

# Bifidobacterium Bifidum Isolated From Chicken Intestine Along With Polyvinylpyrrolidone Showed Synergistic Effects on Reduction of AFB1 Contamination- in Vitro Study

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## Research article

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# Abstract

**Background:** Food contamination with aflatoxin is one of the most important concerns of health professionals. One of the best ways to reduce aflatoxin content in food is to use probiotics. Therefore, this study was performed to isolate *Bifidobacterium* from the chick intestine and its probiotic activities and also its application with Polyvinylpyrrolidone (PVP) to reduce aflatoxin B1 (AFB1) toxin in the medium were investigated.

**Results:** Samples were isolated from the chicken intestine. Biochemical and molecular evaluations indicated isolation of *Bifidobacterium bifidum* strain from chicken intestine. To measure probiotic activities, pH, bile, and salt tolerance tests were used. The selected *B. bifidum* had good probiotic activity. Then, the antimicrobial activity of isolate against gastrointestinal pathogens and the antibiotic susceptibility test were done. The selected strain showed antimicrobial activities on *S. enterica*, *E. coli*, and *P. vulgaricus* and was found to be resistant against Amikacin, Ampicillin, Erythromycin, and Ceftazidime antibiotics. Then, the effect of selected isolate and PVP on reducing AFB1 in the medium was studied using ELISA and HPLC. The selected strain showed the ability to reduce the concentration of AFB1 in the medium (50% reduction) and when used in combination with PVP showed the synergistic effects in reducing the concentration of AFB1 from the medium (up to 90%).

**Conclusion:** In conclusion, it was found that selected *B. bifidum* strain together with PVP could have synergistic effects in reducing AFB1 toxin in medium up to 90%.

## Background

Contamination of food products with aflatoxins is one of the most serious concerns of the health system of today's societies (1). Aflatoxin B1 (AFB1) is the most potent carcinogen whose hepatocarcinogenic effects have been studied (2). The most important biochemical effects of AFB1 are inhibition of DNA replication and RNA synthesis (3). In addition to the liver, aflatoxins have been reported to cause significant pathological changes in other organs, such as the kidneys and spleen (4).

Chemical, physical and biological methods have been used to remove AFB1 in food products (5). Chemical methods are mainly based on the use of acids, alkalis, oxidants, and substances that can degrade mycotoxins, and of the physical methods, we can mention heat and UV treatments (5). The use of many physical and chemical methods to remove mycotoxins from contaminated food is limited due to safety issues and the possibility of losing product quality, low efficiency, and high cost (5). These problems have led researchers to focus on the use of microorganisms and plant extracts, nanoparticles, polymers, and biopolymers (6-7). The biological approach, using microorganisms and their metabolites, is recommended as a promising alternative to the detoxification of mycotoxins (8). The use of bacteria to eliminate AFB1 is more practical due to some advantages such as more removal in a short period as well as no pigment production (9). In recent years, various bacteria including lactic acid bacteria (LAB) such as *Lactobacillus*, *Bifidobacterium*, *Propionibacterium*, and *Lactococcus* in the AFB1 removal have been

reported (10). The researchers showed that some species of LAB and bifidobacteria isolated from dairy products were able to remove aflatoxins (11). It has been suggested that the binding of mycotoxins to LAB is a physical phenomenon related to the bacterial cell wall structure and that peptidoglycans and polysaccharides are two important factors for the binding of toxins to LAB (12). However, several components may be involved in the binding of AFB1 to LAB (13).

An important approach to prevent aflatoxin toxicity is the use of absorbers (14). The adsorbents can bind to AFB1 and reduce the toxicity of the toxin. As a result, these adsorbents reduce the absorption of mycotoxins and reduce their distribution in the blood and target organisms. The adsorbents are high molecular weight compounds that can bind to mycotoxins and can form a stable mycotoxin adsorbent (15). Polymers including cholestyramine, styrene, divinylbenzene, polyvinylpyrrolidone, and its modified form, polyvinylpolpyrrolidone are such adsorbents. Polyvinylpyrrolidone (PVP) is one of the acetylene derivatives of vinylpyrrolidone monomer, which is supplied in powder and aqueous solution. Its powder form is white or milky in color, which can absorb moisture up to 40% of its weight (16). Due to the presence of a highly polar amide ring and the possibility of hydrogen bond formation, this polymer dissolves in water and many other polar solvents (17). The use of PVP has expanded rapidly in sensitive applications including pharmaceuticals and food industries due to its unique properties such as range of solubility and wide compatibility with other materials, physiological inertia, ability to form complexes with other materials, and inherent adhesion (18). Polyvinylpyrrolidone is recognized by the World Health Organization (WHO) as a polymer without side effects that is fully compatible with the human body (19). This polymer can be used as an additive in all edible and non-edible products in solution, suspension, gel, and solid forms (tablet form) such as syrups and pharmaceutical tablets, gels, and creams.

Therefore, due to the harmful effects of AFB1 on health, the present study was performed to study the effects of *Bifidobacterium* and PVP on the reduction of AFB1.

