

Highly Efficient Biosynthesis of Indole-3-acetic acid by *Enterobacter xiangfangensis* BHW6

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Abstract

Background: Indole-3-acetic acid (IAA) plays an important role in the growth and development of plants. Various bacteria in the rhizosphere are capable to produce IAA that acts as a signaling molecule for the communication between plants and microbes to promote the plant growth. Due to the low IAA content and various interfering analogs, it is difficult to detect and isolate IAA from microbial secondary metabolites.

Results: A predominant strain with a remarkable capability to secrete IAA was identified as *Enterobacter xiangfangensis* BHW6 based on 16S rRNA gene sequence, the determination of average nucleotide identity (ANI) and digital DDH (dDDH). The maximum IAA content (134-1129 µg/mL) was found with the addition of 0.2-15 g/L of L-tryptophan at pH 5 for 6 days, which was 4-40 fold higher than that in the absence of L-tryptophan. The highest yield of IAA was obtained at the stationary phase of bacterial growth. An acidic culture medium was preferred for the IAA biosynthesis of the strain. The strain was tolerant and stable to produce IAA in the presence 2.5%-5% (w/v) of NaCl. IAA was then isolated through column chromatography with a mobile phase of hexane/ethyl acetate (1/2, v/v) and characterized by ¹H Nuclear Magnetic Resonance (¹H NMR).

Conclusions: A remarkable IAA production was obtained from *E. xiangfangensis* BHW6 that was tryptophan-dependent. According to genomic analysis, the *ipdC* gene coding for the key enzyme (indole-3-pyruvate decarboxylase) was identified indicating that IAA biosynthesis was mainly through the indole-3-pyruvia acid (IPyA) pathway, which was further confirmed by intermediate assay. *E. xiangfangensis* BHW6 with an important economic value has great prospect in agricultural and industrial application.

Introduction

Phytohormones including abscisic acid (ABA), gibberellins (GAs), ethylene, indole-3-acetic acid (IAA), and cytokinins (CKs), and brassinosteroids (BRs) participate in many physiological and biochemical processes in plants (Fahad et al. 2015). Indole-3-acetic acid (IAA) as one of the main phytohormones plays a key role in the regulation of various physiological processes, such as cell division and elongation, vascular differentiation, gravitropism and phototropism. It inhibits or delays abscission of leaves and induces flowering and fruiting (Boopathi et al. 2013).

IAA can be synthesized in plants and also in microorganisms. A large number of bacteria such as *Bacillus*, *Pseudomonas*, *Enterobacter*, *Azospirillum*, *Agrobacterium* and *Rhizobium* has been reported to generate the capability to synthesize IAA (Oberhänsli, et al. 1991; Lim & Kim, 2009). These microbes act as a communication system with the host plants. Exogenous application of IAA can improve the endogenous levels of plants and thus alter the plant ontogeny (Lin et al. 2018).

The biosynthesis of IAA in microorganisms is accomplished by a variety of pathways. Tryptophan is an efficient precursor for the biosynthesis of IAA. In general, the followings are the main pathways for the conversion of tryptophan to IAA (Patten and Glick, 1996). The IPyA pathway

(tryptophan→IPyA→IAAld→IAA) has been studied in detail in *Enterobacter cloacae* (Koga et al. 1991) and *Bacillus amloliquefaciens* FZB42 (Idris et al. 2007). The IAM pathway (tryptophan→IAM→IAA) has been discovered in *Streptomyces spp* (Manulis et al. 1998). The TAM pathway (tryptophan→TAM→IAAld→IAA) has been reported in *Bacillus cereus* (Perley and Stowe, 1966). The TSO pathway (tryptophan→IAAld→IAA) has been demonstrated in *Pseudomonas fluorescens* CHA0 (Oberhänsli et al. 1991). The IAN pathway (tryptophan→IAN→IAA) has been confirmed in several strains of *Agrobacterium* and *Rhizobium* (Kobayashi et al. 1995).

Due to the low IAA content and various interfering analogs, it is difficult to detect and isolate IAA from microbial secondary metabolites. Cultivation surrounding is crucial to improve the capability of the microorganisms to synthesize IAA. L-tryptophan supplementation, pH, and NaCl concentration have an important effect on bacterial growth and IAA yield. Ultraviolet spectrometry is widely used to determine the IAA content. High performance liquid chromatography is a well-recognized method to enable the sensitive and accurate measurement of the substances in crude extracts (Li et al. 2018). Common purification procedures including liquid-liquid extraction and column chromatography are employed for the isolation and purification of IAA products (Lim and Kim, 2009).

In this study, highly efficient biosynthesis of IAA was found in a strain identified as *Enterobacter xiangfangensis* BHW6, which was tryptophan-dependent. Cultivation parameters including L-tryptophan supplementation, fermentation time, initial pH, NaCl concentration were determined in order to improve the IAA production. Genome analysis was conducted to investigate the IAA biosynthesis of the strain. Immediate assay was also employed to identify the IAA synthetic pathways. IAA was further isolated and purified using column chromatography, which was characterized by ¹H NMR for its chemical structure.

Materials And Methods

Sample collection

Soil samples were collected from the corn farm in the Jining of the Shandong province, P. R. China, which was stored in sealed and sterile bags at 4°C. All chemicals were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China).

Isolation of the strain and determination of IAA content

10 g of the soil sample was mixed with 90 mL of distilled sterile water, which was stirred at 30 °C and 150 rpm for 30 min. The resultant soil solution was diluted and plated on Luria-Bertan (LB) agar medium including NaCl (10 g), yeast extract (5 g), tryptone (10 g), agar (18 g). IAA content of the strains was determined according to the method of Gordon and Weber (1951) with some modifications. The strain was incubated in LB liquid medium supplemented with 0.2 mg/mL of L-tryptophan at 30 °C for 6 days. Uninoculated LB liquid medium was used as a control. Each experiment was conducted in triplicate. After that, the fermentation broth was centrifuged at 10000 rpm for 10 min. 2 mL of supernatant was combined with 4 mL of Salkowski reagent (50 mL of 35% HClO₄ & 1 mL of 0.5 M FeCl₃), which was

incubated in darkness for 30 min at 40 °C. The absorbance of Indole-3-acetic acid (IAA) was measured at 530 nm using a UV-VIS spectrophotometer (UV-6100, Metash, China).

Molecular identification

The DNA of the enriched bacteria was extracted using a DNA kit according to the manufacturer's instructions (OMEGA, USA). 16S rRNA gene sequence was amplified by PCR with 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3'). The polymerase chain reaction (PCR) was performed in a 50 µL system (template DNA 2 µL, forward primer 2 µL, reverse primer 2 µL, 2×mastermix 25µL, and DdH₂O 19 µL) (Tiangen, China). PCR products were sequenced at the Huada gene company (Beijing, China). The sequences were deposited into Genbank and the accession numbers were obtained as MN696244.

