

Full Length Research Paper

Biodegradation of swainsonine by five types of plasmid-transformants from genomic library of *Arthrobacter* sp. HW08

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The aim of this study is to analyze the ability of swainsonine (SW) biodegradation by five types of transformants from the genomic library of *Arthrobacter* sp. HW08, explore preliminarily metabolites and deduce the metabolic pathway. Using 1000 mg·l⁻¹ SW as the sole carbon source, MSM containing five transformants at a proportion for 1:1(V:V) was incubated at 30°C, 180 r·min⁻¹. The degradation rate of SW was 99.78% in 48 h, and SW could be completely degraded in 72 h. Compared with samples containing SW in 0 h, two special spots of metabolites were visualized with iodine vapor by TLC. Two kinds of primary metabolites (stearic acid and palmitic acid) and two kinds of intermediate metabolites appeared in the process of metabolism of SW by GC and GC-MS whose m/z was 388.2 and 314.3 amu respectively. Ultimately, four kinds of end-products appeared in the process of metabolism of SW. This work demonstrates the preliminary results on metabolites of degrading SW by five transformants from *Arthrobacter* sp. HW08. This work also provides further information on metabolic pathway of SW from strain HW08.

Key words: Swainsonine, biodegradation, metabolites, transformants, *Arthrobacter* sp. HW08.

INTRODUCTION

Locoweed is a common name for leguminous plant of the *Astragalus* spp. and *Oxytropis* spp. containing the toxic indolizidine alkaloid swainsonine (SW), (Figure 1) (Molyneux and James, 1982). SW is the main toxin causing locoism in animals (Colegate et al., 1979;

Tulsiani et al., 1984; Cao et al., 1989). SW can inhibit the activity of α -mannosidase (Dorling et al., 1980), affect the synthesis of glycoproteins containing 'high manose' and hybrid asparagines-linked glycans in cultured cells (Elbein et al., 1981, 1982) and the processing of cell glycoprotein *in vivo* (Abraham et al., 1983), cause oligosaccharide accumulation, depress cell function, and result in a series of poisoning symptoms (James et al., 1970; Tulsiani et al., 1985; Cao et al., 1989; Hartley et al., 1989; James and Panter, 1989; Stegelmeier et al., 1999).

For the past decades, these poisonous plants have turned into dominant species on western rangelands in China and have covered up to 11 million hectares (Zhao et al., 2003). Aside from traditional manual eradication to decrease the amount of locoweed (Li, 2003), supplementation with bentonite or mineral elements in daily ration to be bound to SW and thus reduce intoxication

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Abbreviations: SW, Swainsonine; TLC, thin-layer chromatography; GC, gas chromatography; GC-MS, gas chromatography-mass spectrometry; Me-Gal, methyl- α -D-mannopyranoside; BSTFA, N,O-bis(trimethylsilyl) trifluoroacetamide; TMCS, trimethylchlorosilane; LB, Luria-Bertani medium; MSM, mineral salts medium; m/z, mass-to-charge ratio.

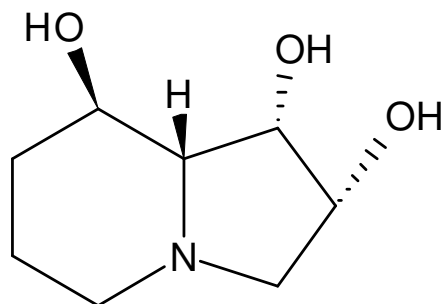


Figure 1. Structure of swainsonine.

(Pulsipher et al., 1994) and immunology to protect animals from lesion when consuming locoweed (Tong et al., 2007, 2008), there are no other reports on effective methods to resolve locoweed intoxication. Nowadays, seeking for a method of SW biodegradation is the hotspot in the world. Chinese scholars buried *Oxytropis kansuensis* Bunge in the soil, and six months later extracted and identified two strains of bacteria which were capable of degrading SW (Zhao, 2008; Zhao et al., 2009). These bacterial strains were further studied to optimize their conditions for degradation (Wang et al., 2010b), identify their SW degradation mechanism, and were hoped to be used in the elimination or reduction of SW concentration in animal bodies to reduce losses due to animal locoweed intoxication. However, the degradation performance of the isolated strains was unsteady after passage. Our research team collected *Oxytropis ochrocephala* Bunge from Nanhua Mountain, Haiyuan County, Ningxia Hui Autonomous Region in Oct, 2008 and buried them in the soil for 6 months. A strain of *Arthrobacter* sp. HW08 capable of SW degradation with high efficiency was obtained after enrichment culture and pure cultivation (Wang et al., 2010a). It was kept in China General Microbiological Culture Collection Center (CGMCC NO: 3313) with the Patent No. 200910218983.5 (Wang et al., 2010). Its 16S rDNA sequence has been included in Genbank under the Registry No. GQ921838. Under the optimized temperature (30°C) and pH value (7.0), HW08 (OD₆₀₀ = 0.3) could degrade about 2 mg SW in 5 ml degrading reaction within 4 h (Wang et al., 2010a). And then, we have constructed a genomic library of strain HW08, screened for five types of degradative plasmids. Based on this work, we have studied the effects on SW degradation by the mixture of transformants of these degradative plasmids and preliminarily analyzed the metabolites of SW. The findings of this study lay the foundation for the research on metabolic pathway or degradation mechanism of SW.

