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Full Length Research Paper

Diversity and phylogenetic analyses of nicotine-degrading bacteria isolated from tobacco plantation soils

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Eighteen nicotine-degrading bacteria, which could utilize nicotine as the sole carbon and nitrogen sources, were isolated from tobacco plantation soils in Mile, Yunnan Province, China. These bacteria were identified as *Arthrobacter* sp. (six strains), *Ochrobactrum* sp. (five strains), *Sphingobacterium* sp. (three strains), *Sinorhizobium* sp. (one strain), *Alcaligenes* sp. (one strain), *Pseudoxanthomonas* sp. (one strain) and *Delftia* sp. (one strain) based on their partial 16S rRNA gene sequences. The member of genera *Pseudoxanthomonas*, *Sphingobacterium*, *Sinorhizobium* and *Delftia* were reported to degrade nicotine for the first time. A phylogenetic tree was constructed based on the 16S rRNA gene sequences of different nicotine-degrading bacteria, and these bacteria clustered into three clades (A, B and C). Moreover, the nicotine-degrading abilities of three bacteria were determined, among them, *Sinorhizobium* sp. 5-28 showed the highest nicotine-degrading ability, 72.5% nicotine in tobacco leaves was degraded after treating for 12 h. Meanwhile, 47.2 and 51.5% nicotine in tobacco leaves were degraded by *Ochrobactrum* sp. 4-40 and *Pseudoxanthomonas* sp. 5-52 under the same conditions, respectively. In summary, many nicotine-degrading bacteria were identified in tobacco plantation soils, and these bacteria showed potential applications in tobacco production and environmental bioremediation.

Key words: Tobacco leaves, nicotine-degrading bacteria, phylogenetic analysis, bacteria diversity.

INTRODUCTION

Nicotine is the principal alkaloid in leaves of most *Nicotiana* species (Doolittle et al., 1995), and it contributes significantly to smoking properties. Nicotine is not a direct cause of most tobacco smoking-related diseases, but it is highly addictive (Benowitz et al., 1999). Most often, nicotine is the main accumulated toxic compound and cannot be recycled. Because nicotine is soluble in water, it can easily contaminate ground water (Civilini et al., 1997). During the manufacturing and processing of tobacco products, powdery solid or liquid wastes are generated in high concentrations (Li et al., 2010). As a result, it has been designated as a Toxic Release Inventory chemical by the U.S. Environmental Protection Agency since 1994 (Novotny and Zhao, 1999). Tobacco waste also has been classified as “toxic and hazardous” by European Union regulations when the nicotine content exceeds 0.05% (w/w) (Civilini et al., 1997). Therefore, reducing nicotine content in tobacco and tobacco waste is the urgent requirement for cigarette production and environmental remediation.

Compared with physical and chemical methods, biological methods using microbes are preferable because they are more efficient and less expensive

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(Lenkey, 1989; Meher et al., 1995). Several bacteria, such as *Arthrobacter nicotinovorans* (Schenk et al., 1998), *Pseudomonas putida* (Wang et al., 2005, 2007), *Rhodococcus* sp. Y22 (Gong et al., 2009), *Acinetobacter* sp. ND12 (Li et al., 2011), *Ochrobactrum intermedium* DN2 (Yuan et al., 2007) and *Agrobacterium tumefaciens* S33 (Wang et al., 2009), have all shown capability of degrading nicotine. Moreover, the metabolic pathway and molecular mechanism of nicotine degradation by *P. putida* and *A. nicotinovorans* have been elucidated gradually (Brandsch et al., 2006; Li et al., 2010). However, little is known about the distribution and diversity of nicotine-degrading bacteria in tobacco plantation soils. In this study, the nicotine-degrading bacteria were isolated from tobacco plantation soils, the 16S rRNA gene sequences of these bacteria were amplified, and the phylogenetic tree was constructed based on the 16S rRNA gene sequences. Moreover, the biochemical properties and nicotine-degrading abilities of partial strains were analyzed.

MATERIALS AND METHODS

Samples and culture conditions

(S)-Nicotine (>99.0% purity) was purchased from Sigma Company (USA). All other chemicals were of analytical grade. The soil samples were taken from tobacco plantation soils in Mile, Yunnan Province, China.