## Materials And Methods

### 5.1. Sampling and culture of intestinal contents

Thirty local chicks over 15 days old and weighed over 100 g, were collected from the villages of Ardabil province, Iran. Sampling was performed by the Convenience Sampling method (Non-random Sampling). Cloacal swabs were taken from intestine content was collected under the aseptic technique, and all samples were sent to the microbiology laboratory. All animal experimental procedures were approved by the Islamic Azad University- North Tehran Branch (Biomedical Research Ethics Committee. Approval ID: IR.IAU.TNB.REC.1400.002). After sampling chickens disinfected with 70% alcohol and betadine, their intestinal contents were collected in sterile plates and diluted with physiological serum. Based on the Karami et al. (2017) method (20), the initial identification of lactic acid bacteria (LAB) was performed by culturing 100 µl of diluted intestinal contents in broth MRS culture medium (Sigma Aldrich 69964) for 96 hrs at 37 °C in anaerobic conditions and finally the colony-forming unit (CFU) was done.

### 5.2. Identification of *Bifidobacterium*

Gram staining, catalase, oxidase, capsule, flagella, and spore staining, gelatin hydrolysis, gas, indole, motility in SIM medium, nitrate reduction, oxidative-fermentative, urease, and Simmon citrate assays were used to identify the isolates in the colonies (21).

### **5.3. Biochemical test of carbohydrate fermentation by *Bifidobacterium* species**

2% solutions of Amylose, Cellobiose, Fructose-6-phosphate, Galactose, Inulin, Lactose, Maltase, Mannitol, Mannose, Melibiose, Raffinose, Ribose, Salicin, Sorbitol, Starch, Trehalose, Xylan and Xylose in glucose-free MRS medium (meat extract (10 g/L), peptone protease (10 g/L), yeast extract (4 g/L), sodium acetate (5 g/L), diammonium citrate (2 g/L), magnesium sulfate (0.2 g/L), manganese sulfate (0.05 g/L), potassium phosphate (2 g/L), tween 80 (1 ml/L) and bromocresol purple (0.05 g/L)) were sterilized separately with a 0.22-micron needle filter (Merck, Germany). Fermentation of carbohydrates was evaluated with bromothymol blue reagent and the media were yellow (weakly acidic solution) or blue (weakly base solution).

### **5.4. Probiotic activity evaluation**

#### **5.4.1. pH Tolerance Test**

The selected *Bifidobacterium* isolate was cultured in MRS broth culture medium with different pHs ranged from 1, 2, 3, 4, 5, 6, 7, 8, and 9 and incubated at 37 °C for 48 hrs in anaerobic conditions. The growth of *Bifidobacterium* was measured with a spectrophotometer at 620 nm.

#### **5.4.2. Bile tolerance test**

The *Bifidobacterium* selected isolate was cultured on MRS broth supplemented with 0.1, 0.3, 0.6, 0.9, and 1.2 % ox gall (pH6) and incubated at 37 °C for 48hrs in anaerobic conditions. The growth of *Bifidobacterium* was measured with a spectrophotometer at 620 nm.

#### **5.4.3. Salt tolerance test**

The *Bifidobacterium* was cultured on MRS broth supplemented with 2.5%, 4.5%, 6.5%, and 8.5% NaCl and incubated at 37 °C for 48hrs in anaerobic conditions. The growth of *Bifidobacterium* was measured with a spectrophotometer at 620 nm (21).

#### **5.4.4. Antibacterial activity and selected *Bifidobacterium* isolate**

Antibacterial activities of *Bifidobacterium* produced metabolite were studied against gastrointestinal pathogens including *Salmonella enterica*, *Escherichia coli*, and *Proteus vulgaricus* using disc diffusion method, and the diameters of growth inhibition zone were measured.

#### **5.4.5. Antibiotics resistance**

The susceptibility and resistance of isolated bacteria to Amikacin, Fusidic acid, Ampicillin, Erythromycin, Ceftazidime, Amphotericin, and Chloramphenicol antibiotics were also assessed by disk diffusion method.

## 5.5. Molecular identification

Bacterial DNA extraction was performed using DBA extraction kit (SINACLON, Iran) and their quality was evaluated using the Nanodrop device (Nanodrop 2000c, Thermo Scientific, Waltham, USA). The PCR reaction mixture consisted of Mastermix solution (Yekta Tajhiz Azma, Iran, Cat No: YT1553), enzyme buffer, MgCl<sub>2</sub>, and four dNTPs nucleotides. The 16S rRNA gene was used for molecular identification. Agarose gel electrophoresis was used to evaluate the accuracy of PCR products and to determine the length of amplified fragments. Gene sequencing was performed at Fanavaran Gene Company (Tehran, Iran) (22).

## 5.6. In vitro study of the effect of *Bifidobacterium* and PVP on AFB1

### 5.6.1. Preparation of the standard AFB1

Aflatoxin B1 purchased from Sigma (Germany) was dissolved in the benzene-acetonitrile organic solvent according to manufacture instructions. Phosphate buffer was used to dilute the sample. To remove the organic solvent, a water bath was used at 80 °C for 15 minutes.

### 5.6.2. Preparation of aflatoxin from *Aspergillus flavus*

First, *A. flavus* (PTCC 5018) purchased from Iran Scientific and Industrial Research Center was cultured in PDB medium in several flasks and incubated at 26 °C for two weeks. To extract aflatoxin from the PDB medium, the contents of each flask were first mixed uniformly. The contents of the flasks were then passed through a Whatman 42 paper filter (with a porosity of 2 to 3 µm). For every 100 ml of filtered solution, 40 ml of chloroform solvent was added and the resulting mixture was stirred in a decanter funnel for 20 min. After 24 hrs, the lower phase containing chloroform solvent and aflatoxin was isolated. The solvent was separated by a rotary apparatus at 45°C under vacuum. The residue was dissolved in 10 ml of HPLC purity methanol solvent and passed through a 0.22 µm nozzle filter. The concentrated sample was stored in a freezer at -20 °C. Then HPLC was performed for qualitative identification and quantitative measurement of AFB1 (23).