MATERIALS AND METHODS

Plasmids and strains

pUCSW-5 were obtained from genomic library of *Arthrobacter* sp. HW08 and constructed by us. It was proved that SW (400 mg·l⁻¹), as the unique carbon source, cultivated with the mixture of the five plasmid-transformants could be degraded within 6 h. The degrading capability was equivalent to that of strain HW08 (Hu et al., 2011). *Escherichia coli* DH5α [*supE44*, *ΔlacU169* (*Φ80 lacZΔM15*), *hsdR17*, *recA1*, *endA1*, *gyrA96*, *thi-1*, *relA1*] was kept in the laboratory.

Culture medium

Luria-Bertani medium (LB) for enrichment culture contained (g·l⁻¹) 5 g yeast extract, 10 g peptone, and 10 g NaCl (pH 7.2). The mineral salts medium (MSM) used in degradation tests comprised (g·l⁻¹) 5.0 g NH₄NO₃, 1.5 g MgSO₄, 5.0 g (NH₄)₂SO₄, 5.0 g KH₂PO₄, 5.0 g NaCl, and 1.5 g K₂HPO₄ (pH 7.2); SW was added to the medium after autoclaving. Media were solidified, if necessary, by the addition of 15 g agar per liter.

Reagents

SW standard substance, Methyl-α-D-mannopyranoside (me-Gal) and N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) + trimethylchlorosilane (TMCS) were purchased from SIGMA. Other chemicals used in this study were of analytical grade and were obtained from commercial sources.

Plasmids transformation and cultivation

Plasmids pUCSW-1, pUCSW-2, pUCSW-3, pUCSW-4 and pUCSW-5 were transferred to *E. coli* DH5α and were grown in LB solid plate, respectively, at 37°C for 12~16 h, and ampicillin-resistant transformants of *E. coli* DH5α were inoculated into LB slant by steak cultivation at 37°C for 48 h (Sambrook et al., 2001).

The capability of degrading SW for transformants

Transformants of LB slant were washed off with MSM and mixed at a proportion for 1:1(V:V). SW was added to a final concentration of 1000 mg·l⁻¹, and the mixture was incubated at 30°C for 72 h. Samples were collected for detection of bacterial solution concentration and SW content within 0, 2, 4, 6, 8, 10, 12, 24, 48 and 72 h, respectively.

Analyses to metabolites of SW

SW and their metabolites were determined by thin-layer chromatography (TLC) as described previously (Molyneux and Roitman, 1980; Molyneux et al., 1988, 1991; Wang et al., 2011). Briefly, supernatant of samples was lyophilized after centrifugation at 10,000 × g for 5 min, and the lyophilized powder was dissolved in 20 μl methanol and developed on silica gel plates in CHCl₃:CH₃OH:NH₄OH:H₂O (V:V=70:26:2:2). The plates were stained with iodine vapor or Ehrlich's reagent. Metabolites of SW were visualized comparing the spots color and retention factor values (*R_f* value) of samples with that of the samples in 0 h.

GC-FID analysis was used for quantitative detection by our previous study (Zhao et al., 2009). Briefly, the culture supernatant was lyophilized after centrifugation at 10,000 × g for 5 min, and the lyophilized powder was used for derivatization with BSTFA + TMCS. SW concentration was analyzed by injecting 2 μl of derivatization sample into GC-14C gas chromatography spectrometer (Shimadzu,

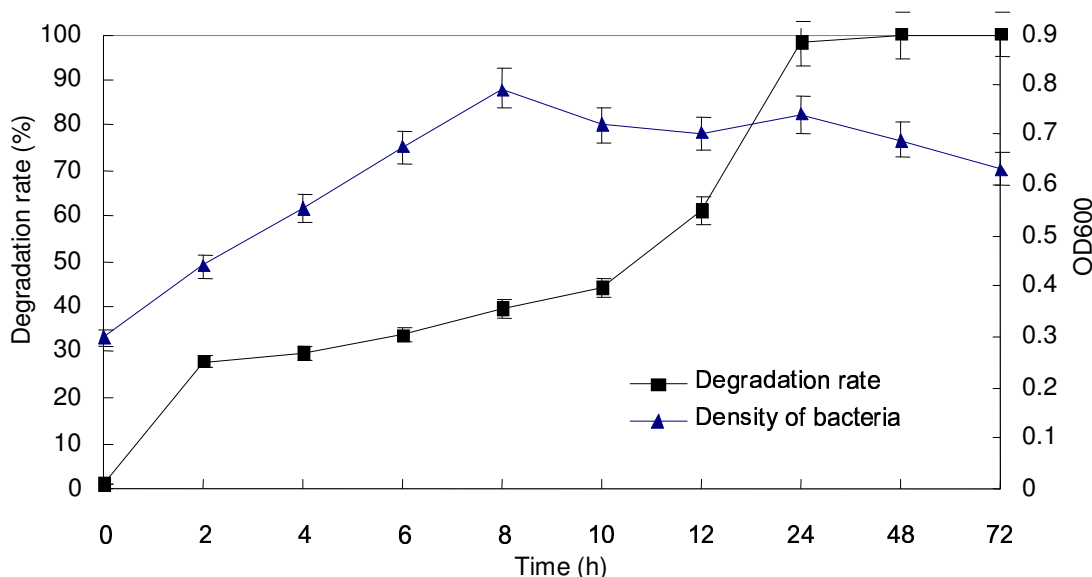


Figure 2. The curve of SW degradation and growth of 5 types of transformants in 72 h.

split ratio was adjusted to 30:1. AT.SE—54 quartz capillary column(30 m × 0.25 mm × 0.25 μm), injector, and FID temperatures were 210, 280 and 300°C, respectively.

