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Research Article

Biotransformation of Indole to 3-Methylindole by *Lysinibacillus xylanilyticus* Strain MA

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An indole-biotransforming strain MA was identified as *Lysinibacillus xylanilyticus* on the basis of the 16S rRNA gene sequencing. It transforms indole completely from the broth culture in the presence of an additional carbon source (i.e., sodium succinate). Gas-chromatography-mass spectrometry identified indole-3-acetamide, indole-3-acetic acid, and 3-methylindole as transformation products. Tryptophan-2-monooxygenase activity was detected in the crude extracts of indole-induced cells of strain MA, which confirms the formation of indole-3-acetamide from tryptophan in the degradation pathway of indole. On the basis of identified metabolites and enzyme assay, we have proposed a new transformation pathway for indole degradation. Indole was first transformed to indole-3-acetamide via tryptophan. Indole-3-acetamide was then transformed to indole-3-acetic acid that was decarboxylated to 3-methylindole. This is the first report of a 3-methylindole synthesis via the degradation pathway of indole.

1. Introduction

Indole is an industrially important heterocyclic aromatic compound that is an environmental pollutant due to its worldwide occurrence [1]. Major contamination sources are industrial waste, coal tar waste, and wastewater from coking plants, coal gasification, and refineries and cigarette smoke [1].

Bacterial aerobic degradation of indole proceeds via diverse mechanisms including (i) the catechol pathway proceeding through indoxyl, 2,3-dihydroxyindole, isatin, N-formylanthranilic acid, anthranilic acid, salicylic acid, and catechol [2]; (ii) the gentisate pathway proceeding through indoxyl, isatin, anthranilic acid, and gentisic acid [3]; and (iii) the anthranilate pathway proceeding through 2,3-dihydroxyindole, N-carboxyanthranilic acid, and anthranilic acid [4].

Bacterial transformation of indole to indigo has been characterized in a variety of bacteria [2, 5, 6]. Initially, indole is oxidized to indoxyl that spontaneously transforms to indigo. Another transformation mechanism involves conversion of indole to indole-3-acetic acid, indole-3-glyoxylic acid, and indole-3-aldehyde [7].

Anaerobic bacterial degradation of indole was studied under denitrifying, sulfate-reducing, or methanogenic conditions by forming oxindole as the main metabolite [8–10]. A sulphate-reducing bacterium, *Desulfobacterium indolicum*, degraded indole via oxindole, isatin, isatoic acid, and anthranilic acid [11, 12].

In this study, we investigated a new mechanism of aerobic transformation of indole via a newly isolated bacterium, *Lysinibacillus xylanilyticus* strain MA.

2. Materials and Methods

2.1. Chemicals and Media. Indole and its derivativeswere purchased from Sigma-Aldrich. All other chemicals, reagents,

and solvents were purchased from Fisher Scientific. Minimal medium was prepared as described previously [13].

2.2. Bacterial Strain. Twenty bacteria were isolated from the contaminated soil using minimal medium containing 10 mM sodium succinate and 0.5 mM indole. For isolation, 1 g soil was added to minimal medium containing 10 mM sodium succinate and 0.5 mM indole. After 48 h of incubation, the medium was serially diluted and plated on the minimal agar plates containing 10 mM sodium succinate and 0.5 mM indole. After incubation period, twenty different morphotypes were selected and streaked for purity. These bacteria were screened for their ability to mineralize or transform indole by growing on minimal media containing 0.5 mM indole in the presence or absence of 10 mM sodium succinate. Samples were collected at regular intervals to monitor indole depletion and extracted with ethyl acetate. The extracted samples were analyzed by high performance liquid chromatography by a previously described method [7], and the results showed that there was no indole depletion by any of the bacteria growing on minimal medium containing 0.5 mM indole. However, one bacteria-designated strain (MA) was able to deplete indole in the presence of additional carbon sources (i.e., sodium succinate). This bacterium was selected for further study.

2.3. 16S rRNA Gene Sequencing and Phylogenetic Analysis. The genomic DNA extraction for PCR amplification of the 16S rRNA gene of strain MA was carried out as described previously [14]. Amplification and sequencing conditions for the 16S rRNA gene were done exactly the same as described previously [15]. The 16S rRNA gene sequence of strain MA was aligned with other sequences obtained from the EzTaxon databases [16]. The phylogenetic tree was constructed using neighbor-joining algorithms and evolutionary distance matrices were calculated with the Kimura two-parameter model [17]. Bootstrap replications (1000) were performed with the MEGA6 program [17].