### 5.6.3. Measurement of free and *Bifidobacterium bifidum* attached aflatoxin B1

*Bifidobacterium* selected isolate was cultured in broth MRS medium and after a maximum growth of 48 hours (growth was measured by spectrophotometry at 620 nm), the tubes containing the bacteria were

centrifuged for 15 minutes at 3000 rpm. *Bifidobacterium* precipitate was washed 3 times each time with 5 ml of phosphate buffer solution (PBS) and added to 5 ml of standard aflatoxin B1 solution extracted in separate vials. Aflatoxin B1 samples in PBS were used as a control. Falcons were incubated for 72 hrs at 37 °C. The samples were collected at different time intervals (0, 24, and 48 hrs), then each centrifuged for 15 minutes at 4000 rpm. Free aflatoxin was isolated for measurement. Samples were screened with an aflatoxin B1 ELISA kit and the optimal sample was analyzed by HPLC and finally, the percentage of AFB1 bound to yeast was calculated (ELISA kit, ZellBio, Germany) (12, 23).

#### **5.6.4. Measurement of free and PVP-attached aflatoxin B1**

The PVP polymer solution was prepared according to international standards and serially diluted. After preparing different dilutions in each vial, 5 ml of standard and extracted AFB1 solution were added in separate vials for 48 hrs at 37 °C. Samples were collected at different time intervals (0, 24, 48 hrs) and then each was centrifuged for 15 minutes at 4000 rpm to measure free aflatoxin and supernatant were screened with aflatoxin B1 ELISA kit and the optimal sample was analyzed by HPLC and finally the percentage of aflatoxin bound to PVP was calculated.

To evaluate the effect of pH, the experiments were conducted at pH 5.5 and 8, and the percentage of polymer adsorption at different pHs was calculated by HPLC.

#### **5.6.5. Measurement of the synergistic effect of *Bifidobacterium bifidum* and PVP in reducing the aflatoxin B1**

*Bifidobacterium* selected isolate was cultured in MRS broth medium and incubated for 48 hrs. The growth of *Bifidobacterium* was evaluated by spectrophotometry at 620 wavelength and then the medium centrifuged for 15 min at 3000 rpm. *Bifidobacterium* precipitate was added to 5 ml of standard and extracted AFB1 solution after washing three times with 5 ml of phosphate buffer solution (PBS). Also, PVP (with the optimal concentration obtained in the previous step) was added to the solution and incubated for 24 hrs at 37 °C. Then centrifuged for 15 min at 4000 rpm and finally, the percentage of aflatoxin bound to bacteria and PVP was calculated by HPLC.

### **5-7. Statistical analysis**

Data were expressed as means± SD. A two-way analysis of variance was used for statistical analysis. SPSS software version 26 was used for data analysis. Tukey's Multiple Range Test was used to compare the means. P <0.05 was considered as a significant level.

## **Results**

### **2.1. Isolation and identification of *Bifidobacterium bifidum***

In the present study, 25 isolates were isolated from 50 samples of chicken intestine samples that were studied using microbiological and biochemical tests. Using different probiotic tests, 4 strain was selected

as the preferred *Bifidobacterium* strains. (Figure 1). The results showed that the isolated strains *Bifidobacterium bifidum* are gram-positive, pleomorphic rod shape, anaerobic, catalase-negative, capsule negative, flagella negative, gelatin hydrolysis negative, indole negative, lacking motility, nitrate reduction negative, oxidase negative, spores negative, and Simmon Citrate negative.

The results of the sugar fermentation test showed that the isolated strains *Bifidobacterium bifidum* can ferment sugars such as Fructose-6-phosphate, Galactose, Glucose, Lactose, and Maltose but in the presence of Cellobiose, Inulin, Mannitol, Raffinose, Ribose, Salicin, Sorbitol, Starch, Sucrose, Trehalose, Xylan and Xylose sugars fermentation did not occur. Variable results were observed for the sugars including Amylose, Mannose, and Melibiose.

## **2.2. Probiotic activities**

### **2.2.1. pH tolerance**

At low pH (below 2) the isolated isolates did not show growth in acidic medium, but at higher pH ( $3 \leq \text{pH} \leq 5$ ) bacterial growth was seen, and the highest growth of isolates was observed at pH 4.5 and 5 in acidic medium (Figure 2). Also, the isolated *Bifidobacterium* strains grew in pH medium 8 and 9 and no growth arrest was observed at high pH. In general, these results indicate that the I3 isolated bacterial strain has a high tolerance to a wide range of pH, indicating its activity at the pH of the gastrointestinal tract is one of the important properties of probiotics.

### **2.2.2. NaCl tolerance**

The growth of the selected bacterial strains at different NaCl concentrations was examined and the results showed that the selected I3 strain has a high tolerance to high concentrations of NaCl (Figure 3a), which indicates the appropriate probiotic activity of this selected strain.

### **2.2.3. Bile salt tolerance**

*Bifidobacterium* strain (I3) showed good tolerance to different concentrations of Ox-gall from 0.1% to 1.2% (figure 3b), which indicates a high tolerance of this strain to bile salt. Tolerance to different concentrations of bile salt is one of the important properties of probiotics and thus, the selected strain can be considered as a probiotic due to its high tolerance to bile salts.