GC-MS analysis was performed on a Finnigan Trace GC ultra coupled to a quadrupole mass selective detector Finnigan Trace DSQ (ThermoFinnigan). The MS interface temperature was set to 320°C and the source temperature to 250°C. Full-scan mass spectra were recorded at an electron energy of 70 eV within a scan range of 40-700 mu at a scan rate of 2.5 scans·s⁻¹. Helium was used as the carrier gas at a constant flow rate of 1 ml·min⁻¹. The chromatographic conditions were as described for GC-FID analysis. Metabolite constituents were identified by comparing retention times with those of silylated reference compounds and by comparing mass spectra with the entries of the mass spectral library (NIST, 2002).

RESULTS

SW degradation ability of transformants

Figure 2 shows the curve of SW degradation and the growth of the mixture with five types of transformants in 72 h. It was found that SW could be degraded in MSM, and could promote physiology activity of transformants as unique carbon source. With the coculture time lasted, SW slowly degraded, transformants swiftly grew and reached plateau phase in 8 h. And the rate of degradation of 1000 mg·l⁻¹ SW was 98.01% in 24 h, 99.78% in 48 h, 100% in 72 h, respectively.

Detection of metabolites by TLC

Fifty microlitre of samples in 0, 6, 12, 18, 24, 48 and 72 h were stained with Iodine vapor or Ehrlich's reagent

(Figures 3A and B). R_f value of SW was 0.612, and R_f value of metabolites I, II was 0.388, 0.125, respectively, which the plate stained with Iodine vapor (Figure 3A). The spots color of SW was purple and its metabolite had no spots color appeared which stained with Ehrlich's reagent (Figure 3B). The results showed that SW contents clearly decreased with the extension of reaction time and spots of SW disappeared after 48 h. But 2 kinds of spots (I and II) of metabolite were clearly visualized during the process of cultivation in 48 h compared with the samples in 0 h, indicating that at least two kinds of metabolites produced in the process of SW biodegradation by five transformants. The spot I and II became lighter in 72 h, indicating that they were probably intermediate metabolites.

GC analysis

Figure 4 shows the gas chromatogram of SW and its metabolites when 1000 mg·l⁻¹ SW was utilized by the mixture of five transformants in 72 h compared with the samples in 0 h. It found that the retention time of me-Gal (internal standard) and SW was 4.64 and 5.03 (or 5.02) min, respectively. SW peak gradually decreased with the extension of culture time and the peak of intermediate metabolite A appeared in 2 h. Metabolite A peak gradually increased with the progress of reaction, and intermediate metabolite B and C peak appeared in 10 h. At the same time, D and E peak appeared while A peak reached the highest and B peak disappeared in 12 h. Then, A peak stepped down gradually with the peak of SW striking decrease, and F, G peak appeared while D peak disappeared in 24 h. It deduced that metabolite F, G

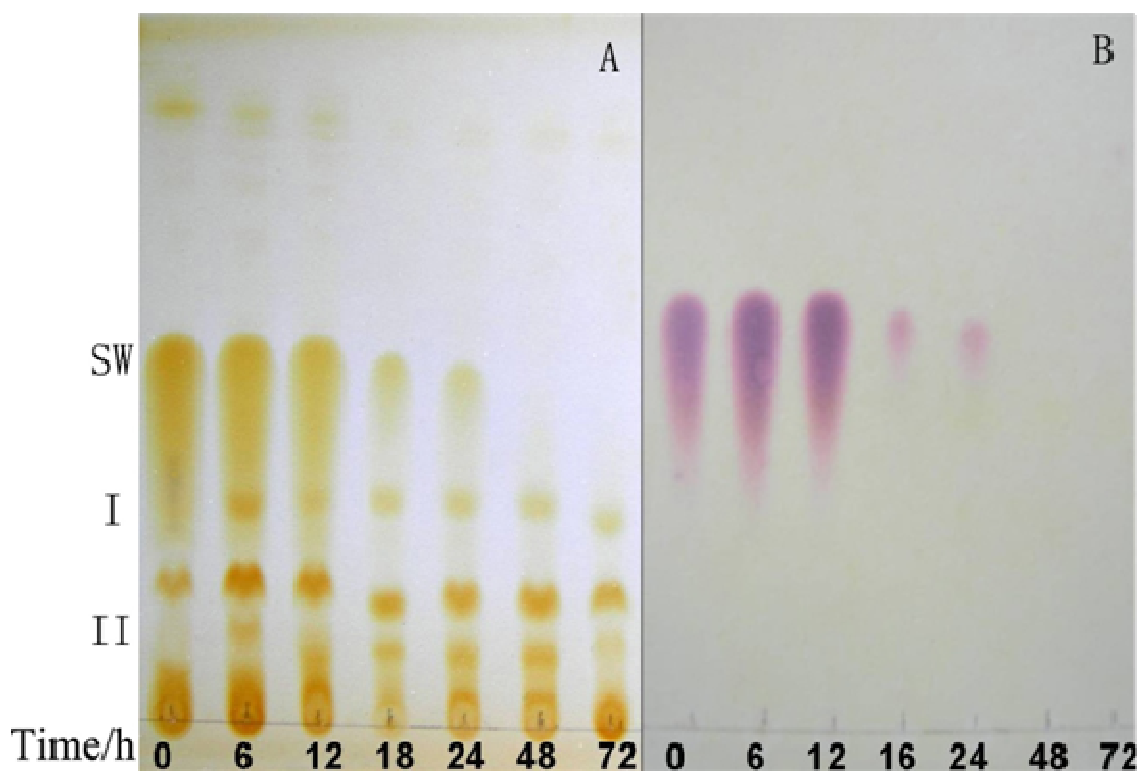


Figure 3. Thin-layer chromatography analysis of SW degradation stained with iodine vapor (A) and Ehrlich's reagent (B). The spots of I and II are visualized as intermediate metabolites of SW.

