# THESIS

## SCREENING OF FUMONISIN B1 BIODEGRADABLE BACTERIA

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**GRADUATE SCHOOL, KASETSART UNIVERSITY**
**Academic Year 2021**

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THESIS

# SCREENING OF FUMONISIN B1 BIODEGRADABLE BACTERIA

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A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
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Pisut Keawmanee : Screening of Fumonisin B1 Biodegradable Bacteria. Doctor of Philosophy (Plant Pathology), Major Field: Plant Pathology, Department of Plant Pathology.
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Fumonisins are an emerging mycotoxin problem in agricultural products, especially in Southeast Asian maize products. Fumonisin contaminates raw materials for food and feed, potentially affecting the health of both humans and animals. The biological degradation of fumonisin has an interesting target for utilized microbial enzymes. There is a high possibility that fumonisins contamination can be reduced using microbial enzymes.

Screening of 95 potent natural sources against the fumonisins crude toxins resulted in 5.3% of natural samples yielding five degrading bacterial isolates. The selected bacteria promised a solution to reducing approximately 10.0-30.0% of fumonisin B1 (FB1). *Serratia marcescens*, one of the dominant reducing bacteria, was identified through molecular identification (16s rRNA gene), protein identification (MALDI-TOF/TOF MS), and biochemical identification (VITEX-2). The bacterial cell-free extract showed the highest fumonisin reduction rates: 30.3% in solution and 37.0% in ground maize.

The antagonistic potential of *S. marcescens* 329-2 against the fumonisin-producing *Fusarium* sp. was investigated. The mycelia of fumonisin-producing *Fusarium* sp. was slightly inhibited on the dual culture test.

The high-throughput transcriptomic and proteomic analyses were used to elucidate the degrading process of FB1 by *S. marcescens* 329-2. RNA-sequencing was used to observe the relevant genes, and label-free quantification was manipulated to determine the relevant enzymes during the degradation. Both studies confirm that hydrolase (EC3) and transferase enzyme groups (EC2) were more highly expressed than the control levels. The hydrolase enzyme can be involved in FB1 conversion to hydrolyzed fumonisin B1 (HFB1) and tricarballylic acids (TCA). The transferase enzyme can react with one end of fumonisin's backbone. The degrading of functional activity points to a further potential for *S. marcescens* 329-2 crude extract as a new bacterial strain for FB1 reduction.

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Student's signature Thesis Advisor's signature

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# LIST OF ABBRIVATIONS

| | | |
|---|---|---|
| ºC | = | degree celcius |
| DNA | = | deoxyribonucleic acid |
| dNTP | = | deoxyribonucleotide triphosphate |
| ELISA | = | enzyme linked immunosorbent assay |
| FB1 | = | fumonisin B1 |
| GC/MS | = | gas chromatography mass spectrometry |
| GB | = | gigabyte |
| g | = | gram |
| h | = | hour |
| kDa | = | kilodalton |
| kb | = | kilobase pair |
| µL | = | microliter |
| µm | = | micrometer |
| µM | = | micromolar |
| mL | = | milliliter |
| mg | = | milligram |
| min | = | minute |
| M | = | molar |
| ng | = | nanogram |
| ppb | = | part per billion |
| ppm | = | part per million |
| % | = | percentage |
| RNA | = | ribonucleic acid |
| rpm | = | revolutions per minute |
| sec | = | second |

# SCREENING OF FUMONISIN B1 BIODEGRADABLE BACTERIA

## INTRODUCTION

Mycotoxins, which are secondary metabolites produced by fungi (Shibamoto & Bjeldanes, 1993), causing serious problems to animal and human health. Plant pathogenic fungi are one of the fungal groups causing crop health problems. This damage directly affects agricultural production and the economy (Pohland, 1993). Mycotoxins are accumulated during the fungal colonization of plants before harvest. Fungi such as *Fusarium graminearum, F. verticillioides, F. proliferatum,* and sometimes, *Aspergillus flavus* are present before the harvest. Another group of fungi can occur after harvesting, as reported for so-called storage fungi such as *Penicillium verrucosum* and *A. flavus* (Ayalew, 2010; Tola *et al.*, 2016)

Fumonisins are mycotoxins mainly produced by *F. verticillioides* (Sacc.) Nirenberg (previously *F. moniliforme,* Sheldon) and *F. proliferatum* (Matsush.) Nirenberg (Leslie & Summerell, 2006). To date, 28 structurally related fumonisin analogs have been identified. Three of the fumonisins, B1, B2, and B3, occur abundantly (EFSA, 2018). Fumonisin B1 (FB1) is highly toxic to fumonisin analogs and causes equine leukoencephalomalacia (ELEM) in horses, hepatocarcinogenesis in rats, and pulmonary edema (PPE) in swine (Rheeder *et al.*, 2002). Maize products are primarily contaminated with fumonisins related to starburst symptoms (Munkvold & Desjardins, 1997; Presello *et al.*, 2008; Sydenham *et al.*, 1991). The BIOMIN World Mycotoxin Survey 2020 reported that fumonisins contaminated various commodities, especially maize. Analyzed maize sample were found to be contaminated by fumonisins in Asia, North America, and Europe with high concentrations (BIOMIN, 2021).

Physical, chemical, and biological principles are used to develop strategies to eliminate fumonisin contamination in food and feed. Even so, physical and chemical

approaches have certain drawbacks in terms of costly instrumentation and nutritional losses. Therefore, biological detoxification using enzyme technology is a promising strategy. Enzymes can reduce mycotoxin toxicity by transforming mycotoxins into less toxic metabolites. In some cases, the use of an enzyme can provide a practical approach to feed detoxification (Loi *et al.*, 2017; Lyagin & Efremenko, 2019; Zhao *et al.*, 2019; Zhu *et al.*, 2017).

The first report of fumonisin microbial detoxification was reported by Duvick *et al.* (1998). Microbes that can grow with FB1 as their sole carbon source were isolated from moldy maize kernels and stalk tissue. They were gram-negative bacteria identified as *Exophiala spinifera* and *Rhinocladiella atrovirens*. *In* 1999, Blackwell *et al*. reported that the fungal species *E. spinifera* produces soluble extracellular esterase and can transform FB1 into the amino polyol AP1 and free tricarballylic acid. Moreover, hydrolyzed FB1 has been demonstrated to greatly reduce toxicity compared to FB1 (Collins *et al.*, 2006). Benedetti *et al.* (2006) reported that the bacterial strain NCB 1492, isolated from soil samples using an enrichment culture technique, can degrade FB1 as the sole carbon and nitrogen source in phosphate buffer. The sequences identified using 16S rDNA analysis were related to the gram-negative bacteria *Delftia/Comamonas*. Heinl *et al.* (2010) investigated two genes involved in fumonisin degradation from *Sphingopyxis* sp. MTA144. The deesterification of FB1 to hydrolyzed FB1 was catalyzed by recombinant carboxylesterase in the same manner as the deamination of hydrolyzed FB1 in the presence of pyruvate and pyridoxal phosphate. *In* 2016, Masching *et al*. noted that a commercial FUMzyme feed supplement that includes fumonisin carboxylesterase FumD prevented changes in the sphinganine-to-sphingosine (Sa/So) ratio of turkeys and pigs. Hence, only a few microorganisms and enzymes have been successful in reducing FB1.

This study intends to screen a fumonisin-degrading bacterial strain and determine the potential influence of bacterial enzymes in FB1 degradation.

# OBJECTIVES

1. To screen for fumonisin-degrading bacterial strains in natural sources.
2. To determine the transcriptomic and proteomic profile of bacterial enzymes with regard to the fumonisin B1 degrading process.

# LITERATURE REVIEW

## 1. Mycotoxins

Mycotoxins are secondary metabolites produced by various filamentous fungi (Shibamoto & Bjeldanes, 1993). The contamination of mycotoxin in food and feed is a health risk for animals and humans (Pohland, 1993). It occurs in various stages of food and feed production due to the invisible spoilage in the field during plant growth, harvesting, storage, and processing stage (Anklam *et al.*, 2002). More than 300 different mycotoxins produced by about 200 different fungi have been identified. The fungi can be divided into two groups: a) those colonizing the plant before harvesting called field fungi such as *F. graminearum*, *F. verticillioides*, and, sometimes, *Aspergillus flavus* and b) those occurring only after harvesting called storage fungi such as *P. verrucosum* and *A. flavus* (Ayalew, 2010; Tola *et al.*, 2016). The appropriate conditions for mycotoxin production are induced by poor hygienic conditions during transportation and storage, high temperature, moisture content, and heavy rains (Cole & Cox, 1981).

**Table 1** Common mycotoxins, mycotoxins producing fungi, and their toxicity.

| Mycotoxins | Fungi | Toxicity |
|---|---|---|
| Aflatoxins | *A. flavus*, *A. parasiticus* | potent carcinogens, association with hepatitis B virus, causing acute liver damage, liver cirrhosis, induction of tumors, and teratogenic effects. |
| Ochratoxin A | *A. ochraceus*, *P. verrucosum* | immunosuppressive, embryonic, probably carcinogenic effects and a cause of nephritis (kidney disease) in pigs. |

**Table 1** (Continued)

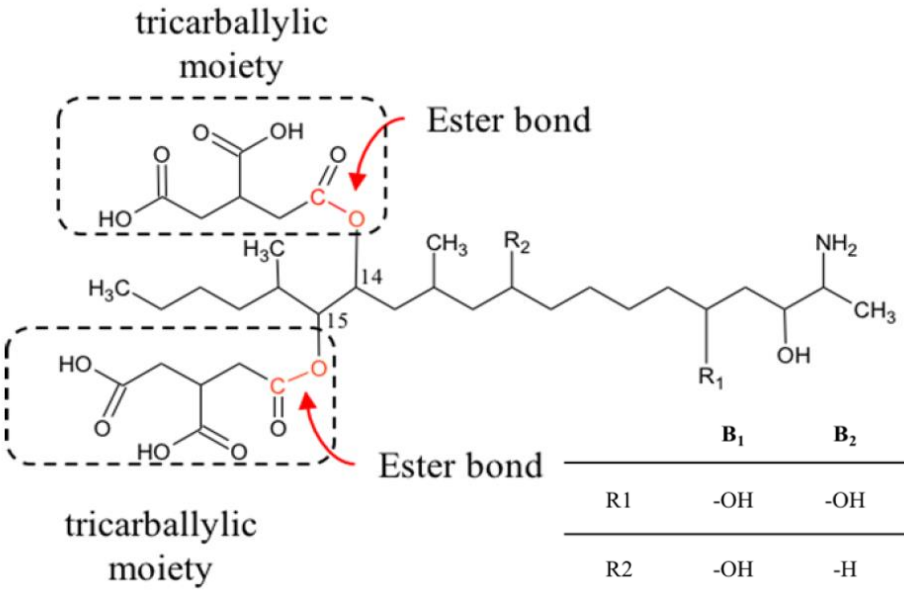
| Mycotoxins | Fungi | Toxicity |
|---|---|---|
| Fumonisins | *F. verticillioides*, *F. proliferatum* | a cause of esophageal cancer, neurotoxin causing of equine leucoencephalomalacia in horse, a cause of pulmonary edema in pigs. |
| Trichothecene toxins: deoxynivalenol and nivalenol | *F. graminearum* | anorexia, nausea, vomiting, headache, abdominal pain, diarrhea, chills, giddiness, and convulsions in human, highly immunosuppressive causes vomiting and feed refusal in pigs. |
| Zearalenone | *F. graminearum* | estrogenic effects in animals enlarged uterus, swelling of the vulva and vagina, enlarged mammary glands, anestrus and abortion. |

**Source:** Schmale and Munkvold (2009)

## 2. Fumonisins

Fumonisins are a group of mycotoxins mainly produced by *F. verticillioides* and *F. proliferatum* (Schmale & Munkvold, 2009). Fumonisins consist of a long chain of amine alcohol with two ester-linked tricarballylate (TCA) groups. Their biological activity is likely through the disruption of sphingolipid biosynthesis (Duvick *et al.*, 1998). Fumonisins have been well known in South Africa since 1970 due to the equine leukoencephalomalacia (ELEM) outbreak in horses, which occurred because of the consumption of maize contaminated with *F. verticillioides* (Starkl & Nahrer, 2015). In 1984, it was confirmed that the feed material caused ELEM in a horse. The

feed is poisoned with fumonisins, which can lead to the development of a fatal disease with symptoms of drowsiness, blindness, staggering, and liquefaction of brain tissue. A pig poisoned with fumonisins may experience reduced feed intake and weight gain, liver damage. It can also result in a fatal disease for pigs, known as pulmonary edema (PPE), in which their lungs are filled with fluid, and may cause liver cancer in rats (Marasas, 2001). Fumonisins were found to have at least 28 different homologs, designated as A-series, B-series, C-series, and P-series (Rheeder *et al.*, 2002). The B-series (FB) is the most abundant and important for toxicity. Structurally, FB1 resembles sphingoid bases such as sphinganine (Sa) and sphingosine (So), with tricarballylic acid groups added at the C14 and C15 positions (Figure 1). It was observed to disturb sphingolipid metabolism by inhibiting the enzyme ceramide synthase, leading to accumulation of sphinganine in cells and tissues (Merrill *et al.*, 1996).




| | $B_1$ | $B_2$ | $B_3$ | $B_4$ |
|---|---|---|---|---|
| R1 | -OH | -OH | -H | -H |
| R2 | -OH | -H | -OH | -H |

**Figure 1** Type B fumonisins chemical structure.

**Source:** Loi *et al.* (2017)

FB1 is the most common and economically important homolog, followed by B2 and B3. In 1993, The International Agency for Research on Cancer (IARC) reported the toxins produced by *F. verticllioides* as group 2B carcinogen (i.e., possibly carcinogenic to humans). The maximum tolerated levels of fumonisin in foods and feeds are regulated in most countries worldwide, commonly being in the range from 1-10,000 ppb (Food and Agriculture Organization of the United Nations, 2004), while the European Union has established the limit of 0.2-60 ppm for fumonisins B1 and B2 in food and feed (Table 2-3).

**Table 2** European Union maximum limits for fumonisins B1 and B2 in foodstuff.

| Foodstuff | Maximum levels in mg/kg (ppm) |
|---|---|
| Unprocessed maize | 2 |
| Maize flour, maize meal, maize grits, maize germ and refined maize oil | 1 |
| Maize based foods for direct human consumption | 0.4 |
| Processed maize-based foods and baby foods for infants and young children | 0.2 |

**Source:** European Commission (2006)

**Table 3** European Union maximum limits for fumonisins B1 and B2 in cereals and cereal-based products for animal feed.

| Products intended for animal feed | Guidance value in mg/kg (ppm) relative to a feeding stuff with a moisture content of 12 % |
|---|---|
| Feed materials (*) | |
| - maize and maize products | 60 |
| Complementary and complete feeding stuffs for: | |
| - pigs, horses, rabbits and pet animals | 5 |
| - fish | 10 |
| - poultry, calves (< 4 months), lambs and kids | 20 |
| - adult ruminants (> 4 months) and mink | 50 |

(*) Particular attention has to be paid to cereals and cereals products fed directly to the animals that their use in a daily ration should not lead to the animal being exposed to a higher level of these mycotoxins than the corresponding levels of exposure where only the complete feeding stuffs are used in a daily ration

**Source:** European Commission (2006)

## 3. Fumonisin-producing fungi

The most common fumonisin-producing fungi are *F. verticillioides* and *F. proliferatum*, although a few other *Fusarium* sp. may produce fumonisin. Fumonisin-producing fungi cause a disease in maize known as *Fusarium* ear rot (Schmale & Munkvold, 2009). The maize ear rot produced by fungi was correlated significantly with high levels of fumonisin contamination. Moreover, maize damaged by insects could promote the penetration of fungi into the kernel. It was earned the fumonisin accumulation in the kernel. The optimum temperature for the growth of fumonisin-producing fungi and fumonisin production is 20-25ºC (Starkl & Nahrer, 2015).

### *Fusarium* ear rot

*Fusarium* ear rot has been frequently found in warmer and drier climate regions. *F. moniliforme* has been reported as the most common pathogen causing *Fusarium* ear rot. Currently, *F. verticillioides* (syn. *F. moniliforme*) is considered the predominant species. Other causative agents such as *F. proliferatum* and *F. subglutinans* have been reported as well (Munkvold, 2003). The fungus can infect corn seedlings and developing kernels and grow for a time in the ear without producing disease symptoms. Once symptoms appear, the plants have a stalk rot and/or ear and kernel rot. The infection occurring mainly in wounds is associated with insects such as European corn borer, thrips, or corn earworms. The fungus can be infected via the silks too. It can grow down through the silk channel to the ear tip (Scarpino *et al.*, 2015). Moreover, Duncan and Howard (2010) reported that a path for infection might be possible through the stylar canal to a developing kernel at the time of pollination in maize. It also increases the incidence of fumonisin contamination (Mazzoni *et al.*, 2011).

**Figure 2** *Fusarium* ear rot symptom on maize kernel.

**Morphology of *Fusarium verticillioides***

In the *Fusarium* laboratory manual, Leslie and Summerell (2006) described the morphological characteristics of *F. verticillioides.* The mycelial culture on Potato Dextrose Agar (PDA) has white mycelia but may develop violet pigment with age. On carnation leaf agar, macroconidia are relatively long and slender, with slightly falcate or straight and thin walls. Microconidia are oval to club shaped with a flattened base, always formed from monophialide and found in chains that may be quite long. Chlamydospores are not produced.