Cultivation factors for IAA production

The reactions were performed in LB medium with 1% inoculation and 150 rpm. L-tryptophan as a precursor was examined at the concentrations of 0, 0.2, 0.5, 1, 2, 5, 10 and 15 (mg/mL) at pH 7.0 and 30 °C for 6 days. Incubation time of 1-10 days was investigated at pH 7.0 and 30 °C. Initial pH value was detected in the range of 3.0-11.0 at 30 °C for 6 days. NaCl concentration was determined at 0%, 2.5%, 5.0%, 7.5% and 10.0% (w/v) at 30°C and initial pH 5 for 6 days.

Genomic sequencing analysis

Genome analysis was performed by Personalbio Company (Beijing, China). The DNA was prepared using a genomic DNA extraction kit (Tiangen, China). The whole genome shotgun (WGS) and next generation sequencing (NGS) were employed. Illumina NovaSeq Sequencing platform was used to sequence the paired-end (PE). GeneMarkS software was used to predict the protein coding genes in the bacterial genome. Protein coding gene was annotated by NR, KEGG, Swiss-Prot, eggNOG, and Go and Pfam.

Intermediate assay for IAA biosynthesis

Intermediate assay for IAA synthetic pathway was based on monitoring the utilization of an intermediate and the determination of the resulting product after enzyme reactions as described by Bunsangiam *et al.* (2019) with some modifications. The precultured strains were resuspended in LB medium were supplemented with 0.2% (w/v) of one of IAA precursors such as L-tryptophan, IPyA, IAM, TAM and IAN, which were incubated at 30 °C for 24 h. The metabolites were quantified by HPLC analysis (Waters 2695, America).

Isolation and purification of IAA

The strain was incubated at 30 °C and pH 5 with 1% inoculation and 150 rpm for 6 days in LB liquid medium supplemented with 10 mg/mL of L-tryptophan. After that, the solution was centrifuged at 5000 rpm for 15 min. The supernatant was acidified to pH 2.5 with 1 mol/L HCl, which was then extracted

twice with double volume of ethyl acetate. The ethyl acetate was evaporated at 40-50 °C by rotary evaporation (EYELAN-1100, Japan) to give the crude product. The crude product was then subjected to column chromatography (1.5×30 cm), which was eluted using hexane/ethyl acetate (1/2, v/v). The solvent was evaporated at 40°C-50 and the resulting product was dried in a vacuum for 2 h. ¹H nuclear magnetic resonance (¹H NMR) of IAA was conducted using a spectrometer (Bruker AVI 400 MHz, Germany).

Results

Determination of IAA production of the strain

Han *et al.* isolated 55 strains with the capability of producing IAA in a range of 1-10 µg/mL with L-tryptophan as a precursor from rice root endophytes. Xu *et al.* (2018) screened 12 strains with the capability of producing IAA in 0.2 g/L of L-tryptophan-supplemented medium from watermelon rhizosphere soil with a high yield of IAA (115.3 µg/mL) obtained from *streptomycete* CL05. In current studies, IAA productions of 14 strains were obtained in a range of 4.62 µg/mL to 109.72 µg/mL with a supplement of L-tryptophan (0.2 g/L) (Fig.1a).

Particularly, 28.36 µg/mL of IAA was obtained in the absence of L-tryptophan, whereas 115.72 µg/mL was achieved in the presence of 0.2 g/L of L-tryptophan. An increase of 4.1-fold was observed with the addition of L-tryptophan, thus the biosynthesis IAA in strain BHW6 was tryptophan-dependent. Similar results were found in the previous study. IAA production in a plant-beneficial bacterium *Arthrobacter pascens* ZZ21 was increased 4.5-fold with the addition of 0.2 mg/L of L-tryptophan, indicating that IAA biosynthesis in ZZ21 was induced by L-tryptophan (Li *et al.* 2018). The low quantity of IAA was also detected in *Enterobactersp.* I-3 in the culture medium without L-tryptophan and the high concentration of IAA (approximately 220 µg/mL) was observed in 2 g and 3 g of L-tryptophan-supplemented medium (Park *et al.* 2015).

Molecular identification of the strain

A number of microorganisms such as *Agrobacterium*, *Pseudomonas*, *Bacillus*, *Rhizobium* and *Azospirillum* are known to be capable to produce IAA (Mohite, 2013). Microbes such as *Bacillus* Tp. 1B-7B and *Penicillium* Tp. 1F-5F could produce IAA, especially when the growth media are supplemented with L-tryptophan as a precursor (Hassan, 2017). In this study, molecular identification of the strain was based on 16S rRNA gene sequence and the phylogenetic tree was shown in Fig. 2. The strain BHW6 showed 99.94% similarity with *Enterobacter xiangfangensis* 72231 (accession No. MN304301, GenBank). Thus the strain was identified as *Enterobacter* sp. BHW6. The obtained nucleotide sequences were submitted to NCBI GeneBank with accession No. MN696244.

Average nucleotide identity (ANI) and digital DDH (dDDH) have been most widely used to identify the microbes. Draft genomes were sequenced and assembled in this study. ANI value was calculated according to the orthoANlu algorithm (Lee *et al.* 2016). ANI value between two genome sequences and

the DNA G+C content of a single genome sequence was calculated based on an online ANI calculator (www.ezbiocloud.net/tools/ani). As shown in Table 1, the ANI value between *Enterobacter* sp. BHW6 and the type strain of phylogenetically related species were more than 97.12%. Moreover, dDDH value was calculated according to Genome-to-Genome Distance Calculator of dDDH Web service (<http://ggdc.dsmz.de/ggdc.php#>). The dDDH value between *Enterobacter* sp. BHW6 and the type strain of phylogenetically related species was more than 77.6%. Therefore, the strain was identified as *Enterobacter xiangfangensis* BHW6.

L-tryptophan concentration for IAA production and bacterial growth

L-tryptophan is considered as a precursor of IAA. IAA production was improved with an increase of L-tryptophan concentration. *Klebsiella pneumoniae* Psn8 was found to have the maximum IAA production (277 µg/mL) at 1.6% (w/v) of L-tryptophan concentration. IAA production (1.5 µg/mL) of *R. benzoatilyticus* JA2 was obtained in the absence of L-tryptophan, which was enhanced to 12-14 µg/mL with 0.5-2 µg/mL of L-tryptophan (Mujahid *et al.* 2011).

Different concentrations of L-tryptophan were studied for *E. xiangfangensis* BHW6. An increase of IAA production was found with the increase of L-tryptophan concentration (Fig. 3). Significantly, the maximum IAA content (1078 µg/mL) was found with the addition of 15 g/L of L-tryptophan at pH 7 for 6 days, which was 38-fold higher than the IAA content (28.33 µg/mL) in the absence of L-tryptophan. Therefore, IAA biosynthesis in *E. xiangfangensis* BHW6 was mainly dependent on the exogenous L-tryptophan concentration.