degraded. C, E peak increased gradually with the decrease of peak A. After that, H, I peak appeared when C reached the highest peak height in 48 h. In 72 h, the peak of SW, A and C were all disappeared, and E, F, G, H peak existed. The results indicate that compound A, C are main intermediate metabolites and compound E, F, G, H are end-products in the process of metabolism of SW by five transformants.

GC-MS analysis

Samples of SW degradation by five transformants in 0, 12, 24 and 48 h were determined by GC-MS. Figure 5 shows the gas chromatogram by Trace GC. Six kinds of compound peaks appeared. According to mass spectrum scanogram (Figures 6A and B) with the entries of the mass spectral library (NIST, 2002), the retention time of Me-Gal, SW, palmitinic acid and stearic acid was 2.90, 3.20, 6.10 (or 6.09), 11.22 (or 11.23, 11.24, 11.26), respectively. Two special compound peaks (retention time: 5.72 or 5.73, 10.20 or 10.22) appeared after 12 h, named as compound α (Figure 6A) and β (Figure 6B), respectively. Their peak heights increased with the extension of culture time, and reached the top in 24 h. Then, the peak heights gradually decreased even disappeared with the SW peak change, indicating that

they were main intermediate metabolites of SW degradation. Their molecular ion ($M+H$) peaks were very abundant and m/z was 388.2 and 314.3 amu respectively.

DISCUSSION

Arthrobacter strains are metabolically diverse and are capable of catabolizing a variety of chemical and environmentally relevant compounds because they contain genes or pathways for the catabolism of any of these compounds (Mongodin et al., 2006). We have proved that *Arthrobacter* sp. HW08 is particularly well-endowed genetically to metabolize SW by selecting for degradative plasmid-transformants from genomic library of strain HW08 and have screened for 5 types of degradative plasmids which laid the foundation of this study.

TLC is the method currently most used for the analysis of polyhydroxy alkaloids (Molyneux et al., 1991). SW may be visualized as a pyrrolidine ring fused to a piperidine ring moiety, yielding a bicyclic five/six ring system because it is a toxic polyhydroxy indolizidine alkaloid which stained with Ehrlich's reagent producing a purple color (Molyneux et al., 2002). Figure 2B shows the effect on degradation of SW by the mixture of five types of transformants. The purple color spots of samples become