### **2.2.4. Antimicrobial activities of selected *Bifidobacterium* isolate**

Antibacterial activities of selected *Bifidobacterium* isolate were studied against *S. enterica*, *E.coli*, and *P. vulgaricus* using the disc diffusion method the results showed the highest growth of inhibition on *S. enterica*, but the lowest growth inhibition showed for *E. coli* (Figure 4a).

### **2.2.5. Antibiotics resistance**

The results of antibiotic resistance test indicated that the selected strain of *Bifidobacterium* was resistant to Amikacin, Ampicillin, Erythromycin, and Ceftazidime antibiotics but was sensitive to Amphotericin and Chloramphenicol antibiotics (Figure 4b).

### 2.3. Molecular identification

DNA extraction of the selected *Bifidobacterium* strain was done and the sequencing results of 16S rRNA gene indicated that the isolated strain was from *Bifidobacterium bifidum* and showed 100% similarity to ZT-B1 strain (Figure 5).

### 2.4. Detoxifying of AFB1 by selected *Bifidobacterium* strain and PVP

The selected *Bifidobacterium* strain caused a nearly two-fold decrease in standard and extracted AFB1 content over 24 hrs, indicating a high uptake of AFB1 into the surface of the strain wall (Figure 6a, b). This optimal sample was analyzed by HPLC and the results showed that the isolate can adsorb and reduce standard and extracted AFB1 up to 48.32% and 47.47%, respectively.

The results of the present study showed a decrease in the concentration of standard and extracted AFB1 by PVP in the medium and also the relatively quickly binding of AFB1 to PVP occurred. After 24 hrs, a 50% decrease in standard and extracted AFB1 content was observed (Figure 6c, d).

The optimal sample, ie 2.5 mg/ml PVP during 24 hrs was analyzed by HPLC. HPLC results showed that PVP polymer with the above concentration can absorb and reduce aflatoxin up to 52.78% and 55%, respectively.

The effect of using the isolated *Bifidobacterium* strain from the intestines of chickens and PVP (25 mg/ml) on the standard and extracted AFB1 content was studied and the results showed that it caused a severe reduction of the AFB1 (About 90%) content in the medium (Figure 6e, f). This indicates that the use of *Bifidobacterium* strain with PVP has a synergistic effect in reducing AFB1 content.

The results of AFB1 adsorption to PVP at pHs of 5.5 and 8 showed that there was no significant difference in terms of AFB1 adsorption to PVP polymer at different pHs.

## Discussion

Identification of new species with probiotic activity is of great importance (24) and the probiotic activity of *Bifidobacterium bifidum* strains has been reported in various studies (25, 26). In the present study, the *Bifidobacterium bifidum* strain was isolated from the intestine of chickens and was shown to have good probiotic activities. Also, the desired strain was able to reduce the concentration of AFB1 in the medium and when used in combination with PVP in the environment, synergistic effects were observed in reducing the concentration of AFB1 in the medium.

Although the use of biochemical methods is still widely used in the identification of bacteria (27), the use of molecular identification methods such as polymerase chain reaction (PCR) in the detection of bacterial strains allows identifying of them more accurately (28). In the present study, in addition to biochemical methods, the 16S rRNA gene amplification method was used to make a more accurate identification, and the results showed that the selected *Bifidobacterium bifidum* has 100% identity with ZT-B1.

As mentioned in recent studies, the antimicrobial properties of *Bifidobacterium* are important probiotic features (29, 30) that showed in the present study in a selected strain isolated from chicken intestine, and antimicrobial effects were seen on all three pathogens *S. enterica*, *E. coli*, and *P. vulgaricus* using the disc diffusion method. Such a feature has been reported in other studies (31, 32). This may be due to the production of bacteriocins and mechanisms including the production of bacterial inhibitory compounds, the regulation of intestinal pH, the strengthening of the immune system, the blockage of bacterial binding sites, competition for nutrient uptake, the production of organic acids such as acetic acid, propionic acid, phenyl lactic acid, formic acid or free fatty acids and so on (32, 33). Most bacteriocins are low molecular weight proteins that are resistant to heat, acid, and cold, and block DNA biosynthesis in bacteria (33).

The results of the present study indicated resistance of the selected strain to Amikacin, Ampicillin, Erythromycin and Ceftazidine antibiotics and sensitivity to Amphotericin and Chloramphenicol antibiotics. Antibiotic resistance in probiotic bacteria has been reported in other studies (34, 35), which is similar to the findings of the current study.

The good resistance of selected bacterium strain to different concentrations of Ox-gall bile salt was observed in the present study, which can be attributed to the ability of the strain to reduce the detergent effects of bile salts (36). This indicates the probiotic activity of the selected strain that can resist and continue to grow at different concentrations of bile salts. Also, high resistance of the selected strain to a wide range of pHs was observed in the present study, which is considered an important characteristic of probiotics, indicating their resistance to gastrointestinal conditions (37). Survival of other probiotic strains even at pH 1 has been reported in other studies (38). Also, another feature of probiotics is their tolerance to different concentrations of NaCl, which was observed in the current study. Overall, these results indicate the appropriate probiotic activities of the selected strain that can be considered in future studies.