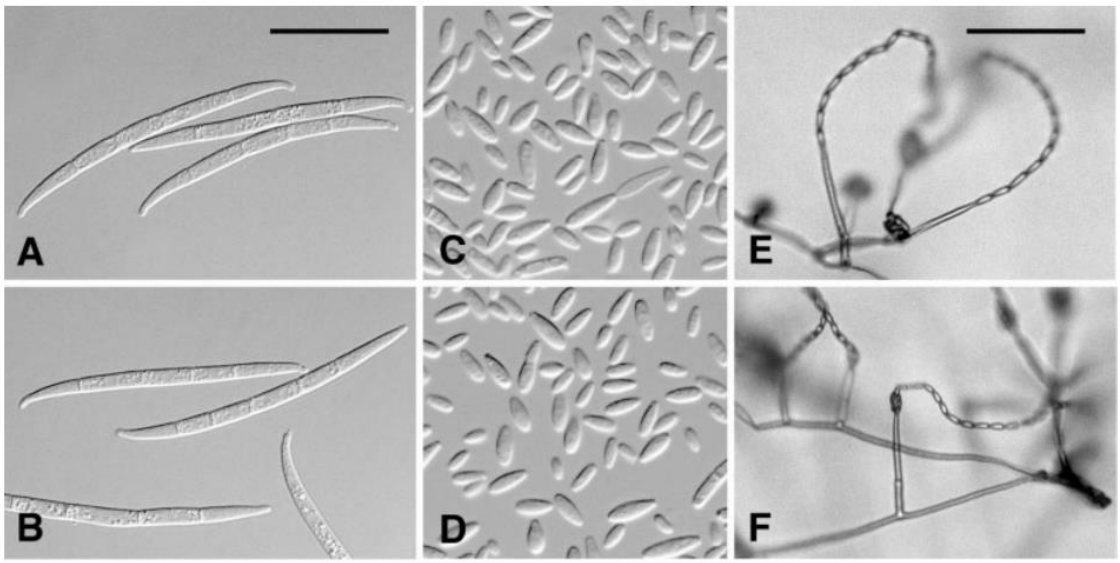



**Figure 3** Morphology of *Fusarium verticillioides*.
(A-B) Macroconidia, (C-D) Microconidia, (E-F) Microconidia on carnation leaf agar, scale bar 50 µm.

**Source:** Leslie and Summerell (2006)

**Morphology of *Fusarium proliferatum***

Leslie and Summerell (2006) illustrated the morphological characteristics of *F. proliferatum* on PDA culture. The colony demonstrated white mycelia, which might become purple violet with age. On carnation leaf agar, macroconidia are slender and almost straight and usually have 3-5 septum. Microconidia form in chains and less commonly in false heads from monophialide and polyphialide. The microconidia chains are of moderate length and usually are shorter than *F. verticillioides*. Microconidia are club shaped with a flattened base. Chlamydospores are absent.

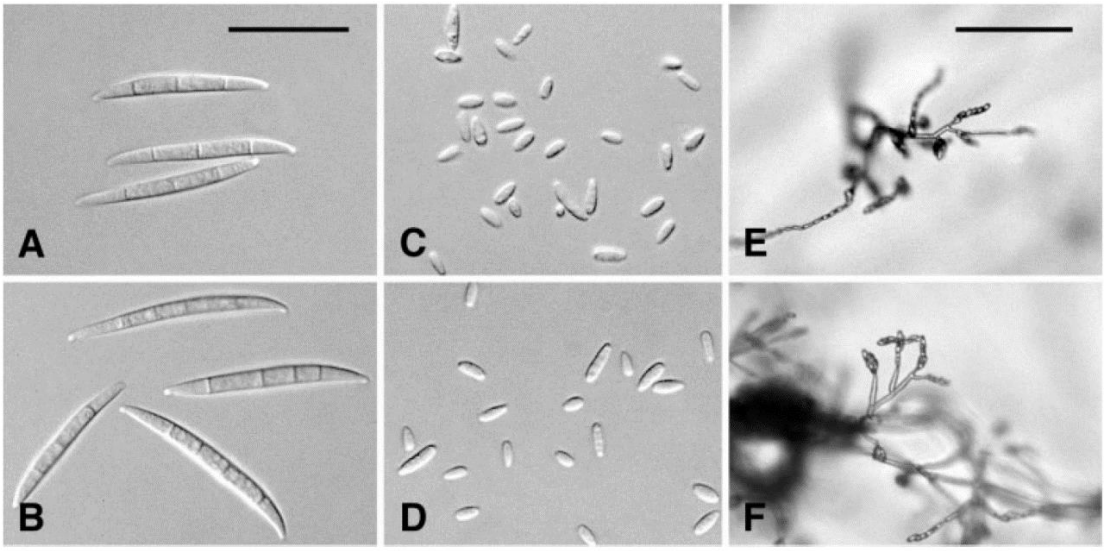



**Figure 4** Morphology of *Fusarium proliferatum.*
**(**A-B) Macroconidia, (C-D) Microconidia, (E-F) Microconidia on Carnation leaf agar, scale bar 50 µm.

**Source:** Leslie and Summerell (2006)

## 4. Fumonisin detoxification

### Chemical method

Several studies investigated chemical food processing methods for their suitability to destroy or inactivate mycotoxins in various treatments such as acid treatments, base treatments, oxidizing agents, reducing agents, food ingredients, and medical plants (Karlovsky *et al.*, 2016). Fumonisin detoxification with chemicals was reported only in base treatment. Treatment of FB1 contaminated corn with $Ca(OH)_2$ can simulate nixtamalization (soaking/cooking in an alkaline solution) and can completely hydrolyze FB1 (Park *et al.*, 1996). Additionally, ammonization reduces the concentration of FB1 in wheat by 79% (Park *et al.*, 1992) but it is inefficient in corn according to Norred *et al.* (1991). Pujol *et al.* (1999) reported that steeping corn kernels in 0.2% solution of $SO_2$ at 60 °C for 6 h was effective in reducing FB1.

### Physical method

The physical removal of fumonisin contamination has been reported in limited studies. A few physical methods were applied with fumonisin detoxification such as sorting, flotation, and steeping (Karlovsky *et al.*, 2016). In 1960, optical sorting was established. The operation principle is to direct streams of grains along an array of optical sensors. When a grain differing in color is detected, the detector triggers a magnetic valve, and a jet of pressurized air blows the kernel from the stream (Grenier *et al.*, 2014). The infection with *F. verticillioides* often causes no signs (Munkvold & Desjardins, 1997), and the correlation between fumonisin content and signs is weak (Afolabi *et al.*, 2007). Thus, grain sorting might not reduce fumonisin content efficiently, though successful attempts have been reported (Karlovsky *et al.*, 2016). The different physical properties of mold-damaged kernels compared to non-damaged kernels can be exploited to separate them by density segregation with a floating technique. In a later study, fumonisin reduction of 86% was achieved by removing maize kernels buoyant in saturated brine, with about 20% material loss (Shetty & Bhat, 1999). In maize processing, steeping is the first step in the wet milling of maize

and involves soaking maize for 36-50 h at 50°C in water containing 0.1-0.2% $SO_2$ to facilitate germ separation and breaking down of protein matrix.

**Biological method**

Only a few microorganisms are known to degrade and thereby detoxify fumonisins. The main mode of action is the removal of the two TCA side groups as well as the free amino group at one end (Vanhoutte *et al.*, 2016). The first report of fumonisins microbial detoxification was revealed by Duvick *et al.* (1998). They isolated microbes from field-grown, moldy maize kernels and stalk tissue. Two microbes are capable of growing on FB1 as a sole carbon source, identified as *Exophiala spinifera* and *Rhinocladiella atrovirens*, belonging to the "black yeasts" found widely in plant debris and a gram-negative bacterium from stalk tissue. Blackwell *et al.* (1999) reported that the pure cultures of *E. spinifera* transform FB1 to the amino polyol AP1 plus free tricarballylic acid through the activity of a soluble extracellular esterase. Later on, the FB1 toxin degrading bacteria was regarded by Benedetti *et al.* (2006). The bacterial strain NCB 1492 was isolated from the soil sample using an enrichment culture. The degrading procedure used FB1 as the sole carbon and nitrogen source in phosphate buffer. The analysis of 16S rDNA was related to the *Delftia/Comamonas* group. Analysis of metabolites by GC/MS of the culture supernatant after 24 h of growth of NCB 1492 with FB1 revealed the presence of four compounds, tentatively identified as heptadecanone ($C_{17}H_{34}O$), isononadecene ($C_{19}H_{38}$), octadecenal ($C_{18}H_{34}O$), and eicosane ($C_{20}H_{42}$).

Heinl *et al.* (2010) studied two genes responsible for fumonisin degradation from *Sphingopyxis* sp. MTA144 bacteria. The recombinant carboxylesterase was demonstrated to catalyze the deesterification of FB1 to hydrolyzed FB1. The heterologously expressed aminotransferase was shown to deaminate hydrolyzed FB1 in the presence of pyruvate and pyridoxal phosphate. The aminotransferase *fuml* gene from *Sphingopyxis* sp. MTA144 was expressed by *E. coli* at 30 °C from a T7 promoter and the characterization of the enzyme together with its kinetics was studied (Hartinger *et al.*, 2010; Hartinger *et al.*, 2011). Masching *et al.* (2016) noted that feed

supplementation with the commercial application of *FumD* (commercial namely FUMzyme®), when included with *FUM1* gene product, prevented alterations of the Sa/So ratio in turkeys and pigs.

The probiotic bacteria *Lactobacillus* sp. was interesting as an alternative bacterial source for FB1 reduction. In 2017, Niderkirn *et al.* noted that the fermentative bacteria could potentially be utilized to detoxify corn silage contaminated with deoxynivalenol (DON), zearalenone (ZEN), FB1, and FB2. Eight *Lactobacilli* and three *Leuconostoc* biotransformed ZEN into α-zearalenol. *Streptococcus* and *Enterococus* are capable of binding up to 33, 49, 24, and 62% of DON, ZEN, FB1, and FB2, respectively, from a total of 202 strains. Deepthi *et al.* (2016) reported that *L. plantarum* MYS6 has potent probiotic attributes and antifungal activity against fumonisin-producing *F. proliferatum*. The isolate has broad application as a potential probiotic and biocontrol agent in feed and crop growth. The cell-free suspension of *L. plantarum* MYS6 has the potential to control conidia germination, distorting the morphology of hyphae and conidia. It could be used as a promising bio-alternative to the chemical preservatives in poultry feeds.

Moreover, the biological microorganism inhibiting the fumonisin-producing fungi was involved with the biocontrolled detoxification of fumonisins. Dalie *et al.* (2010) reported that *Pediococcus pentosaceus* (L006) isolated from maize leaf has the most efficient antifungal activity against *F. proliferatum* and *F. verticillioides*. The antifungal metabolites were shown to be heat stable, resistant to proteolytic enzyme treatments, and pH-dependent. Pereira *et al.* (2010) reported that the effects of four bacterial biocontrol agents on *F. verticillioides* infection and fumonisin accumulation in the maize agroecosystem were evaluated in a two-year field study. In the study, seed treatment with *Bacillus amyloliquefaciens* and *Enterobacter hormaechei* reduced the infection by the fungus and FB1 contents in both the years of the study, while the ear inoculation produced highly variable results. This bacterial treatment has shown its efficiency, and it may improve the quality of maize grains obtained at the harvest by reducing toxin content.

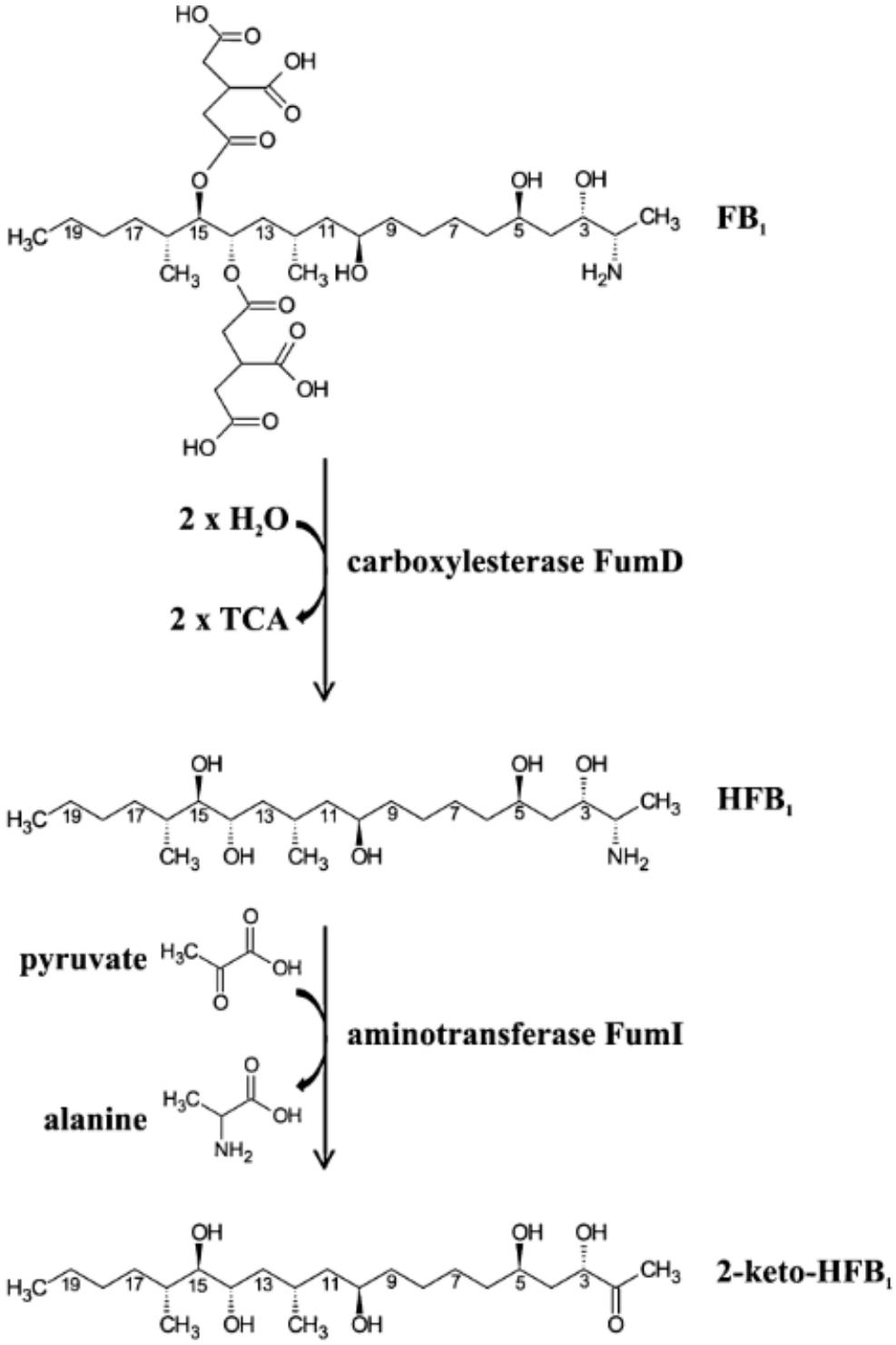



**Figure 5** Fumonisin B1 degradation pathway of *Sphingopyxis* sp. MTA144.

**Source:** Hartinger *et al.* (2011)

# MATERIALS AND METHODS

## 1. Screening of fumonisin B1 reducing bacteria

### 1.1 Acclimatization of bacteria from natural sources

Natural sources such as maize (starburst symptom and fumonisin-contaminated samples), rice (bakanae disease samples), soil, and fermented fluid were collected randomly from the continuous production area in Thailand. Subsequently, 5 g or mililiers of sample was added to 50 mL of nutrient glucose broth (NGB, 3 g of beef extract, 5 g of peptone, and 5 g of glucose) and incubated for 24 h. Then one milliliter of bacterial culture was transferred into 20 mL of NGB with crude fumonisins at 3 ppm. The crude fumonisins were prepared by the inoculation of ground maize with *F. verticillioides* (fumonisin-producing strain from the Physiological Plant Disease Laboratory, Kasetsart University). After 45 days, the crude fumonisins were extracted with 70% methanol and filtrated samples were diluted 1:20 with distilled water before taking into ELISA assay (AgraQuant® Total Fumonisin Assay, Romer Lab®, Singapore), range 0.25-5.0 ppm with LOD = 0.20 ppm and LOQ = 0.25 ppm. Two hundred microliters of conjugation solution were mixed with 100 µL of each standard or sample. One hundred microliters of the mixture were transferred into the reaction wells and incubated for 10 min. The reaction wells were washed 5 times with distilled water and added with 100 µL of substrate solution. After 5 min of incubation at RT, one hundred microliters of stop solution were added into the mixture. Absorbance was measured by a microplate reader using a 450 nm filter (Tecan, Switzerland). The data was interpreted by the AQ FUM form provided by the company. The samples were incubated at room temperature on a shaker in the dark for 15 days, after which 100 µL of bacterial suspension was isolated using an agar spread plate on nutrient glucose agar (NGA) plates. The plates were incubated with alternating periods of 12 h darkness/light at 25±2°C for 24 h. A single colony of bacteria was cross-streaked on NGA until the pure culture was obtained. The pure culture was used for further study.

1.2 Fumonisin B1 reduction activity by a bacterial isolate

Bacteria were studied for the FB1 reduction activity as described by Niderkorn *et al.* (2007) with some modification. Overnight cultures of the selected bacteria on NGB were prepared, and the bacterial cell was harvested by centrifugation at 8,000 rpm for 10 min. The bacterial pellet was adjusted to 0.2 OD using phosphate buffer solution pH 7 (PBS; 8 g of NaCl, 0.2 g of KCl, 0.2 g of $KH_2PO_4$, and 1.44 g of $Na_2HPO_4$). One hundred microliters of bacterial suspension were mixed with FB1 standard solution (Biopure, Tulln, Austria) to a final concentration of 5 ppm in a 1,000 µL reaction. A positive control containing only FB1 standard in PBS, and a negative control containing only a bacterial suspension in PBS was used. All tubes were incubated in the dark for 24 h. After incubation, the supernatants were qualitatively analyzed for FB1 toxin using ELISA (AgraQuant® Total Fumonisin Assay 0.25/5.0, Romer Lab®, Singapore) range 0.25–5.0 ppm with LOD = 0.20 ppm and LOQ = 0.25 ppm. The rate of FB1 reduction was calculated using the following formula: [(concentration of FB1 control-concentration of FB1 residual)/concentration of FB1 control)] × 100%.

1.3 Determination of bacterial cell component as an active component

The 24-48 h bacterial culture on NGB was used. The cultures were separated into the culture supernatant, cell suspension, and cell-free extract parts. All components were used to screen for fumonisin-degrading activity according to Wang *et al*. (2018).

The culture supernatant part was prepared by the centrifuged bacterial culture at 10,000 rpm and 4 ℃ for 15 min and filtered through 0.22 µm sterile cellulose acetate filters.