Luria-Bertani medium (LB), the enrichment medium (EM), nicotine selective medium (NSM), and an inorganic salt medium (ISM) were prepared according to our previous report (Gong et al., 2009). ISM was used for determining the nicotine-degrading activity of bacteria. The initial pHs of both NSM and ISM were adjusted to 7.0, and a certain amount of nicotine was added to the media after sterilization, according to experimental design. All experimental liquid cultures were aerated at 150 rpm and a fixed temperature designated by the specific test.

Isolation of nicotine-degrading bacteria

2 g of soils collected from tobacco plantation soils were inoculated into 100 ml of EM medium containing 0.5 g/L nicotine, and incubated at 28°C and 150 rpm for 7 days. Enrichment of nicotine-degrading bacterium was performed by transferring 10 ml of the culture to fresh EM medium for 7 days, and gradually increasing the concentration of nicotine from 0.5 to 1.0 g/L. After four cycles of enrichment in the EM medium, the culture was transferred into ISM medium (containing 1.0 g/L nicotine), spread onto LB plates using the dilution plate method, and incubated at 28°C for 48 h. Purified colonies were then inoculated into NSM plate (containing 1.0 g/L nicotine) for preliminary screening, and then transferred to liquid ISM containing 1.0 g/L nicotine for secondary screening.

Identification of nicotine-degrading bacteria

The morphological characters of nicotine-degrading bacteria were observed under a light microscope (Olympus BX51, Japan). Physiological and biochemical characteristic assays were determined according to the methods described by Dong and Cai (2001). The genomic DNA of bacterium was extracted using the method described by Wilson (1987). The partial 16S rRNA gene was amplified by PCR using Taq polymerase and the universal primer pair of 20F (5'-GAGTTTGATCCTGGCTCAG-3') and 1500R (5'-GTTACCTTGTTACGACTT-3') described by Weisburg et al. (1991). The 16S rRNA gene sequence of nicotine-degrading bacteria and reference sequences obtained from GenBank were aligned using multiple sequence alignment software Clustal_X version 1.83 (Thompson et al., 1997). A phylogenetic tree was constructed using Mega 4.0 (Kumar et al., 2008) based on the 16S rRNA gene sequences of different nicotine-degrading bacteria and reference bacteria.

Analytical methods

Cell growth was determined using a Nucleic Acid and Protein Analyzer (Beckman DU 640, USA). After the cells in the samples were removed by centrifugation at 12,000 g for 10 min at 4°C, the supernatant was used for nicotine concentration analysis. The nicotine concentration was determined by high-performance liquid chromatography (HPLC, Agilent 1200 Series, USA), equipped with a SB C-18 column (5 µm, 4.6 × 250 mm) and an ultraviolet detector operating at a wavelength of 259 nm (Chen et al., 2008). The column was eluted with a mixture of methanol: 20 mM phosphate buffer (0.46% triethylamine, pH 6.5) (60:40, v/v) at a flow rate of 1.0 ml/min and at 35°C. Quantitative data were obtained by comparing the peak area of the query compound with that of standard of known concentration.

Preparing of resting cells and nicotine degradation

Resting cells cultured in ISM medium were prepared according to the reported methods (Gong et al., 2009; Wang et al., 2005). 5 ml of ISM at an initial pH 7.0 containing 0.5 to 4.5 g/L nicotine, respectively, were inoculated with 100 µl cell suspension of nicotine-degrading bacterium and incubated at 28°C for 28 h, and the cell concentration was determined at OD600.

Degradation of nicotine in tobacco leaves was carried out in a 250 ml flask containing 5 ml resting cells, 2 g tobacco leaves (Baoshan, Hongda, C_3F) and 25 ml sterilized sodium phosphate buffer (50 mM, pH 7.0) with a constant shaking at 150 rpm at 28°C. The cultures were sampled at 4, 8, and 12 h, respectively, and the nicotine concentration in the culture was determined by HPLC.

RESULTS

Isolation and identification of nicotine-degrading bacteria

Nicotine-degrading bacteria were isolated by using nicotine as the sole carbon and nitrogen sources, and 18 bacteria were isolated from nine soil samples. Different colours were observed when these bacteria were incubated on ISM medium, which suggested that different pigments and metabolites were produced when nicotine was degraded by different bacteria.