*Aspergillus flavus* grows on a wide range of agricultural products and foodstuffs and infects them by producing AFB1 toxin. Aflatoxins, as one of the most toxic fungal metabolites, weaken the body's immune system and cause cancers and tumors in humans (39). Aflatoxin contamination of crops is a devastating global problem in agriculture, food and livestock industries. The use of biological methods, such as the use of antagonistic bacteria, can inactivate and degrade aflatoxins by producing certain biological compounds. The results of the current study showed a decrease in AFB1 concentration with the application of selected strain *Bifidobacterium bifidum* in the medium. Also, the greatest decrease in AFB1 occurred in the next 24 hrs, indicating that binding of the toxin to the selected strain is a rapid process. Specific binding of aflatoxin M1 in dairy products to LAB has been reported in studies (40). In

this study, the ability of bacteria to adsorb toxins in the medium was investigated and the results showed that the adsorption process is rapid. This adsorption appears to be due to the reaction of AFB1 with the bacterial surface wall without chemical modification of the toxin (41). The findings of the present study show that *Bifidobacterium bifidum* has a high ability to adsorb high amounts of AFB1 and this can be attributed to the composition of the bacterial cell wall.

One of the most important strategies used to reduce the risk of aflatoxin contamination is the use of toxin binders to reduce the adsorption of aflatoxin in the gastrointestinal tract. In the present study, it was shown that PVP in a concentration-dependent manner could reduce the concentration of AFB1 in the medium and the highest percentage of AFB1 adsorb from the medium was observed at a concentration of 25 mg/ml. Polyvinylpyrrolidone, which dissolves in water and biofluids, is a non-toxic and indigestible polymer (42). With such special properties, this polymer has found wide applications in the preparation of pharmaceutical compounds and medical research (42). The current study is the first to investigate the effects of PVP on AFB1 contamination, and the results showed that it can reduce the AFB1 content in the medium that can be attributed to AFB1 binding to the structure of this compound.

The synergistic effect of simultaneous application of PVP and selected strains was observed in reducing standard and extracted AFB1 in the medium. It seems that the cell walls of selected bacterial strains together with the structural properties of PVP are effective in drastically reducing the concentration of AFB1 in the medium. To the best of our knowledge, this is the first study to examine the effect of a combination of *Bifidobacterium bifidum* strain and PVP in reducing AFB1. However, more studies are needed to confirm this.

## Conclusion

In conclusion, it can be concluded that the selected *Bifidobacterium bifidum* strain has probiotic properties and the ability to reduce AFB1 in the medium, and when used in combination with PVP, shows a synergic effect on reducing AFB1 in the medium. However, in vivo studies are needed to confirm its effectiveness.

## Abbreviations

*B. bifidum*: *Bifidobacterium bifidum*; PVP: Polyvinylpyrrolidone; AFB1: Aflatoxin B1; S-AFB1: Standard Aflatoxin B1; E-AFB1: Extract Aflatoxin B1; *S. enterica*: *Salmonella enterica*; *E. coli*: *Escherichia coli*; *P. vulgaricus*: *Proteus vulgaris*; ELISA: The enzyme-linked immunosorbent assay; HPLC: High Performance Liquid Chromatography; DNA: Deoxyribonucleic Acid; RNA: Ribonucleic acid; UV: Ultraviolet; LAB: lactic acid bacteria; WHO: World Health Organization; PCR: polymerase chain reaction; 16S rRNA: 16S ribosomal RNA; MRS: De Man, Rogosa and Sharpe; PDB: Potato dextrose agar; PBS: phosphate buffer solution; CFU: colony-forming unit; dNTPs: Deoxynucleoside triphosphate

## Declarations

## **Ethics approval and consent to participate**

We obtained written informed consent to use the animals in our study from the owners of the animals. All animal experimental procedures were approved by the Islamic Azad University- North Tehran Branch (Biomedical Research Ethics Committee. Approval ID: IR.IAU.TNB.REC.1400.002).

## **Consent for publication**

Not applicable

## **Availability of data and materials**

All data generated or analyzed during this study are included in this published article [and its supplementary information files]. Raw sequence data on 16s RNA gene had been submitted to the NCBI Sequence Read Archive (SRA) with the accession number PRJNA730567 (<https://www.ncbi.nlm.nih.gov/sra/PRJNA730567>).

## **Competing interests**

The authors have declared no conflict of interest.

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## **Authors' contributions**

MRF and AAS managed the project and conceptualized it. FSM provided the samples for the study. SAA designed and performed the experiments, completed the data analysis, submitted sequence data to GenBank, and writing the manuscript. All authors reviewed and agreed to the published version of the manuscript.