2.4. Growth and Indole Depletion. The bacteria were grown on minimal medium containing 0.5 mM indole and 10 mM sodium succinate. Samples were collected at every 4 h interval up to 32 h. For growth measurement, the optical density of the culture was measured at 600 nm using a spectrophotometer. For indole depletion, samples were centrifuged and extracted with ethyl acetate. The extracted samples were dissolved in 20 μL methanol and analyzed by HPLC (Waters 600 HPLC model) as described previously [7].

2.5. Identification of Metabolites. The samples (0 h, 12 h, 24 h, and 32 h) were analyzed by gas-chromatography-mass spectrometry (GC-MS) to identify metabolites via an Agilent gas chromatography system model 7890A equipped with a high throughput time-of-flight mass spectrometer and HP-5 column (30 m × 0.320 mm × 0.25 μm) [7]. The column temperature was initially increased from 50°C to 280°C at the rate of 20°C/min and then held for 5 min [7]. Helium was used as a carrier gas at 1.5 mL/min and the samples (1 μL) were injected in splitless mode [7]. The ion-source temperature and transfer line temperature were maintained at 250°C and 225°C, respectively [7]. The electron energy was set at 70 eV [7].

2.6. Tryptophan 2-Monooxygenase Activity. Enzyme activity was determined in a 1 mL reaction mixture containing 100 mM Tris buffer (pH 7.8), 0.5 mM of L-tryptophan, and crude extracts. After the incubation at 10 min at room temperature, the reaction was stopped by adding 100 μL of 5 N HCl. The reaction mixture without crude extracts served as the control. The reaction mixture was centrifuged, extracted with ethyl acetate, and dissolved in 20 μL of methanol and analyzed with GC-MS to identify the product.

3. Results and Discussion

Strain MA was identified as a member of the genus *Lysinibacillus xylanilyticus* on the basis of the 16S rRNA gene sequencing. The 16S rRNA gene sequence of strain MA has been deposited in NCBI under the GenBank accession number KT030900. Phylogenetic analysis showed that strain MA fell within other members of *Lysinibacillus* with a cluster near *Lysinibacillus xylanilyticus* strain XDB9 (Figure 1). On the basis of the 16S rRNA gene sequencing and phylogenic analysis, strain MA was identified as *Lysinibacillus xylanilyticus* strain MA.

Figure 2 showed that strain MA grew well on minimal medium supplemented with 0.5 mM indole and 10 mM sodium succinate. During the initial 4 h, there was no bacterial growth due to lag phase whereas bacteria grow rapidly after 8 h due to the exponential phase. There was very slow growth after 24 h when the bacteria reached stationary phase. The maximum optical density of the culture was 1.7 after 32 h of incubation. No bacterial growth was observed on minimal medium supplemented with 0.5 mM indole because it is the sole source of carbon and energy. These data indicate that strain MA did not utilize indole as its sole source of carbon and energy. Strain MA transforms indole in the presence of additional carbon source (i.e., sodium succinate). Indole transformation was measured by HPLC, and the results showed complete indole depletion within 32 h.

The GC-MS studies showed transformation of indole into three metabolites. These metabolites were identified on the basis of their mass spectra comparisons with those of authentic standards. The mass spectrum of metabolite I had a molecular ion at m/z 174 and quinolinium ion at m/z 130. This metabolite was identified as indole-3-acetamide (Figure 3(a)). The mass spectrum of metabolite II contains a parent ion at m/z 175 and quinolinium ion at m/z 130. This metabolite was identified as indole-3-acetic acid (Figure 3(b)). The mass spectrum of metabolite III had ions at m/z 131, 130, 103, 102, 77, and 78. This metabolite was identified as 3-methylindole (Figure 3(c)).

The GC-MS analysis of the enzyme reaction mixture indicated the formation of a product with a mass spectrum corresponding to indole-3-acetamide. However, this product was not detected in the control.