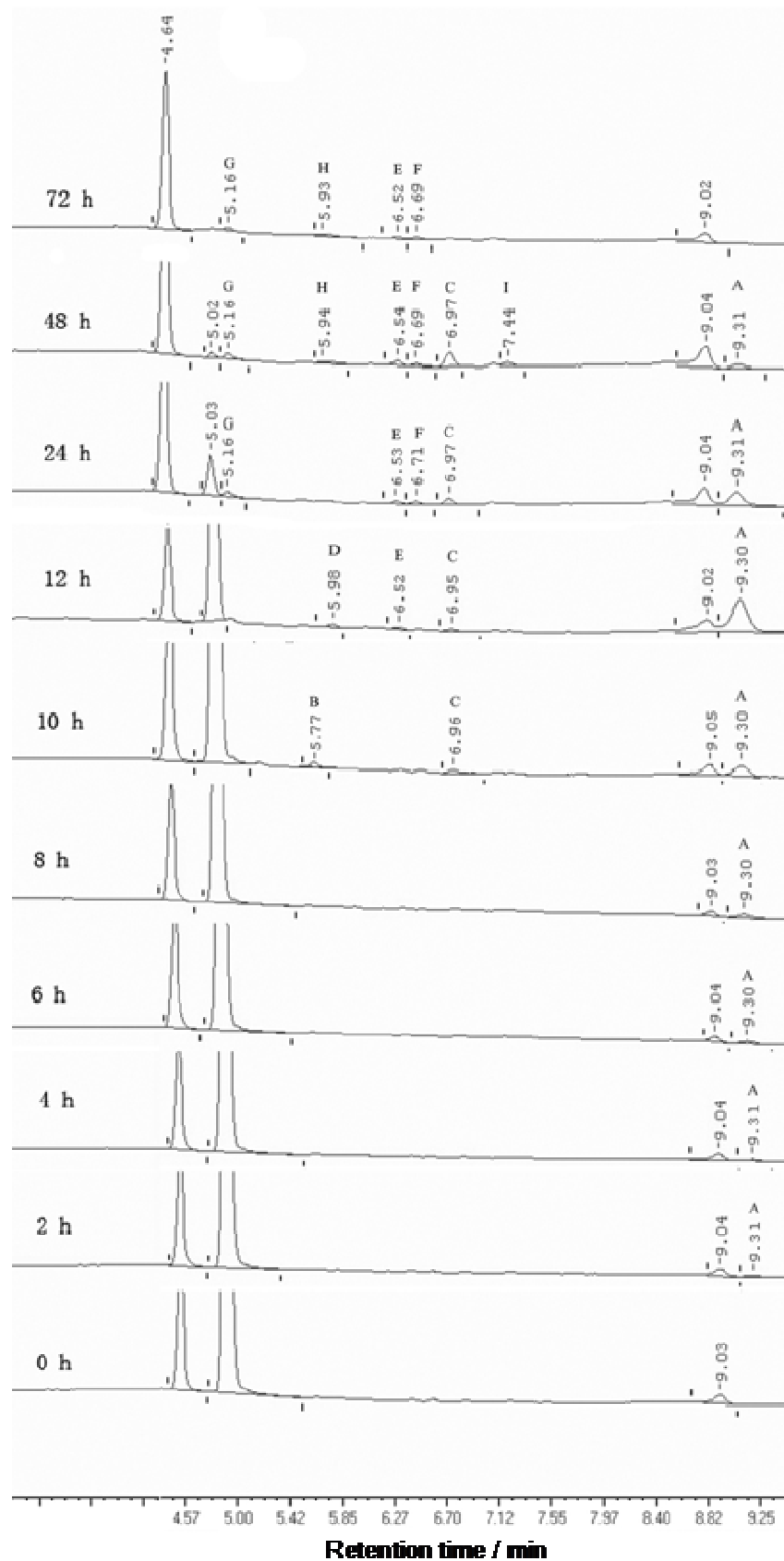


Figure 4. The gas chromatography of 1 g^{-1} SW degradation by the mixture of 5

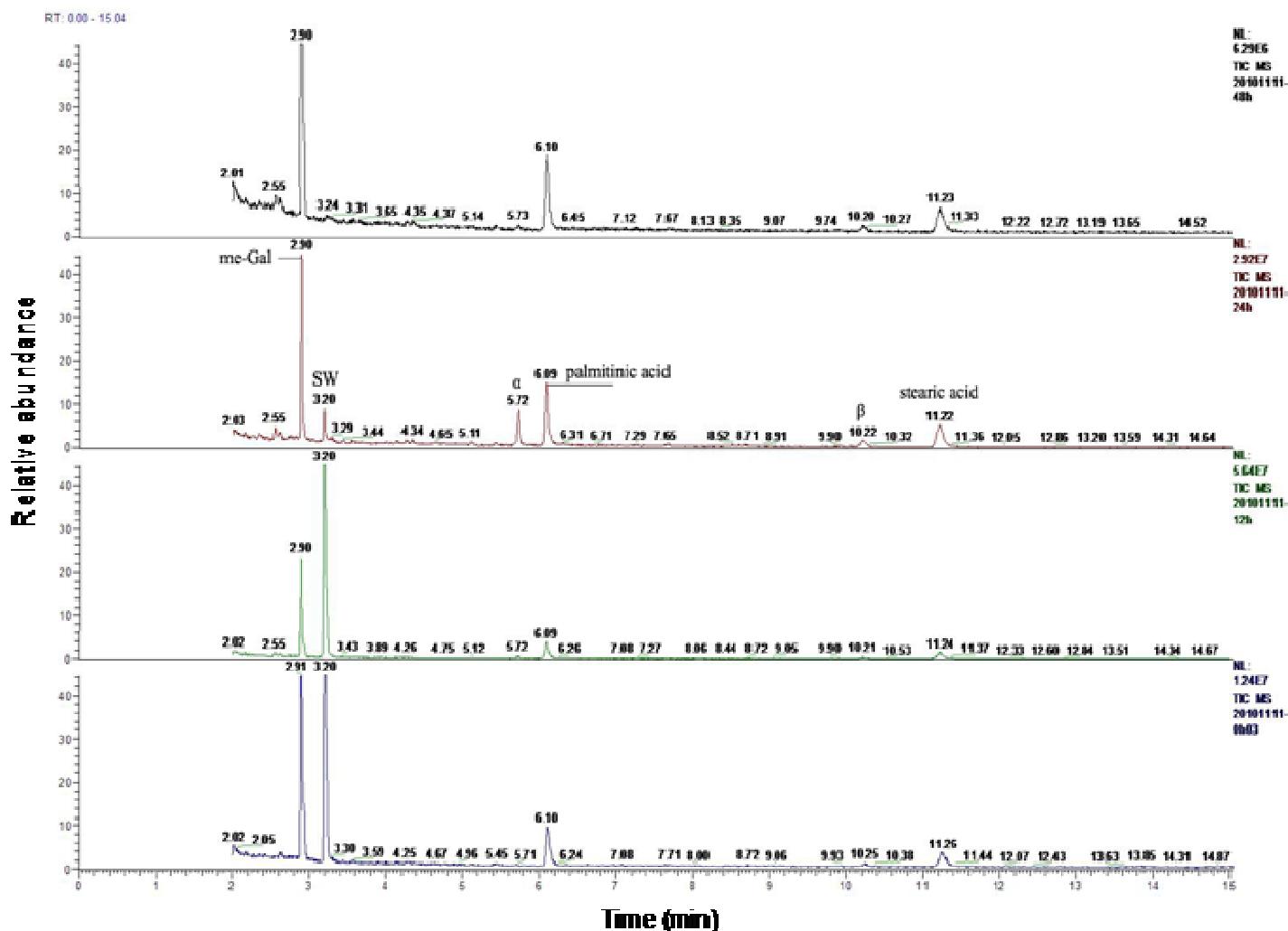


Figure 5. The gas chromatography of $1 \text{ g}\cdot\text{l}^{-1}$ SW degradation by 5 transformants with Trace GC.

the indole ring system of SW was changed. That is to say, metabolites of SW by five transformants probably do not contain indole ring. Whereas metabolites can be stained with Iodine vapor which are applied to most of organic compounds containing heteratom, double or triple bond, aromatic ring, polyalcohols, etc (Akhrem and Kuznetsova, 1963; Sherma, 2000).

