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Full Length Research Paper

Isolation and characterization of *Arthrobacter* sp. HW08 capable of biodegrading swainsonine

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The aim of this study was to isolate bacteria capable of degrading swainsonine, an indolizidine alkaloid in poisonous plants of *Oxytropis* and *Astragalus*. The bacterium HW08 was isolated and enriched from soil and its physiological and biochemical properties, 16S rRNA gene and the ability of swainsonine-degradation were investigated. The results showed that HW08 was a species of *Arthrobacter* sp. Under the optimized temperature (30°C) and pH value (7.0), HW08 (OD_{600} = 0.3) could degrade about 2 mg swainsonine in 5 ml degrading reaction within 4 h. In conclusion, HW08 would be a better potential candidate to be considered for SW-degrading in practice.

Key words: Locoweeds, *Arthrobacter*; swainsonine, biodegradation.

INTRODUCTION

Locoweeds, poisonous plants of genus Oxytropis and Astragalus are widely distributed in most rangelands of the world, including China (Zhao et al., 2003), the United States (Ralphs and James, 1999), Canada (Harries et al., 1972), Australia (Huxtable and Dorling, 1982) and Brazil (Medeiros et al., 2003). The locoweed is a serious problem to worldwide livestock industry because contains the toxin swainsonine (SW) leading to locoism. The syndromes of locoism include lack of weight gain and reproductive ability loss, immune systems impairment and even animal death (James et al., 1981; Stegelmeier et al., 1995).

Currently, the strategies of managing locoweeds include air-borne herbicides (McDaniel, 1999), feeding aversion (Wyatt-Eric-Allen, 2006), employing special machines to remove locoweeds and immunologic method (Tong et al., 2007), all of which are either impractical or profitless. Developing a microbial system to deal with locoism remains a promising alternative to minimize, even resolve SW intoxication of livestock. Similar and successful cases had been applied to degrade mimosine *of Leucaena leuc-ocephala* by transferring bacteria between goats (Jones and Megarrity, 1986) . Therefore, it is reasonable to explore bacteria with capacity of degrading SW in natural environment for purpose of detoxifying SW in practice.

In this study, we isolated and identified a strain of *Arthrobacter* sp. HW08 with capacity of degrading SW and investigated some of its biochemical and physiological properties.

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Abbreviations: SW, Swainsonine; **Me-Gal,** methyl- -D-mannopyranoside; **BSTFA,** N O-bis(trimethylsilyl) trifluoroacetamide; **TMCS,** trimethylchlorosilane; **LB,** Luria-Bertani medium; **MSM,** mineral salts medium.

MATERIALS AND METHODS

Chemicals

Methyl- -D-mannopyranoside(Me-Gal),N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) + trimethylchlorosilane (TMCS) were purchased from SIGMA. SW was provided by the laboratory of biotoxin and molecular toxicology of Northwest Agriculture and Forestry University, China. All chemicals were analytical reagents.

Culture medium

Luria-Bertani medium (LB) for enrichment culture, contains (grams per liter) 5 g yeast extract, 10 g peptone and 10 g NaCl (pH 7.2).

Table 1. Physiological and biochemical characteristics of HW08.

Test item	Results	Test item	Result
G stain	+	Nitrate reduction	–
O/F test	Oxidized	Use of citrate	–
M.R.experiment	–	Catalaes	+
V-P experiment	+	Ornithine decarboxylase	–
Acid production from Raffinose	–	H_2S production	–
Acid production from lactose	–	Lysine decarboxylase	+
Acid production from adonitol	–	Lipase (Tween 20)	–
Acid production from glucose	–	Lipase (Tween 80)	–
Acid production from sucrose	+	Urase	–
Acid production from xylose	–	Oxidase	+
Acid production from D-mannose	+	Phenylmalonic acid dehydrogenase	–
Indole production	+	Cellulose decomposition	–
Souring and peptonize	+	Gelatin liquefaction	+
Litmus milk	–	NaCl 7%	+
Starch hydrolysis	–	NaCl 10%	–

+ means positive reaction or growth; – means negative reaction or no growth.

Mineral salts medium (MSM) for degradation tests, contains (grams per liter) 5.0 g NH_4NO_3, 1.5 g $MgSO_4$, 5.0 g $(NH_4)_2SO_4$, 5.0 g KH_2PO_4, 5.0 g NaCl, 1.5 g K_2HPO_4 (pH 7.2); MSM-1 contains 100mg/l SW; MSM-2 contains 400 mg/l SW.