Meanwhile, the strains grew well at 10 g/L of L-tryptophan and produced good yield of IAA. Although the bacterial growth was reduced remarkably at 15 g/L, IAA content was still increase to the maximum amount. Therefore, the highest IAA production was obtained at the stationary phase of bacterial growth. With the further increase of L-tryptophan concentration to 20 g/L, bacterial growth was inhibited with a significant reduced OD₆₀₀ value, which was also accompanied by the decreased IAA production.

Incubation time for IAA production and bacterial growth

Swain *et al.* (2007) described that IAA production of *B. subtilis* was increased linearly from 2 days to 8 days with the maximum IAA content at 8th day of incubation, which was accompanied with the growth of the strain. Bharucha *et al.* (2013) found that IAA production was improved from 12 h to 96 h and then was reduced from 96 h to 144 h for *Pseudomonas putida* UB1, which was consistent with that for bacterial growth.

As shown in Fig. 4a, IAA production of *E. xiangfangensis* BHW6 was increased notably from 1 day to 6 days with a high IAA yield at 6th in the presence of 0.2 g/L of L-tryptophan. After 6 days, the ability of the strain to produce IAA was decreased slightly. However, the growth of strain was increased remarkably within 2 days of cultivation.

Similar results were obtained in the presence of 15 g/L of L-tryptophan (Fig. 4b). The maximum amount of IAA was observed at 6-8 days of cultivation. Meanwhile, the growth of strain was reduced after 2 days, which indicated that IAA was accumulated mainly during stationary phase of the growth of *E. xiangfangensis* BHW6.

Initial pH for IAA production and bacterial growth

Initial pH has a crucial effect on the growth of the strains and their metabolic activity. Acuña *et al.* (2011) found that the highest IAA production was obtained at pH 6.0 for *Bacillus* spp. MQH-19. However, 62% of reduction was observed at pH 5.0. In this study, the strain has good growth over a broad range of pH 5.0-10.0, with a strong ability to secrete IAA at pH 5.0-8.0.

As reported, the highest IAA yield for *B. pyrrocina* JK-SH007 was achieved at pH 7.0 and the fastest growth of this strain was observed at pH 4.0 (Liu *et al.* 2019). In our study, the highest yield of IAA was found at pH 5.0 and the optimal growth of the strain was found at pH 5.0-7.0 (Fig. 5). An acidic culture medium was preferred for the IAA biosynthesis indicating that pH have a reasonably effect on the metabolic activity of *E. xiangfangensis* BHW6. When the pH value was below 5.0, the strains hardly survived without the capacity to produce IAA. Therefore, the maximum amount of IAA (134 µg/mL and 1129 µg/mL) was obtained at pH 5 with the addition of L-tryptophan at 0.2 g/L and 15 g/L, respectively (Fig. 5b).

NaCl tolerance of the IAA producing strain

As reported, IAA production under salt stress was observed to be 250 µg/ml, 220µg/ml and 200 µg/ml for *Rheinheimera* sp. *Rhizobium* sp. and *Bacillus subtilis* respectively (Rupal K, 2020). The salt tolerance was determined by selecting NaCl concentrations of 2.5%, 5.0%, 7.5% and 10.0% (w/v). *E. xiangfangensis* BHW6 can grow well and generate good capability to produce IAA in the range of 0%-5% (w/v) NaCl concentration. When the medium was supplemented with 2.5%-5% (w/v) of NaCl, IAA content was stable at 117-115 µg/mL (Fig. 6a) and 1260-1053 µg/mL (Fig. 6b) in the presence of 0.2 g/L and 15 g/L of L-tryptophan, respectively. However, the bacterial growth was inhibited with addition of 7.5%-10.0% (w/v) of NaCl, which caused the poor yield of IAA and the bacterial growth.

Genomic analysis of IAA biosynthesis pathways

There are five main pathways for the conversion of tryptophan to IAA including IPyA pathway, IAM pathway, TAM pathway, TSO pathway, and IAN pathway. The indole-3-pyruvate acid (IPyA) pathway is the most important pathway for IAA biosynthesis, which has been described in various bacteria such as *Enterobacter cloacae*, *Azospirillum brasilense*, *Bacillus amloliquefaciens* (Zakharova *et al.* 1999). According to genomic analysis, *E. xiangfangensis* BHW6 contains the gene (*ipdC*) encoding the key enzyme (IPDC) that can catalyze the conversion of IPyA to IAAld in the lpyA pathway (Fig.7a). The length of this gene is 1659 bp, encoding 559 amino acid sequences (Fig.7b). The similarity of the gene sequence is 99.82% with the IPDC enzyme of *Enterobacter cloacae* (KGY930.98.1). The *ipdC* gene coding

for the IPDC enzyme has been isolated and identified from *Azospirillum brasilense*, *Pseudomonas putida* and *Pa. agglomerans* (Zakharova *et al.* 1999; Patten & Glick, 2002b; Costacurta *et al.* 1994).

Intermediates assay for IAA biosynthesis

IAA biosynthetic pathway was investigated by intermediates assay using different IAA precursors. The accumulation of IAA was found when the medium was supplemented with lpyA indicating that the strain was tryptophan-dependent lpyA pathway. IAAld and IET were also detected in the medium containing lpyA. In IPyA pathway, tryptophan is converted into IPyA by an aminotransferase. IPyA is then decarboxylated to indole-3-acetaldehyde (IAAld) by indole-3-pyruvate decarboxylase (IPDC), which is the rate-limiting step. After that, IAAld is oxidized in IAA (Fig. 8). The intermediates assay confirmed that IPyA pathway was the main IAA biosynthetic pathway.

Purification and characterization of IAA

The IAA produced by *E. xiangfangensis* BHW6 was extracted by ethyl acetate. The IAA product could be detected by thin-layer chromatography using n-butanol/ammonia/water (10/1/10, v/v) as the solvent system, which was visualized when the plate was sprayed with a reagent (50 mL of 35% HClO₄ & 1 mL of 0.5 M FeCl₃) under 254 nm of ultraviolet light (Bharucha, *et al.*, 2013). The crude product was then applied to a column chromatography eluted by hexane/ethyl acetate (1/2, v/v). The chemical structure of the IAA product from *E. xiangfangensis* BHW6 was characterized as shown in Fig.9. ¹H NMR (400 MHz, DMSO-d₆) (ppm): 12.23 (s, 1H), 10.97 (d, 1H), 7.55 (d, 1H), 7.41 (s, 1H), 7.28 (s, 1H), 7.13 (t, 1H), 7.03 (t, 1H), 3.69 (s, 1H).

Discussion

IAA plays a central role in modulating plant growth and development, which can be synthesized by both plants and microbes. Various bacteria in the rhizosphere are capable to produce IAA that acts as a signaling molecule for the communication between plants and microbes to promote the plant growth. Generally, IAA production was obtained in a range of 10–200 µg/mL with the addition of 0.2–2 µg/mL of L-tryptophan (Mujahid *et al.* 2011; Park *et al.* 2015; Xu *et al.* 2018). In this study, a predominant strain was identified as *Enterobacter xiangfangensis* BHW6 that was capable of producing 134–1129 µg/mL of IAA in the presence of 0.2–15 g/L of L-tryptophan indicating that the strain was highly tryptophan-dependent. A good IAA production of *E. xiangfangensis* BHW6 was beneficial for agricultural application. In the presence of L-tryptophan, a remarkable IAA yield of *E. xiangfangensis* BHW6 as a cell factory has great prospect in industrial application.