The partial 16S rRNA gene sequences of these nicotine-degrading bacteria were amplified and sequenced, and these bacteria were preliminary identified by aligned the 16S rRNA gene sequences in GenBank. These bacteria were identified to *Arthrobacter* sp., *Ochrobactrum* sp., *Sphingobacterium* sp.,

Table 1. Isolation and identification of nicotine-degrading bacteria from the soils.

Strain nos.	GenBank accession nos.	The closest bacterial strains/genera	16S rRNA identity (%)
3-9	JQ955552	*Arthrobacter* sp.	98
3-18	JQ955553	*Alcaligenes paradoxus*	100
3-21	JQ955554	*Sphingobacterium* sp.	99
3-22	JQ955555	*Delftia* sp.	100
3-24	JQ955567	*Arthrobacter* sp.	100
3-45	JQ955557	*Sphingobacterium* sp.	99
3-46	JQ955558	*Sphingobacterium* sp.	99
3-53	JQ955568	*Arthrobacter* sp.	100
3-55	JQ955559	*Arthrobacter* sp.	100
4-40	JN700178	*Ochrobactrum* sp.	100
4-50	JQ955560	*Arthrobacter* sp.	100
4-83	JQ955561	*Arthrobacter* sp.	100
5-28	JN700177	*Sinorhizobium* sp.	100
5-29	JQ955562	*Ochrobactrum* sp.	100
5-30	JQ955563	*Ochrobactrum* sp.	100
5-31	JQ955564	*Ochrobactrum* sp.	100
5-52	JQ955565	*Pseudoxanthomonas* sp.	100
5-56	JQ955566	*Ochrobactrum* sp.	100

Table 2. Morphological properties of nicotine-degrading bacteria*.

Strains properties	*Sphingobacterium* sp. 3-21	*Sinorhizobium* sp. 5-28	*Ochrobactrum* sp. 4-40	*Pseudoxanthomonas* sp. 5-52
Colony	Round	Round	Round	Round
Surface	Smooth, ridgy	Smooth, convex	Smooth, convex	Smooth, convex
Border	Tidiness	Tidiness	Tidiness	Tidiness
Character	Wet, sticky	Very wet, sticky	Wet, sticky	Very wet, sticky
Diameter	1.0 mm	0.5 - 1.5 mm	1.0 mm	1.5 - 2.5 mm
Color	Waxy yellow	Milky yellow	Pale yellow	Milky yellow
Transparency	Opacity	Translucency	Opacity	Translucency
Cell morphology	Long rod-shaped	Rod-shaped	Short rod-shaped	Rod-shaped
Gram stain	Negative	Negative	Negative	Negative

*The colony morphology was observed after bacteria were cultured in LB medium for 48 h.

Sinorhizobium sp., *Alcaligenes* sp., *Pseudoxanthomonas* sp. and *Delftia* sp., respectively (Table 1). Several bacteria of them have been reported to degrade the nicotine previously, such as *Arthrobacter* (Schenk et al., 1998), *Ochrobactrum* (Yuan et al., 2007) and *Alcaligenes* (Uchida et al., 1976), while other isolated bacteria in this study have not yet been reported to degrade nicotine.

The morphological properties of four bacteria including *Sphingobacterium* sp. 3-21, *Pseudoxanthomonas* sp. 5-52, *Sinorhizobium* sp. 5-28 and *Ochrobactrum* sp. 4-40 were observed (Table 2), they are all Gram-negative bacteria. The partial biochemical properties of four bacteria were determined (Table 3) and their biochemical properties are consistent with bacteria in corresponding genus.

Phylogenetic analysis of nicotine-degrading bacteria

A phylogenetic tree was constructed based on 16S rRNA gene sequences (Figure 1). From the tree, these nicotine-degrading bacteria clustered into three clades (A, B and C), and most of them clustered into the clade A. Clade A was further divided into two subclades (A1 and A2), subclade A1 consisted of five bacteria in different genera, such as *Alcaligenes* sp., *Delftia* sp., *Acinetobacter* sp., *Pseudomonas* sp., *Pseudoxanthomonas* sp., *Alcaligenes* sp. and *Delftia* sp.,

Table 3. Physiological and biochemical properties of nicotine-degrading bacteria.

