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FULL LENGTH ARTICLE

Aquatic *Bacillus cereus* JD0404 isolated from the muddy sediments of mangrove swamps in Thailand and characterization of its cellulolytic activity

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KEYWORDS

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Abstract This study aimed to conduct the isolation, screening and identification of bacteria with a high level of cellulolytic activity from the muddy sediments of mangrove swamps in Thailand. One hundred and ninety aquatic bacterial isolates were isolated from different muddy sediments and eighty one isolates were determined to be cellulolytic bacteria. The cellulolytic bacterium identified as *Bacillus cereus* JD0404 showed maximum hydrolysis activity on carboxymethylcellulose agar plates. Its cellulolytic performance for CMCase activity, Avicelase activity and β-glucosidase activity was 1.778 ± 0.003 U/mL, 0.079 ± 0.001 U/mL and 0.048 ± 0.002 U/mL, respectively. The optimum temperature and pH for the enzyme activity were determined to be 50 °C and 7.0 respectively. The cellulolytic activity was greatly enhanced by Mn^{2+} and considerably inhibited by EDTA and toluene. Preliminary bioconversion application showed that the *B. cereus* JD0404 could be used for the hydrolysis of cellulose-based biomass. This study demonstrated a feasible bacterium for environmentally friendly industries and biotechnology.

Introduction

Mangrove swamps or mangrove forests are the unique coastal wetland ecosystems which are found along tropical and sub-tropical coastlines dominated by halophilic plants in the genera *Rhizophora* and *Avicennia* (Mitsch and Gosselink, 2015). The mangrove swamps and neighbouring coastal environments provide many ecological benefits (Ghosh et al., 2010; Sandilyan and Kathiresan, 2014), including coastal protection against natural disasters, storage of organic material, habitats for estuarine organisms and mitigation of the global warming phenomenon. Mangrove ecosystems can store large amounts of organic carbon and are rich in organic carbon in sediments which mainly originated from litter falls and the underground roots of mangrove plants (Yong et al., 2011). The mangrove microbial communities play a vital role in the organic carbon cycle. Cellulolytic microorganisms can perform the degradation of cellulose-based plant litter, resulting in the production of simple-sugar derivatives in the sediments (Soares-Júnior et al., 2013). Microbial cellulolytic enzymes, called cellulase, are the complex enzymes that consist of endoglucanases

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(E.C. 3.2.1.4), exoglucanases (E.C. 3.2.1.91, and E.C. 3.2.1.176) and β-glucosidases (E.C. 3.2.1.21) which synergistically work to hydrolyse the β-1, 4 glycosidic bonds of cellulose. Cellulase have become focal biocatalysts in various green technology industries, such as textile production, detergent composition, food processing, animal feed production, removal of bacterial biofilm and bioconversion for biofuel production (Juturu and Wu, 2014). Most cellulolytic enzymes isolated from mangrove swamps and related aquatic environments are produced from fungi belonging to the genera *Cladosporium*, *Alternaria* and *Byssochlamys*. (Alsheikh-Hussain et al., 2014; Matondkar et al., 1980; Thatoi et al., 2013), and bacteria belonging to the genera *Micrococcus*, *Bacillus*, *Pseudomonas*, *Xanthomonas* and *Brucella* (Behera et al., 2014). Interestingly, Thailand is one of the Asian countries that contain large areas of mangrove swamps (Sandilyan and Kathiresan, 2014), but the knowledge of cellulolytic microbes isolated from mangrove swamps is limited (Behera et al., 2014). For this study, the aquatic bacteria demonstrating cellulolytic performance were isolated from muddy sediments of mangrove swamps in Thailand and identified. The purpose was to determine a competent aquatic cellulolytic bacterium for possible use in green technology industries and related biotechnological applications.

Materials and methods

Description of sampling sites

Muddy sediment samples were collected from mangrove swamps in Rayong River (12° 39′ 57.40″ N, 101° 14′ 30.79″ E), Rayong Province, Thailand (Fig. 1). Samples were collected twice during the rainy season, once in July 2014 and another in September 2014. Muddy sediments close to the roots of mangrove plants or beneath the decomposed plant litter at a depth of 0–15 cm were taken from sampling sites at different locations, placed in sterile zip-lock plastic bags and kept at 4 °C.