Isolation and enrichment of SW-degrading bacteria

Oxytropis ochrocephala bunge (One species of locoweed) was collected by clipping the plant stems just above the ground from HaiYuan county (Ningxia Province, China). The aerial plant materials, including flowers, stems and leaves, were dried and buried 50 cm deep for half a year since October, 2008. The bacteria were isolated from the centre of the co-buried and aerated yellow-brown soil, and enriched as described (Zhao et al., 2009) with modifications. Briefly, 10 g soil samples were suspended in PBS and well stirred, 10 ml soil-derived- supernatant was firstly co-cultured with 90 ml LB medium for 24 h and the resulting bacterial supernatant was mixed with MSM-1 at ratio of 1 9 (v/v). The mixture was incubated at 30°C and 160 rpm for 4 days. After 6 subcultures in MSM- 1, the bacteria were serially diluted and spread (100 l) over the surface of MSM agar plates with 200 mg/l SW. Colonies were picked up and maintained. One of the selected strains was designated as HW08 for further characterizations.

The physiological and biochemical properties of HW08 were examined according to Bergey's manual of determinative bacteriology (Bergey et al., 1994).

Sequencing of 16S rRNA gene and phylogenetic analysis

Genomic DNA extraction and amplification of 16S rRNA gene of HW08 were performed as described (Ding et al., 2008). PCR reaction conditions were as follows: 95°C 10 min first, then 30 cycles of 95°C 1min, 55°C 1 min, 72°C 1.5 min, and finally 72°C 5 min. The sequences of primers were: 5 -CAGGC-CTAAC-ACATGCAAGTC-3 (63 forward) and 5 - GGGCGGWGTGT ACAAG-GC-3 (1387 reverse) (Marchesi et al., 1998). PCR products were purified and cloned into pMD-18T, then sent to sequencing (JINSITE Company, Nanjing, China). For comparing the similarity of 16S rRNA sequences between HW08 and the related species, blast analysis was performed. Phylogenetic tree was constructed with software Clustal X 1.83 and MEGA4.0.

Degrading SW by HW08

For SW degradation assay, the OD_{600} of HW08 in 5 ml MSM-2 was adjusted to 0.3. The culture was maintained under optimized temperature and initial pH. Samples were collected once per hour from 0 to 4 h and measurement of SW retention by gas chromatography was performed as our previous description (Zhao et al., 2009).

RESULTS AND DISCUSSION

3 strains of bacteria with SW-degrading capacity were isolated and enriched from the soil samples. Of the 3 resulting strains, only 1 was designated as HW08 for complete characterizations because the others had unstable ability of swainsonine-degrading. The physiological and biochemical tests of HW08 are listed in Table 1. 16S rRNA gene sequencing and blast analysis indicated that HW08 was 99% homologous to 16S rRNA gene of *Arthrobacter* sp. The accession number of HW08-16S rRNA sequence is GQ921838. A phylogenetic tree involving HW08 was also constructed using Neighbor-Joining method (Figure 1). The result shows that HW08 is phylogenetically closest to *A. aurescens*. Indeed, HW08 and *A. aurescens* have some physiological properties in common, e.g. both have yellow colony and no motility, both can not grow at low temperature (5°C) but can grow at 30°C. However, *A. aure-scens* can hydrolyze starch, but HW08 can not (Reddy et al., 2002).

To investigate the SW-degrading capacity of HW08, the dynamical changes of SW retention were examined (Figure 2C). Under the optimized temperature (Figure 2A) and pH value (Figure 2B), HW08 could degrade about 2 mg

