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Short communication

A novel Aroclor 1242-degrading culturable endophytic bacterium isolated from tissue culture seedlings of *Salix matsudana* f. *pendula* Schneid

Man Cai[a,b,1], Ge Song[a,b,1], Yuling Li[a,b], Kejiu Du[a,b,*]

[a] Agricultural University of Hebei, Baoding 071000, China
[b] Provincial Key Laboratory of Tree Species Germplasm Resources and Forest Protection of Hebei, Baoding 071000, China

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ABSTRACT

With this study, we aimed to isolate the most promising bacteria to enhance persistent environmental pollutants (Aroclor 1242) phytoremediation from *Salix. matsudana* f. *pendula* Schneid, which has wide distribution and adaptability characteristics. A total of 12 endophytic bacteria strains were isolated and purifed from both *Salix. matsudana* f. *pendula* Schneid and its tissue culture seedlings. Of which, the strains CGL-1, SGL-1, SPL-3, 8, 17 and 24 were further isolated by enrichment culture with Aroclor 1242 as the sole source of carbon and energy. Compared with the growth characteristic of these strains, strain CGL-1 growth well under higher concentration of Aroclor 1242 exposure than others. The strain CGL-1 was identified as *Enterobacter* sp. with respect to 16S ribosomal DNA sequences, colony morphology and cell morphological characteristics. The optimum culture condition of the CGL-1 for removing Aroclor 1242 was optimized as: 27–32 °C, pH 7.0, NaCl ≦ 1%, inoculum 10%. Under these conditions, the removing ratio could reach to 43.2% after 7 d. The strain CGL-1 appears to be the most promising bacterium to enhance Aroclor 1242 phytoremediation. The endophytic bacterium is potentially useful for helping plants to tolerate pollutant stress and for increasing bioremediation resources.

1. Introduction

Polychlorinated biphenyls (PCBs) are a large group of persistent environmental pollutants (Brázová et al., 2012; Murínová et al.,2014a). Since 1920, due to their extremely stable physical and chemical properties, PCBs have been widely used in many fields such as transformers, plasticizers, lubricants, flame retardants and so on (Chamkasem et al., 2016; Klees et al., 2015; Polak et al., 2016). PCBs were entered in soils and the food chain during their manufacture, use and disposal. PCBs have been reported to cause skin toxicity, reproductive toxicity and carcinogenicity in animals and human (Ottinger et al., 2009; Xiao et al., 2010; Li et al., 2013). Because of their toxicity and persistence in the environment, PCBs have been banned in most countries in 1979. In spite of this, today PCBs are still emitted from several sources, such as leaks of existing equipment (e.g., electrical capacitors and ballasts), volatilization from dredging sediments, and sewage sludge soil application (Aken et al., 2010). The problem of PCBs residual is of great social importance to environmental hazard (Alcock et al., 1995).

The microbial degradation is considered as an efficient and cost-effective process for the decontamination of PCBs (Li et al., 2016; Muir and Norstrom, 2000; Borja et al., 2005; Tandlich et al., 2011). Many PCB-degrading bacteria have been isolated from long-term contaminated soil and sediments (Bopp, 1986; Furukawa and Miyazaki, 1986; Bedard et al., 1987; Erickson and Mondello, 1993; Gibson et al., 1993; Seto et al., 1995; Shimura et al., 1999; Goris et al., 2004). These strains only existed in typical contaminated soil, sediments, inhibited by environment restriction and native antibacterial material. In contrast to those soil microorganisms, endophytic bacteria occur naturally in the internal tissues of plants, be beneficial to overcome certain environmental stresses (Sessitsch et al., 2002; Lucía and Ana, 2013).

In this study, Aroclor 1242 with an average of 3 chlorines per biphenyl molecule containing 42% chlorine by weight (Yadav et al., 1995) was selected as a test compound for screening the functional endophytic bacteria from tissure culture seedlings of *Salix. matsudana* f. *pendula* Schneid. These endophytic bacteria are potentially useful for increasing bioremediation resources.

2. Materials and methods

2.1. Chemicals

Aroclor 1242 (AccuStandard Inc, NH, USA, CAS 53469-21-9) was

* Corresponding author at: Agricultural University of Hebei, Baoding 071000, China.
E-mail address: dukejiu@126.com (K. Du).
[1] Man Cai and Ge Song both authors contributed equally to the work.

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Table 1
Colony morphology of strains.

Strains	Medium	Origin	Size (mm)	Morphological	Surface Appearance	Height	Transparency	Color	Margin
CGL-1	GSA	tissue culture seedling	1	circular	rough	platform	opaque	white	fan edge shape
SGL-1	GSA	garden plant	0.5–1	irregular	rough	flat	opaque	brown	radial
SPL-3	PDA	garden plant	1–2	circular	smooth	convex	opaque	white	shipshape
SNL-4	NA	garden plant	0.5–2	circular	smooth	convex	opaque	gold	shipshape
5	NA	leaves from garden plant	1–4	irregular	rough	convex	opaque	oyster white	slightly undulate edge
6	NA	stems from garden plant	0.5–5	concentric circle	smooth	flat	opaque	pink	shipshape
8	NA	roots from garden plant	1–3	irregular	rough	flat	opaque	oyster white	jaggies
17	PDA	stems from garden plant	0.5–1	irregular	rough	flat	opaque	grey	radial
19	GSA	roots from garden plant	1–2	circular	smooth	convex	opaque	light yellow	shipshape
23	PDA	leaves from garden plant	1–3	irregular	rough	flat	opaque	oyster white	jaggies
24	PDA	stems from garden plant	1–4	circular	smooth	concave	transparent	white	shipshape
25	PDA	roots from garden plant	1–5	circular	smooth	convex	opaque	gold	shipshape

diluted with acetone to 20 mg/mL before use. All standards and solutions were stored hermetically sealed in amber glass vials at 4 °C until used. All chemicals and reagents used in this experiment were of analytical reagent grade or better.