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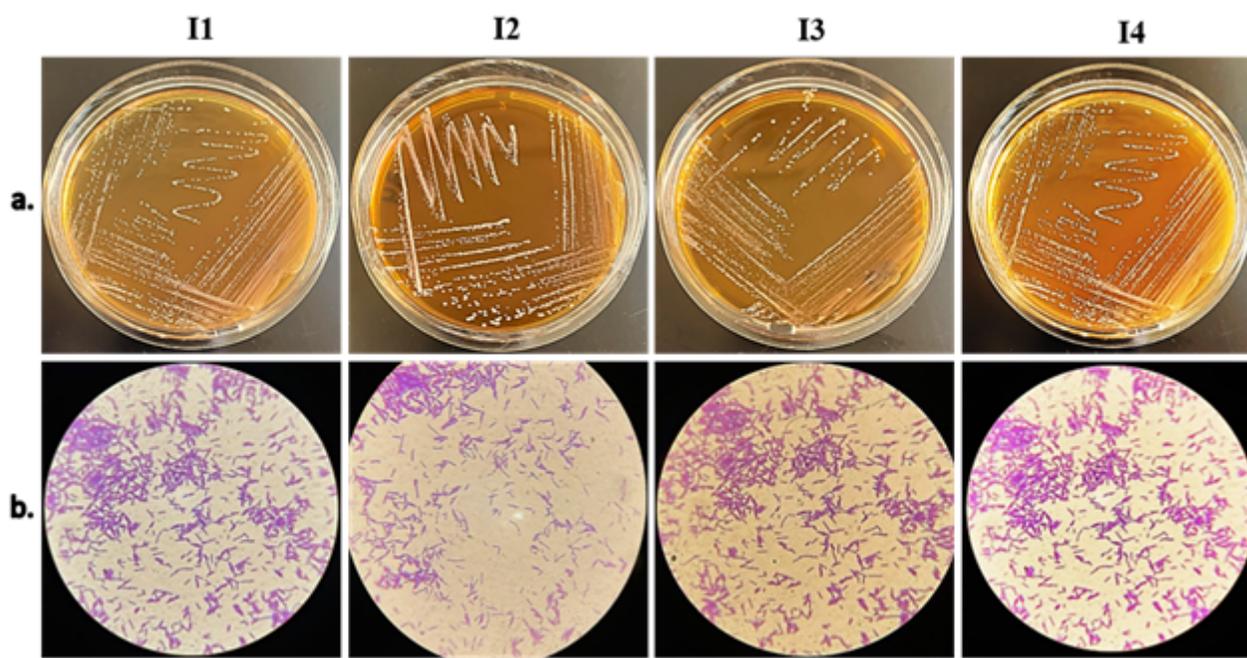
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## Figures



**Figure 1**

a The four selected *Bifidobacterium* spp. isolates colonies morphology on MRS Agar medium. b Microscopic image (1000 ×) of Gram-stained *Bifidobacterium* spp. smears illustrating the Gram-positive bacilli.

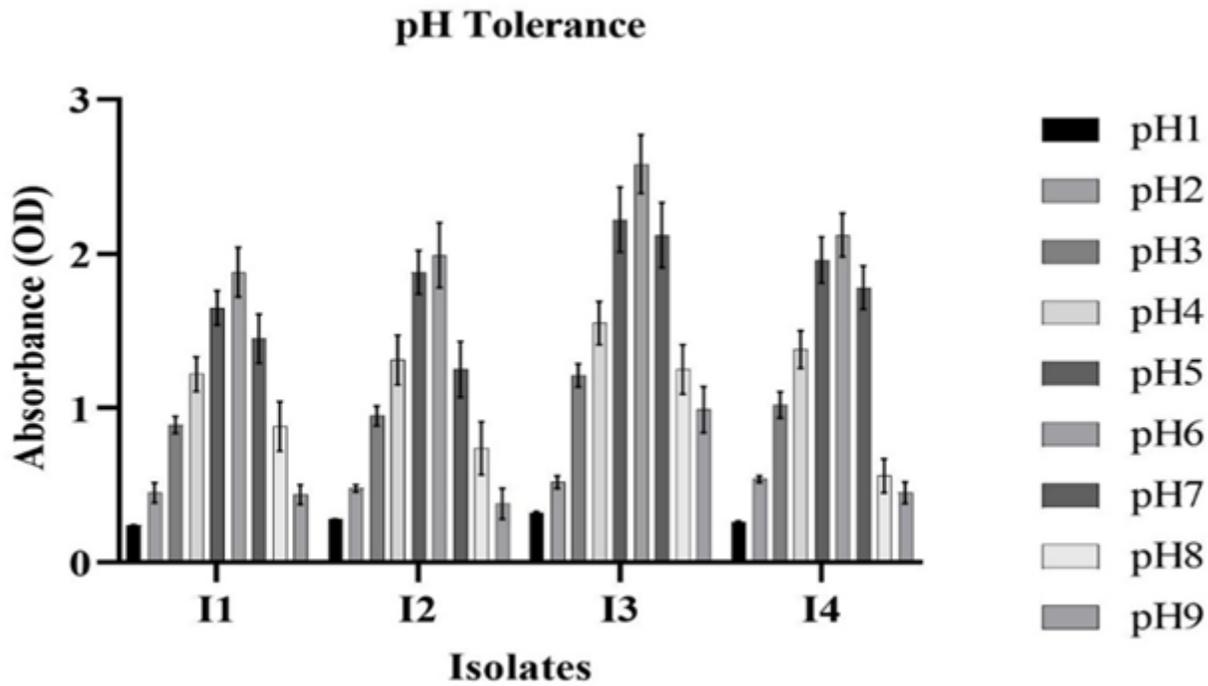


Figure 2

The growth of four selected Bifidobacterium isolates after cultured 48 hours in the MRS broth with different pHs ranging from 1 to 9.