According to TLC, GC and GC-MS analysis, the results illustrated that five types of transformants co-cultured with $1000 \text{ mg}\cdot\text{l}^{-1}$ SW as sole carbon source, and SW could be completely degraded in 72 h. SW had no inhibit bacterial growth and several metabolites produced in the metabolism of SW. Palmitic acid and Stearic acid existed in the sample of 0 h, too. And their peak areas obviously increased with the extension of reaction time, inferring that they were produced as primary metabolites of transformants by continuous stimulation of SW (Wu, 2006). Because of the structural relationship of

polyhydroxy alkaloids to sugars, SW must be derivatized with BSTFA and TMCS in pyridine which provides hydroxyl groups with the necessary volatility and stability for GC and GC-MS analyses (Nash et al., 1986). But the sample must be maintained at 60°C for 1 h to ensure that all of the alkaloids give single peaks. In the course of mass spectrometric analysis of the derivative of SW, the characteristic fragment ions at m/z 120, m/z 185, m/z 260, m/z 299 and m/z 374 are generated (Yu, 2009). But it is difficult to analyze the mass spectra of intermediate metabolite α and β because of their derivatization.

Metabolites pathway of SW would be deduced by gas chromatogram (Figure 4). Figure 7 shows the deduced result, indicating that SW being degraded by five types of transformants at least need two kinds of enzymes (Enzyme 1 and 2) to complete. Intermediate metabolite B, C, D, I are probably converted into end-products E, F, G, H by more than two kinds of enzymes. Further studies