Strains properties	*Sphingobacterium* sp. 3-21	*Sinorhizobium* sp. 5-28	*Ochrobactrum* sp. 4-40	*Pseudoxanthomonas* sp. 5-52
Catalase	+	+	+	+
Nitrate reduction	-	-	+	+
Glucose	+	-	+	+
Lactose	-	-	-	+
Sugar	+	-	-	-
Mannitol	-	-	+	-
Citrate utilization	-	+	+	-
Starch hydrolysis	+	-	-	-
Phenylalanine deaminase	+	-	-	-
Gelatin liquefaction	-	-	-	-
Voges-Proskauer test	-	-	+	-
Methyl red test	-	-	-	-
Indole test	-	+	-	-

+, Positive; –, Negative.

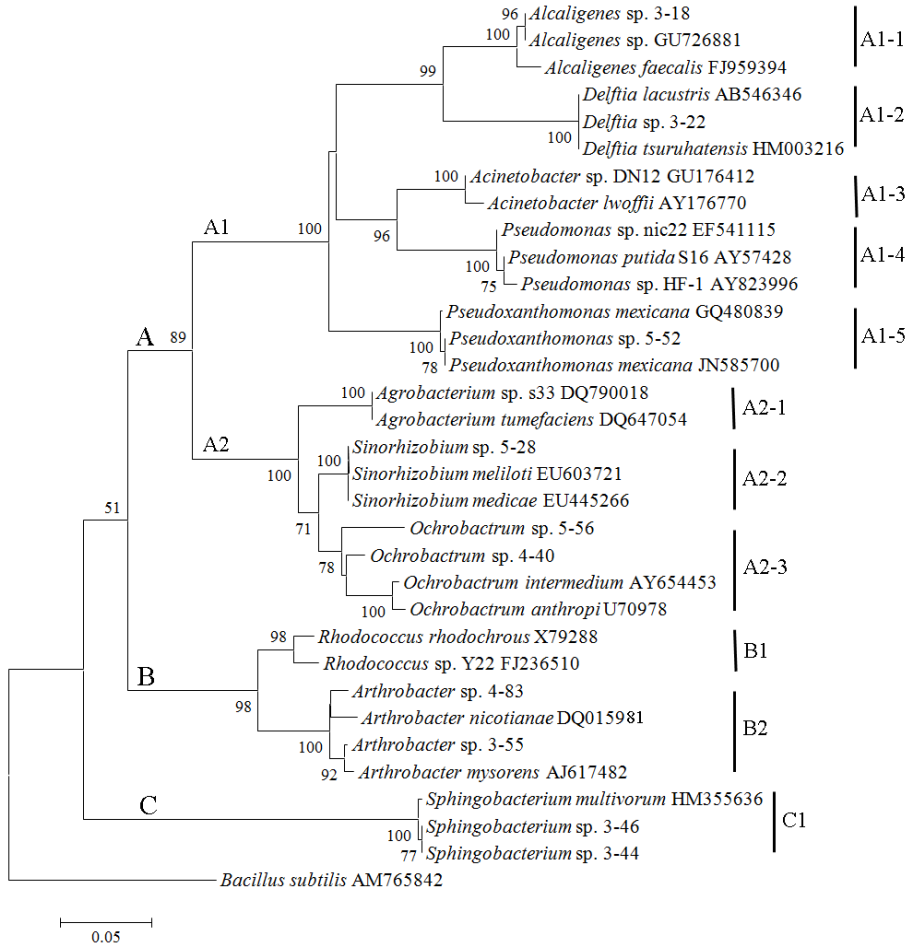

Figure 1. Phylogenetic analysis of nicotine-degrading bacteria and related species based on their 16S rRNA gene sequences. Accession numbers of bacteria isolated in this study were showed in Table 1, and other bacteria were provided in this tree. *Bacillus subtilis* (AM765842) used as outgroup. The program MEGA 4.0 was used to construct a neighbor joining (NJ) tree with 1,000 replicates.

