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SHORT COMMUNICATION

Simple and Rapid Method for Detecting Biofilm Forming Bacteria

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Abstract Biofilm forming bacteria play a vital role in causing infectious diseases and for enhancing the efficiency of the bioremediation process through immobilization. Different media and conditions have been reported for detecting biofilm forming bacteria, however, they are not quite rapid. Here, we propose the use of a simple medium which can be used for detecting biofilm former, and also provide a mechanism to regulate the expression of biofilm formation process.

Keywords Bacteria · Biofilm · Bioremediation · Detection · Infections · Medium

Bacteria form biofilm primarily during infectious diseases, and in effluent treatment plants. It helps to retain a large population, which can withstand harsh environmental stress conditions [1–5]. Bacteria within the biofilm can tolerate up to 1000 times more antibiotic concentrations compared to their planktonic partners [6]. During the effluent treatment process, biofilm enables bacteria to tolerate high concentrations of salts and organic compounds. Biofilm formation can be either regulated by the phenomenon of quorum sensing (high cell density) or it may be independent of it [1, 7, 8]. There are a few methods and specific media, which allow bacteria to form biofilm: Brain heart infusion and Tryptic soy broth, etc. [9–14]. In nature, bacteria exist as communities and it is difficult to detect the biofilm formers among them. In this study, 30 different media (procured from HiMedia India) (Table S1), were screened for isolating bacteria from cattle dung at 37 °C for 24 h for their abilities to produce hydrolytic enzymes, H_2 and Polyhydroxyalkanote (PHA) from glucose and biowastes. Four bacterial strains out of 300 isolates—*Bacillus amyloliquefaciens* 16(1): (KX348272), *B. velezensis* 5(5): (KX621313), *B. tequilensis* 13(2), and *Cronobacter sakazakii* 13(3): (KX621314), were found to form biofilm exclusively on Medium 16 constituted of 20 g/L each of the Casein enzyme hydrolysate (CEH) and Mannitol (Table 1). The bacteria were identified by 16S rDNA amplification and sequencing. In order to define the medium component critical for biofilm formation, the two compounds were mixed in different ratios. The optimization of media components revealed that CEH is the important component which influences biofilm formation abilities of the bacteria. At lower CEH concentrations, biofilm formation reduced drastically (Table 2). On the other hand, reduction in Mannitol in the medium did not influence the biofilm formation process. Since glucose was to be used in further studies such as biofuel (Hydrogen) and PHA production, its effect on biofilm formation was also evaluated. It was observed that addition of glucose at the rate of 0.5 % w/v did not have any significant effect on biofilm formation. Hence, CEH alone is sufficient to detect biofilm forming bacteria (Table 2). In addition, the production of biofilm can be regulated by reducing the concentration of CEH. Thus, this medium can be exploited as a simple and rapid screening method to identify the biofilm forming bacteria.

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Table 1 Effect of media on biofilm forming capacity of bacterial strains

Medium[a]	Biofilm formation			
	Bacillus amyloliquefaciens 16(1)	*Bacillus velezensis* 5(5)	*Bacillus tequilensis* 13(2)	*Cronobacter sakazakii* 13(3)
1	NO[b]	NO	NO	NO
2	NO	NO	NO	NO
3	NO	NO	NO	NO
4	NO	NO	NO	NO
5	NO	NO	NO	NO
6	NO	NO	NO	NO
7	NO	NO	NO	NO
8	NO	NO	NO	NO
9	NO	NO	NO	NO
10	NO	NO	NO	NO
11	NO	NO	NO	NO
12	NO	NO	NO	NO
13	NO	NO	NO	NO
14	NO	NO	NO	NO
15	NO	NO	NO	NO
16	Biofilm formed	Biofilm formed	Biofilm formed	Biofilm formed
17	NO	NO	NO	NO
18	NO	NO	NO	NO
19	NO	NO	NO	NO
20	NO	NO	NO	NO
21	NO	NO	NO	NO
22	NO	NO	NO	NO
23	NO	NO	NO	NO
24	NO	NO	NO	NO
25	NO	NO	NO	NO
26	NO	NO	NO	NO
27	NO	NO	NO	NO
28	NO	NO	NO	NO
29	NO	NO	NO	NO
30	NO	NO	NO	NO

[a] Media details presented in Table S1

[b] No formation of biofilm

Table 2 Effect of media components on biofilm formation by different bacteria

Medium			Biofilm formation			
CEH (x)	M (x)	G (%)	*Bacillus amyloliquefaciens* 16(1)	*Bacillus velezensis* 5(5)	*Bacillus tequilensis* 13(2)	*Cronobacter sakazakii* 13(3)
1.0	1.0	0	High	Medium	High	High
0.5	1.0	0	Medium	Medium	Medium	Medium
0.1	1.0	0	None	None	None	Medium
0.05	1.0	0	None	None	None	Medium
0	1.0	0	None	None	None	None
1.0	0.5	0	High	Medium	Medium	High
1.0	0.1	0	High	Medium	High	Medium
1.0	0.05	0	High	Medium	High	Medium
1.0	0	0	High	Medium	High	Medium
1.0	0	0.5	Medium	Medium	Medium	Medium
1.0	0.05	0.5	Medium	Medium	Medium	Medium
1.0	0.1	0.5	High	High	High	Medium
1.0	0.5	0.5	Medium	Medium	Medium	Medium

CEH 1× Casein enzyme hydrolysate (w/v)—20 g/L

M 1× Mannitol (w/v)—20 g/L

G Glucose (w/v)

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Compliance with Ethical Standards

Conflict of interest Authors declare no conflict of interests.

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