The cell suspension part was prepared by a cell pellet harvested from bacterial culture. The cell pellet was washed twice with PBS of pH 7 before being suspended again in the same buffer.

The cell-free extract part was prepared by disintegrating (5 sec on/off) the cell pellet using a sonicator for 30 min on an ice bath. Then, the cell debris was removed by centrifugation at 13,000 rpm and 4°C for 15 min. To obtain a cell-free extract, the supernatant was filtered through 0.22 µm sterile cellulose acetate filters.

FB1 reduction activity was determined using 250 µL of FB1 standard (10 µg/mL) was mixed with 250 µL of the bacterial culture supernatant, cell suspension, or cell extract. All reaction systems were placed on a rotary shaker at room temperature for 24 h.



## 2. Bacterial identification

### 2.1 Bacterial morphological identification

Gram staining was done following the method reported by Davies *et al.* (1983). One loop of the 24 h bacteria culture cell suspension was smeared on a slide and fixed to the slide by carefully passing the slide with a Bunsen burner three times. Crystal violet solution was added to the smear and incubated for 1 min. The slide was rinsed with sterile water for 5 sec, and an iodine solution was added for 1 min. The slide was rinsed with alcohol for 3 sec followed by sterile water. Safranin O was added to the smear and incubated for 1 min before gently washing with water for 5 sec. The slide was observed under a compound microscope. Bacterial isolates were cultured on NGA for 24 h and the macro characteristic was observed under a compound microscope with 1,000X.

### 2.2 Bacterial molecular identification

Twenty-four hours of bacterial culture suspension was used for DNA extraction with GeneJET Genomic DNA Purification Kit (Thermo Scientific, Lithuania). The bacterial cells were harvested in a 2 mL microtube by centrifugation at 5,000 g for 10 min. The supernatant was discarded, and the pellet was resuspended in 180 µL of digestion solution with the addition of 20 µL of proteinase K solution.

The mixture was mixed thoroughly to obtain a uniform suspension. The sample was incubated at 56°C and vortexed occasionally until the cells were completely lysed for about 30 min. Subsequently, 20 µL of RNase A solution was added and mixed for 10 min at room temperature. The mixture was added with 200 µL of lysis solution and mixed thoroughly by vertexing for 15 sec until homogeneous. The mixture was added with 400 µL of 50% ethanol and transferred to a purification column inserted in a collection tube. Then, the column was centrifuged at 6,000 g for 1 min. The purification column was placed into a new 2 mL collection tube, and 500 µL of wash buffer I was added (with ethanol). The column was centrifuged at 8,000 g for 1 min. After that, the flow-through was discarded and the purification column placed back into the collection tuber, and 500 µL of wash buffer II (with ethanol added) was added into the purification column and centrifuged at maximum speed (≥ 12,000 g) for 3 min. The purification column was placed into a new tube and 200 mL of elution buffer was added to the center of the membrane to elute genomic DNA. After that, the column was centrifuged at 8,000 g for 1 min; the purified DNA was stored at -20°C.

The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using the primers 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) (Lane, 1991.; Turner *et al.*, 1999). In total, 25 µL PCR reaction contained 1 unit of *Taq* DNA polymerase (Thermo scientific), 0.1 micromolar of each primer, 0.2 mM deoxynucleotide triphosphates (Fermentas), 1X *Taq* buffer (Thermo scientific), 2.5 micromolar, $MgCl_2$ (Thermo scientific), and 1 microliter (10 ng) template DNA. Cycling conditions were as follows: pre-denaturation at 94ºC for 3 min, 35 cycles of 94ºC for 1 min, 56ºC for 1 min, and 72ºC for 1 min. The reaction was completed with 10 min extension at 72ºC, and samples were held at 25ºC for 10 min. PCR products were confirmed using agarose gel electrophoresis (1X agarose in TBE buffer). The products were purified and sequenced by Sanger sequencing (Apical Scientific, Malaysia). Nucleotide sequence comparisons were performed using the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/BLAST, accessed date 23 March 2021) and deposited in the GenBank database. Similar 16S rDNA sequences were downloaded from GenBank and manually reviewed, after which all the

sequences were aligned. Furthermore, a phylogenetic tree was constructed using neighbor-joining by MEGA X (Kumar *et al.*, 2018).

2.3 Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/TOF MS) identification

Bacteria were transferred to NGA and incubated at room temperature for 24 h before analysis. The MALDI-TOF MS data were analyzed by the Salaya Central Instrument Facility, Mahidol University, Nakhon Pathom, with Autoflex MALDI-TOF mass spectrometer (Bruker, Germany). The standard Bruker interpretative criteria were applied according to Table 4.

**Table 4** Meaning of score values from MALDI-TOF/TOF MS.

| Rang | Description | Symbols | Color |
|---|---|---|---|
| 2.300-3.000 | highly probable species identification | +++ | green |
| 2.000- 2.299 | secure genus and probable species identification | ++ | green |
| 1.700-1.999 | probable genus identification | + | yellow |
| 0.000-1.699 | unreliable identification | - | red |

**Source:** Ng *et al.* (2012).

2.4 Biochemical identification

Bacteria were transferred to NGA and incubated at room temperature for 24 h before analysis. The biochemical data were analyzed by the Thailand Institute of Nuclear Technology (Public Organization), Pathum Thani. The sample was adjusted to 0.50–0.63 McFarland turbidity in sterile saline and analyzed with GN card via VITEX® 2 compact platform (bioMérieux, France). The identification levels were applied in accordance with Table 5.

**Table 5** Identification levels of the biochemical test by VITEX® 2 compact.

| ID message confidence level | Choice | % Probability (%) | Comment |
|---|---|---|---|
| Excellent | 1 | 96-99 | - |
| Very good | 1 | 93-95 | - |
| Good | 1 | 89-92 | - |
| Acceptable | 1 | 85-88 | - |
| Low discrimination | 2-3 | - | 2-3 taxa exhibit the same biopattern. Separate by supplement testing. |
| Unidentified organism | >3 | - | > 3 biopattern taxa exhibit the same biopattern or very atypical biopattern. Does not correspond to any taxon in the database. Check gram stain and purity. |

**Source:** Pincus (2006)

### 3. *In vitro* application of the fumonisin degrading bacteria

To determine the potential of active components, maize was ground. Twenty grams of 5 ppm FB1-spiked ground maize was set in a 50 mL laboratory bottle. Then, 500 μL of culture supernatant, cell suspension, and cell-free extract of bacteria according to topic 1.3 was dropped in the middle of the ground maize. All active components were incubated for 24 h at room temperature. The ground maize was extracted, analyzed by using ELISA, the compared to the control without active components.

**4. Antagonistic activity of *S. marcescens* 329-2 to fumonisin producing *Fusarium* sp.**

The *in vitro* antagonistic assay was performed according to the dual culture assay on potato dextrose agar (PDA). The overnight NGB culture of *S. marcescens* 329-2 was streaked on PDA medium for 2 cm from the dish edge. A seven-day mycelial disk (5 mL) of fumonisin-producing *Fusarium* sp. was placed opposite the bacterial streak at 2 cm from the dish edge (Figure 6). The antagonistic activity of the studied bacterial strains was observed as the fungal growth inhibition in comparison with the control. The inhibition was observed after 7 and 9 days of incubation with alternating periods of 12 h darkness/light at 25±2°C.

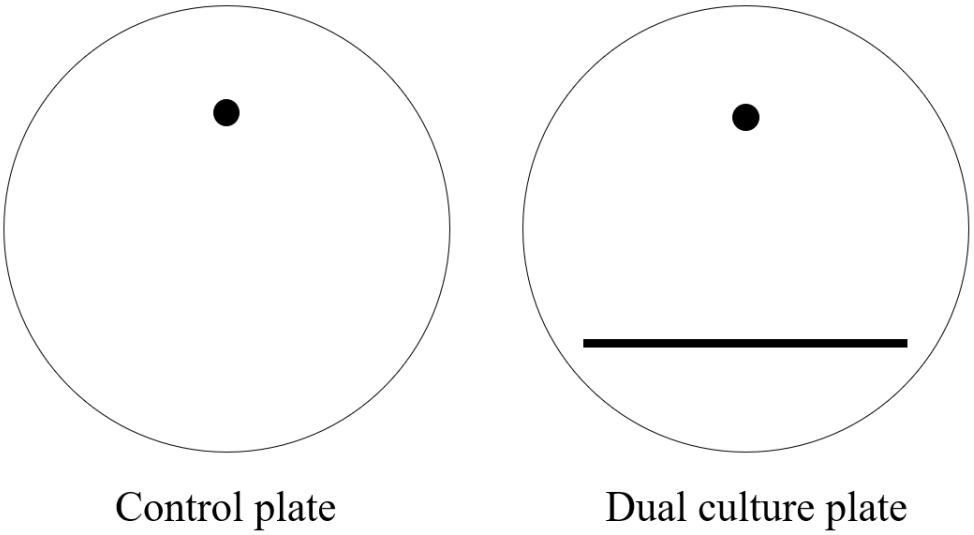



**Figure 6** Dual culture method illustrating measurement.

## 5. Fumonisin degrading process study

### 5.1 Transcriptome analysis of RNA expression from *Serratia marcescens* during fumonisin B1 reduction

#### 5.1.1 Sample preparation and total RNA extraction

A bacterial pellet was harvested from a two-day-old bacterial in NGB. The pellet was washed twice with PBS and centrifuged at 10,000 rpm for 15 min. The bacterial pellet was incubated in 5 ppm FB1 standard solution. The control was inoculated in PBS without FB1 standard. After 24 h of incubation, a bacterial pellet was collected for RNA extraction with RNeasy Kits (Qiagen, Germany). The bacterial cells were harvested in a 2 mL microtube by centrifugation at 5,000 g for 5 min. The supernatant was discarded, and the pellet was added to 50 mg of acid-washed glass beads and resuspended in 700 µl of buffer RLT (with mercaptoethanol added). The mixture was mixed thoroughly for 5 min to obtain a uniform suspension and centrifuged at maximum speed for 10 sec. After that, the mixture was transferred into a new tube and an equal volume of 70% ethanol was added. After being mixed well by pipetting, the mixture was transferred to RNeasy spin column inserted in a collection tube. Then, the column was centrifuged at 8,000 g for 15 sec. The purification column was placed into a new 2 mL collection tube and 80 µl of DNase I added. After incubation for 20 min, 350 µL of wash buffer RW1 was added into the purification column and centrifuged at 8,000 g for 15 sec. After that, the flow-through was discarded and the purification column placed back into the collection tube. Subsequently, 500 µL of buffer RPE was added into the purification column and centrifuged at 8,000 g for 15 sec. Another 500 µL of buffer RPE was added to the purification column and centrifuged at 8,000 g for 2 min. The purification column was placed into a new tube and 50 mL of RNase-free water was added to the center of the membrane to elute total RNA. Finally, the column was centrifuged at 8,000 g for 1 min to elute the total RNA samples; the total RNA was stored at -80°C.

### 5.1.2 RNA quantification and qualification.

RNA degradation and contamination were monitored on 1% agarose gels. RNA purity was checked using the NanoPhotometer® spectrophotometer (IMPLEN, USA). RNA integrity and quantitation were assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, USA).

### 5.1.3 Library preparation for transcriptome sequencing

A total of 1 μg RNA per sample was used as input material for the RNA sample preparations. Sequencing libraries were generated using NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB, USA) following the manufacturer's recommendations, and index codes were added to attribute sequences to each sample. Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was performed using divalent cations under elevated temperature in NEBNext First Strand Synthesis Reaction Buffer (5X). First-strand cDNA was synthesized using random hexamer primer and M-MuLV Reverse Transcriptase (RNase H-). Second strand cDNA synthesis was subsequently performed using DNA Polymerase I and RNase H. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities. After adenylation of 3' ends of DNA fragments, NEBNext Adaptor with hairpin loop structure was ligated to prepare for hybridization. To select cDNA fragments preferentially of 150~200 bp in length, the library fragments were purified with the AMPure XP system (Beckman Coulter, Beverly, USA). Then 3 μl USER Enzyme (NEB, USA) was used with size-selected, adaptor-ligated cDNA at 37 °C for 15 min followed by 5 min at 95 °C before PCR. The PCR was performed with Phusion High-Fidelity DNA polymerase, Universal PCR primers, and Index (X) Primer. Ultimately, PCR products were purified (AMPure XP system), and library quality was assessed on the Agilent Bioanalyzer 2100 system.

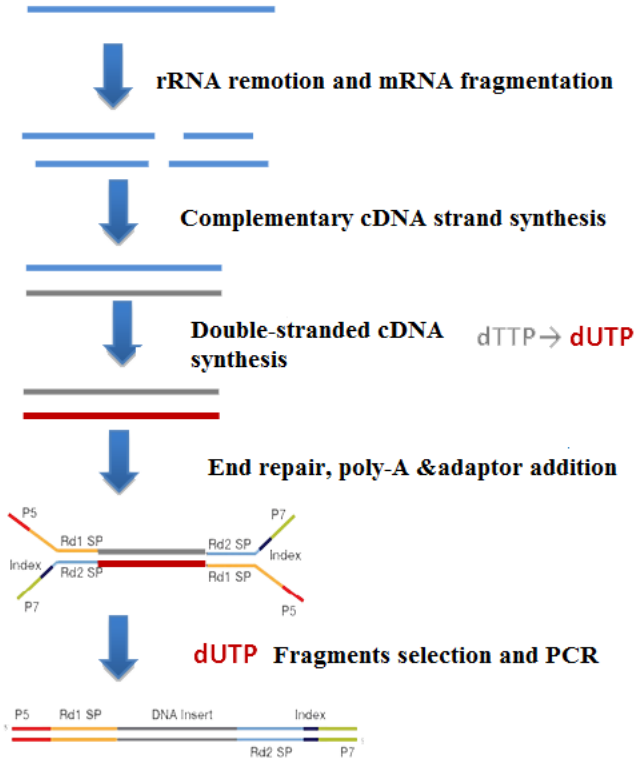



**Figure 7** Transcriptome library construction protocol.

**Source:** Novogene (2020)

### 5.1.4 Quality control

Raw data of the FASTQ format were processed through fastp. In this step, clean data (clean reads) were obtained by trimming reads containing adapter and removing poly-N sequences, and reads from raw data. Simultaneously, the Q20, Q30 and, GC content of the clean data were calculated. All the downstream analyses were based on high-quality clean data.

### 5.1.5 Reads mapping to the reference genome

Reference genome and gene model annotation files were downloaded from the genome website directly. Both building index of and aligning clean reads to the reference genome were performed using Bowtie2 (Langmead & Salzberg, 2012).

### 5.1.6 Differential expression analysis

Before differential gene expression analysis, for each sequenced library, the read counts were adjusted by Trimmed Mean of M-values (TMM) through one scaling normalized factor. The differential expression analysis of the two conditions was performed using the edgeR package. The p-values were adjusted using the Benjamini and Hochberg methods. The corrected p-values of 0.005 and |log2 (Fold Change)| of 1 were set as the threshold for significantly differential expression.

### 5.1.7 GO analysis of differentially expressed genes

Gene Ontology (GO) enrichment analysis of differentially expressed genes was implemented by the cluster profile R package, in which gene length bias was corrected. GO terms with a corrected p-value of less than 0.05 were considered significantly enriched by differential expressed genes.

## 5.2 Proteome analysis of protein expression during fumonisin B1 reduction by *Serratia marcescens* 329-2

### 5.2.1 Sample preparation

A bacterial pellet was harvested from a two-day-old bacterial culture in NGB. The pellet was washed twice with PBS and centrifuged at 10,000 rpm for 15 min. The bacterial pellet was resuspended in 5 ppm FB1 standard solution with shaking. The control was inoculated in PBS without FB1. The bacterial pellet was collected at 3, 5, and 7 days after incubation for screening protein differentiation with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A seven-day-old bacterial pellet after incubation with FB1 was determined to be proteomic by label-free quantification.

### 5.2.2 SDS-PAGE of bacterial cell component

SDS-PAGE was performed according to the Invitrogen SureCast (Thermo Fisher, USA) specification. Proteins from the bacterial pellet were separated using a 12% resolving gel at pH 8.8 and a 4% stacking gel at pH 6.8 (Table 6). The gel was performed by SureCast Mini Gel Tank electrophoresis systems (Thermo Fisher, Israel) with running buffer ((250 mM Tris (Vivantis, Malaysia), 192 mM Glycine (Sigma-Aldrich, USA), 1% SDS (Merck, Japan)). Protein samples were dissolved in 2X SDS PAGE loading buffer (0.125 M Tris-HCl (Vivantis, Malaysia), 4% SDS (Merck, Japan), 20% Glycerol (Sigma-Aldrich, USA), 0.01% bromophenol blue (Ajex Finechem, Australia)), and 5% 2-Mercaptoethanol (Sigma-Aldrich, Switzerland). Protein samples were heated at 100 °C for 5 min and run at a constant 50 V and 100 V until the bromophenol blue reached the bottom of the gel.

The SDS-PAGE gel was fixed with a fixing solution (50% methanol, 10% acetic acid, and 40% $H_20$) for 2 h with gentle agitation. After that, SDS-PAGE gel was stained with Coomassie Blue-staining solution (0.1% coomassie R-250 (Merck, USA), 50% methanol, 10% acetic acid, 40% water) for 4 h with gentle agitation and destain with destaining solution (50% methanol, 10% acetic acid, and 40% $H_20$) until the background is nearly clear.