Tryptophan is the main precursor for IAA biosynthesis. Tryptophan could be obtained from root exudates or released from degrading roots and microbial cells, which are the natural sources of tryptophan for the rhizosphere microbes that may enhance auxin biosynthesis in the rhizosphere (Marten & Frankenberger, 1994). In plants, four tryptophan-dependent pathways have been identified including indole-3-acetamide (IAM), indole-3-pyruvic acid (IPyA), tryptamine (TAM), and indole-3-acetaldoxime (IAOX) pathway (Mano &

Nemoto, 2012). Actually, IAA can be synthesized via tryptophan-dependent pathway and tryptophan-independent pathway in microbes. However, tryptophan-independent pathway was still unclear. In bacterial, five pathways including IAM pathway, IPyA pathway, TAM pathway, IAN (indole-3-acetonitrile) pathway and tryptophan side-chain oxidase pathway were identified using tryptophan as a precursor. A combination of genetic analysis and chemical analysis was employed in this study. A candidate gene (*ipdC*) was identified for *E. xiangfangensis* BHW6 based on genomic analysis. This gene is similar with the IPDC enzyme of *Enterobacter cloacae*. The key IPDC enzyme is capable to convert IPyA into IAAld in the lpyA pathway. The following intermediate assay confirmed the lpyA pathway for *E. xiangfangensis* BHW6, as the accumulation of desired products was observed in the medium with the addition of precursors. Therefore, lpyA pathway is the major IAA biosynthetic pathway for *E. xiangfangensis* BHW6.

Environmental factors have an important effect on the IAA synthesis of microbes. An acidic pH is typical for the rhizosphere environment due to proton extrusion through membranes of root cells. Thus IAA biosynthesis of *E. xiangfangensis* BHW6 is probably tuned to encounter environmental stresses associated with the soil and plant environment (Spaepen et al. 2007). An alternative sigma factor that is acid-regulated could be responsible in passing on the pH response to *ipdC* expression (Ona et al. 2003). With the increase of culture time, the nutrient in the medium was gradually depleted and the proportion of each nutrient was maladjusted, which resulted in the reduction of multiplication of the strain. Meanwhile, a number of harmful metabolite was possibly accumulated, which caused the cell death. Herein, the physiological metabolic activity of the strain gradually slows down and IAA production tends to stagnate. Moreover, bacteria such as rhizosphere growth-promoting bacteria, nitrogen-fixing bacteria, phosphorus bacteria, potassium bacteria and rhizobia are very limited in their ability to resist salinity and alkali, leading to their seriously restricted application in agricultural production. A high NaCl tolerance and a broad pH value of *E. xiangfangensis* BHW6 are promising in agricultural application. Furthermore, as the low IAA content and various interfering analogs, it is difficult to isolate IAA from microbial secondary metabolites. HPLC, HPLC-MS, GC-MS are always applied to measure IAA content and determine its chemical structure. In this study, IAA was successfully isolated and purified by column chromatography, which was characterized by NMR.

Conclusions

E. xiangfangensis BHW6 was identified to possess a predominant ability to secrete IAA in the presence of L-tryptophan. The maximum IAA content (134–1129 µg/mL) was found with the addition of 0.2–15 g/L of L-tryptophan at pH 5 for 6 days, which was 4–40 fold higher than the IAA content in the absence of L-tryptophan. IAA was produced at the stationary phase of bacterial growth. The strain was tolerant and stable to 2.5%-5% (w/v) of NaCl. Genomic sequencing analysis indicated that IAA biosynthesis of the strain was mainly through the IPyA pathway, which was confirmed by intermediates assay as the accumulation of desired products was found in the media containing IAA precursors of lpyA pathway. IAA was isolated with column chromatography and characterized with NMR. *E. xiangfangensis* BHW6 with a crucial economic value has great prospect for the effective and inexpensive production of IAA.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

BX and XM designed research. YY and XL conducted experiments. XY and JJ analyzed data. BX wrote the manuscript. All authors read and approved the manuscript.

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References

Acuña JJ, Jorquera MA, Martínez OA, Menezes-Blackburn D, Fernández MT, Marschner P, Greiner R, Mora ML. Indole acetic acid and phytase activity produced by *rhizosphere bacilli* as affected by pH and metals. *J Soil Sci Plant Nut.* 2011; 11(3): 1-12. doi: 10.1007/s42729-020-00295-x

Bharucha U, Patel K, Trivedi UB. Optimization of Indole acetic acid production by *Pseudomonas putida* UB1 and its effect as plant growth-promoting rhizobacteria on Mustard (*Brassica nigra*). *Agric Res.* 2013; 2(3): 215-221. doi: 10.1007/s40003-013-0065-7

Boopathi T, Balamurugan V, Gopinath S, Sundararaman M. Characterization of IAA production by the Mangrove Cyanobacterium *Phormidium* sp. MI405019 and its influence on tobacco seed germination and organogenesis. *J Plant Growth Regul.* 2013; 32(4): 758-766. doi:10.1007/s00344-013-9342-8