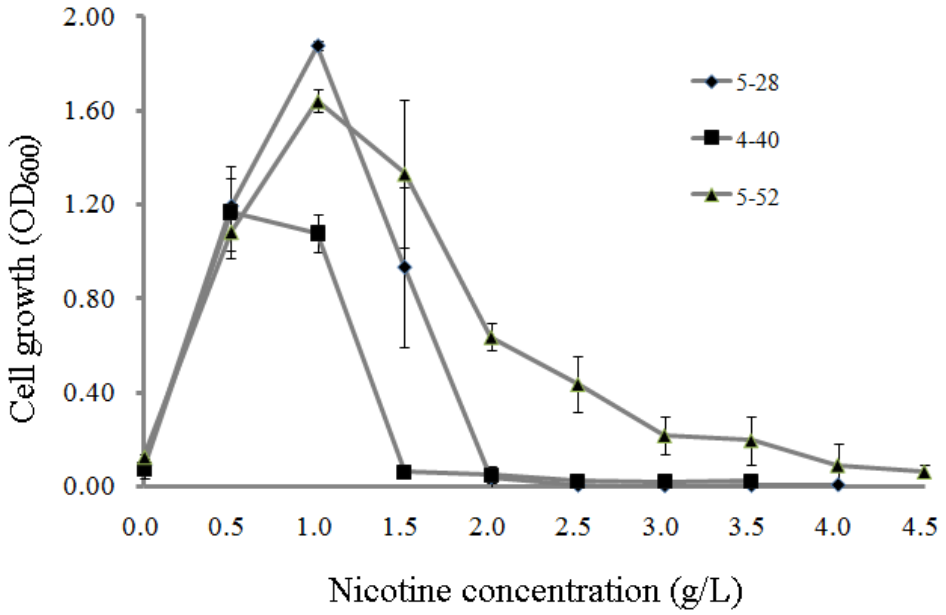

Figure 2. Effect of nicotine concentration on the growth of strains 5-28, 4-40 and 5-52.

meanwhile, *Pseudomonas* sp. and *Acinetobacter* sp. showed closely genetic relationship; while subclade A2 consisted of three bacteria in different genera including *Agrobacterium* sp., *Sinorhizobium* sp. and *Ochrobactrum* sp.. Clade B contained two reported nicotine-degrading bacteria *A. nicotianae* and *Rhodococcus* sp.. Clade C only contained *Sphingobacterium* sp.

Effect of nicotine concentration on nicotine-degrading bacteria

Pseudoxanthomonas sp. 5-52, *Sinorhizobium* sp. 5-28 and *Ochrobactrum* sp. 4-40 were cultured in LB medium for 12 h, and 100 µl broth was transferred into ISM medium containing 0.5 to 4.5 g/L nicotine, respectively, and incubated at 28°C for 28 h. The optimum nicotine concentration for the growth of strains 5-28, 4 40 and 5-52 was 1.5, 0.5 and 1.0 g/L (Figure 2), and the highest nicotine-tolerance concentration was 2.5, 2.5 and 4.5 g/L, respectively.

Comparison of nicotine degradation by three typical strains

Resting cells of *Pseudoxanthomonas* sp. 5-52, *Sinorhizobium* sp. 5-28 and *Ochrobactrum* sp. 4-40 were prepared, and the nicotine-degrading activities were analyzed. Among them, strain 5-28 showed the highest activity to degrade nicotine in tobacco leaves, 72.5% nicotine was decomposed after treating for 12 h (Table 4); strains 4-40 and 5-52 also showed obvious effect to degrade nicotine in tobacco leaves, 51.5 and 47.2% nicotine was decomposed under the same condition, respectively; while, the nicotine was not decomposed in the control samples.

