Acta Ecologica Sinica xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Acta Ecologica Sinica

journal homepage: www.elsevier.com/locate/chnaes

Phenotyping using semi-automated BIOLOG and conventional PCR for identification of *Bacillus* isolated from biofilm of sink drainage pipes

Mohamed Azab El-Liethy *, Bahaa A. Hemdan, Gamila E. El-Taweel

Environmental Microbiology Lab., Water Pollution Research Department, National Research Centre, Dokki, Giza 12622, Egypt

ARTICLE INFO

Article history:
Received 22 October 2017
Received in revised form 18 January 2018
Accepted 22 January 2018
Available online xxxx

Keywords:
Bacillus spp.
Sink drainage pipes biofilm
BIOLOG
PCR

ABSTRACT

The presence of *Bacillus* in natural biofilms which develop in sink drainage pipes is not widely studied. Therefore, the main aim of this study was to isolate and identify *Bacillus* spp. using the BIOLOG GEN III system as a phenotypic fingerprint and polymerase chain reaction (PCR). A total of 61 biofilms samples were collected from sink drainage pipes in a kitchen and bathroom of different households in Helwan area and both laboratory and hospital collected from National Research Centre (NRC). *Bacillus* was isolated from the biofilms using HiCrome Bacillus Agar followed by isolates identification by both BIOLOG to the species level and PCR using genus specific primers to the genera level. *Bacillus* was detected in all tested biofilm samples (61 samples). The highest counts were observed in hospital sink drainage pipes (10^5 CFU/10 cm^2) while; the lowest counts were observed in both bathroom and laboratory sink drainage pipes (10^2 CFU/10 cm^{-2}). In total, 61% *Bacillus* isolates were identified by BIOLOG while, 67% isolates were confirmed by PCR. The diversity of *Bacillus* among species level using BIOLOG were 34% *B. cereus*, 23% *B. subtilis ss subtilis*, 17% *B. thuringiensis*, 16% *B. licheniformis* and 13% *B. amyloliquefaciens*. It can be concluded that; PCR is more sensitive than BIOLOG for identification of *Bacillus*. However, BIOLOG can identify *Bacillus* at species level and test 94 carbon and chemical sources on a microplate in one shot. Thus, the combination between phenotyping by BIOLOG and molecular approaches such as PCR for identification of bacterial isolates is recommended.

1. Introduction

Bacillus species are Gram-positive, strictly aerobic or facultative anaerobic encapsulated bacilli. They produce endospores that unusually resistant to unfavorable conditions [1]. *Bacillus* is frequently detected in natural mineral, ground and drinking waters [2]. Also, many *Bacillus* species including; *B. subtilis*, *B. cereus*, *B. anthracis* and *B. thuringiensis* have been detected in wastewater and sludge and they are able to form biofilm [3–6]. Biofilms that develop in drainage pipes usually harbor large microbial populations which may include, bacterial indicators and some pathogenic microbes such as *E. coli*, *Salmonella*, *Staphylococci*, *Pseudomonas*, *Listeria*, *Bacillus* and *Candida* spp. [6,7]. When these biofilms form and colonize in kitchens and a bathroom sinks where there are great changes of human exposure to them, this could represent, a potential health threat due to the wide diversity of microbial pathogens that could be found in them [3,6,8]. Humans could be exposed to these potential microbial pathogens through direct routes such as the handling, preparing and eating of food and/or indirectly through touching contaminated surfaces that contain huge numbers of these microbes [9,10]. Traditional culture based methods involving the morphological characterization of colonies, Gram staining followed by biochemical and serological tests; may provide useful information about the structures of microbial communities. These methods have numerous disadvantages as they are time-consuming, laborious and provide ambiguous results due to differences in the culture and environmental conditions [11–13]. Thus, recently great attention has been focused on the application of the BIOLOG system for the identification and characterization of over 2900 species of bacteria, yeasts and fungi [14]. This system can also give phynotyping fingerprint and full picture by testing 94 different carbon and chemical utilizing sources only after incubation period extended from 4 to 24 h [15,16]. Molecular methods like PCR are still considered as rapid and sensitive tools for identification of microorganisms in water and biofilm environmental samples [17,18]. Therefore, the scope of this study is to identify *Bacillus* species isolated from biofilm of sink drainage pipes by using both BIOLOG and PCR.