- Bunsangiam S, Sakpuntoon V, Srisuk N, Ohashi T, Fujiyama K, Limtong S. Biosynthetic Pathway of Indole-3-Acetic Acid in Basidiomycetous Yeast *Rhodospiridiobolus fluvialis*. *Mycobiol.* 2019; doi: [10.1080/12298093.2019.1638672](https://doi.org/10.1080/12298093.2019.1638672)
- Costacurta A, Keijers V, Vanderleyden J. Molecular cloning and sequence analysis of an *Azospirillum brasilense* indole-3-pyruvate decarboxylase gene. *Mol Genet Genomics.* 1994; 243(4): 463-472. doi:[10.1007/BF00280477](https://doi.org/10.1007/BF00280477).
- Fahad S, Hussain S, Bano A, Saud S, Hassan S, Shan D, Khan FA, Khan F, Chen YT, Wu C, Tabassum M A, Chun M X, Afzal M, Jan A, Jan M T, Huang J L. Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: consequences for changing environment. *Environmental Environ Sci Pollutr.* 2015; 22(7): 4907-4921. doi:[10.1007/s11356-014-3754-2](https://doi.org/10.1007/s11356-014-3754-2)
- Gordon SA, Weber RP. Colorimetric estimation of indoleacetic acid. *Plant Physiol.* 1951; 26(1): 192-195. doi: [10.1104/pp.26.1.192](https://doi.org/10.1104/pp.26.1.192).
- Han RY, Li RR, Yang F, Li HY, Qian YD, Zheng GP, Guo YX, Jing RY. Isolation and identification of an endophytic and antagonistic bacterium from rice roots. *Microbiology China.* 2019; 46(5): 1030-1040. doi:[10.13344/j.microbiol.china.180471](https://doi.org/10.13344/j.microbiol.china.180471)
- Hassan SED. Plant growth-promoting activities for bacterial and fungal endophytes isolated from medicinal plant of *Teucrium polium* L. *J Adv Res.* 2017; 8(6): 687-695. doi: [10.1016/j.jare.2017.09.001](https://doi.org/10.1016/j.jare.2017.09.001)
- Idris EE, Iglesias DJ, Talon M, Borriss R. Tryptophan-dependent production of Indole-3-acetic acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. *Mol Plant Microbe In.* 2007; 20(6): 619-626. doi: [10.1094/MPMI-20-6-0619](https://doi.org/10.1094/MPMI-20-6-0619) M
- Kobayashi M, Izui H, Nagasawa T, Yamada H. Nitrilase in biosynthesis of the plant hormone indole-3-acetic acid from indole-3-acetonitrile: cloning of the *Alcaligenes* gene and site-directed mutagenesis of cysteine residues. *P Natl Acad Sci USA.* 1993; 90(1): 247-251. doi: [10.1073/pnas.90.1.247](https://doi.org/10.1073/pnas.90.1.247)
- Koga J, Adachi T, Hidaka H. Molecular cloning of the gene for indolepyruvate decarboxylase from *Enterobacter cloacae*. *Mol Genet Genomics.* 1991; 226(1-2): 10-16. doi: [10.1007/BF00273581](https://doi.org/10.1007/BF00273581)
- Lee I, Ouk Kim Y, Park SC, Chun J. OrthoANI: An improved algorithm and software for calculating average nucleotide identity. *Int J Syst Evol Micr.* 2016; 66:1100–1103. doi: [10.1099/ijsem.0.000760](https://doi.org/10.1099/ijsem.0.000760)
- Li MS, Guo R, Yu F, Chen X, Zhao HY, Li HX, Wu J. Indole-3-Acetic Acid Biosynthesis Pathways in the Plant-Beneficial Bacterium *Arthrobacter pascens* ZZ21. *Int J Mol Sci.* 2018; 19(2): 443-457. doi: [10.3390/ijms19020443](https://doi.org/10.3390/ijms19020443)
- Lim JH, Kim SD. Synergistic plant growth promotion by the indigenous auxins-producing PGPR *Bacillus subtilis* AH18 and *Bacillus licheniformis* K11. *J Korean Soc for Appl Bi.* 2009; 52(5): 531-538. doi: [10.3839/jksabc.2009.090](https://doi.org/10.3839/jksabc.2009.090)

- Lin HR, Shu HY, Lin GH. Biological roles of indole-3-acetic acid in *Acinetobacter baumannii*. Microbiol Res. 2018; 216, 30-39. doi: [10.1016/j.micres.2018.08.004](https://doi.org/10.1016/j.micres.2018.08.004)
- Liu WH, Chen FF, Wang CE, Fu HH, Fang XQ, Ye JR, Shi JY. Indole-3-acetic acid in *Burkholderia pyrrocinia* JK-SH007: Enzymatic Identification of the Indole-3-acetamide synthesis pathway. Front Microbiol. 2019; 10. doi: [10.3389/fmicb.2019.02559](https://doi.org/10.3389/fmicb.2019.02559)
- Mano Y, Nemoto K. The pathway of auxin biosynthesis in plants. J. Exp. Bot. 2012; 63: 2853-2872.
- Manulis S, Haviv-Chesner A, Brandl MT, Lindow SE, Barash I. Differential involvement of indole-3-acetic acid biosynthetic pathways in pathogenicity and epiphytic fitness of *Erwinia herbicola* pv. *gypsophila*. Mol Plant Microbe In. 1998; 11(7): 634-642. doi: [10.1094/MPMI.1998.11.7.634](https://doi.org/10.1094/MPMI.1998.11.7.634)
- Martens, DA, & Frankenberger, WT. Assimilation of exogenous 2'-14C- indole-3-acetic acid and 3'-14C-tryptophan exposed to the roots of three wheat varieties. Plant soil. 1994; 166: 281-290. Doi: [10.1007/s11104-020-04626-w](https://doi.org/10.1007/s11104-020-04626-w)
- Mohite B. Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. J Soil Plant Nut. 2013; 13(3), 638-649. doi: [10.4067/S0718-95162013005000051](https://doi.org/10.4067/S0718-95162013005000051)
- Mujahid M, Sasikala C, Ramana CV. Production of indole-3-acetic acid and related indole derivatives from L-tryptophan by *Rubrivivax benzoatilyticus* JA2. Appl Microbiol Biot. 2011; 89: 1001-1008. doi: [10.1007/s00253-010-2951-2](https://doi.org/10.1007/s00253-010-2951-2)
- Oberhänsli T, Defago G, Haas D. Indole-3-acetic-acid (IAA) synthesis in the biocontrol strain CHA0 of *Pseudomonas fluorescens*: role of tryptophan side chain oxidase. Journal of General Microbiology. 1991; 137(10): 2273-2279. doi: [10.1099/00221287-137-10-2273](https://doi.org/10.1099/00221287-137-10-2273)
- Ona O, Smets I, Gysegom P, Bernaerts K, Van Impe J, Prinsen E & Vanderleyden J. The effect of pH on indole-3-acetic acid (IAA) biosynthesis of *Azospirillum brasilense* Sp7. Symbiosis. 2003; 35: 199-208.
- Park JM, Radhakrishnan R, Kang SM, Lee IJ. IAA Producing Enterobacter sp. I-3 as a Potent Bio-herbicide Candidate for Weed Control: A Special Reference with Lettuce Growth Inhibition. Indian J Microbiol. 2015; 55(2): 207-212. doi: [10.1007/s12088-015-0515-y](https://doi.org/10.1007/s12088-015-0515-y)
- Patten CL, Glick BR. Bacterial biosynthesis of indole-3-acetic acid. Can J Microbiol. 1996; 42(3): 207-220. doi: [10.1139/m96-032](https://doi.org/10.1139/m96-032)
- Perley JW, Stowe BB. On the ability of *Taphrina deformans* to produce indoleacetic acid from tryptophan by way of tryptamine. Plant physiol. 1966; 41(2): 234-237. doi: [10.1104/pp.41.2.234](https://doi.org/10.1104/pp.41.2.234)
- Rupal K S, Raval VH, Saraf M. Biosynthesis and purification of indole-3-acetic acid by halotolerant rhizobacteria isolated from Little Runn of Kachchh. Biocatal Agr Biotechnol. 2020; 23:101435.

doi:10.1016/j.bcab.2019.101435

Spaepen S, Vanderleyden J & Remans R. Indole-3-acetic acid in microbial and microorganism-plant signaling, *Fems Microbiol Rev.* 2007; 31: 425-448. doi: 10.1093/femsre/fuz024

Swain MR, Naskar SK, Ray RC. Indole-3-acetic acid production and effect on sprouting of yam (*Dioscorea rotundata* L.) minisets by *Bacillus subtilis* isolated from culturable cow dung microflora. *Pol J Microbiol.* 2007; 56(2): 103–110. doi: 10.33073/pjm-2020-001

Xu WH, Lyu ZH, Shi YR, Jiang JY, Hu YL, Wang ZG. Establishment of complex growth-promoting rhizobacteria for watermelon and promotin effect on watermelon roots. *Acta Agriculturae Zhejiangensis.* 2018; 30(5): 778-786.