**Table 6** Composition for SDS-PAGE gel.

| Solution | Polyacrylamide (%) | |
|---|---|---|
| | **Resolving gel (12%)** | **Stacking gel (4%)** |
| Acrylamide (40%) | 2.40 ml | 0.30 ml |
| Resolving buffer | 2.00 ml | - |
| Stacking buffer | - | 0.75 ml |
| Distilled water | 3.42 ml | 1.89 ml |
| 10% sodium dodecyl sulphate (SDS) | 80 µl | 30 µl |
| 10% ammonium persulfate (APS) | 80 µl | 30 µl |
| Tetramethylethylenediamine (TEMED) | 8 µl | 3 µl |



5.2.3 Label-free quantification

The cell pellet was washed in PBS 100 µl twice. The cell pellet was resuspended in lysis buffer (10% sodium deoxycholate (TCI, Japan), 10 mM Tris (2-carboxyethyl) phosphine hydrochloride, (Sigma-Aldrich, USA), 40 mM 2-chloroacetamide (Sigma-Aldrich, USA), and 50 mM phosphate buffer, pH 8.0) twice, then boiled at 95°C for 10 min, and sonicated for 15 min. The cell debris was pelleted by centrifugation at 10,000 rpm for 5 min, and the clarified lysate was transferred into a new tube. Crude protein was extracted with trypsin digestion following a protocol. The lysate was diluted 1:10 for trypsin digestion using Trypsin Gold, Mass Spectrometry Grade (Promega, USA) at an enzyme:substrate ratio of 1:50, and digestion was performed overnight at 37°C. The digested sample was acidified to a final concentration of 0.5% trifluoroacetic acid (Sigma-Aldrich, USA), and sodium deoxycholate was extracted by adding an equal volume of ethyl acetate and vigorous shaking. The organic phase was removed after centrifugation at 10,000 rpm for 5 min. The aqueous solution was transferred to a new tube and submitted to lyophilization. Label-free quantification and data analysis were performed by the Salaya Central

Instrument Facility, Mahidol University, Nakhon Pathom, with NanoLC (Ultimate 3000, Thermo Scientific) using an Acclaim PepMap RSLC C18 column (75 μm × 150 mm, Thermo Scientific). The mobile phases were 2% (v/v) acetonitrile with 0.1% (v/v) formic acid (phase A) and 80% (v/v) acetonitrile with 0.1% (v/v) formic acid (phase B). The linear gradient elution was as follows: 0-5 min, 3% B; 5-45 min, 3%-45% B; 45-50 min, 90% B; and 50-60 min, 3% B. The masses of the peptides were determined using a Sciex Triple TOF 6600+ instrument (AB Sciex, USA).

# RESULTS AND DISCUSSION

## 1. Screening of biodegradable toxin bacteria

### 1.1 Acclimatization of the bacterial from natural sources

Fumonisin B1 (FB1) is one of the most important mycotoxins produced by several species of *Fusarium*, mainly *F. verticillioides* or *F. proliferatum*. *Fusarium* sp. frequently occurs in maize kernels and affects grain quality (Logrieco *et al.*, 2003; Voss & Riley, 2013). FB1 is a potential natural contaminating mycotoxin. The number of fumonisin-contaminated products increase yearly with global warming, inducing increases in some *Fusarium* sp. populations. Food and feed factories are very concerned about avoiding this low-quality raw material from agricultural products. The degradation of fumonisins is necessary for reducing the contamination of feed products. Many researchers are trying to find biodegradable microorganisms to decrease FB1 contamination in agricultural products. In these studies, screening of bacteria for fumonisin degradation was performed through an acclimatization process. After acclimatizing, natural sources such as maize, rice, soil, and fermented fluid were tested for FB1, and the degradation efficiency was determined after 15 days of incubation. From 95 natural source samples, the studied discovered five potential samples in which FB1-reducing bacteria were isolated. The FB1-reducing bacteria were in approximately 5.3% of the natural source samples from our study. Four were found in maize samples, and one was from fermented fluid (Table 7). The bacterial isolates were named S2, 302-2, 329-2, P1, and 412 (Table 8). All bacteria were purified and collected for further study.

**Table 7** Number of samples collected from various natural sources used for fumonisin degrading potential.

| Natural source | Number of screened samples | Potential degrading samples | % potential degradable microorganism |
|---|---|---|---|
| Maize | 37 | 4 | 10.8 |
| Rice | 12 | 0 | 0.0 |
| Soil | 8 | 0 | 0.0 |
| Fermented fluid | 38 | 1 | 2.6 |
| Total | 95 | 5 | 5.3 |

**Table 8** Source of potential bacterial isolates after acclimatization.

| Isolate | Natural source |
|---|---|
| S2 | maize |
| 302-2 | maize |
| 329-2 | maize |
| 412 | maize |
| P1 | fermented fluid |

1.2 Fumonisin B1 reduction activity by bacterial isolates

A single isolate of the selected bacterium was studied for FB1 reduction activity under FB1 solution. FB1-degrading activities were determined using ELISA. The result on reduction efficacies of selected bacteria is shown in Figure 8. The reduction rates of these isolates ranged from 7.72-31.34% after 24 h of incubation. The highest FB1 reduction rate (31.34%) was exhibited by bacterial isolate 302-2, followed by isolate 329-2 at 26.48%. The previous study of biodegradable microorganisms to FB1 was quite limited. Only some microorganisms have been reported to effectively degrade FB1 in the post-harvest stage, such as *Exophiala spinifera* isolate 2141.10, *Rhinocladiella atrovirens*, and the gram-negative bacterium

2412.1 isolated from maize (Blackwell *et al.*, 1999; Duvick *et al.*, 1998), the *Delftia/Comamonas* group isolated from soil (Benedetti *et al.*, 2006), and the microorganism consortium SAAS79 isolated from spent mushroom compost (Zhao *et al.*, 2019).

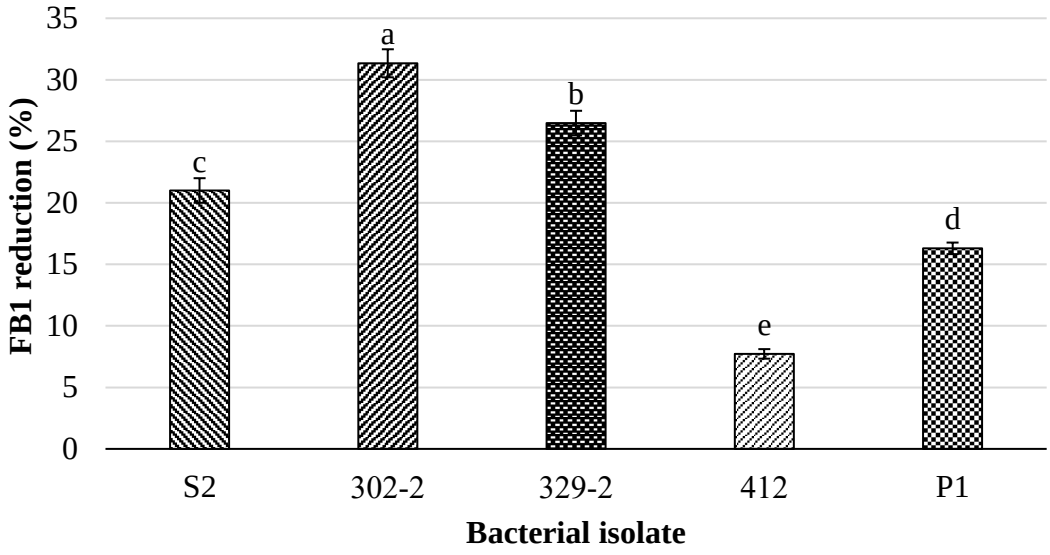



**Figure 8** Percentage of FB1 reduction by all isolates after 24 h of incubation with the FB1 standard at 5 ppm.
Different lowercase letters above the columns represent significant differences by ANOVA ($p < 0.05$).

1.3 Determination of the active component from bacteria

To determine the active component of bacteria for FB1 reduction, the selected bacterial culture was separated into the culture supernatant, cell suspension, and cell-free extract fractions. The efficiency of FB1 degradation was determined for each portion. The percent reduction ranged from 0-30.29%. The highest reduction occurred with the treatment by the isolate 329-2 cell-free extract, which resulted in a reduced rate of 30.29%, followed by reductions of 25.80% using the cell suspension of 302-2 and 22.13% using the cell suspension of S2. The reduction rate of 302-2 culture supernatant was 13.82%, while the reduction rates using cell suspensions of 412 and 329-2 were 12.02% and 10.55%, respectively. Culture supernatants and cell-

free extract of bacterial isolates S2, 412, and P1 revealed no reduction; moreover, the culture supernatant of isolate 329-2 was effective (Figure 9). Similar to Zhao *et al.* (2019) studied on the FB1 reduction contained in a crude enzyme from inside bacterial cells. The result of different portions showed a different FB1 reduction rate. In our study, isolate 329-2 was chosen for a bacterial cell component study. This result means that the active protein involved in FB1 degradation was present in different proportions in different bacterial parts.



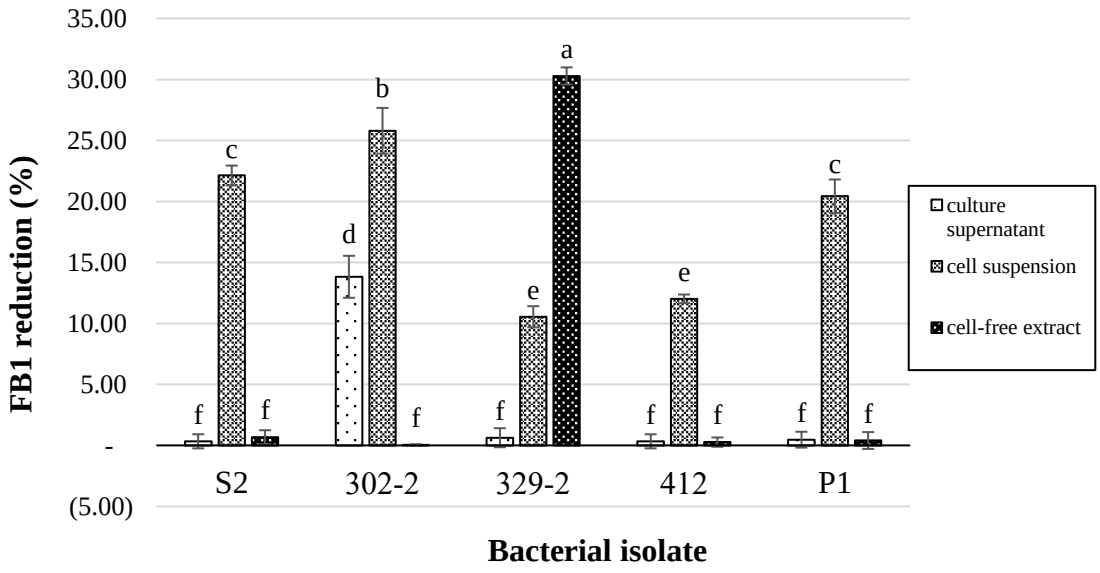



**Figure 9** Percentages of FB1 reduction by the different bacterial parts as culture supernatant, cell suspension, and cell-free extract in solution after 24 h of incubation with the FB1 standard at 5 ppm.
Different lowercase letters above the columns represent significant differences by ANOVA ($p < 0.05$).

Various bacterial portions and bacterial strains showed different FB1 reductions. In 329-2, the cell-free extract showed a high reduction of FB1. This action similar to Zhao *et al.* (2019) reported that enzymes from cell-free extracts of bacterial consortium SAAS79, isolated from the spent mushroom compost eliminated about 90% of 10 microgram/mL FB1 in 3 h. Additionally, Wang *et al.* (2018) reported that

the efficacy of culture supernatant from *Lysinibacillus* sp. ZJ-2016-1 for zearalenone was decreased after heat treatment, declining from 95.8% without heat treatment to 10.4% after heat treatment. The effect of temperature on culture supernatant indicates that the removal of zearalenone might be enzymatic.

**2. Bacterial identification**

2.1 Gram strain

Gram staining is a common technique used to differentiate two large groups of bacteria based on their different cell wall constituents. Five isolates of degradable bacteria were tested with Gram stain and colony morphology on NGA after 24 h. All five isolates were gram-negative (Table 9).

Bacterial isolate S2 was gram-negative and straight rod-shaped, had round colonies and an entire margin, and produced red pigmentation. Bacterial isolate 302-2 was gram-negative, rod-shaped, white color, smooth, low, convex, and moist. Bacterial isolate 329-2 was gram-negative and straight rod-shaped, had round colonies and an entire margin, and produced red pigmentation. Bacterial isolate 412 colony was gram-negative, straight rod-shaped, capsulated, white color, large mucoid, circular, convex, and smooth. Bacterial isolate P1 colony was rod-shaped, white-color, opaque, and flat (Table 9, Figure 10-11)

**Table 9** Gram stain and colony characteristics of potential bacteria.

| Isolate | Gram staining | Shape | Colony characteristics on NGA |
|---|---|---|---|
| S2 | negative | straight rod-shaped | round colonies, an entire margin, and produce red pigmentation |
| 302-2 | negative | rod-shaped | white color, smooth, low, convex, and moist |
| 329-2 | negative | straight rod-shaped | round colonies, an entire margin, and produce red pigmentation |
| 412 | negative | straight rod-shaped, capsulated | white color, large mucoid, circular, convex, and smooth colony |
| P1 | negative | rod-shape | white color, opaque, flat colony |

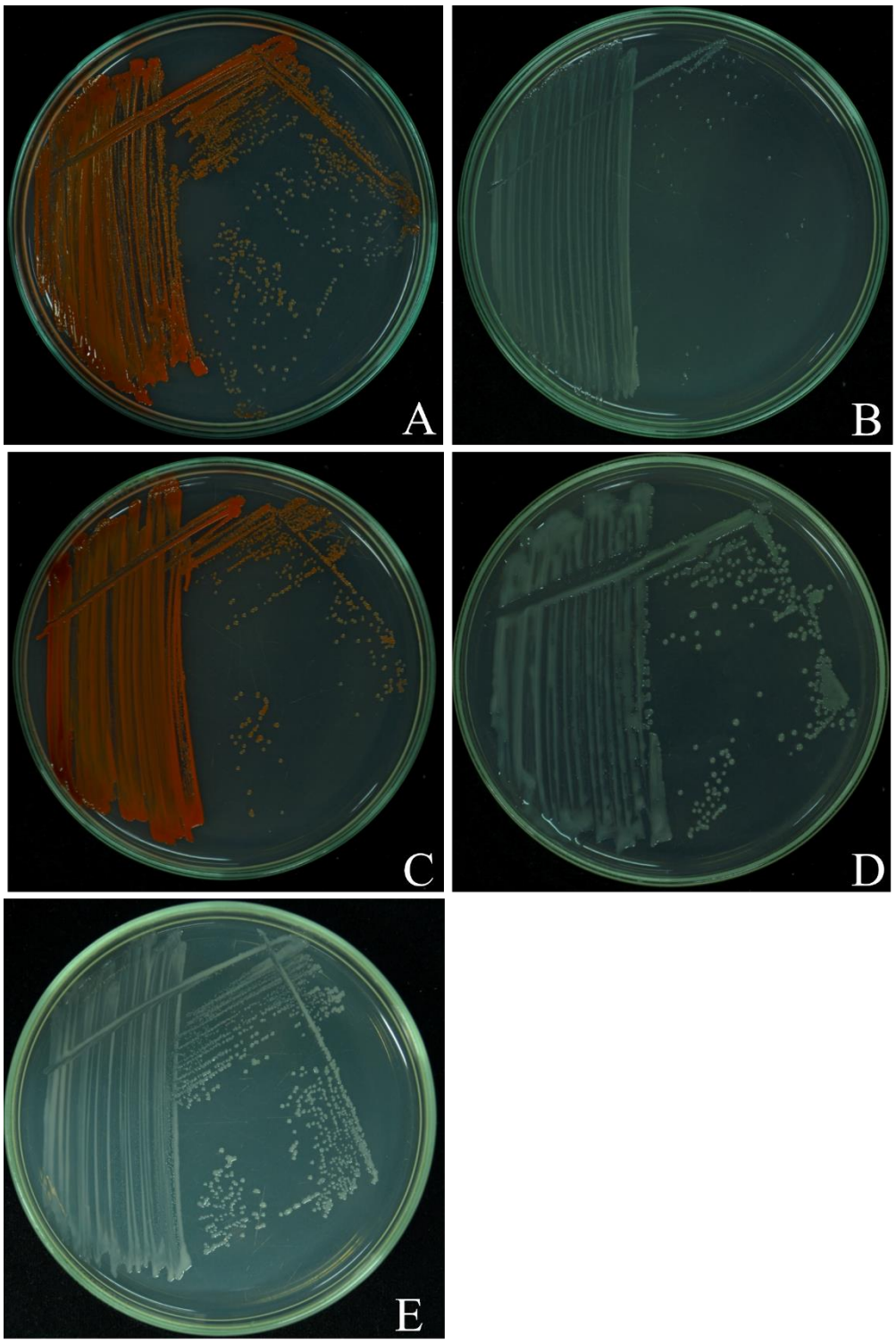



**Figure 10** Bacterial colony on NGA after 24 h of incubation.
(A) S2, (B) 302-2, (C) 329-2, (D) 412, (E) P1.

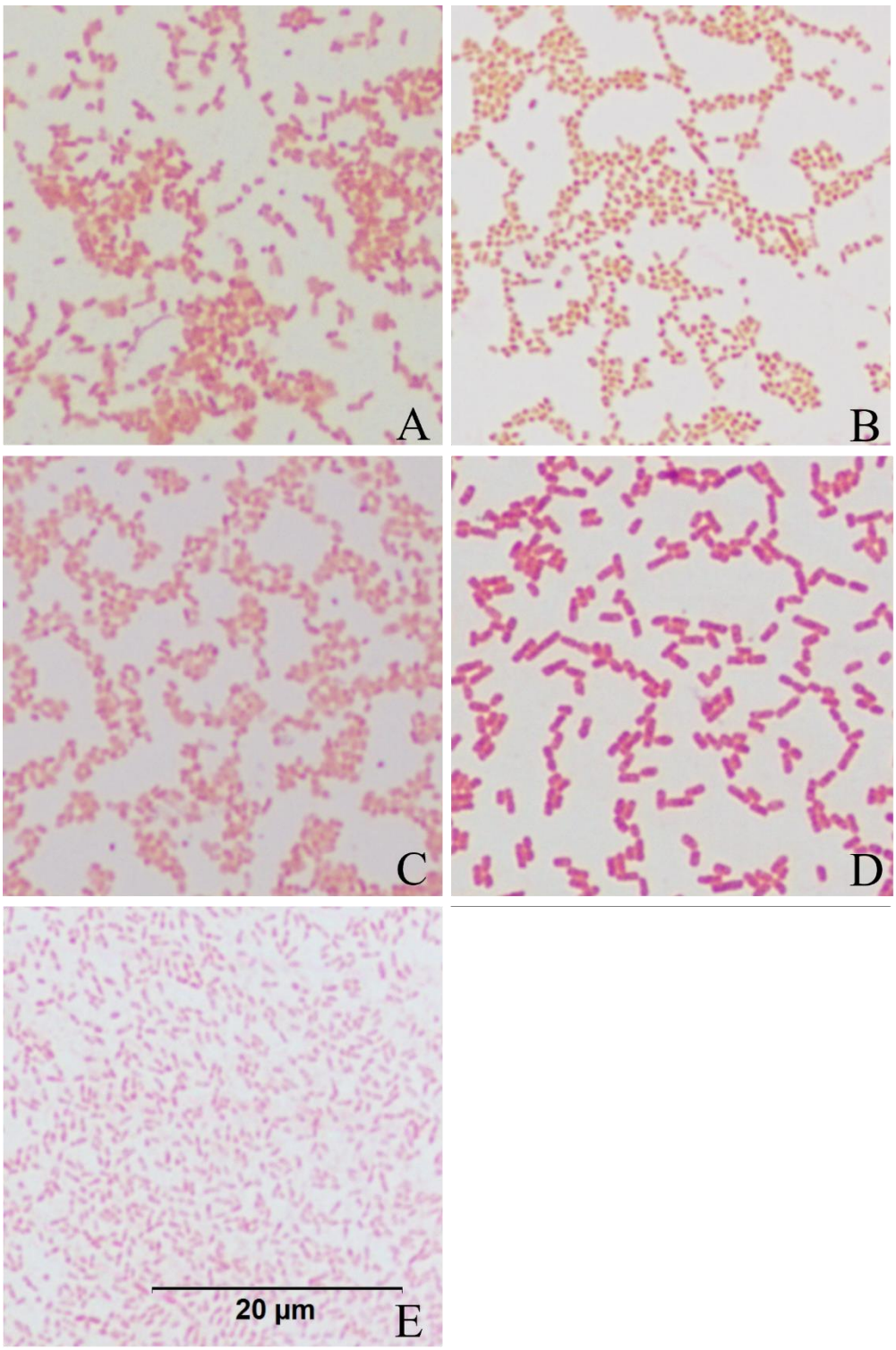



**Figure 11** The microscopic of bacterial cell character under microscope after 24 h of incubation on NGA.
(A) S2, (B) 302-2, (C) 329-2, (D) 412, (E) P1.