DISCUSSION

The bacterial community residing in the tobacco rhizosphere has presumably adapted to use nicotine as a growth substrate, including biochemical pathways to decompose this organic heterocyclic compound (Brandsch, 2006). In this study, 18 nicotine-degrading bacteria were isolated from tobacco plantation soils by enrichment culture; these bacteria were distributed into seven different genera according to their 16S rRNA gene sequences. Among them, the number of bacteria *Arthrobacter* and *Ochrobactrum* are the most, which suggests that they are the dominant nicotine-degrading bacteria in tabocco plantation soils in Mile, Yunnan province. Meanwhile, many *Sphingobacterium* spp. were also found in the soils. While, few bacteria in genera *Alcaligenes*, *Pseudoxanthomonas*, *Pseudoxanthomonas* and *Delftia* were found in the soils. Moreover, *Arthrobacter* (Schenk et al., 1998), *Ochrobactrum* (Yuan et al., 2007) and *Alcaligenes* (Uchida et al., 1976) have been reported to degrade nicotine, while *Pseudoxanthomonas*, *Sphingobacterium* and *Delftia* are reported to utilize nicotine for the first time. Although the pathway and mechanism of nicotine degradation by these bacteria are unknown, our results provide a basis for understanding the metabolic mechanism and their potential application. Moreover, the nicotine-degrading

Table 4. Comparison of nicotine degradation by three strains in tobacco leaves.

Strains	Times (h)	Peak area		Nicotine concentration (%)	Degradation rate of nicotine (%)
		Control	Sample		
Ochrobactrum sp. 4-40	0	18542.3	18542.3	100	0
	4	19366	12056.1	62.3	37.7
	8	19534.5	10863.9	55.6	44.4
	12	19499.4	9453.5	48.5	51.5
Pseudoxanthomonas sp. 5-52	0	18542.3	18542.3	100	0
	4	19366	11358.1	58.6	41.4
	8	19534.5	10659.9	54.6	45.4
	12	19499.4	10290.5	52.8	47.2
Sinorhizobium sp. 5-28	0	18542.3	18542.3	100	0
	4	19366	9883.8	51	49
	8	19534. 5	7755.5	39.7	60.3
	12	19499.4	5369.6	27.5	72.5

bacteria *Pseudomonas* sp. (Ruan et al., 2005; Wang et al., 2005; Chen et al., 2008), *Rhodococcus* sp. (Gong et al., 2009) and *Agrobacterium* sp. (Wang et al., 2009) were not isolated in this study, which may be result from soil samples and the isolation method.

From the phylogenetic tree (Figure 1), these nicotine-degrading bacteria were evolved into three clades, those bacteria clustered in the same clade may have conserved pathway of nicotine degradation. At present, the nicotine degradation pathways in *P. putida* (Wang et al., 2005, 2007; Li et al., 2010) and *A. nicotinovorans* (Schenk et al., 1998; Brandsch, 2006) have been elucidated, while little is known in other nicotine-degrading bacteria. *P. putida* and *Acinetobacter* sp. ND12 are clustered in a subclade, which suggested that they may share close nicotine degradation pathway. In our previous report, similar nicotine metabolites were identified in *P. putida* and *Acinetobacter* sp. ND12 (Li et al., 2011), which provides evidence to support the aforementioned conjecture. Moreover, similar blue pigment was observed during the degradation of nicotine by *A. nicotinovorans* and *Rhodococcus* sp. Y22 (Gong et al., 2009), which suggested that they may break down nicotine by similar pathway. Interestingly, they were clustered in the same clade (clade B).

Pseudoxanthomonas sp. 5-52, *Sinorhizobium* sp. 5-28 and *Ochrobactrum* sp. 4-40 can tolerate high nicotine concentration, and decompose nicotine effectively; especially, *Sinorhizobium* sp. 5-28 can degrade 72.5% nicotine in tobacco leaves, which suggested that these bacteria have a potential application in tobacco industry and environmental bioremediation. In summary, we reported the diversity of nicotine-degrading bacteria in tobacco plantation soils, and it is provides a basis for understanding the evolution and systematic relationship of these bacteria, and the metabolic pathway and related degradation mechanism.

REFERENCES

Benowitz NL, Perez-Stable EJ, Fong I, Modin G, Herrera B, Jacob P (1999). Ethnic differences in N-glucuronidation of nicotine and cotinine. J. Pharmacol. Exp. Ther. 291:1196–1203.

Brandsch R (2006). Microbiology and biochemistry of nicotine degradation. Appl. Microbiol. Biotechnol. 69:493–498.