Zakharova EA, Shcherbakov AA, Brudnik VV, Skripko NG, Bulkin NS, Ignatov VV. Biosynthesis of indole-3-acetic acid in *Azospirillum brasilense* insights from quantum chemistry. *Eur J Biochem.* 1999; 259(3): 572-576. doi: 10.1046/j.1432-1327.1999.00033.x

Tables

Table 1 ANI and dDDH values

Strain	ANI value	dDDH value
<i>Enterobacter hormaechei</i> subsp. <i>xiangfangensis</i> LMG 27195T CP017183	97.12%	77.6%

Figures

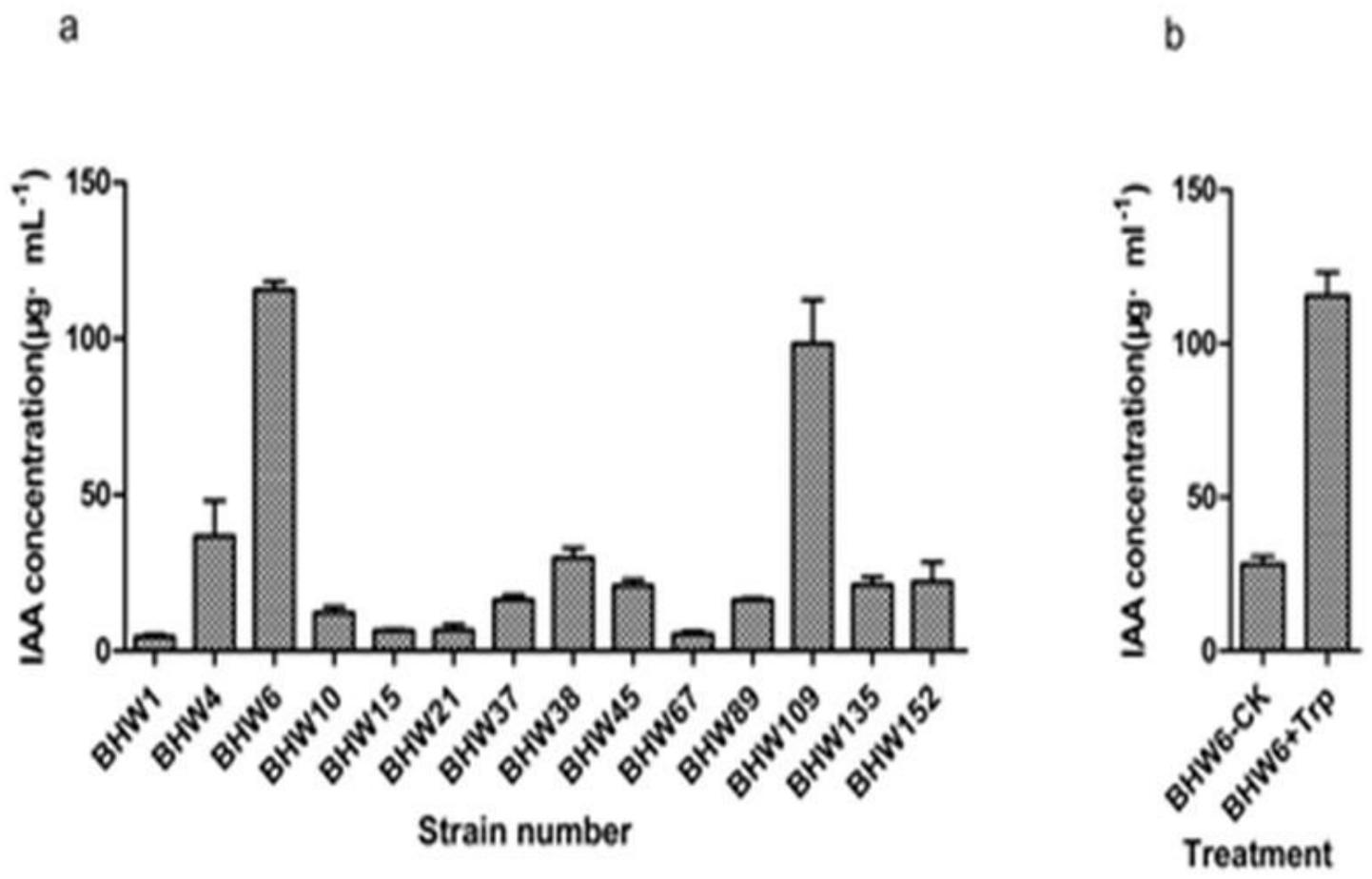