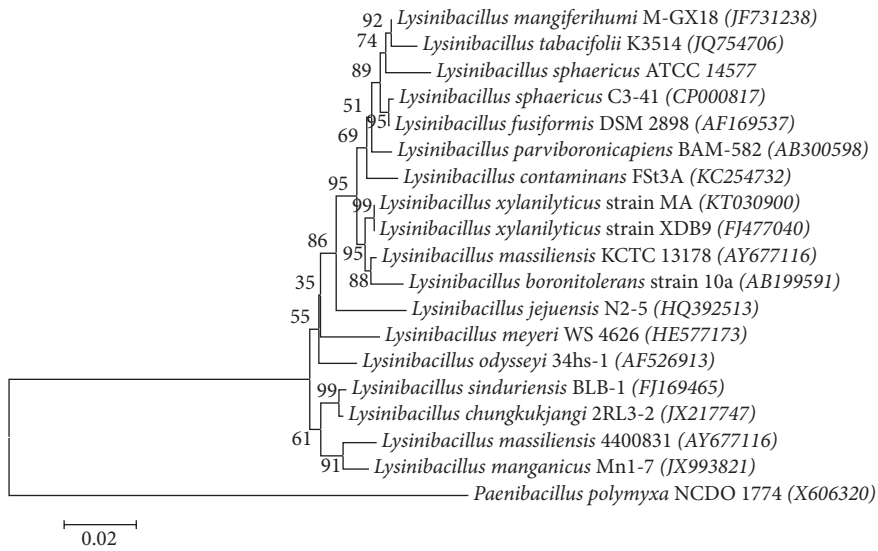

Figure 1: Neighbor-joining tree of *Lysinibacillus xylanilyticus* strain MA based on the 16S rRNA gene sequences.

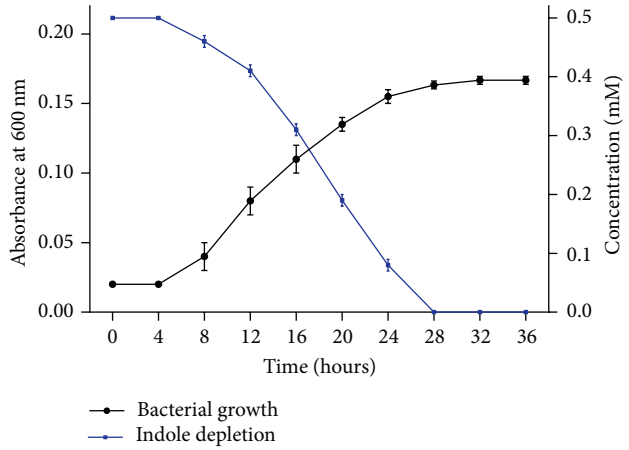

Figure 2: Growth of *Lysinibacillus xylanilyticus* strain MA on minimal medium containing 10 mM sodium succinate and 0.5 mM indole and indole depletion by *Lysinibacillus xylanilyticus* strain MA.

On the basis of transformation products, we proposed a new pathway of indole transformation. Indole is initially converted to indole-3-acetamide via tryptophan. Indole-3-acetamide was then transformed into indole-3-acetic acid, which was decarboxylated to 3-methylindole (Figure 4). This is the first report of formation of 3-methylindole from indole.

Previous studies showed that indole-3-acetic acid formation occurs via either tryptophan-dependent pathway [18, 19] or tryptophan-independent pathway [7, 20, 21]. The *Arthrobacter* sp. SPG converted indole to indole-3-acetic acid without forming tryptophan. This suggested the involvement of a tryptophan-independent pathway [7]. However, in this study, we observed tryptophan 2-monooxygenase activity in the crude extracts of indole-induced cells of strain MA suggesting the involvement of a tryptophan-dependent pathway. Two mechanisms are known for formation of indole-3-acetic acid from tryptophan [18, 19]. The first mechanism involves a tryptophan aminotransferase-catalyzed conversion of tryptophan to indole-3-pyruvic acid, which is decarboxylated to indole-3-acetaldehyde by an indole-3-pyruvic acid decarboxylase. This is then further oxidized to indole-3-acetic acid [18]. We have not detected indole-3-pyruvic acid and indole-3-acetaldehyde as metabolites of indole degradation, suggesting that this mechanism is not involved in the transformation. In the second mechanism, the initial step is catalyzed by tryptophan 2-monooxygenase and involves conversion of tryptophan to indole-3-acetamide that is then transformed to indole-3-acetic acid by indole-3-acetamide hydrolase [19]. In this study, indole-3-acetamide was detected as a metabolite, indicating involvement of the indole-3-acetamide pathway. Furthermore, the tryptophan 2-monooxygenase activity confirmed the formation of indole-3-acetamide from L-tryptophan.

In this study, 3-methylindole was also detected as a transformation product. It may be formed from decarboxylation of indole-3-acetic acid. Several researchers have reported the formation of 3-methylindole from indole-3-acetic acid [1, 22–25]. Many anaerobic bacteria including *Lactobacillus* sp. [22], *Clostridium scatologenes* [23], and *Clostridium drakei* [23] transformed indole-3-acetic acid to 3-methylindole. A mixed population of pig fecal bacteria has been reported to convert indole-3-acetic acid to 3-methylindole [24]. Attwood et al. [25] reported that six rumen microorganisms (similar to *Prevotella* sp., *Clostridium* sp., *Actinomyces* sp., and *Megasphaera* sp.) isolated from grazing ruminants produced 3-methylindole in the presence of indole-3-acetic acid [1].