* Corresponding author at: Environmental Microbiology Lab., Water Pollution Research Department, Environmental Research Division, National Research Centre, Dokki, Giza 12622, Egypt.
E-mail address: ma.el-liethy@nrc.sci.eg (M.A. El-Liethy).

https://doi.org/10.1016/j.chnaes.2018.01.011
1872-2032/

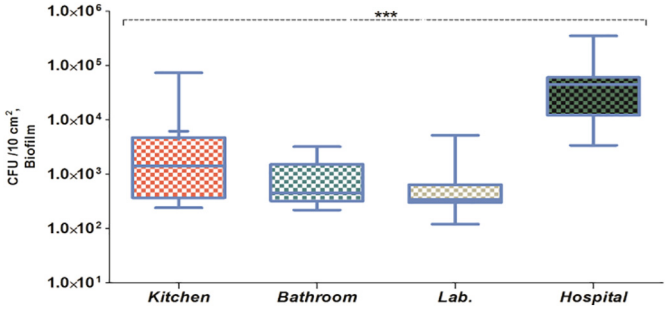

Fig. 1. Counts of *Bacillus* spp. in biofilms from different sink drainage pipes.

2. Material and methods

2.1. Biofilm sampling and preparation

Sixty one natural biofilm samples were aseptically scraped from bathroom, kitchen, laboratory and hospitals sink drainage pipes. The natural biofilm samples were preserved in ice box and immediately transferred to the Environmental Microbiology Lab, Water Pollution Research Dept., National Research Centre (NRC). The biofilm samples were harvested by scraping 10 cm^2 from the inner surface of pipes using sterile cotton swabs. Swabs were submerged into tubes each containing 10 ml sterile water and homogenized by using vortex agitator for 5 min [19].

2.2. Isolation of Bacillus spp. from biofilm samples of sink drainage pipes

Enumeration of *Bacillus* was carried out by transferring 100 μl of a suitable dilution of biofilm samples under aseptic condition onto HiCrome Bacillus Agar (HiMedia, India). Two typical *Bacillus* colonies form each sample were isolated and kept on tryptic soy broth (TSB) (BD, Germany) at −40 °C for phenotyping and PCR confirmation.

2.3. Phenotyping identification of Bacillus isolates using BIOLOG GEN III

The kept *Bacillus* colonies were inoculated into TSB and incubated at 37 °C for 24 h. A loopful of the 24 h culture was streaked into tryptic soy agar (TSA) (BD, Germany) plates and incubated at 37 °C for 24 h. Following incubation, a single colony was picked using a sterile disposable inoculator swab and inoculated into 10 ml of inoculating fluid (IF-A) (Biolog Inc., USA). The inoculated IF-A was dispensed into 96 wells of a microplate (100 μl per well) using a multichannel repeating pipettor. The microplate was incubated at 37 °C for 24 h. The reading was carried out automatically by the computerized MicroStation™ system (Biolog Inc., USA) with the fingerprint data which was previously fed into the software (OmniLog® Data Collection) and used to identify the bacteria from their phenotypic patterns in the GEN III MicroPlate.

2.4. Confirmation of Bacillus isolates by PCR

2.4.1. Bacterial preparation and DNA extraction of biofilm Bacillus isolates

The preserved *Bacillus* isolates were resuspended on TSB for identification and kept for 18–24 h at 37 °C. Following incubation, the enriched bacterial suspension was inoculated again into TSB for 3–4 h at 37 °C. The wavelength of the enriched bacterial cultures was measured using a spectrophotometer (Cary 100 UV/Vis, Agilant) at 460 nm. After that, 1 ml of the enriched bacterial cultures was centrifuged at 12,500 rpm for 5 min at 7 °C. The resulting pellet was obtained and used for DNA extraction using the Presto™ Mini gDNA bacterial kit (Geneaid, Taiwan) according to the manufacturer's instructions.