Figure 1

IAA productions of various strains

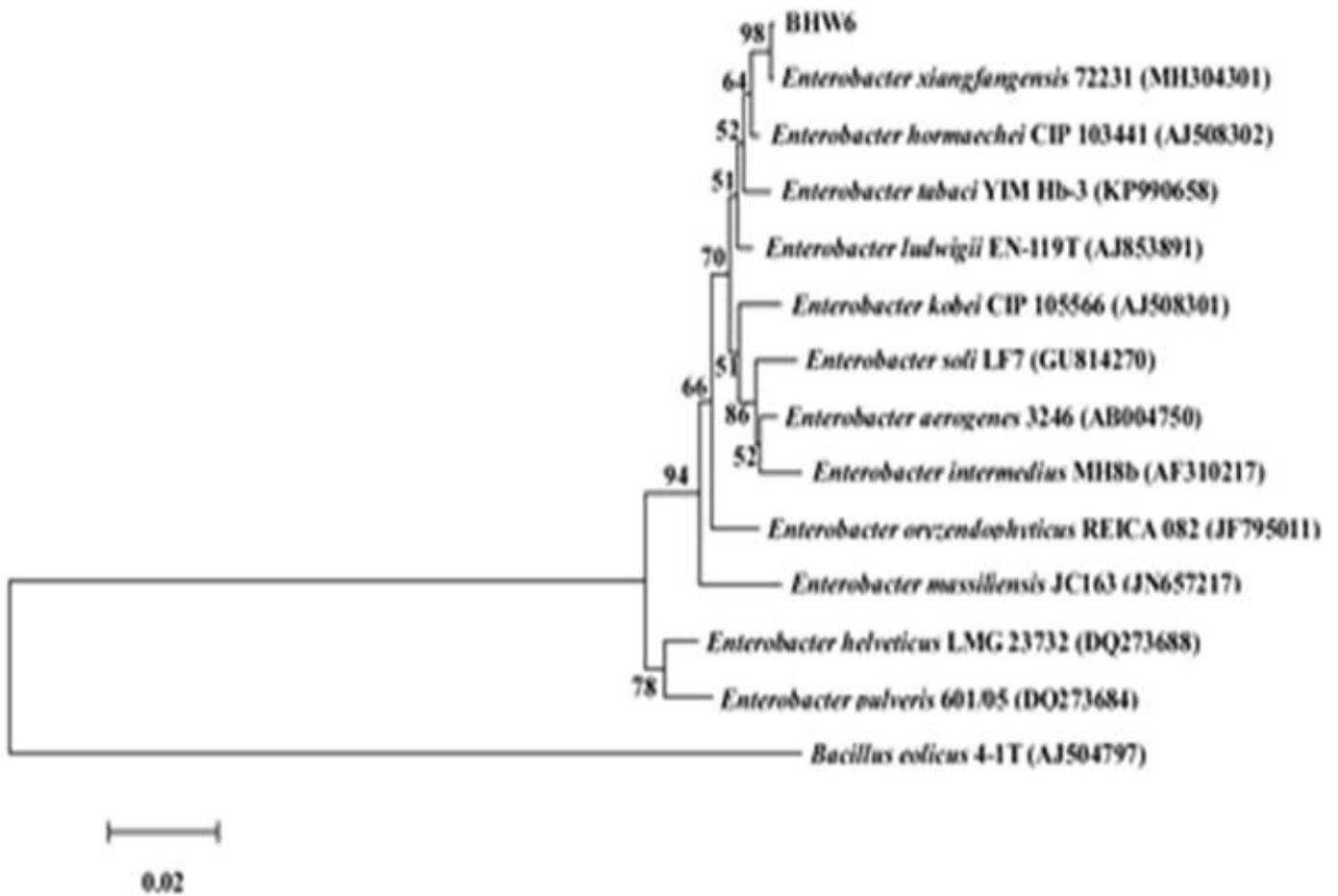


Figure 2

Phylogenetic tree of the strain

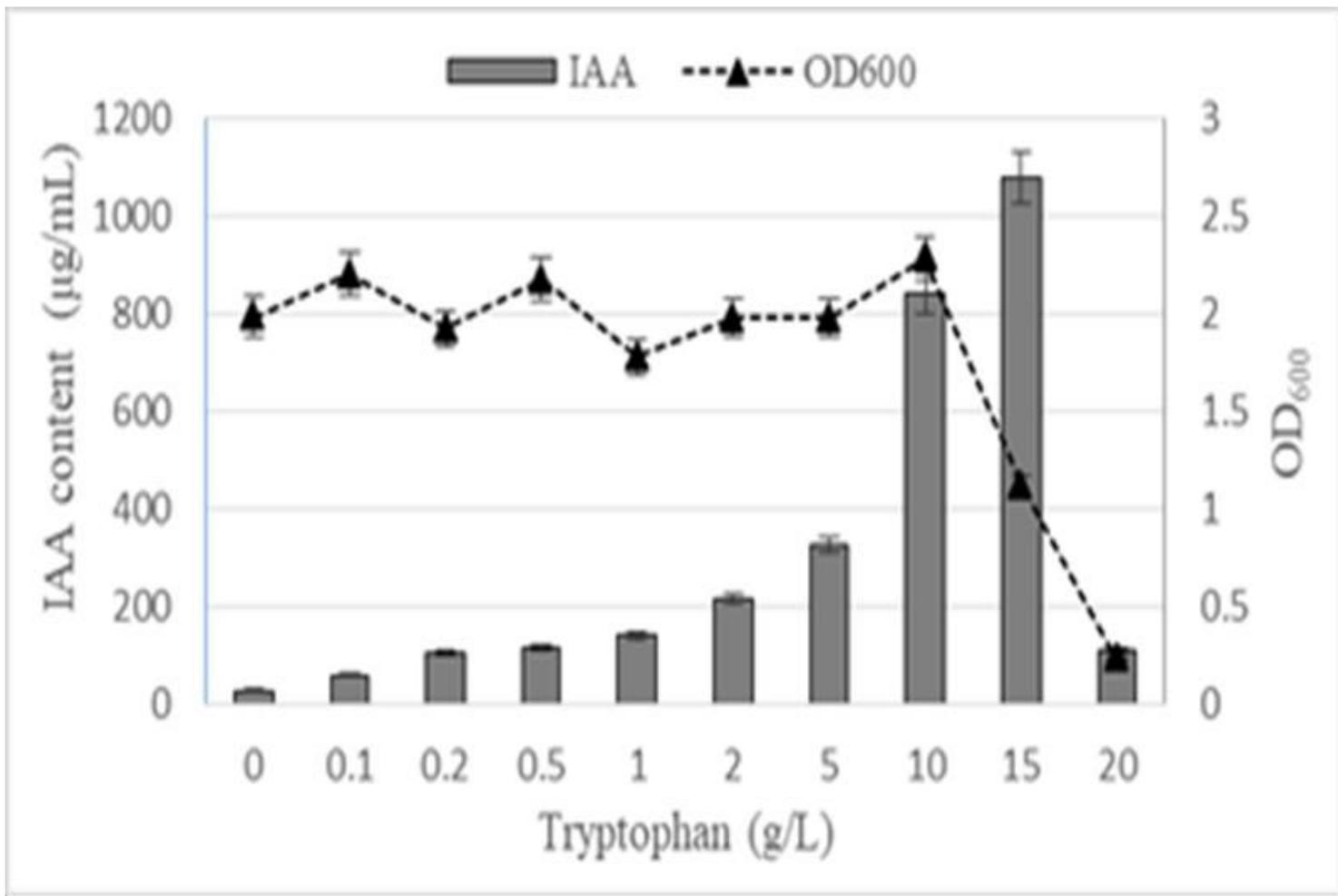


Figure 3

IAA production and bacterial growth with different L-tryptophan concentration

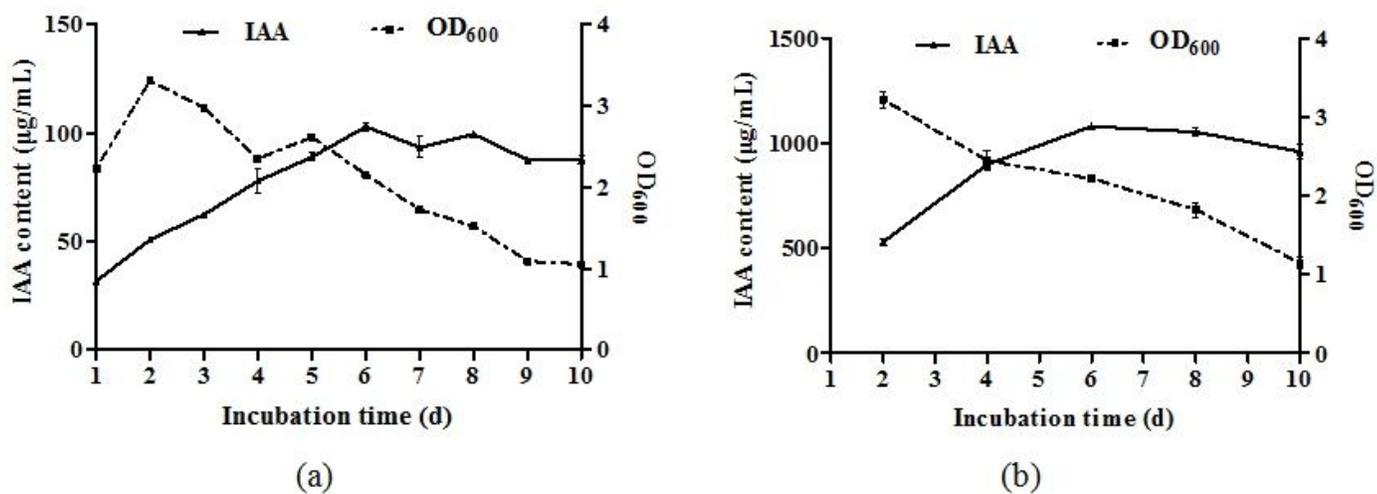
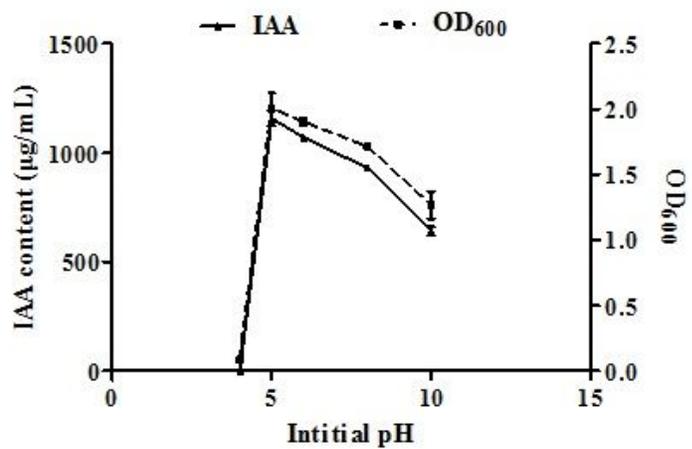
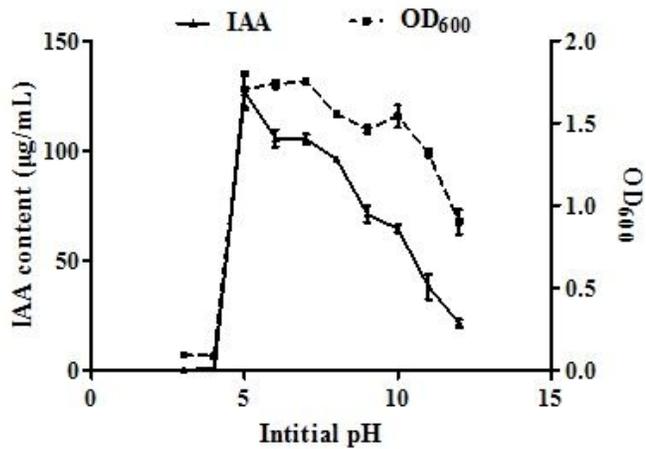


Figure 4

IAA production and bacterial growth during different incubation time