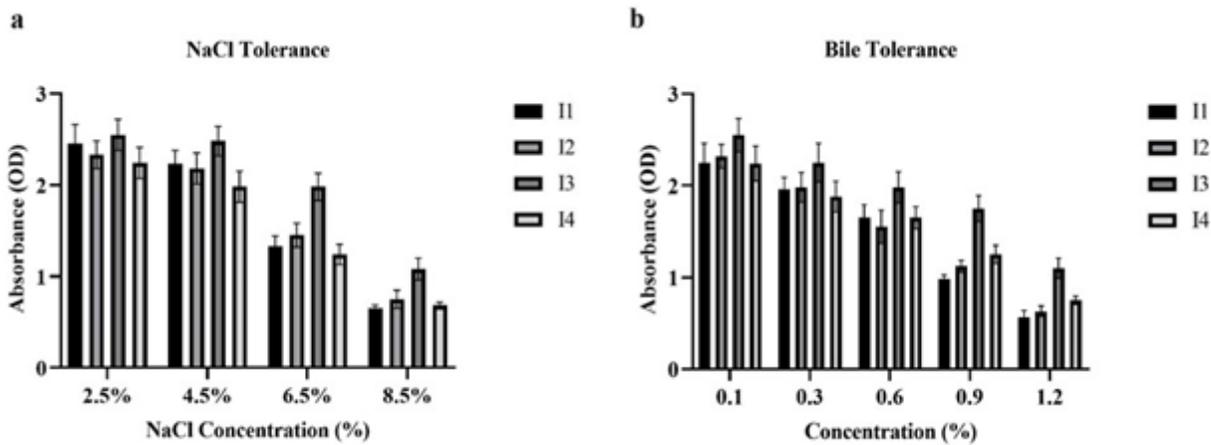
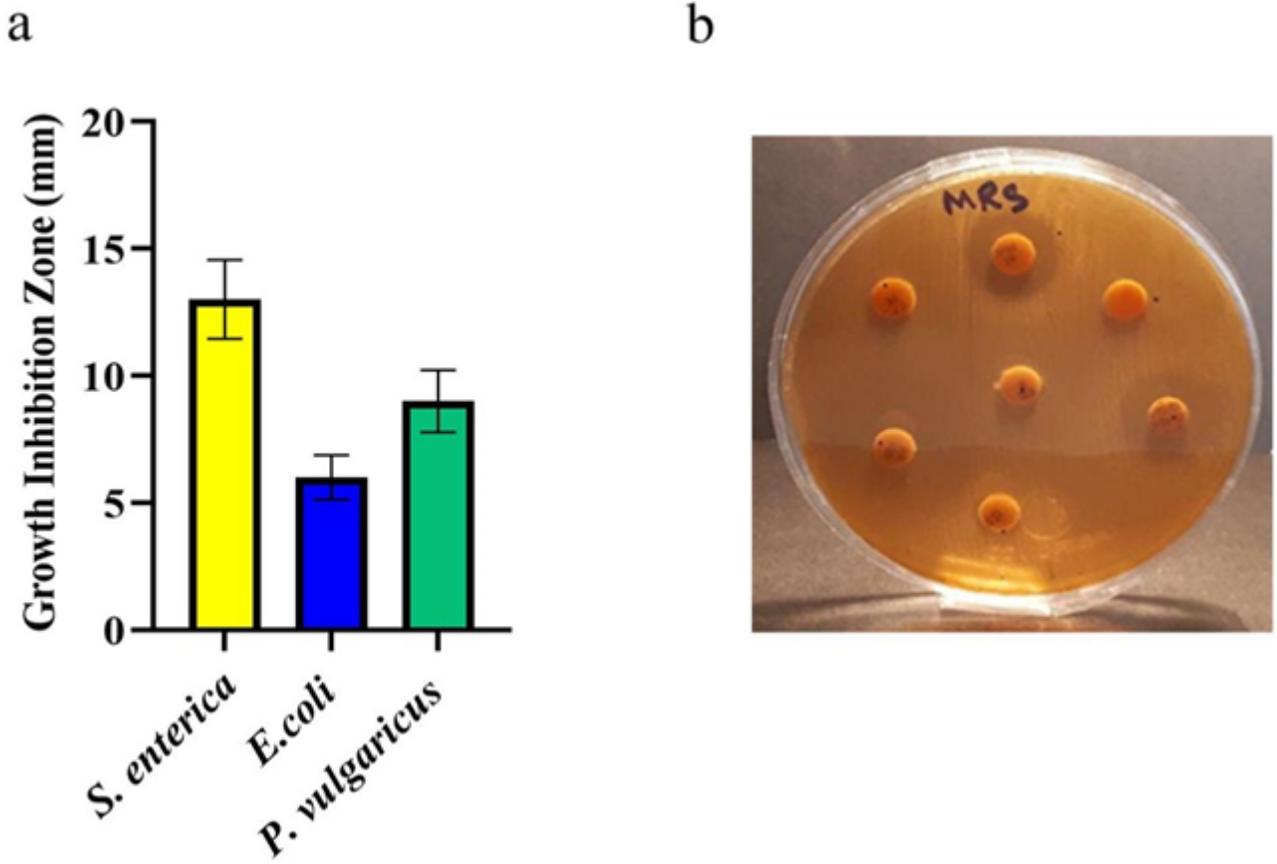