2.2. Plant materials

Tissues of roots, stems and leaves were obtained from the 20-years old *S. matsudana* f. *pendula* Schneid in Agricultural University of Hebei, and its tissue culture seedlings were obtained from the phytoremediation lab of Agriculturl University of Hebei.

2.3. Culture medium

Plant materials were incubated on Nutrient Agar (NA) Media to isolate culturable endophytic bacteria. NA medium was supplemented with peptone (10 g/L), beef extract (3 g/L), NaCl (5 g/L), and 1.5% agar. The pH was adjusted to 7 before sterilization at 121 °C for 30 min.

2.4. Isolation and identification of Aroclor 1242-degrading culturable endophytic bacteria

Plant materials were subjected to surface sterilization by sequential steps. The stems, roots and leaves were washed with tap water and distilled water and dried on absorbent paper. After surface sterilization with 70% alcohol for 1 min, then treated with 5.5% NaClO for 15–20 min, separately washed with sterile distilled water at least 6 times. The collected roots, stems and leaves samples were cut into small pieces (about 1 cm length) under aseptic conditions using a sterile scalpel. These materials were spread on plates containing bacterial growth basal medium (NA) described by and Barraquio et al. (1997). The last time washed water was used as a negative control. Tissue culture seedlings were grinded fully with sterile water; the leach liquor was spread on plates containing bacterial growth media, incubated at 30 °C for two days. If bacterial growth occurred, isolating and purifying them by streaking inoculation until single colonies occurred. Saves strains at −60 °C.

The identification of Aroclor 1242-degrading endophytic bacteria were firstly examined by morphological properties including the shape of the cell, Gram-stain, the presence of spores, and colony morphology on nutrient agar medium.

2.5. 16S rDNA and phylogenetic analysis

Bacteria isolated from plant materials as above were used for colony PCR to amplify 16S rDNA sequences using universal primer pair 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492r (5′-GGTTACCTTGTTACGACTT-3′). PCR was performed in a 25 μL mixture containing 1 U Taq polymerase, 20 pmol of each primer, and 10 nmol of dNTP. The PCR conditions include; initial denaturation (95 °C for 5 min); 30 cycles [94 °C for 50 s, 55 °C for 1 min, 72 °C for 2 min] and a final extension at 72 °C for 10 min. The PCR products were purified using PCR purification kit (Real Jimer) and sent to BGI Sequencing, Inc. (Beijing, China) for sequencing. The sequences were analyzed by BLAST of the NCBI to find the closest matches. The multiple sequence alignment was generated using the Clustalx 1.83 program. The phylogenetic dendrogram was generated by MEGA 5.0 software with a bootstrap consensus of 1000 replicates.

2.6. Temperature, inoculum density, pH and salt optimum for bacteria growth

Strain CGL-1 was subcultured in liquid minimal medium added 20 mg/L Aroclor 1242 at different temperature (22 °C, 27 °C, 32 °C, 37 °C and 42 °C), with various inoculums (1%, 5%, 10%, 15% and 20% the amount of the bacterium of OD600 = 1), various pH (4, 5, 6, 7, 8, 9 and 10) and various concentration of NaCl (1%, 2%, 3%, 4%, 5%, 6%, 7% and 8%). After 5–7 days of incubation (32 °C, 200 rpm), the concentration of bacterium was measured (OD600).

2.7. Aroclor 1242 detection

The bacterium (OD600 = 0.1) was cultured in liquid minimal media with or without 20 mg/L Aroclor 1242 on a rotary shaker 200 rpm for 5–7 d at 32 °C. After 5–7 d of cultivation, Aroclor 1242 of

Table 2
Cell morphological characteristics of strains.

Strains	Simple stain	Gram staining	Spore	Motility	Size (μm)
CGL-1	Bacillus	G^-	without	no	2–3
SGL-1	Bacillus	G^-	without	no	0.5–1.3
SPL-3	Bacillus	G^+	without	no	1–3
SNL-4	Coccus	G^+	without	no	1–5
5	Bacillus	G^+	exist	no	1–5
6	Bacillus	G^+	without	no	2–5
8	Bacillus	G^+	exist	no	1–6
17	Bacillus	G^+	exist	no	2–3
19	Coccus	G^+	without	no	2–15
23	Bacillus	G^+	exist	no	1–6
24	Coccus	G^-	without	no	1
25	Coccus	G^+	without	no	1–2

+, positive reaction; −, negative reaction.