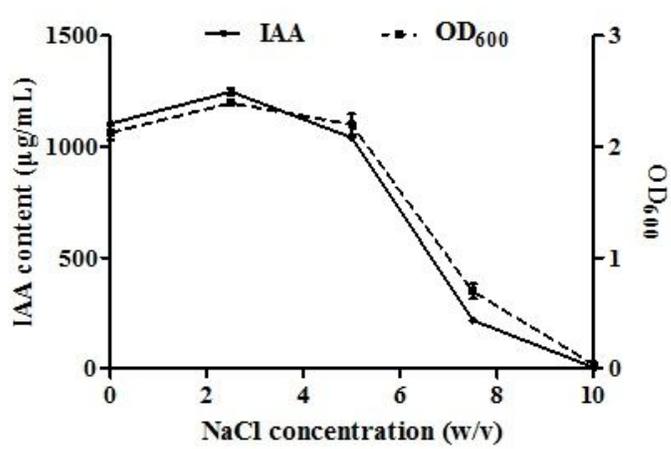
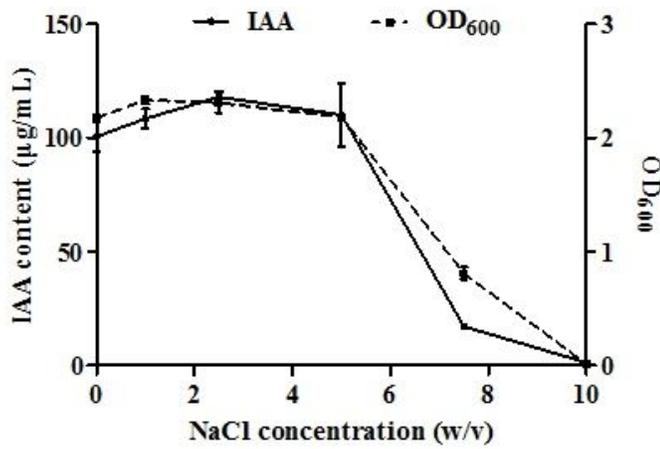


(a)

(b)

Figure 5

IAA production and bacterial growth at different initial pH



(a)

(b)

Figure 6

IAA production and bacterial growth under different NaCl concentration

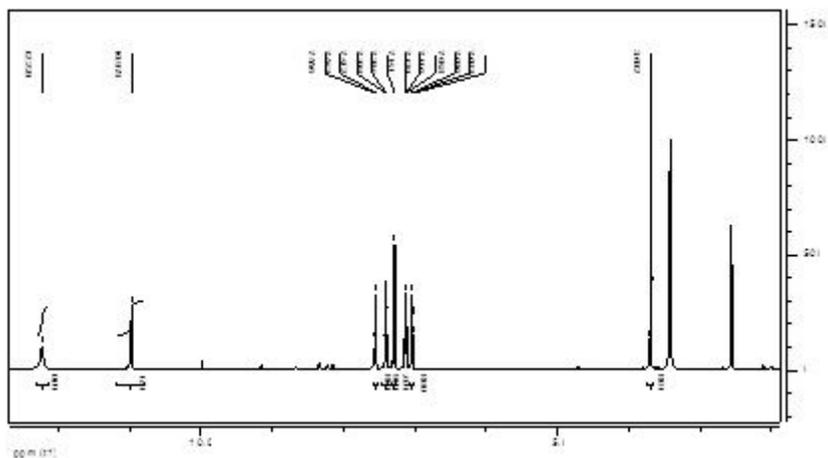


Figure 9

¹H NMR spectrum of IAA from *Enterobacter* sp. BHW6

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