## 2.2 Molecular identification

Molecular identification with 16S ribosomal DNA was used for bacterial identification. The 16S rRNA gene used to investigate bacterial phylogeny and taxonomy is the most frequent housekeeping genetic marker and is more reliable than other genes for various reasons. First, it is present in all bacteria, typically as part of a multigene family or operon. Second, it is a more accurate measurement over time and is not altered, implying that random sequence changes are a more accurate measure of time. Finally, it is large enough for informatics purposes (Jin *et al.*, 2010; Patel, 2001; Woese, 1987).

The result of 16S ribosomal DNA amplification using 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) was the production of a single amplicon fragment of 1,500 bp (Figure 12). The amplicon fragments were sequenced and deposited in the GenBank database (Table 10). The nucleotide sequencing was analyzed with the BLASTn search (Basic Local Alignment Search Tool) at the NCBI database (National Center for Biotechnology Information). The closely related sequence of each sample was collected and analyzed with the MEGA X program. The bacterial isolates S2 and 329-2 were related to *S. marcescens*, 302-2 to *Citrobacter freundii*, 412 to *Klebsiella variicola*, and P1 to *Pseudomonas* sp. (Table 11-15). The phylogenetic tree of the sequence from the unweighted pair group method with arithmetic mean (UPGMA) from 16S ribosomal DNA showing the relationships of the bacterial isolates is seen in Figure 13.

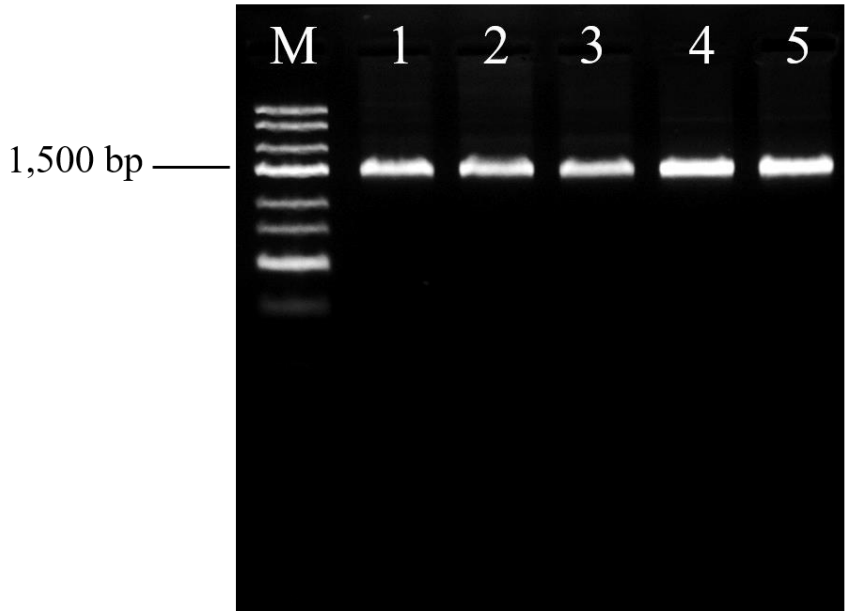



**Figure 12** DNA amplification of isolate S2, 302-2, 329-2, 412 and P1 using 27F/1492R primer on agarose gel electrophoresis.
1= S2, 2 = 302-2, 3 = 329-2, 4 = 412, 5 = P1,
M = O'GeneRuler Express DNA Ladder.

**Table 10** Deposited accession number on GenBank of FB1 reducing bacteria.

| Isolate | Accession number | Scientific name |
|---|---|---|
| S2 | LC627326 | *Serratia marcescens* |
| 302-2 | LC627324 | *Citrobacter freundii* |
| 329-2 | LC625784 | *Serratia marcescens* |
| 412 | LC627325 | *Klebsiella variicola* |
| P1 | LC627323 | *Pseudomonas* sp. |

**Table 11** Comparing the result with a BLASTn search on the NCBI database of bacterial isolate S2.

| Accession number | Description | Query cover | Similarity (%) |
|---|---|---|---|
| MK319946.1 | *Serratia marcescens* strain UMBR 0044 16S ribosomal RNA gene, partial sequence | 1410/1411 | 99.93% |
| KJ443714.1 | *Serratia marcescens* strain SEN 16S ribosomal RNA gene, partial sequence | 1410/1411 | 99.93% |
| AY514434.1 | *Serratia marcescens* strain N2.4 16S ribosomal RNA gene, partial sequence | 1409/1410 | 99.93% |
| MT949651.1 | *Serratia marcescens* strain ZTP1 16S ribosomal RNA gene, partial sequence | 1411/1413 | 99.86% |
| KF700093.1 | *Serratia marcescens* strain TC-1 16S ribosomal RNA gene, partial sequence | 1410/1412 | 99.86% |

**Table 12** Comparing the result with a BLASTn search on the NCBI database of bacterial isolate 302-2.

| Accession number | Description | Query cover | Similarity (%) |
|---|---|---|---|
| MN420979.1 | *Citrobacter freundii* strain EBS8 16S ribosomal RNA gene, partial sequence | 1380/1384 | 99.71% |
| AB548831.1 | *Citrobacter freundii* gene for 16S ribosomal RNA, partial sequence, strain: JCM 24066 | 1380/1387 | 99.50% |
| MK561018.1 | *Citrobacter freundii* strain FC18565 16S ribosomal RNA gene, partial sequence | 1382/1389 | 99.50% |
| MN220576.1 | *Citrobacter freundii* strain CZ-2 16S ribosomal RNA gene, partial sequence | 1382/1389 | 99.50% |
| MN208196.1 | *Citrobacter freundii* strain 14-a blue 16S ribosomal RNA gene, partial sequence | 1382/1390 | 99.42% |

**Table 13** Comparing the result with a BLASTn search on the NCBI database of bacterial isolate 329-2.

| Accession number | Description | Query cover | Similarity (%) |
|---|---|---|---|
| AY514434.1 | *Serratia marcescens* strain N2.4 16S ribosomal RNA gene, partial sequence | 1384/1384 | 100.00% |
| KT964299.1 | *Serratia marcescens* strain DD274 16S ribosomal RNA gene, partial sequence | 1382/1382 | 100.00% |
| KJ443714.1 | *Serratia marcescens* strain SEN 16S ribosomal RNA gene, partial sequence | 1383/1384 | 99.93% |
| MT949651.1 | *Serratia marcescens* strain ZTP1 16S ribosomal RNA gene, partial sequence | 1381/1382 | 99.93% |
| MK319946.1 | *Serratia marcescens* strain UMBR 0044 16S ribosomal RNA gene, partial sequence | 1381/1382 | 99.93% |

**Table 14** Comparing the result with a BLASTn search on the NCBI database of bacterial isolate 412.

| Accession number | Description | Query cover | Similarity (%) |
|---|---|---|---|
| KC853302.1 | *Klebsiella variicola* strain XF6 16S ribosomal RNA gene, partial sequence | 1378/1378 | 100.00% |
| JF690980.1 | *Klebsiella variicola* strain JDM-14 16S ribosomal RNA gene, partial sequence | 1378/1378 | 100.00% |
| MK929064.1 | *Klebsiella variicola* strain NM43_TS4-1 16S ribosomal RNA gene, partial sequence | 1377/1378 | 99.93% |
| MH111590.1 | *Klebsiella variicola* strain HCD26-1 16S ribosomal RNA gene, partial sequence | 1377/1378 | 99.93% |
| MF370894.1 | *Klebsiella variicola* strain Y37R 16S ribosomal RNA gene, partial sequence | 1377/1378 | 99.93% |

**Table 15** Comparing the result with a BLASTn search on the NCBI database of bacterial isolate P1.

| Accession number | Description | Query cover | Similarity (%) |
|---|---|---|---|
| DQ118954.1 | *Pseudomonas* sp. JQ2-6 16S ribosomal RNA gene, partial sequence | 1388/1388 | 100.00% |
| EU099380.1 | *Pseudomonas* sp. J14 16S ribosomal RNA gene, partial sequence | 1387/1388 | 99.93% |
| KY039270.1 | *Pseudomonas* sp. strain S102 16S ribosomal RNA gene, partial sequence | 1386/1388 | 99.86% |
| LC133613.2 | *Pseudomonas* sp. JCM 28266 gene for 16S ribosomal RNA, partial sequence | 1386/1388 | 99.86% |
| MN007089.1 | *Pseudomonas* sp. strain LAM1902 16S ribosomal RNA gene, partial sequence | 1386/1388 | 99.86% |

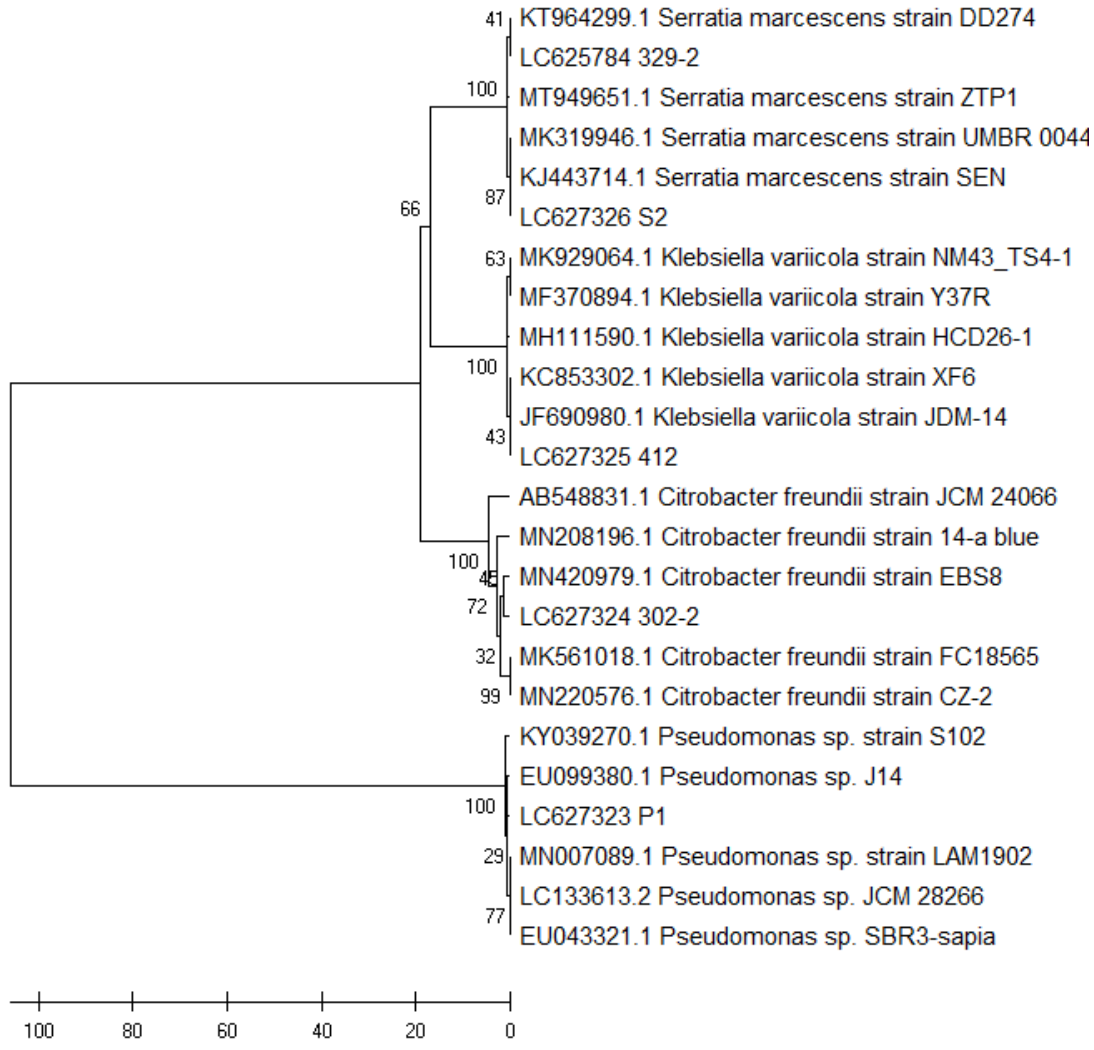



**Figure 13** Phylogenetic tree based on 16S rRNA gene sequences and related taxa.

## 2.3 Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/TOF MS)

MALDI-TOF/TOF MS is an efficient high-throughput technology for identifying and evaluating proteins (Neville *et al.*, 2011; Timperio *et al.*, 2017). It can identify microorganisms from prepared samples in about 10 min, and the obtained results show a high rate of concordance with 16S rRNA gene identification. MALDI-TOF/TOF MS allows the identification of microorganisms at the species level by the analysis of the amount of total protein. This technique is based on the generation of mass spectra from whole cells and their comparison to reference spectra. It successfully identifies whole bacterial cells from various sources (Dingle & Butler-Wu, 2013; Li *et al.*, 2019; Sauget *et al.*, 2017)

In this study, MALDI-TOF/TOF MS was used to identify the outstanding FB1-reducing bacteria as bacterial isolate 329-2. The result of the MALDI-TOF/TOF MS confirmed that bacterial isolate 329-2 belonged to *S. marcescens*, and the highest score (2.361) was for *S. marcescens* DSM 12481 (Table 16). According to Othman *et al.* (2019); Rödel *et al.* (2019) successfully identified *S. marcescens* by MALDI-TOF/TOF MS from soil samples and humans.

**Table 16** Score and matched pattern result from the MALDI-TOF/TOF MS analysis of *Serratia marcescens* 329-2.

| Rank | Quality | Matched Pattern | Score | Color |
|---|---|---|---|---|
| 1 | +++ | *Serratia marcescens* DSM 12481 | 2.361 | green |
| 2 | +++ | *Serratia marcescens* DSM 12485 | 2.345 | green |
| 3 | +++ | *Serratia marcescens* 13103_1 CHB | 2.319 | green |
| 4 | +++ | *Serratia marcescens* subsp. *marcescens* DSM 30121T | 2.306 | green |
| 5 | ++ | *Serratia marcescens* subsp. *sakuensis* CIP 107489T | 2.240 | green |
| 6 | ++ | *Serratia ureilytica* DSM 16952T | 2.155 | green |
| 7 | ++ | *Serratia marcescens* DSM 30122 | 2.147 | green |
| 8 | ++ | *Serratia marcescens* (PX) 24086109 | 2.062 | green |
| 9 | ++ | *Serratia marcescens* DSM 12483 | 2.039 | green |
| 10 | + | *Serratia entomophila* DSM 12358T | 1.942 | yellow |

2.4 Biochemical identification

The VITEK® 2 compact is an automated microbiology system utilizing growth-based technology. The reagent card has wells that can contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalinization, enzyme hydrolysis, and growth in the presence of inhibitory substances (Pincus, 2006).

In this study, VITEK® 2 compact was used for the biochemical identification of isolate 329-2. The GN card for gram-negative bacteria was used for the biochemical test. The result of 47 biochemical tests and one negative control is shown in Table 17. The result concluded that bacterial isolate 329-2 belonged to *S. marcescens* with excellent identification (99%). According to Melissa *et al.* (2017) and Rafii (2014) successfully identified *S. marcescens* by MALDI-TOF/TOF MS from clinical samples.