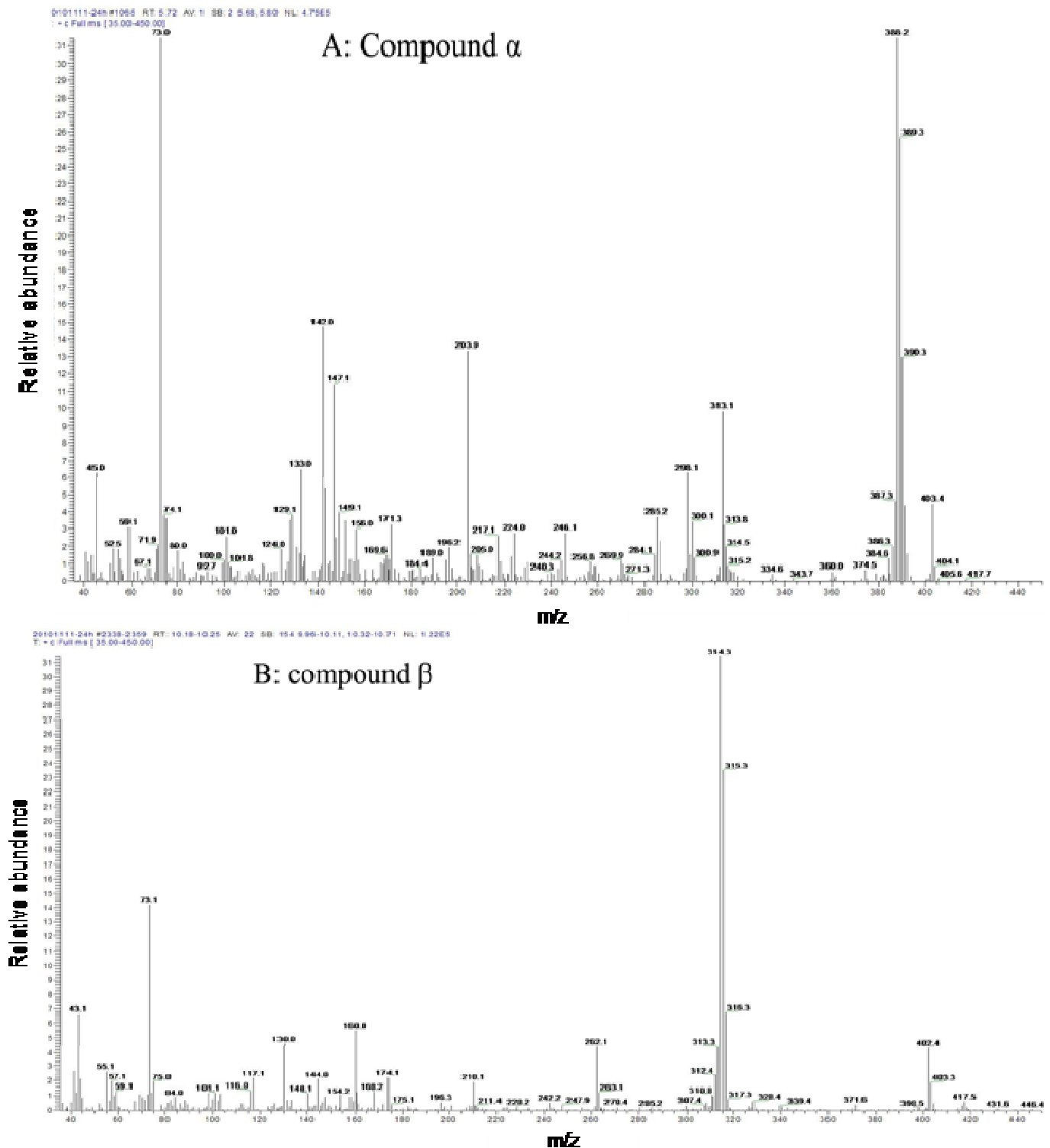


Figure 6. The mass spectrogram of metabolites for SW degradation by 5 types of transformants detected by Trace DSQ. Intermediate metabolite: (A) compound α and (B) compound β .

chromatography and NMR so as to prove the metabolism pathway of SW and explore SW degradation mechanism

by five types of transformants.

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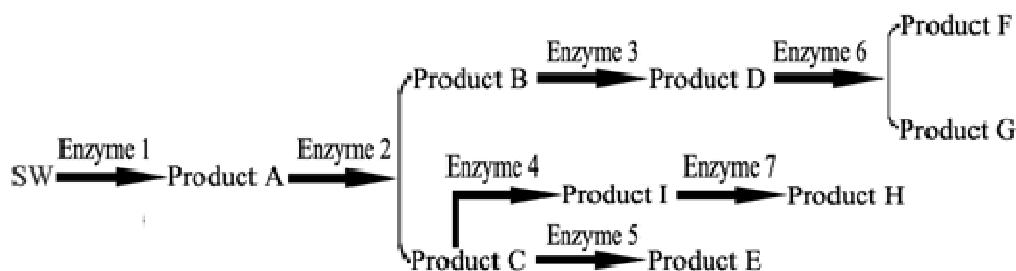


Figure 7. The deduced metabolic pathway of SW degradation with 5 types of transformants by GC-FID analysis of figure 4.