2.4.2. The PCR amplification and condition of Bacillus isolates

The primer pairs used in PCR to identify *Bacillus* isolates were BacF "AGGGTCATTGGAAACTGGG" and BacR "CGTGTTGTAGCCCAGGTCATA" [20]. The primers used in this study were synthesized by Macrogen Co. (Soul, Republic of Korea). The PCR assay was performed in a total volume of 20 μl consisting of 4 μl of 1× FIREPol® Master Mix Ready to Load with 12.5 mM $MgCl_2$ (FIREPol, Estonia), 0.5 μl of each primer (final concentration, 10 pmol), 12.5 μl of nuclease free water and 2.5 μl of template DNA. The PCR was carried out under the following conditions: 94 °C for 5 min followed by 35 cycles of 94 °C for 1 min, 65 °C for 1 min and 72 °C for 2 min, and final extension at 72 °C for 10 min. The amplified products were analyzed by agarose gel electrophoresis. Gels were stained with ethidium bromide (0.005%, w/v) and visualized under a UV transilluminator with UVP BioDoc-it Imaging System (UVP, UK).

Table 1
The biochemical analysis of *Bacillus* isolates using BIOLOG GEN III.

Properties	Results	Properties	Results	Properties	Results	Properties	Results
Negative control	−	A-D-glucose	+	Gelatin	−	P-hydroxy-phenylacetic acid	−
Dextrin	+	D-mannose	+	Glycyl-L-prolline	+	Methyl pyruvate	+
D-maltose	+	D-fractose	+	L-alanine	+	D-lactic acid methyl ester	+
D-trehalose	+	D-galactose	+	L-arginine	+	L-lactic acid	+
D-cellobiose	+	3 methyl glucose	−	L-aspartic acid	+	Citric acid	+
Gentiobiose	+	D-fucose	+	L-glutamic acid	+	A-keto-glutaric acid	+
Sucrose	+	L-fucose	−	L-histidine	+	D-mallc acid	−
D-turanose	+	L-rhamnose	+	L-pyroglutamic acid	+	L-mallc acid	−
Stachyose	+	Inosine	+	L-Serine	+	Bromo-succinic acid	+
Positive control	+	1%sodium lactate	+	Lincomycin	−	Nalidixic acid	−
pH 6	+	Fusidic acid	−	Guanidine HCl	+	Lithium chloride	+
pH 5	+	Serine	−	Niaproof 4	−	Potassium tellurite	+
D-raffinose	+	D-sorbitol	+	Pecin	−	Tween 40	+
α-D-lactose		D-mannitol	+	D-galacturonic	+	γ-Amino-butryric acid	+
D-mellbiose	+	D-arabitol	+	L-galactonic acid lactone	−	α-Hydroxy-butyric acid	−
β-Methyl-D-glucoside	+	Myo-inostol	+	D-gluconic acid	+	β-Hydroxy-D,L-butyric acid	−
D-sallcin	+	Glycerol	+	D-glucuronic acid	+	A-keto-butyric acid	−
N-acetyl-D-glucoseamine	+	D-Glucose 6-PO_4	−	Glucuronamide	−	Acetoacetic acid	+
N-actyl-β-D-mannosamine	−	D-Fructose 6-PO_4	−	Mucic acid	+	Propionic acid	−
N-actyl-D-galactosamine	−	D-aspartic acid	+	Quinic acid	−	Acetic acid	−
N-acetyl neuraminic acid	−	D-serine	−	D-saccharic acid	+	Formic acid	+
1%NaCl	+	Troleandomycin	−	Vancomycin	−	Aztreonam	−
4%NaCl	+	Rifamycin SV	−	Tetrazollum violet	−	Sodium butyrate	+
8% NaCl	+	Minocyclline	−	Tetrazollum blue	−	Sodium bromate	−

Please cite this article as: M.A. El-Liethy, et al., Phenotyping using semi-automated BIOLOG and conventional PCR for identification of *Bacillus* isolated from biofilm of sink drainage pipes..., Acta Ecologica Sinica (2018), https://doi.org/10.1016/j.chnaes.2018.01.011