**Table 17** The result of the biochemic al reaction of *S. marcescens* from VITEX-2 with GN card.

| Well | Reaction | Result |
|---|---|---|
| 2 | Ala-Phe-Pro-arylamidase | Negative |
| 3 | Adonito | Positive |
| 4 | L-pyrrolydonyl-arylamidase | Positive |
| 5 | L-arabitol | Negative |
| 7 | D-cellobiose | Negative |
| 9 | Beta-galactosidase | Negative |
| 10 | H2S production | Negative |
| 11 | Beta-N-acetyl-glucosaminidase | Positive |
| 12 | Glutamyl arylamidase pNA | Negative |
| 13 | D-glucose | Positive |
| 14 | Gamma-glutamyl-transferase | Negative |
| 15 | Fermentation/glucose | Positive |
| 17 | Beta-glucosidase | Positive |
| 18 | D-maltose | Negative |
| 19 | D-mannitol | Positive |
| 20 | D-mannose | Positive |
| 21 | Beta-xylosidase | Negative |
| 22 | BETA-Alanine arylamidase pNA | Negative |
| 23 | L-proline arylamidase | Positive |
| 26 | Lipase | Negative |
| 27 | Palatinose | Negative |
| 29 | Tyrosine arylamidase | Negative |
| 31 | Urease | Negative |
| 32 | D-sorbitol | Positive |
| 33 | Saccharose/sucrose | Positive |
| 34 | D-tagatose | Negative |
| 35 | D-trehalose | Positive |

**Table 17** (Continued)

| Well | Reaction | Result |
|---|---|---|
| 33 | Saccharose/sucrose | Positive |
| 34 | D-tagatose | Negative |
| 35 | D-trehalose | Positive |
| 36 | Citrate (sodium) | Positive |
| 37 | Malonate | Negative |
| 39 | 5-keto-D-gluconate | Negative |
| 40 | L-lactate alkalinization | Positive |
| 41 | Alpha-glucosidase | Negative |
| 42 | Succinate alkalinization | Negative |
| 43 | Beta-N-acetyl-galactosaminidase | Negative |
| 44 | Alpha-galactosidase | Negative |
| 45 | Phosphatase | Positive |
| 46 | Glycine arylamidase | Negative |
| 47 | Ornithine decarboxylase | Positive |
| 48 | Lysine decarboxylase | Positive |
| 53 | L-Histidine assimilation | Negative |
| 56 | Courmarate | Positive |
| 57 | Beta-glucoronidase | Negative |
| 58 | O/129 resistance (comp.vibrio.) | Positive |
| 59 | Glu-Gly-Grg-arylamidase | Positive |
| 61 | L-malate assimilation | Negative |
| 62 | Ellman | Negative |
| 64 | L-lactate assimilation | Negative |

From the results of the bacterial identification involving morphological, biochemical, molecular, and protein identification, bacterial isolate 329-2 was identified as *S. marcescens*.

### 3. *In vitro* application of the fumonisin reducing bacteria.

Contaminated maize was found at high levels of 96% in Asia, 70% in North America, and 71% in Europe. Moreover, some positive samples were reported to contain maximum concentrations of fumonisins at 30,872 ppb, 66,588 ppb, and 13,902 ppb, respectively (BIOMIN, 2021). The high potential bacterium, *S. marcescens* 329-2, was selected for studied *in vitro* application of the fumonisin reduction in maize.

In an *in vitro* study of fumonisin reduction in ground maize for 24 h of incubation, the cell-free extract of *S. marcescens* 329-2 had the highest reduction rate at 37.00%, followed by the culture supernatant at 31.30%, and the cell suspension at 13.40%. The FB1 reduction factor may be a crude enzyme from inside bacterial cells (Zhao *et al*., 2019).

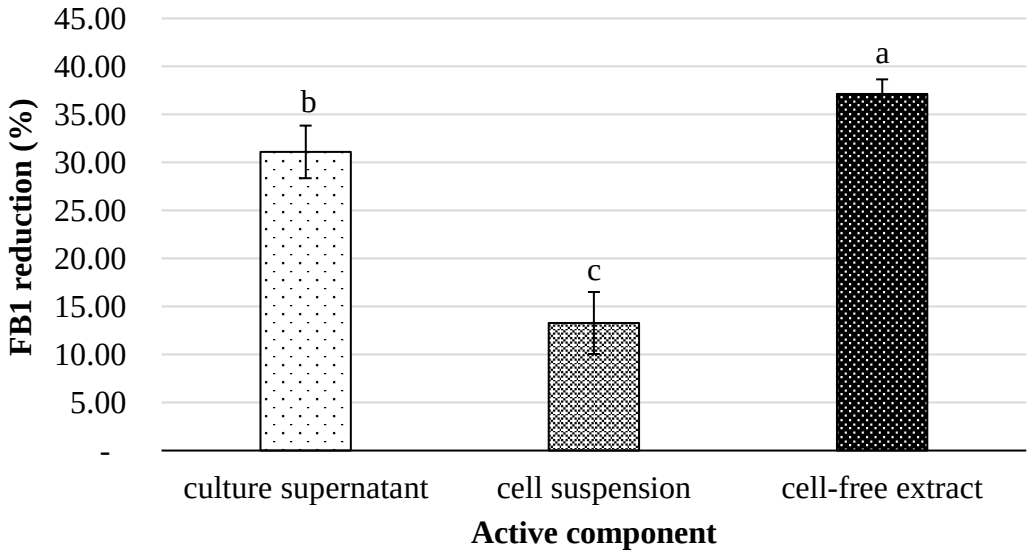



**Figure 14** Percentage of FB1 reduction by the culture supernatant, cell suspension, and cell-free extract from in ground maize after 24 h of incubation with the FB1 standard at 5 ppm.
Different lowercase letters above the columns represent significant differences by ANOVA ($p < 0.05$).

## 4. Antagonistic activity of *Serratia marcescens* 329-2 to fumonisin producing *Fusarium* sp.

Biological control of plant diseases is the suppression of plant pathogens by living organisms (Heimpel & Mills, 2017). It is considered a complementary approach for the control of plant pathogens. Additionally, the biological control of plant pathogens is an attractive agricultural practice as it is non-chemical and supports sustainable agriculture.

*S. marcescens* has been reported to be a potential biocontrol agent for plant pathogens causing several diseases such as damping-off disease in cyclamen caused by *Rhizoctonia solani* (Someya *et al.*, 2000), damping-off disease in cucumber caused by *Phytophthora capsici* (Okamoto *et al.*, 1998), and blast disease caused by *Pyricularia oryzae* in rice (Jaiganesh *et al.*, 2007). Moreover, *S. marcescens* has been demonstrated to be a plant growth-promoting agent inducing systemic resistance in cucumber against *Fusarium* wilt disease caused by *F. oxysporum* (Press *et al.*, 2001), and it can induce systemic resistance, enhance salinity tolerance, and inhibit *F. graminearum* infection in wheat (Singh & Jha, 2016). Guo *et al.* (2020) reported that *S. marcescens* inhibited the germination of *F. proliferatum* and suppressed fumonisin accumulation in an *in vitro* study.

However, this study found that the mycelia of all isolates of fumonisin-producing *Fusarium* sp. showed slightly inhibition zone between *Fusarium* sp. and *S. marcescens* 329-2 on the dual culture test (Figure 15-16). After seven days of incubation period, the *Fusarium* sp. grew slower than control, however, after nine days of incubation period, the *Fusarium* sp. grew over the *S. marcescens* isolate 329-2 grown line on an agar plate.

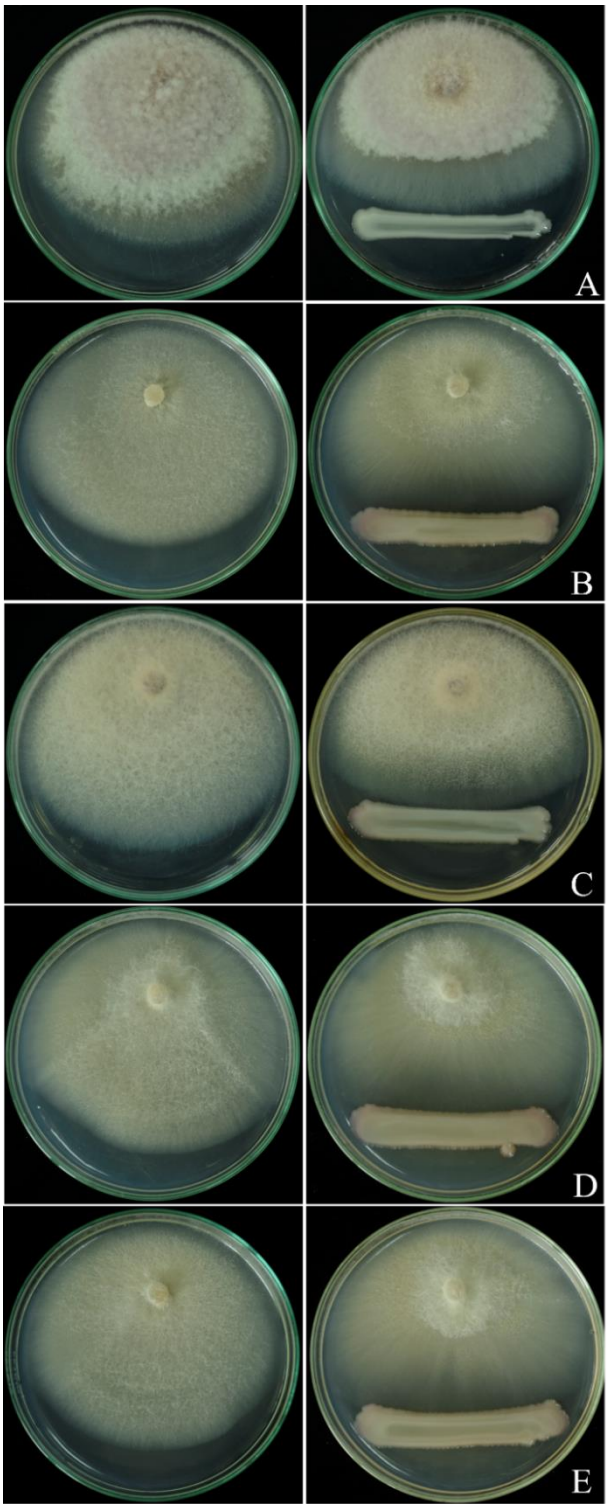



**Figure 15** Dual culture assay of *S. marcescens* 329-2 and fumonisin-producing *Fusarium* sp. at 7 days after incubation.
Left = control, Right = treatment, (A-C) *F. vercillioides*,
(D-E) *F. proliferatum*.

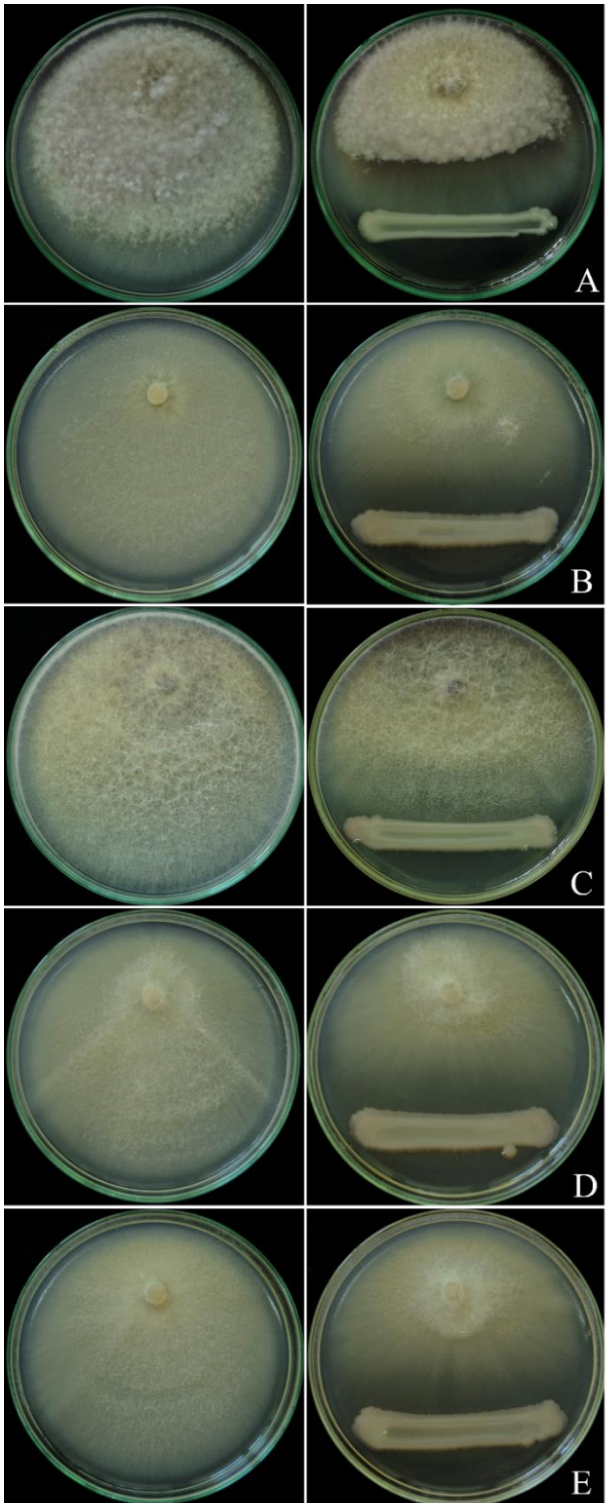



**Figure 16** Dual culture assay of *S. marcescens* 329-2 and fumonisin-producing *Fusarium* sp. at 9 days after incubation.
Left = control, Right = treatment, (A-C) *F. vercillioides,*
(D-E) F. *proliferatum.*

## 5. Fumonisin B1 degradation process study

The Fumonisin B1 structure corresponds to the amino polyol AP1 (sphingoid base backbone) and the tricarballylic acids. In consideration of the FB1 degradation by enzymes, the decomposition reaction possibility of FB1 was summarized on the breakdown site as in Figure 17 (Hartinger *et al.*, 2011). The modern strategy of the transcriptome was used to elucidate the relevant genes, and the proteomic data was used for determining the relevant enzymes involved in the degrading of tricarballylic acids.

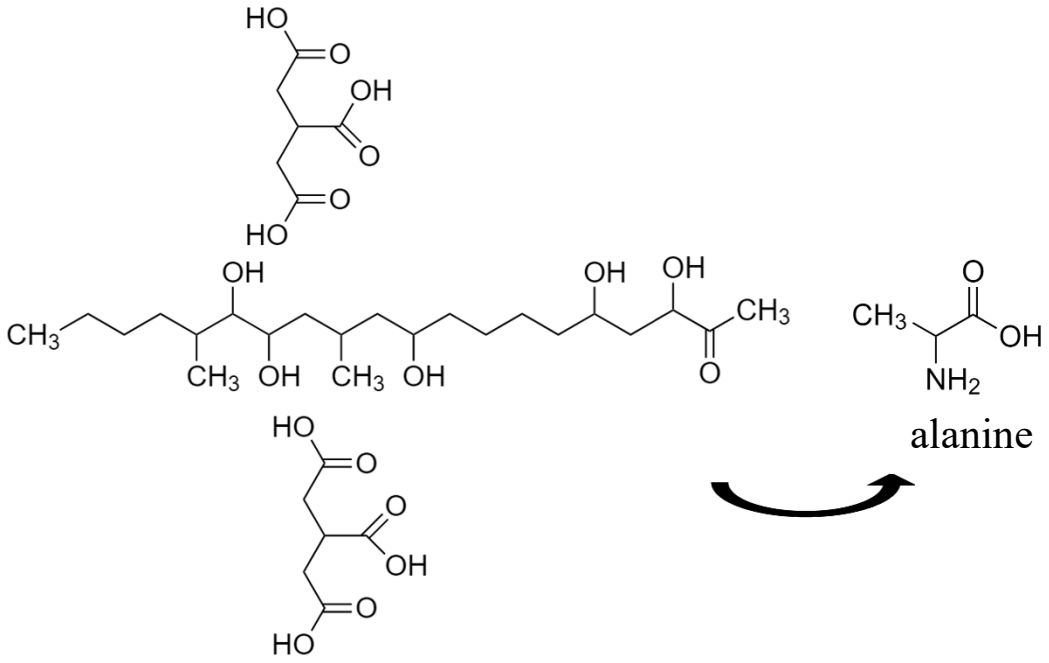



**Figure 17** Decomposition reaction possibility of fumonisin B1.

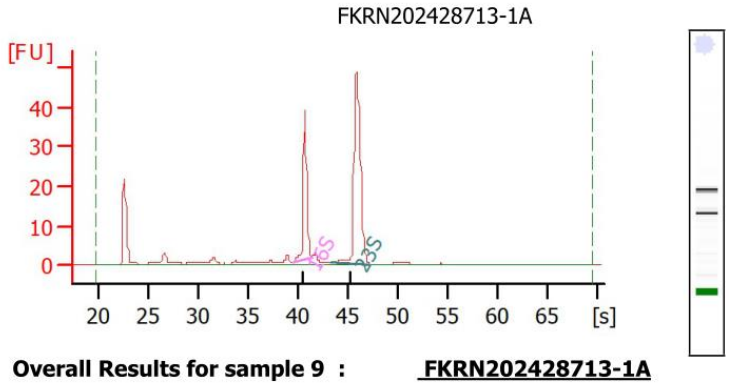
### 5.1 Transcriptome analysis of RNA expression from *Serratia marcescens* during fumonisin B1 reduction

A transcriptome pertains to the RNA transcribed from the particular genes under investigation in a given condition at a particular time. The RNA content of a cell provides direct knowledge of gene regulation and protein content information (Gupta & Gupta, 2014). In this study, the total RNA of *S. marcescens* was used as a

control, while the FB1 treated sample was used as a treatment. A transcriptome was used to determine the mRNA expression during FB1 reduction. Hence, high RNA transcription will result in the transcriptome data that can show the expression of genes during the studied reaction. In this manner, the RNA quality, the transcription reaction, and the RNA expression quality were important.

Total RNA was isolated from *S. marcescens* 329-2 after 24 h during FB1 reduction. The RNA quality was first evaluated by the rRNA ratio (23S/16S), showing no degradation and contamination. The RNA integrity number values from control and treatment samples were 9.8 and 9.9 (Figure 18), indicating that all RNA samples were of high quality for an Illumina Novaseq 6000 platform.

