Research in Microbiology 166 (2015) 408–418

www.elsevier.com/locate/resmic

Original article

Degradation of abamectin by newly isolated *Stenotrophomonas maltophilia* ZJB-14120 and characterization of its abamectin-tolerance mechanism

Yuan-Shan Wang [a,b], Xing-Chang Zheng [a,b], Qi-Wei Hu [a,b], Yu-Guo Zheng [a,b,*]

[a] *Institute of Bioengineering, Zhejiang University of Technology, No. 18, Chaowang Road, Hangzhou, Zhejiang 310014, PR China*

[b] *Engineering Research Center of Bioconversion and Biopurification of Ministry of Education, Zhejiang University of Technology, No. 18, Chaowang Road, Hangzhou, Zhejiang 310014, PR China*

Received 28 December 2014; accepted 20 April 2015
Available online 6 May 2015

Abstract

An abamectin (ABM)-degrading bacterium, *Stenotrophomonas maltophilia* ZJB-14120, was isolated and identified. This strain is capable of degrading 84.82% of ABM at an initial concentration of 200 mg/L over a 48 h incubation period. This strain showed efficient biodegradation ability (7.81 mg/L/h) to ABM and high tolerance (1000 mg/L) to all macrolides tested. In addition to ABM, emamectin, erythromycin and spiramycin can also be degraded by this strain. Modifications involving either reduction of the double bond between C22–C23 or replacement of the C25-group of ABM with a cyclohexyl group can completely inhibit biodegradation of ABM. The ABM-degrading capability of strain ZJB-14120 is likely to be intrinsic to its metabolism and could be inhibited by incubating with erythromycin, azithromycin, spiramycin or rifampicin. A new and successive degradation pathway was proposed based on metabolite analysis. Although there is evidence for metabolite inhibition, this strain has high ABM degradation activity and reusability. Further investigation showed that activated macrolide efflux pump(s) and an undetermined mechanism for regulating the intracellular ABM concentration are responsible for normal uptake of essential metabolites while pumping out excess harmful compounds. Strain ZJB-14120 may provide efficient treatment of water and soil contaminated by toxic levels of abamectin and emamectin.

Keywords: Bioremediation; *Stenotrophomonas maltophilia*; Macrolides; Tolerance; Drug efflux pump

1. Introduction

Abamectin (ABM), also called avermectin (AVM), is a series of natural macrocyclic lactones produced by *Streptomyces avermitilis*. It is commercially available as a mixture of avermectin B1a (>90%) and avermectin B1b (<10%) [1,2]. ABM is used worldwide in animal husbandry and agriculture as a broad-spectrum, highly effective antiparasitic agent and acaricide [3,4]. In the past decades, many commercial abamectin derivatives with new bioactivities and higher stability, e.g. ivermectin, emamectin and doramectin, were developed through chemical modification of ABM [5–7]. Recently, ABM was found to have the potential to reduce malaria transmission by killing *Anopheles* mosquitoes [8].

The toxicity of macrocyclic lactones in terrestrial and aquatic environments, and specifically, the chronic ecotoxicological impact on invertebrates as well as plants and algae, has been reviewed in recent years [9]. In contrast to low ABM toxicity documented for mammals, earthworms and birds [10], ABM has been shown to be extremely toxic to bees [11] and aquatic organisms, especially fish (causing degenerative changes to brain, kidney, and liver function) and daphnids (Supplementary Table 1). ABM could be classified as very

* Corresponding author. Institute of Bioengineering, Zhejiang University of Technology, No. 18, Chaowang Road, Hangzhou, Zhejiang 310014, PR China.
E-mail addresses: yuanshan@zjut.edu.cn (Y.-S. Wang), zhengxingchang@qq.com (X.-C. Zheng), hudei92@sina.com (Q.-W. Hu), zhengyg@zjut.edu.cn (Y.-G. Zheng).

http://dx.doi.org/10.1016/j.resmic.2015.04.002
0923-2508/

toxic to aquatic organisms according to criteria for the EU classification of dangerous substances (EC 2001) [12,13].

Generally, the commercially available products of ABM used in agriculture and animal farming are dissolved in the presence of co-solvents prior to use due to the poor solubility of pure ABM in water [14]. Therefore, the readily available commercial form poses a threat to aquatic environments with the development and increased utilization of ABM and its derivatives.