This study differs from all previous studies of indole biotransformation due to involvement of new transformation mechanism. In the previous study, *Arthrobacter* sp. SPG also transformed indole to indole-3-acetic acid that was further converted to indole-3-glyoxylic acid and indole-3-aldehyde [7]. In this case, indole-3-acetic acid is transformed to 3-methylindole. Several bacteria transformed indole to

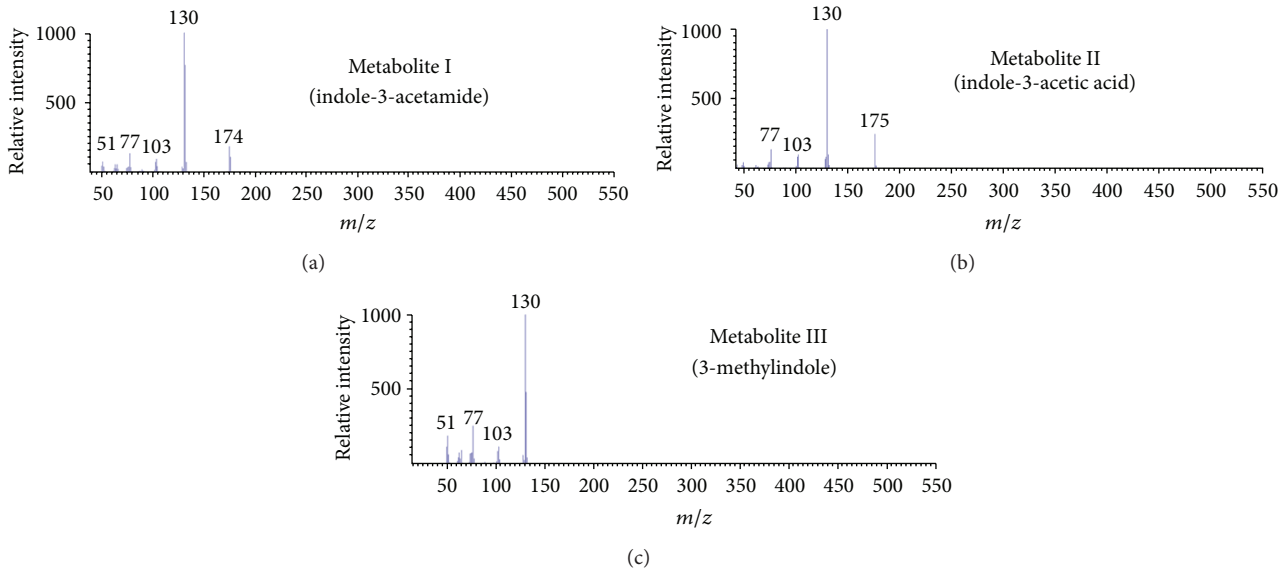

Figure 3: Mass spectra of metabolites I (a), II (b), and III (c).

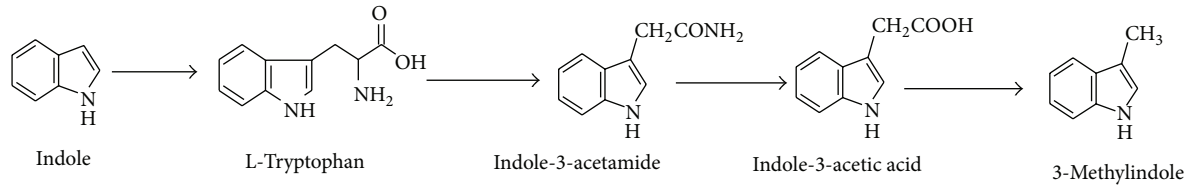

Figure 4: Pathway of indole transformation by *Lysinibacillus xylanilyticus* strain MA.

indigo via indoxyl [1]; however, strain MA did not produce indoxyl or indole. Recently, Fukuoka et al. [26] reported biotransformation of indole in *Cupriavidus* sp. strain KK10 via an N-heterocyclic ring cleavage or carbocyclic aromatic ring cleavage of indole; however, in this study, neither N-heterocyclic ring cleavage nor carbocyclic aromatic ring cleavage occurred.

4. Conclusion

Lysinibacillus xylanilyticus strain MA transforms indole to 3-methylindole via L-tryptophan, indole-3-acetamide, and indole-3-acetic acid. This is the first report of 3-methylindole bacteria-based formation from indole.

Conflict of Interests

The authors declare that they have no conflict of interests.

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