The transcriptome analysis via RNA-Seq of control and treatment FB1 degrading samples were conducted. Control sample produced raw reads of 20,891,970, clean reads of 20,669,294 (98.93%), raw bases of 3.1 G, clean bases of 3.1 G, an error rate of 0.02%, Q20 of 98.71%, Q30 of 95.76%, GC content of 57.11%, uniquely mapping rate of 71.48%, and multiple mapping rate of 23.38%. The treatment sample produced raw reads of 21,892,746, clean reads of 21,559,270 (98.48%), raw bases of 3.3 G, clean base of 3.2 G, an error rate of 0.02%, Q20 of 98.58%, Q30 of 95.54%, GC content of 57.18%, uniquely mapping rate of 92.20%, and multiple mapping rate of 5.21%, indicating successful library construction and RNA sequencing (Figure 19, Table 18).



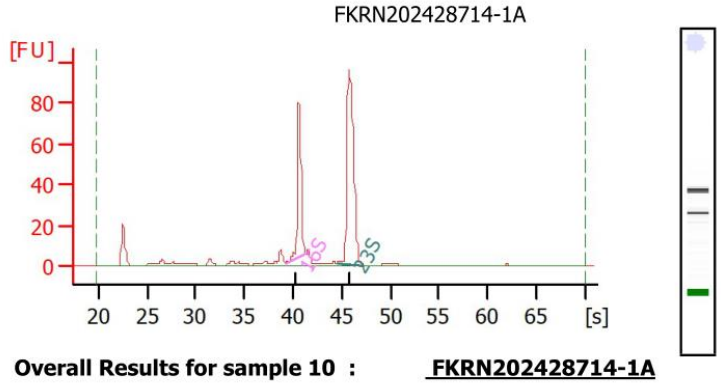

**Overall Results for sample 9 : FKRN202428713-1A**

| | |
|---|---|
| RNA Area: | 172.8 |
| RNA Concentration: | 87 ng/µl |
| rRNA Ratio [23s / 16s]: | 2.1 |
| RNA Integrity Number (RIN): | 9.8 (B.02.10, Anomaly Threshold(s) manually adapted) |
| Result Flagging Color: | |
| Result Flagging Label: | RIN: 9.80 |

**Fragment table for sample 9 : FKRN202428713-1A**

| Name | Start Time [s] | End Time [s] | Area | % of total Area |
|---|---|---|---|---|
| 16S | 39.50 | 41.37 | 38.5 | 22.3 |
| 23S | 43.28 | 47.22 | 79.8 | 46.2 |

A




**Overall Results for sample 10 : FKRN202428714-1A**

| | |
|---|---|
| RNA Area: | 347.3 |
| RNA Concentration: | 174 ng/µl |
| rRNA Ratio [23s / 16s]: | 2.0 |
| RNA Integrity Number (RIN): | 9.9 (B.02.10, Anomaly Threshold(s) manually adapted) |
| Result Flagging Color: | |
| Result Flagging Label: | RIN: 9.90 |

**Fragment table for sample 10 : FKRN202428714-1A**

| Name | Start Time [s] | End Time [s] | Area | % of total Area |
|---|---|---|---|---|
| 16S | 39.26 | 41.24 | 82.7 | 23.8 |
| 23S | 44.49 | 47.29 | 163.1 | 47.0 |

B

**Figure 18** The integrity test of the treatment samples.

(A) control sample, (B) treatment sample.


**Figure 19** Composition of raw reads.

(A) control sample, (B) treatment sample.

**Table 18** Overview of data quality control and mapping status.

| Sample name | Control sample C329_2 | Treatment sample T329_2 |
|---|---|---|
| Raw reads | 20,891,970 | 21,892,746 |
| Clean reads | 20,669,294 | 21,559,270 |
| Raw bases | 3.1G | 3.3G |
| Clean bases | 3.1G | 3.2G |
| Error rate | 0.02% | 0.02% |
| Q20 | 98.71% | 98.58% |
| Q30 | 95.76% | 95.54% |
| GC content | 57.11% | 57.18% |
| Total mapped reads | 19,606,009 | 21,002,010 |
| Uniquely mapped reads | 14,774,246 | 19,878,707 |
| Multiple mapped reads | 4,831,763 | 1,123,303 |
| Total mapping rate | 94.86% | 97.42% |
| Uniquely mapping rate | 71.48% | 92.2% |
| Multiple mapping rate | 23.38% | 5.21% |

To elucidate the differences in gene expression during FB1 degradation, the differentially expressed genes (DEGs) were identified by pairwise comparison (p-value <=0.05 |log2FoldChange|>=1.0 threshold). The comparative analysis identified 557 differentially expressed genes, 206 upregulated genes, and 351 down-regulated genes (Figure 20).

The highly matching gene upregulated in FB1 reduction was categorized into catalytic activity, binding protein function, and metabolic process under molecular function. The protein expression data are shown in Table 19, with the log2fold change values in expression relative to the noninduced protein levels from *S. marcescens*. The upregulated gene in Table 19 indicates the genes were highly related to FB1 degradation by *S. marcescens*. The details of each gene were compared with the UniportKB database. This indicates the gene function during FB1 degradation.

To better understand the functions, metabolic pathways, and interactions of DEGs associated with degradation, gene ontology (GO) enrichment analysis was performed. A total of 561 GO terms were assigned, including 88 cellular component terms, 232 biological process terms, and 241 molecular function terms.

Among these terms, membrane part (GO:0044425), cell (GO:0005623), and cell part (GO:0044464) were the main distributed terms in the cellular component ontology. In the ontology of biological processes, the main categories were gathered in terms of nucleotide biosynthetic process (GO:0009165) and nucleoside phosphate biosynthetic process (GO:1901293). Concerning the ontology of molecular function, the top categories were ion-binding (GO:0043167), nucleic acid-binding (GO:0003676), and small molecule-binding (GO:0036094). The transcriptome data revealed the degrading of FB1 by *S. marcescens* included various cell processes of cellular component and molecular function.

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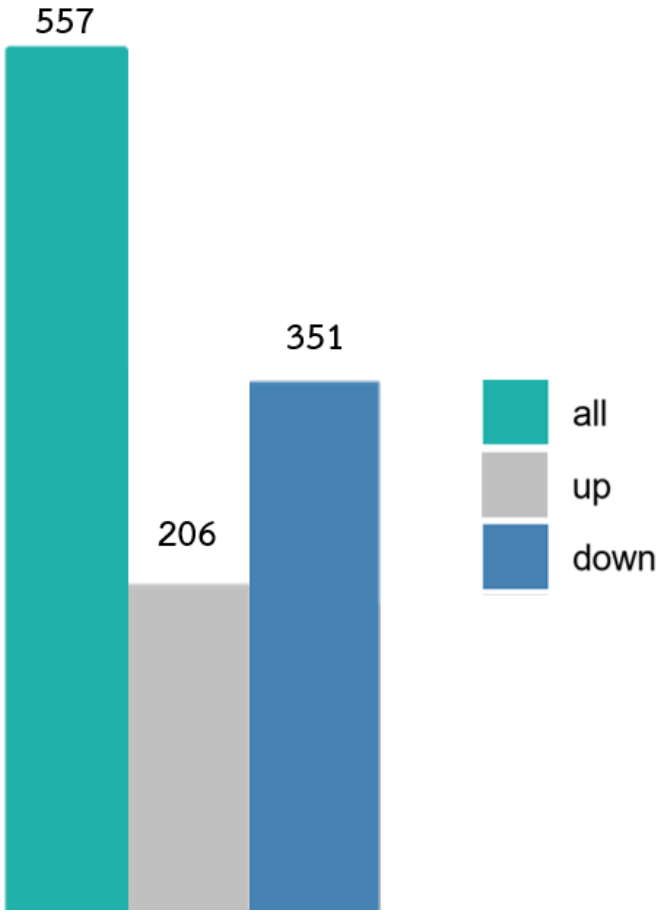



**Figure 20** Number of differentially expressed genes during FB1 reduction.

**Table 19** Identification of the upregulated gene (>1.3 log2fold change) in FB1-treated *S. marcescens* 329-2 compared with the control group.

| Entry | Gene description | Gene names | log2Fold change |
|---|---|---|---|
| E0IWI3 | Diaminopimelate decarboxylase | *lysA* | 2.62 |
| P27508 | Coenzyme PQQ synthesis protein | *pqqF* | 2.46 |
| Q56062 | 2-methylisocitrate lyase | *prpB* | 2.18 |
| P31660 | 2-methylcitrate synthase | *prpC* | 2.14 |
| P0AB40 | Multiple stress resistance protein BhsA | *bhsA* | 2.14 |
| P46319 | Lichenan-specific phosphotransferase enzyme IIA component | *licA* | 2.11 |
| Q2FNR04 | hydroxy-tetrahydrodipicolinate synthase | *dapA* | 2.03 |
| P77243 | 2-methylcitrate dehydratase | *prpD* | 1.94 |
| A8GAA9 | Enolase-phosphatase | *E1* | 1.93 |
| G8QM62 | Putative protein-methionine-sulfoxide reductase subunit YedZ1 | *yedZ1* | 1.92 |
| P0AB17J | Uncharacterized protein YccJ | *yccJ* | 1.91 |
| A8GCL2 | Ribosome modulation factor | *rmf* | 1.85 |
| Q46856 | Alcohol dehydrogenase YqhD | *yqhD* | 1.85 |
| G3XD12 | Hydrogen cyanide synthase subunit HcnC | *hcnC* | 1.82 |
| P76079 | 1,2-phenylacetyl-CoA epoxidase | *paaC* | 1.75 |
| Q46629 | Amylovoran export outer membrane protein AmsH | *amsH* | 1.74 |
| Q9KX40 | Esterase EstB | *estB* | 1.71 |
| Q46634 | Amylovoran biosynthesis glycosyltransferase AmsD | *amsD* | 1.71 |
| A8GF80 | Anthranilate phosphoribosyltransferase | *trpD* | 1.70 |
| Q8FK07 | Universal stress protein | *uspG* | 1.70 |
| P69811 | PTFAH_ECOLI Multiphosphoryl transfer protein | *fruB* | 1.69 |

**Table 19** (Continued)

| Entry | Gene description | Gene names | log2Fold change |
|---|---|---|---|
| P26420 | Fructokinase | *scrK* | 1.69 |
| P75952 | HTH-type transcriptional repressor ComR | comR | 1.67 |
| P75820 | N-acetylmuramoyl-L-alanine amidase AmiD | *amiD* | 1.62 |
| P76078 | 1,2-phenylacetyl-CoA epoxidase, subunit B | *paaB* | 1.62 |
| Q9I747 | Protein hcp1 | *hcp1* | 1.60 |
| 252110 | Methylthioribulose-1-phosphate dehydratase | *mtnB* | 1.58 |
| A9MWZ8 | Phosphonoacetaldehyde hydrolase | *phnX* | 1.58 |
| P56579 | PTS system glucitol/sorbitol-specific EIIC component | *srlA* | 1.57 |
| Q7DDB6 | Probable TonB-dependent receptor NMB1497 | NMB1497 | 1.56 |
| A8GKK6 | Protein TusC | *tusC* | 1.55 |
| P76085 | 1,2-phenylacetyl-CoA epoxidase | *paaB* | 1.53 |
| P16482 | Citrate-proton symporter | *citH* | 1.53 |
| P0AB40\| | BHSA_ECOLI Multiple stress resistance protein BhsA | bhsA | 1.52 |
| P26505 | 5-aminolevulinate synthase | *hemA* | 1.50 |
| Q8UAA8 | Biofilm growth-associated repressor | *bigR* | 1.50 |
| P0AF26 | Nitrate reductase molybdenum cofactor assembly chaperone NarJ | *narJ* | 1.48 |
| Q56989 | Hemin receptor | *hmuR* | 1.48 |
| P00888 | Phospho-2-dehydro-3-deoxyheptonate aldolase, Tyr-sensitive | *aroF* | 1.45 |
| A1JM38 | Deoxyribose-phosphate aldolase | *deoC* | 1.45 |
| PF01261 | Xylose isomerase-like TIM barrel | | 1.44 |
| O34777\| | Organic hydroperoxide resistance transcriptional regulator | *ohrR* | 1.42 |

**Table 19** (Continued)

| Entry | Gene description | Gene names | log2Fold change |
|---|---|---|---|
| P77467 | 1,2-epoxyphenylacetyl-CoA isomerase | *paaG* | 1.42 |
| P0ADM8 | Uncharacterized protein YieE | *yieE* | 1.39 |
| A8GKC9 | Protein PsiE homolog | *psiE* | 1.39 |
| P0AEM9 | L-cystine-binding protein FliY | *fliY* | 1.39 |
| P03841 | Maltose operon periplasmic protein | *malM* | 1.38 |
| P54955 | Uncharacterized hydrolase YxeP | *yxeP* | 1.38 |
| Q58094 | Putative transketolase N-terminal section | MJ0681 | 1.38 |
| P0AF89 | Uncharacterized protein YjfY | *yjfY* | 1.38 |
| Q8G9F9 | Isonitrile hydratase | *inhA* | 1.37 |
| Q7CQM9 | Tetrathionate reductase subunit B | *ttrB* | 1.37 |
| P0A996 | Anaerobic glycerol-3-phosphate dehydrogenase subunit C | *glpC* | 1.37 |
| P77228\| | Putative inner membrane metabolite transport protein YdfJ | *ydfJ* | 1.37 |
| Q9I6Z9 | HTH-type transcriptional activator BauR | *bauR* | 1.36 |
| P43531 | Inner membrane transport protein YnfM | *ynfM* | 1.36 |
| P51066 | Isocitrate lyase | *aceA* | 1.36 |
| Q566U | Kynurenine formamidase | *afmid* | 1.36 |
| O51900 | Enterochelin esterase | *fes* | 1.35 |
| P0A9P9 | 5-keto-D-gluconate 5-reductase | *idnO* | 1.35 |
| O32218 | Disulfide bond formation protein D | *bdbD* | 1.33 |
| P23597 | Proteases secretion protein PrtE | *prtE* | 1.33 |
| A4IPB4 | 5-deoxy-glucuronate isomerase | *iolB* | 1.32 |
| Q7CKG8 | Ribose 1,5-bisphosphate phosphokinase PhnN | *phnN* | 1.32 |
| P76082 | 2,3-dehydroadipyl-CoA hydratase | *paaF* | 1.32 |
| P76082 | 2,3-dehydroadipyl-CoA hydratase | *paaF* | 1.32 |

**Table 19** (Continued)

| Entry | Gene description | Gene names | log2Fold change |
|---|---|---|---|
| P0A9S9 | High-affinity branched-chain amino acid transport ATP-binding protein LivG | *livG* | 1.32 |
| B1JKC0 | Alkanesulfonate monooxygenase | *ssuD* | 1.30 |



5.2 Proteome analysis of protein expression during fumonisin B1 reduction by *Serratia marcescens* 329-2

5.2.1 SDS-PAGE of bacterial cell component

The total amount of proteins of the bacterial component sample can be analyzed after SDS-polyacrylamide gel electrophoresis (SDS-PAGE) for observing the different protein profiles. It is a commonly used technique and can yield information about a protein's size (molecular weight).

To observe the differential protein profile, the SDS-PAGE of bacterial component samples was analyzed. The bacterial component samples 3, 5, and 7 days after incubation period on the FB1 standard solution were observed. The protein profiles of each sample were seen, and different proteins of various molecular weights were found in the samples. However, the protein pattern in each lane did not observe any difference in protein profile between control and treatments (Figure 21). This may be caused by the low protein expression in the sample, and the SDS-PAGE can not show any differentiation.

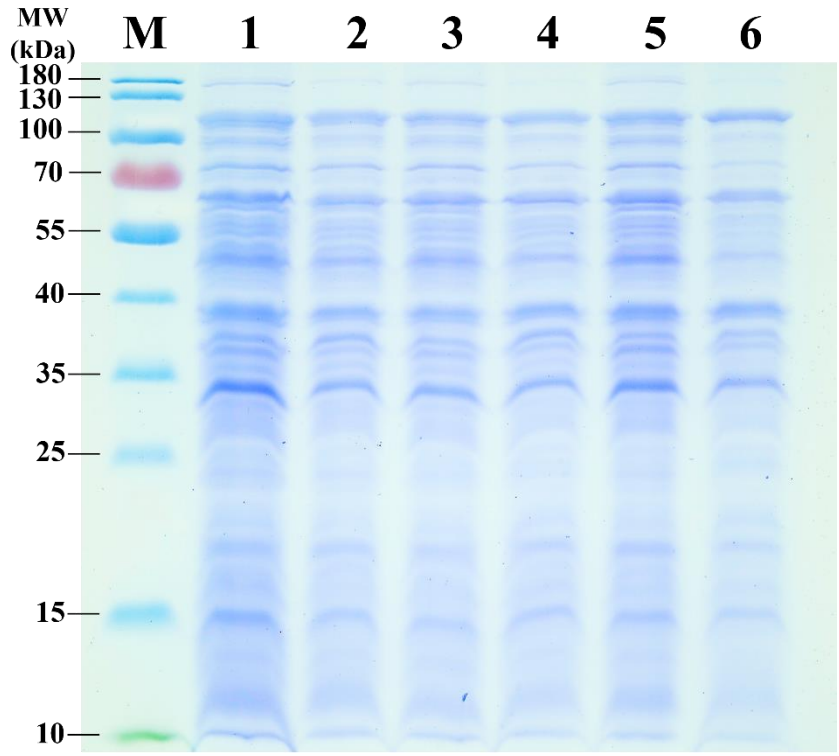



**Figure 21** SDS-PAGE proteins profiles of the induced *S. marcescens* 329-2 with fumonisin B1.

1 = Control sample at 3 days after incubation

2 = Treatment control at 3 days after incubation

3 = Control sample at 5 days after incubation

4 = Treatment sample at 5 days after incubation

5 = Control sample at 7 days after incubation

6 =Treatment sample at 7 days after incubation

M = PageRuler™ Prestained Protein Ladder.

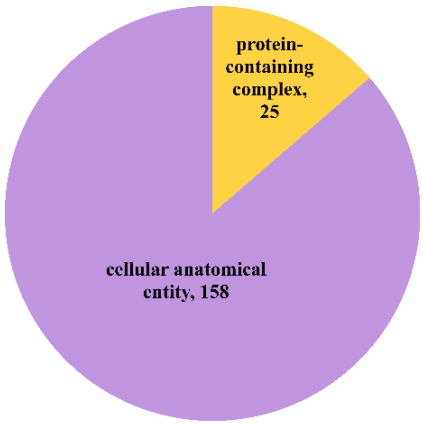
### 5.1.3 Label-free quantification

By observation of the related proteins during the FB1 degradation process, bacterial cell components were analyzed with label-free techniques. Label-free MS-based quantitative proteomic analysis was attempted to further characterize protein expression during fumonisin degradation.

