0.269	0.649	0.230	0.278	0.530	0.785	1.898

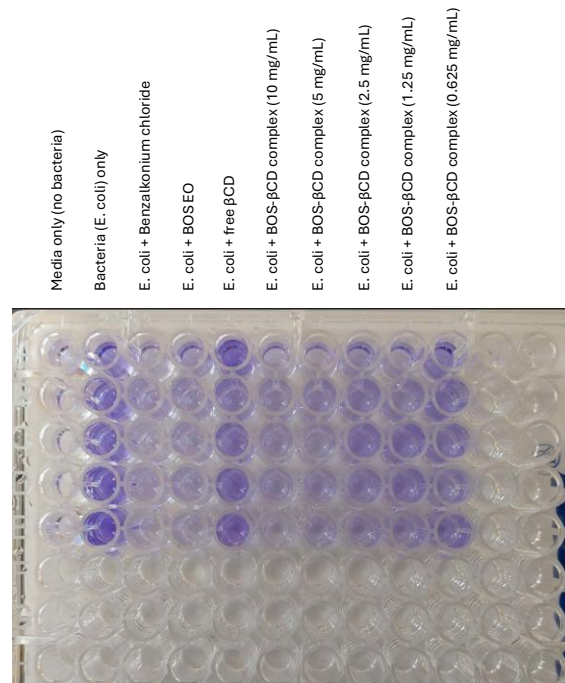


**Figure S8II.** Assay of established biofilm disruption against *P. putida*.

Supplementary Figure S8III.

**Biofilm disruption -  
*E. coli***

	1	2	3	4	5	6	7	8	9	10
0.291	0.834	0.284	0.466	1.320	0.325	0.268	0.496	0.338	0.782	
0.127	1.155	0.377	0.371	0.560	0.344	0.410	0.524	0.402	0.719	
0.114	1.094	0.199	0.311	0.635	0.331	0.233	0.611	0.636	0.606	
0.100	1.366	0.305	0.320	0.750	0.322	0.356	0.484	0.593	0.484	
0.250	1.593	0.173	0.227	0.804	0.217	0.329	0.414	0.449	0.728	



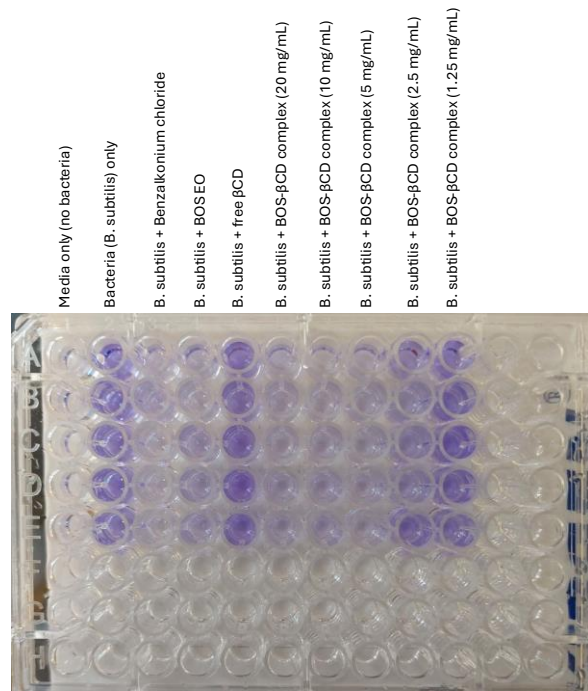
**Figure S8III.** Assay of established biofilm disruption against *E. coli*.



Supplementary Figure S8IV.

**Biofilm disruption -  
*B. subtilis***

	1	2	3	4	5	6	7	8	9	10
A	0.137	0.963	0.215	0.290	0.920	0.514	0.175	0.242	0.674	0.972
B	0.135	0.934	0.335	0.188	0.714	0.213	0.184	0.254	0.367	1.017
C	0.059	0.831	0.147	0.381	0.805	0.177	0.191	0.201	0.496	1.214
D	0.071	0.944	0.148	0.280	0.870	0.233	0.275	0.233	0.388	0.995
E	0.121	0.723	0.161	0.282	0.792	0.247	0.295	0.203	0.808	0.809



**Figure S8IV.** Assay of established biofilm disruption against *B. subtilis*.