Macrocyclic lactones such as erythromecin may be degraded during composting, anaerobic digestion, manure storage and soil [15]. Degradation of ABM in terrestrial environments is mainly caused by photodegradation and aerobic degradation via the metabolic activity of soil microbes [10]. However, ABM could bind strongly to feces in their non-metabolized form. This can significantly delay its degradation in soil-feces [16]. Escalada et al. conducted a kinetic study of ABM photodegradation which indicated that natural light was not sufficient to induce chemical transformations of this molecule [17]. Hence, bioremediation could be a promising alternative tool for removal of ABM in the environment because of its cost effectiveness, inherent eco-friendly characteristics and potential for complete decomposition of harmful compounds. Biodegradation of environmental contaminants has been widely reported and was shown to be highly superior to other methods [18–20]. Despite these advantages, only a few reports are available on the biological degradation of ABM, and only two bacterial strains, *Bacteroidetes endosymbiont* strain LYH [21] and *Burkholderia* sp. GB-01 (ultimately identified as *Burkholderia diffusa* species) [22–24], are capable of sustained growth in the presence of ABM as sole carbon source. Thus far, no report is available for the application of macrolide-tolerant bacteria in biodegradation of ABM, nor in bioremediation. Here we report the biodegradation of ABM by a macrolide-tolerant strain ZJB-14120 that we isolated from soil. Assays were performed to determine a pathway for biodegradation and to test a hypothetical mechanism for ABM tolerance of strain ZJB-14120.

2. Materials and methods

2.1. Chemicals and culture media

Avermectin (16-membered-ring macrolide, 97%) was generously supplied by Zhejiang Qianjiang Biochemical Co., Ltd. (China) and maintained at 4 °C. Ivermectin (97%), doramectin (98%), emamectin (90%), erythromycin (14-membered-ring macrolide, 95%), azithromycin (15-membered-ring macrolide, 98%), spiramycin (16-membered-ring macrolide, 90%) and rifampicin (ansa macrolide, 97%) were purchased from Aladdin Chemical Reagent Co., Ltd. (China). Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), which is used to qualitatively assay the protonmotive-force-dependent pump [25], was purchased from J&K Chemical Ltd. (China). All other chemicals were of analytical grade and commercially available. The mineral salts medium (MSM; pH 7.0) used in microbial enrichment steps, isolation procedures and degradation assays consisted of the following compounds per liter of distilled water: NH_4NO_3 1.0 g, K_2HPO_4 1.5 g, KH_2PO_4 0.5 g, NaCl 1 g, $MgSO_4 \cdot 7H_2O$ 0.1 g [22]. Luria-Bertani medium (LB) was used for cell growth. The *Stenotrophomonas maltophilia* isolate was routinely maintained on LB agar (2% w/v) plates. Antibiotic tolerance was determined using Mueller-Hinton (MH) medium from Aladdin Chemical Reagent Co., Ltd. (China) according to the manufacturer's instruction. All media were sterilized at 121 °C for 20 min.

2.2. Soils

For bacterial isolation, soil samples were collected from surface layer (0–10 cm) soils in vegetable fields or rice fields in Hangzhou, China. In these fields, ABM was widely applied to control pests such as *Cnaphalocrocis medinalis* and *Pieris rapae* Linne. All soil samples were sieved through a 2-mm mesh before use.

2.3. Isolation and identification of ABM-degrading strain ZJB-14120

For isolation of ABM-degrading bacteria, a selective enrichment method was used. Briefly, 1 g of soil was added to flasks that contain 30 mL of MSM with 200 mg/L ABM. After incubation at 30 °C and 150 rpm in the dark for 2 days, 2 mL of the growing culture were transferred to fresh MSM with 400 mg/L ABM and incubated as described above. After two cycles, the enrichment broth were sequentially diluted and plated on LB agar plates with 200 mg/L ABM and grown at 30 °C in the dark for 2 days. After three repeats, pure culture colony was obtained. The colonies were picked up and transferred into MSM with 400 mg/L ABM and then cultivated in the dark at 30 °C, 150 rpm for 2 days. The cells were harvested by centrifugation and their ABM degradation ability was evaluated. Strain ZJB-14120 which exhibited maximum degradation ability, was selected for further study. Morphological characterization was performed after 24 h incubation on LB agar plate. Cell morphology was observed with a Leica DM4000 B light microscope (Leica Microsystems, Germany). The physiological and biochemical characteristics of strain ZJB-14120 were identified using a standardized micromethod with bioMérieux GN card according to the manufacturer's instructions. The data were obtained and analyzed by a BioMérieux Vitek 2 Compact system (bioMérieux, France). The 16S rRNA gene of strain ZJB-14120 was amplified using the universal primer set 27f (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492r (5′-TACGGYTACCTTGTTACGACTT-3′) [26], and then sequenced by Sangon Biotech (Shanghai) Co., Ltd (China).

2.4. Biodegradation experiments

Strain ZJB-14120 was grown in 30 mL MSM containing 200 mg/L ABM for 12 h in the dark at 30 °C and 150 rpm. Two milliliters of the resulting culture (at OD_{600} of 0.35, 0.8 g dry cell weight (dcw)/L) was introduced into 30 mL LB medium supplemented with 200 mg/L ABM and incubated for