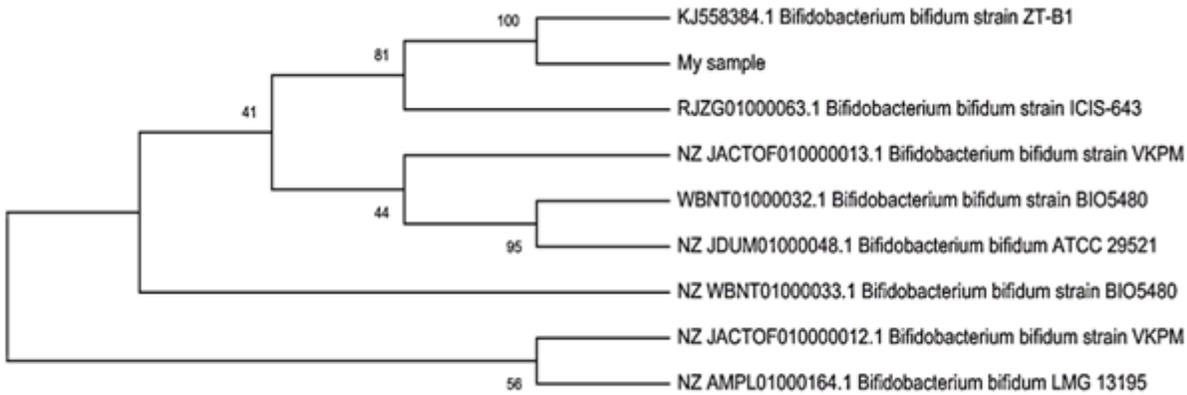
Figure 3

a The growth of selected Bifidobacterium spp. isolates in MRS broth mediums with different concentrations of bile salt and b NaCl.



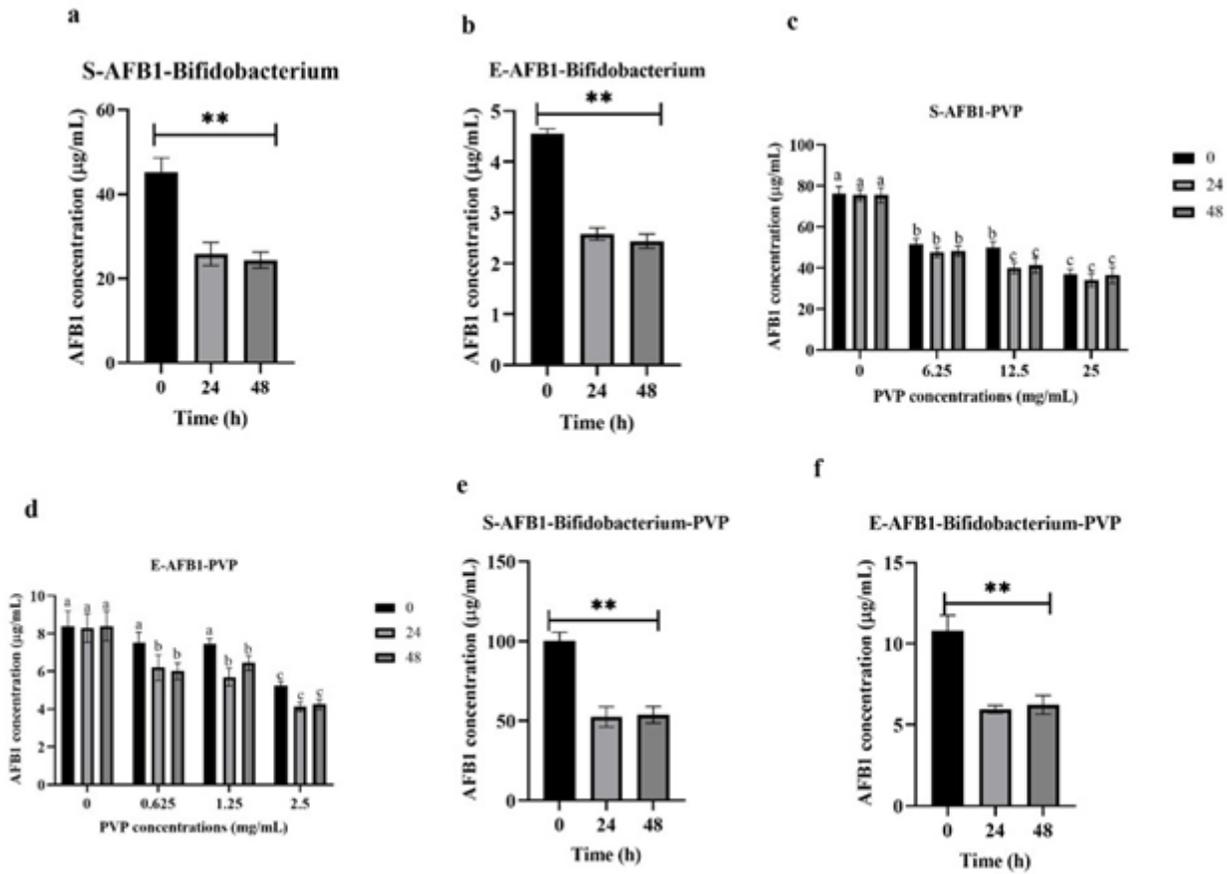
**Figure 4**

a The effects of selected Bifidobacterium spp. isolate on growth on three gastrointestinal pathogens *S. enterica*, *E. coli*, and *P. vulgaricus*. b Antibiotic resistance test



**Figure 5**

The results of blasting of sequences of the 16S rRNA of the isolated Bifidobacterium bifidum strain with 9 strains.



**Figure 6**

a,b The effects of selected Bifidobacterium isolate, and c,d different concentration of PVP on different concentration of standard and extracted AFB1. e,f the synergistic effects of selected Bifidobacterium isolate and PVP on standard and extracted AFB1, respectively.