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REFERENCES

- Abraham DJ, Sidebotham R, Winchester BG, Dorling PR, Dell A (1983). Swainsonine affects the processing of glycoproteins *in vivo*. *FEBS Lett.*, 163(1): 110-113.
- Akhrem AA, Kuznetsova AI (1963). Thin-layer chromatography. *Russ. Chem. Rev.*, 32(7): 366.
- Cao GR, Li SJ, Duan DX, Zhao XW (1989). Isolation and identification of the toxic components from *Oxytropis ochrocephala*. *J. Northwest A and F Univ.*, 17: 1-8.
- Colegate SM, Dorling PR, Huxtable CR (1979). A Spectroscopic Investigation of Swainsonine: An α -Mannosidase Inhibitor Isolated from *Swainsona canescens*. *Aust. J. Chem.*, 32(10): 2257 - 2264
- Dorling PR, Huxtable CR, Colegate SM (1980). Inhibition of lysosomal alpha-mannosidase by swainsonine, an indolizidine alkaloid isolated from *Swainsona canescens*. *Biochem. J.*, 191(2): 649-651.
- Elbein AD, Dorling PR, Vosbeck K, Horisberger M (1982). Swainsonine prevents the processing of the oligosaccharide chains of influenza virus hemagglutinin. *J. Biol. Chem.*, 257(4): 1573-1576.
- Elbein AD, Solf R, Dorling PR, Vosbeck K (1981). Swainsonine: an inhibitor of glycoprotein processing. *Proc. Natl. Acad. Sci. USA.*, 78(12): 7393-7397.
- Hartley WJ, Baker DC, James LF (1989). Comparative pathological aspects of locoweed and Swainsona poisoning of livestock. In: L.F. James, A.D. Elbein, R.J. Molyneux and C.D. Warren, Editors, Swainsonine and Related Glycosidase Inhibitors. Iowa State University Press, Ames, pp. 50-56.
- Hu YC, Wang JN, Wang Y, Yang GD, Geng GX, Wang JH (2011). Screening of degradative plasmids from *Arthrobacter* sp. HW08 and their ability to degrade swainsonine. *Afr. J. Biotechnol.*, in press 10(25): 5027-5032.
- James LF, Panter KE (1989). Locoweed poisoning in livestock. In: L.F. James, A.D. Elbein, R.J. Molyneux and C.D. Warren, Editors, Swainsonine and Related Glycosidase Inhibitors Iowa State University Press, Ames, pp: 23-38.
- James LF, Van Kampen KR, Hartley WJ (1970). Comparative pathology of *Astragalus* (locoweed) and *Swainsona* poisoning in sheep. *Pathol. Vet.*, 7(2): 116-125.
- Li Jk (2003). The present situation and prospect of the studies on locoweed in China. *Scientia. Agric. Sin.*, 36(9): 1091-1099.
- Molyneux RJ, Benson M, Wong RY, Tropea JE, Elbein AD (1988). Australine, a Novel Pyrrolizidine Alkaloid Glucosidase Inhibitor from *Castanospermum australe*. *J. Nat. Prod.*, 51(6): 1198-1206.
- Molyneux RJ, Gardner DR, James LF, Colegate SM (2002). Polyhydroxy alkaloids: chromatographic analysis. *J. Chromatogr. A.*, 967(1): 57-74.
- Molyneux RJ, James LF (1982). Loco intoxication: indolizidine alkaloids of spotted locoweed (*Astragalus lentiginosus*). *Science*, 216: 190-191.
- Molyneux RJ, James LF, Panter KE, Ralphs MH (1991). Analysis and distribution of swainsonine and related polyhydroxyindolizidine alkaloids by thin layer chromatography. *Phytochem. Anal.*, 2(3): 125-129.
- Molyneux RJ, Roitman JN (1980). Specific detection of pyrrolizidine alkaloids on thin layer chromatograms. *J. Chromatogr. A.*, 195: 412-415.
- Mongodin EF, Shapir N, Daugherty SC, DeBoy RT, Emerson JB, Shvartzbeyn A, Radune D, Vamathevan J, Riggs F, Grinberg V, Khouri H, Wackett LP, Nelson KE, Sadowsky MJ (2006). Secrets of soil survival revealed by the genome sequence of *Arthrobacter aurescens* TC1. *PLoS. Genet.*, 2(12): 214.
- Nash RJ, Goldstein WS, Evans SV, Fellows LE (1986). Gas chromatographic method for separation of nine polyhydroxyalkaloids. *J. Chromatogr.*, 366: 431-434.
- Pulsipher GD, Galyean ML, Halford DM, Smith GS, Kiehl DE (1994). Effects of graded levels of bentonite on serum clinical profiles, metabolic hormones, and serum swainsonine concentrations in lambs fed locoweed (*Oxytropis sericea*). *J. Anim. Sci.*, 72: 1561-1569.
- Sambrook J, Fritsch EF, Maniatis T (2001). *Molecular Cloning: A laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, pp. 116-118.
- Sherma J (2000). Thin-layer chromatography in food and agricultural analysis. *J. Chromatogr. A.*, 880(1-2): 129-147.
- Stegelmeier BL, James LF, Panter KE, Gardner DR, Pfister JA, Ralphs MH, Molyneux RJ (1999). Dose response of sheep poisoned with locoweed (*Oxytropis sericea*). *J. Vet. Diagn. Invest.*, 11(5): 448-456.
- Tong DW, Wang JY, Mu PH, Dong Q, Zhao BY, Liu WM, Zhao J, Li L, Zhou T (2008). Analysis of several serum enzymes and blood urea nitrogen of swainsonine-HSA immunized goats. *Anim. Feed Sci. Tech.*, 142(1-2): 74-88.
- Tong DW, Mu PH, Dong Q, Zhao BY, Liu WM, Zhao J, Li L, Zhou T, Wang JY, Sui GD (2007). Immunological evaluation of SW-HSA conjugates on goats. *Colloid. Surface. B.*, 58: 61-67.
- Tulsiani DR, Broquist HP, Touster O (1985). Marked differences in the swainsonine inhibition of rat liver lysosomal alpha-D-mannosidase, rat liver golgi mannosidase II, and jack bean alpha-D-mannosidase. *Arch. Biochem. Biophys.*, 236: 427-434.
- Tulsiani DRP, Broquist HP, James LF, Touster O (1984). The similar effects of swainsonine and locoweed on tissue glycosidases and oligosaccharides of the pig indicate that the alkaloid is the principal toxin responsible for the induction of locoism. *Arch. Biochem. Biophys.*, 232(1): 76-85.
- Wang JH, Hu YC, Wang Y (2010). An *Arthrobacter* sp. strain HW08 of degrading swainsonine and its application to locoweed utilization. In: Patent Office of the PRC (Ed. PO of PRC). China.
- Wang Y, Hu YC, Wang JH, Liu ZB, Yang GD, Geng GX (2011). Ultrasound-assisted solvent extraction of swainsonine from *Oxytropis ochrocephala* Bunge. *J. Med. Plants Res.*, 5(6): 890-894.
- Wang Y, Hu YC, Wang JH, Yu YT, Song YM, Yang GD, Geng GX (2010a). Isolation and characterization of *Arthrobacter* sp. HW08

capable of biodegrading swainsonine. Afr. J. Microbiol. Res., 4(15): 1635-1638.
Wang Y, Hu YC, Yu YT, Song YM, Li HL, Geng GX, Wang JH (2010b).

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- Characteristics of swainsonine-degrading bacteria YLZZ-2 and optimization of conditions for degradation. Acta. Agrestia. Sinica., 18(1): 89-92.
- Wu ZQ (2006). Comparison of bacteria between primary metabolism and secondary metabolism. Biol. Teach., 31(5): 79.
- Yu YT (2009). Isolation, identification and genetic polymorphism of swainsonine-producing fungal endophytes from locoweeds in China. In: College of Veterinary Medicine, Northwest A and F University. Northwest Sci-Tech University of Agriculture and Forestry, Yangling, Shaanxi.
- Zhao BY, Tong DW, Ge PB, Dong Q, Mo CH (2003). Locoweed harm investigation in the west grassland of China. Grassland of China, 25: 65-68.
- Zhao XH (2008). Isolation and identification of swainsonine degrading bacteria and degradation character. In: College of Veterinary Medicine, Northwest A and F University. Northwest Sci-Tech University of Agriculture and Forestry, Yangling, Shaanxi.
- Zhao XH, He X, Wang JN, Song YM, Geng GX, Wang JH (2009). Biodegradation of Swainsonine by *Acinetobacter calcoaceticus* strain YLZZ-1 and its isolation and identification. Biodegradation, 20: 331-338.