Isolation and purification of mangrove bacteria

The isolation of mangrove bacteria and media was modified from Dias et al. (2009). The samples were serially diluted with sterile normal saline solution (0.85% NaCl) within 24 h of collection to obtain 1:10,000 dilutions. One hundred microlitres of each diluted sample was spread plated on Tryptone Soya Agar (Himedia, India) supplemented with 1.8% NaCl and incubated at 28 °C, the average sediment temperature of the sampling sites, for 48 h. The agar plates were investigated in terms of colony morphology including shape, margin, elevation and pigmentation. Morphologically dissimilar colonies were selected and streak plated on Tryptone Soya Agar to obtain pure colonies.

Screening of cellulolytic bacteria

Screening of the cellulolytic bacteria was conducted from that previously described (Kasana et al., 2008) using carboxymethylcellulose (CMC) agar plates and Gram's iodine staining method. Five microlitres of overnight growth culture in the Tryptone Soya Broth (Himedia, India) of each bacterial isolate was spot plated on CMC agar (0.2% $NaNO_3$, 0.1% K_2HPO_4, 0.05% $MgSO_4$, 0.05% KCl, 0.2% CMC sodium salt, 0.02% peptone and 1.7% agar). The agar plates were incubated at 28 °C for 48 h and then flooded with Gram's iodine solution for 10 min. The cellulolytic isolates were detected by the cellulolytic zone around the colonies after Gram's iodine staining. The hydrolysis capacity (HC) value that determined the cellulolytic activity was calculated from the ratio between the diameter of the cellulolytic zone and the diameter of the bacterial colony. The negative control for screening was the

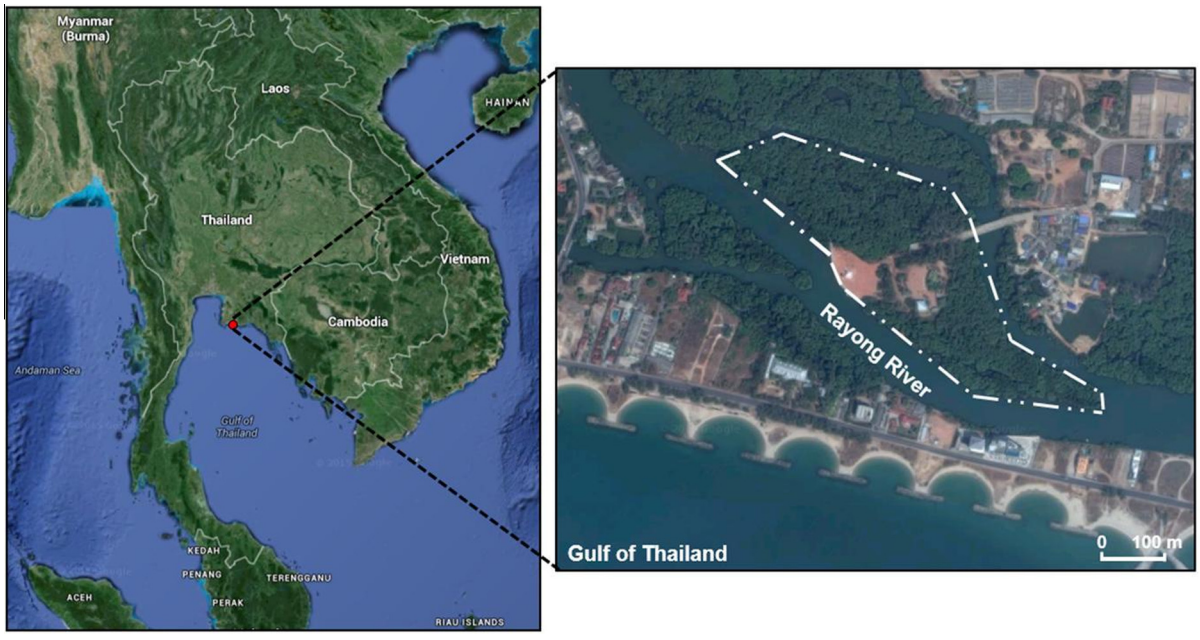

Figure 1 Map of mangrove swamps in Rayong River. The sampling site covered an area of 75,400 m^2. The figure was generated using the Google Maps service.