Chen CM, Li XM, Yang JK, Gong XW, Li B, Zhang KQ (2008). Isolation of nicotine-degrading bacterium *Pseudomonas* sp. nic22, and its potential application in tobacco processing. Int. Biodeter. Biodegr., 62: 226–231.

Civilini M, Domenis C, Sebastianutto N, Bertoldi M (1997). Nicotine decontamination of tobacco agro-industrial waste and its degradation by micro-organisms. Waste Manag. Res. 15:349–358.

Dong XZ, Cai MY (2001). Manual of identification for general bacteriology. Publishing Company of Science, China.

Doolittle DJ, Winegar R, Lee CK, Caldwell WS, Hayes AW, de Bethizy JD (1995). The genotoxic potential of nicotine and its major metabolites. Mutat. Res. 344:95–102.

Gong XW, Yang JK, Duan YQ, Dong JY, Zhe W, Wang L, Li QH, Zhang KQ (2009). Isolation and characterization of *Rhodococcus* sp. Y22 and its potential application to tobacco processing. Res. Microbiol. 160:200–204.

Kumar S, Tamura K, Nei M (2008). MEGA: molecular evolutionary genetics analysis software for microcomputers. Bioinformatics 10:189-191.

Lenkey AA (1989). Nicotine removal process and product produced thereby; mixing with alkaline agent in aerobic environment. US Patent 4,848,373.

Li HJ, Li XM, Duan YQ, Zhang KQ, Yang JK (2010). Biotransformation of nicotine by microorganism: the case of *Pseudomonas* spp. Appl. Microbiol. Biotechnol. 86:11-17.

Li HJ, Duan YQ, Ma GH, Lei LP, Zhang KQ, Yang JK (2011). Isolation and characterization of *Acinetobacter* sp. ND12 capable of degrading nicotine. Afri. J. Microbiol. Res. 5:1335–1341.

Meher KK, Panchwagh AM, Rangrass S, Gollakota KG (1995). Biomethanation of tobacco waste. Environ. Pollut. 2:199–202.

Novotny TE, Zhao F (1999). Consumption and production waste: another externality of tobacco use. Tob. Control 8:75–80.

Ruan AD, Min H, Peng X, Huang Z (2005). Isolation and characterization of *Pseudomonas* sp. strain HF-1, capable of degrading nicotine. Res. Microbiol. 156:700–706.

Schenk S, Hoelz A, Krauss B, Decker K (1998). Gene structures and properties of enzymes of the plasmid-encoded nicotine catabolism of *Arthrobacter nicotinovorans*. J. Mol. Biol. 284:1323–1339.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25:4876–4882.

Wang SN, Xu P, Tang HZ, Meng J, Liu XL, Qing C (2005). ‘‘Green’’ route to 6-hydroxy-3-succinoyl-pyridine from (S)-nicotine of tobacco waste by whole cells of a *Pseudomonas* sp. Environ. Sci. Technol. 39:6877–6880.

Wang SN, Liu Z, Tang HZ, Meng J, Xu P (2007). Characterization of environmentally friendly nicotine degradation by *Pseudomonas putida* biotype A strain S16. Microbiol. 153:1556–1565.

Wang SN, Liu Z, Xu P (2009). Biodegradation of nicotine by a newly isolated *Agrobacterium* sp. strain S33. J. Appl. Microbiol. 107:838–847.

Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991). 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 173:697–703.

Wilson K (1987). Preparation of genomic DNA from bacteria, In: Ausubel FM, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (Eds.), Current Protocols in Molecular Biology, Wiley, New York. pp. 241-245.

Uchida S, Maeda S, Masubuchi K, Shinohara T, Kisaki T (1976). Isolation of nicotine-degrading bacteria and degradation of nicotine in shredded tobacco and tobacco extract. Nippon Sembai Kosha Chou Kenkyusho Kenkyu Hokoku 18:197–201.

Yuan YJ, Lu ZX, Huang LJ, Li Y, Lu FX, Bie XM, Teng YQ, Lin Q (2007). Biodegradation of nicotine from tobacco waste extract by *Ochrobactrum intermedium* DN2. J. Ind. Microbiol. Biotechnol. 34:567-570.