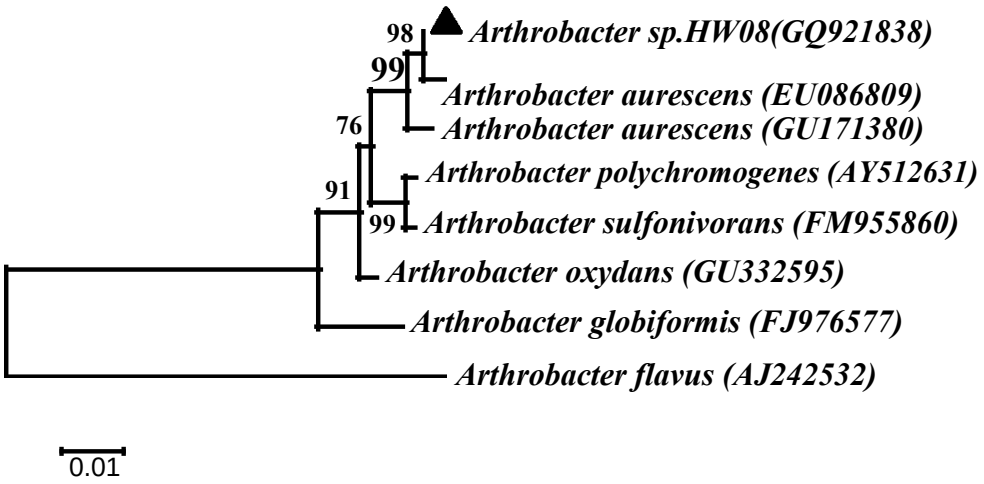

Figure 1. Phylogenetic analysis of 16S rRNA gene of HW08. The scale bar (0.01) represents the genetic distance. The percent numbers at the nodes indicate the levels of bootstrap support based on neighbor-joining analyses of 1000 replicates. Sequence accession numbers are noted in brackets.

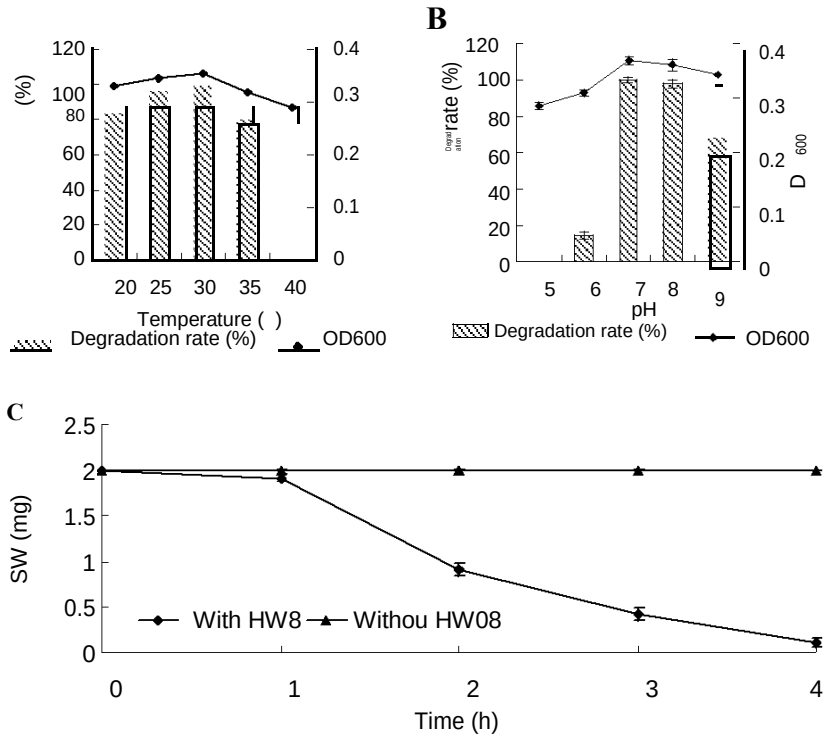

Figure 2a. Degradation of SW by HW08. SW-degrading efficiency of HW08 under different temperature(A) and different pH value (B) is compared; The SW retention (mg) in group with HW08 at 0, 1, 2, 3, 4 h is 2.000 ± 0.000, 1.917 ± 0.042, 0.9185 ± 0.0625, 0.4255 ± 0.066, 0.121 ± 0.048(n = 3), respectively. While SW retention in control group without HW08 keeps unchanged(C).

SW within 4 h, which is about 100 times more efficient than that of YLZZ -1 (Zhao et al., 2009). Although it is not directly related to this study, it may be important to note that swainsonine in locoweeds is an indolizine alkaloid which inhibits golgi alpha-mannosidase II and thus is found to be an anti-cancer drug (Sun et al., 2009) and can also protect cells from chemotherapeutic toxicity (Klein et al., 1999). However, swainsonine is a two edged

sword and can bring about significant economic loss in animal production industry as a toxin. The results here would underlie its further investigation on genetic relationships, metabolic pathways and facilitate the establishment of a bacterial system to harness locoweeds. Once we grasped the in-depth knowledge of HW08, we may utilize locoweeds through the way of bio- control. Similar strategies have been applied successfully to deal with poisoning of leucaena (Akingbade et al., 2001; Jones and Lowry, 1984; Pandey and Dwivedi, 2007). It is supposed that acclimated HW08 could be introduced into the rumen to prevent animals from locoweeds intoxication after careful evaluation of its safety and effectiveness in an animal system.

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