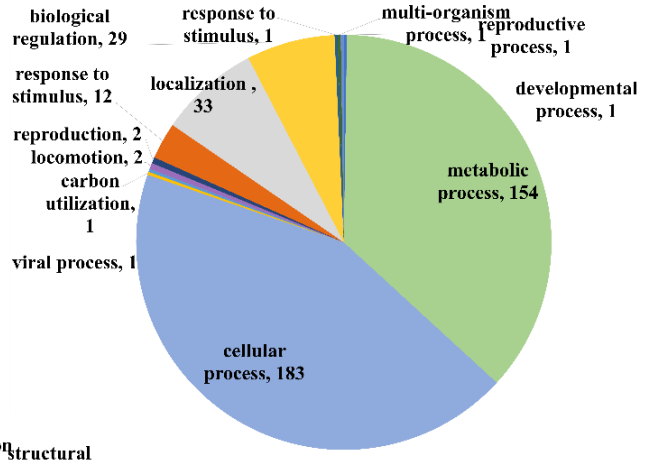


The label-free quantification data showed 461 differentially expressed proteins with $p < 0.05$. Of these proteins, 159 were upregulated and 25 downregulated in the treatment group. To evaluate the expression of protein functions, we annotated these proteins based on the gene ontology (GO) databases. The identified level two GO terms (related to cellular components, molecular functions, and biological processes) associated with the differentially expressed proteins are shown in Figure 22. Gene annotation of the expressed proteins showed their relatedness to cellular components (A) for 183 proteins, biological processes (B) for 421 proteins, and molecular functions (C) for 430 proteins. Major concerns exist regarding the biological process by which FB1 degradation is activated: the proteins involved in the cellular (183) and metabolic processes (154). In the category of the molecular function of FB1 degradation, the majority of the proteins were related to catalytic activity (188 proteins) and protein-binding (177 proteins).

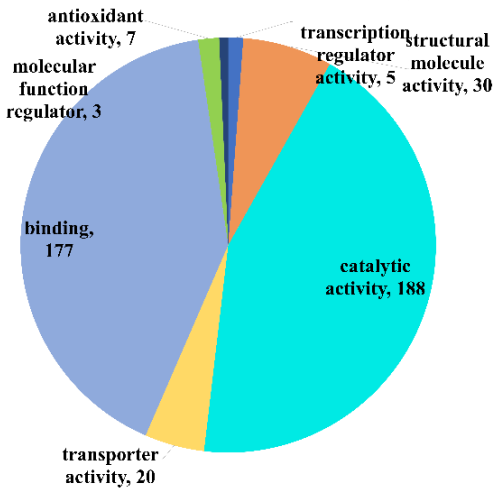
The highly matching proteins upregulated in FB1 degradation were categorized into catalytic activity, binding-protein function, and metabolic process under molecular function (Figure 22). The protein expression data are shown in Tables 20, with the more than 3-fold change values in expression relative to the noninduced protein levels from *S. marcescens*. The upregulated proteins in Table 20 indicate that proteins were highly related to FB1 degradation by *S. marcescens*. The details of each protein were compared within the UniProt database. This indicates the function related to the protein degradation of FB1.




A. Cellular component




B. Biological process




C. Molecular function

**Figure 22** Gene ontology (GO) classifications of the differentially expressed proteins during fumonisin degradation by *S. marcescens* 329-2.
(A) cellular component, (B) molecular function, (C) biological process.

**Table 20** Identification of the upregulated proteins (>3-fold change) in FB1-treated *S. marcescens* 329-2 compared with the control group.

| Entry | Protein names | Gene names | Fold change |
|---|---|---|---|
| A0A6G9UZ48 | ABC transporter substrate-binding protein | HCG50_10660 | 8.20 |
| A0A6N0D898 | Porin OmpC | *ompC* | 7.94 |
| A0A3E2ENK9 | Amino acid ABC transporter substrate-binding protein | *gltI* | 7.12 |
| V5YV29 | Maltodextrin-binding protein | *malE* | 6.89 |
| A0A080V044 | Universal stress protein | *uspA* | 5.42 |
| A0A6M5HVT2 | 4-hydroxy-tetrahydrodipicolinate synthase | *dapA* | 5.33 |
| A0A6G9UQE2 | ABC transporter substrate-binding protein | HCG50_08880 | 5.33 |
| A0A1Q4NZ53 | Superoxide dismutase | BHU62_14220 | 4.99 |
| A0A6G9UU24 | Phosphate-binding protein PstS | *pstS* | 4.98 |
| A0A6N3ZRH0 | Fumarylacetoacetate hydrolase family protein | G3M84_1332 | 4.91 |
| A0A656VL53 | Alpha/beta hydrolase | AB868_03825 | 4.90 |
| A0A6M5HYD0 | Phenylacetate-CoA oxygenase/reductase subunit PaaK | *paaK* | 4.78 |
| A0A3E2EF40 | Malate dehydrogenase | *mdh* | 4.68 |
| A0A6N0D0Q5 | Neutral metalloproteinase | F0335_18215 | 4.49 |
| A0A086FBX8 | Transcription termination/antitermination protein NusG | *nusG* | 4.39 |
| A0A2V4FJ05 | Peptide deformylase | *def* | 4.35 |
| V5YU98 | Extracellular solute-binding protein | E4655_11925 | 4.27 |
| A0A5Q8BY15 | YtfJ family protein | EGJ31_19890 | 4.20 |

**Table 20** (Continued)

| Entry | Protein names | Gene names | Fold change |
|---|---|---|---|
| A0A1Q5WAZ4 | Oligopeptide ABC transporter substrate-binding protein OppA | A8A12_03045 | 4.18 |
| A0A6H1E4N5 | Acetylornithine/succinyldiaminopimelate aminotransferase | *argD* | 4.10 |
| A0A6N3ZYZ9 | 2,3-diphosphoglycerate-dependent phosphoglycerate mutase | *gpmA* | 4.06 |
| A0A0P0Q8S3 | ABC transporter substrate-binding protein | AR325_02675 | 4.06 |
| A0A6N0CVJ7 | Superoxide dismutase | *sodB* | 4.03 |
| A0A5Q8C0J8 | Organic hydroperoxide resistance protein | EGJ31_14360 | 4.02 |
| V5YUS0 | Periplasmic serine endoprotease DegP-like | *degQ* | 4.01 |
| Q6MXC8 | Methyltransferase | SMR0272 | 3.94 |
| A0A2S4XAJ8 | Surface composition regulator | *glgS* | 3.89 |
| A0A1Q5WEW3 | Antibiotic biosynthesis monooxygenase | A8A12_06980 | 3.87 |
| A0A6I4GZS8 | Hydrolase | GMA22_24835 | 3.80 |
| A0A221FKL4 | UPF0234 protein BVG93_01845 | BVG93_01845 | 3.75 |
| A0A6H3S2C0 | ATP-dependent protease subunit HslV | *hslV* | 3.71 |
| A0A6M5I193 | MBL fold metallo-hydrolase | HMI62_20840 | 3.64 |
| A0A6N0DB57 | Protein deglycase HchA | *hchA* | 3.62 |
| A0A2V4G7I4 | Amino acid ABC transporter substrate-binding protein | *glnH* | 3.62 |
| A0A5C7CH16 | VOC family protein | FOT62_15570 | 3.58 |
| A0A0G8B4P9 | Peptidyl-prolyl cis-trans isomerase | *fkpA* | 3.58 |
| A0A656VU86 | Long-chain fatty acid transport protein | AB868_00683 | 3.55 |
| A0A2S4X857 | Histidine ABC transporter substrate-binding protein HisJ | *hisJ* | 3.47 |

**Table 20** (Continued)

| Entry | Protein names | Gene names | Fold change |
|---|---|---|---|
| A0A656V5R8 | 5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase | *metE* | 3.46 |
| A0A0G8BFE1 | Cystine ABC transporter substrate-binding protein | *tcyJ* | 3.43 |
| A0A1C3HIZ5 | Aconitate hydratase B | *acnB* | 3.35 |
| A0A0U6KIH4 | GTP cyclohydrolase 1 | *folE* | 3.34 |
| A0A6N0CW48 | Branched-chain amino acid ABC transporter substrate-binding protein | F0335_15805 | 3.32 |
| A0A6N3ZXZ8 | ABC transporter substrate-binding protein | G3M84_09620 | 3.32 |
| A0A6G8TTH4 | Autoinducer 2-binding protein LsrB | G5643_21680 | 3.29 |
| A0A656VPU2 | Uncharacterized protein | AB868_00798 | 3.26 |
| A0A1C3HHX7 | Nitrogen regulatory protein P-II | *glnB* | 3.23 |
| A0A1Q5WH71 | Thiol:disulfide interchange protein | *dsbA* | 3.22 |
| A0A0G8B466 | 2-dehydro-3-deoxygluconokinase | AR325_02155 | 3.22 |
| A0A0M5K334 | Transaldolase | *tal* | 3.18 |
| A0A080UWJ0 | Peptidyl-prolyl cis-trans isomerase | *fklB* | 3.15 |
| A0A6N0D450 | Two-component system response regulator BaeR | *baeR* | 3.13 |
| A0A6N0CZA0 | Glucose-6-phosphate isomerase | *pgi* | 3.10 |
| A0A0F6KTS7 | 2-dehydro-3-deoxy-phosphogluconate aldolase | *eda* | 3.10 |
| V5YUY6 | Stringent starvation protein A | *sspA* | 3.09 |
| A0A6M5HTX8 | Uncharacterized protein | HMI62_14785 | 3.08 |
| A0A1Q4NZT3 | DUF1471 domain-containing protein | BHU62_12635 | 3.03 |

The transcriptomic and proteomic data regarding the degrading of FB1 were important. The related genes or enzymes involved in some specific functions of 1) eliminating an atom with hydrolase activity and 2) moving an atom as activated transferase were important in the FB1 degradation. The cell functions involved in cell catalysis, especially hydrolase lyase and aminotransferase, were highlighted. The fumonisin-degrading enzymes were indicated in a report from Blackwell *et al.* (1999). A soluble extracellular esterase from *E. spinifera* isolate 2141.10 transformed FB1 to the amino polyol AP1 and free tricarballylic acid. Moreover, carboxylesterase and aminotransferase for fumonisin degradation by esterification and hydrolysis were described in the bacterium *Sphingopyxis* sp. (Hartinger *et al.*, 2010; Heinl *et al.*, 2010). From the data of the pre-published research, our study compared the gene data from the transcriptome and the protein data from the proteome concerning the related enzyme functions.

The two enzyme groups of interest were interpreted. First, the hydrolase enzyme which breaks down a chemical bond with the $H_20$ molecule divides a larger molecule into smaller molecules. The hydrolase enzyme commonly performs as a biochemical catalyst, and it is classified as EC3 in the EC number of enzyme classification (BRENDA, 2021; Hafner Česen *et al.*, 2016). Second, the transferase is an enzyme that catalyzes and transfers specific functional groups from one molecule to another. The enzyme was classified as EC2 in the EC number of enzyme classification (BRENDA, 2021; Goesaert *et al.*, 2008). The enzyme catalyzes and transfers specific functional groups from one molecule to another. Both enzymes group were interpreted in accordance with the transcriptome and proteome data.

The related RNA transcriptome with upregulated data of hydrolase function was evidenced by E0IWI3 (diaminopimelate decarboxylase, 2.62), Q9KX40 (esterase EstB, 1.71), A9MWZ8 (phosphonoacetaldehyde hydrolase, 1.58), P0AF89 (uncharacterized hydrolase YxeP, 1.38), Q566U (kynurenine formamidase, 1.36), O51900 (enterochelin esterase, 1.35), P21367 (probable hydrolase YcaC, 1.23), and P76084 (acyl-coenzyme A thioesterase PaaI, 1.13). The transferase enzymes can shift the chemical groups out from the FB1 structure. It was possibly included with P26505

(5-aminolevulinate synthase, 1.50), O85746 (tyrosine aminotransferase, 1.22), A8GC78 (histidinol-phosphate aminotransferase, 1.01), and P31660 (2-methylcitrate synthase).

Regarding the proteome data of FB1 degradation, different proteins and enzymes were displayed. The data on the cell catalysis process with hydrolase proteins and aminotransferase proteins was observed, which was parallel to the transcriptome data. The upregulated hydrolase function was evidenced by entries A0A6N3ZRH0 (fumarylacetoacetate hydrolase family protein, 4.91), A0A656VL53 (alpha/beta hydrolase, 4.90), A0A6I4GZS8 (hydrolase, 3.80), and A0A6M5I193 (MBL fold metallo-hydrolase, 3.64), and the transferase enzymes that can activate chemical groups in FB1 were included with A0A6H1E4N5 (Acetylornithine/succinyl-diaminopimelate aminotransferase, 4.1), Q6MXC8 (methyltransferase, 3.94), A0A656V5R8 (5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, 3.46), and A0A0U6KIH4 (GTP cyclohydrolase, 3.34).

Hydrolases are a group of enzymes that act as biochemical catalysts. They catalyze the hydrolysis of C-O, C-N, C-C, and phosphoric anhydride bonds. The enzymes use $H_2O$ to break a chemical bond, which typically degrades a larger molecule into smaller molecules. Hydrolases are classified as EC3 enzymes. One common example of hydrolase enzymes is esterase, which includes enzymes such as lipases, phosphatases, glycosidases, peptidases, and nucleosidases (Devlin, 2002; McKee & McKee, 2003). Montella *et al.* (2012) reported that esterases hydrolyze ester bonds, which are present in a wide range of insecticides, including fumonisin B1 esterase (EC 3.1.1.87) (Heinl *et al.*, 2010). Later, fumonisin B1 esterase is named the gene encoding carboxylesterase activity *fumD*. It assumes that hydrolase (A0A656VL53, 4.90) may be involved in FB1 conversion to HFB1 and tricarballylic acids (Figure 23).

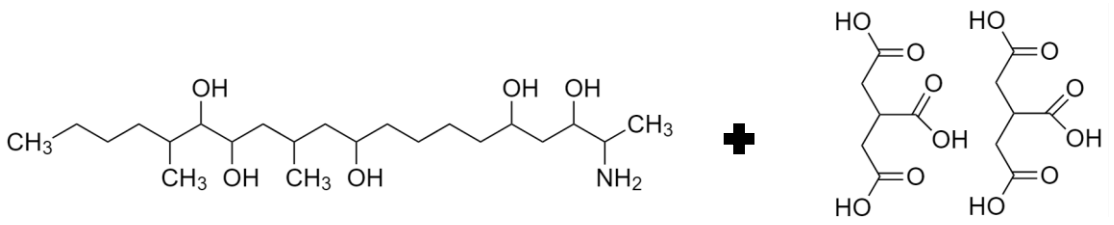



**Figure 23** Possibility of fumonisin B1 degradation area by hydrolase enzyme.

Transferases, an enzyme class, can transfer various chemical groups from one compound to another. The enzymes work with functional groups such as the amino group (-$NH_2$, transferred from amino acids to keto groups in the case of transaminase), phosphate, methyl (-$CH_3$), and sulfur-containing groups. The enzymes may react with one end of fumonisin (-$CHNH_2CH_3$). One of the enzymes identified was acetylornithine/succinyldiaminopimelate aminotransferase (A0A6H1E4N5, 4.10), which is related to the -$CHNH_2CH_3$ end of the fumonisin structure (Figure 25).

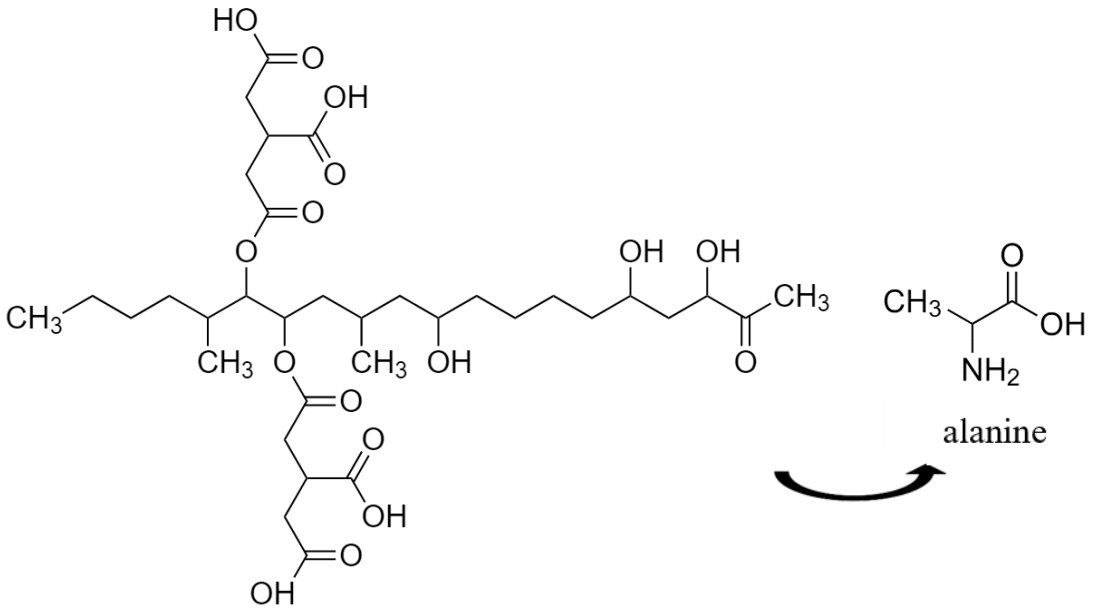



**Figure 24** Possibility of fumonisin B1 degradation area by transferase enzyme.

Additionally, the data expressed by other genes or enzymes were also in focus. Within the transcriptome data, the bacterial genes involved those in various enzyme activities of degrading by-products such as the tricarballylic moiety with the lyase activity (breaking of chemical bonds by eliminating some functional group) and Q56062 (2-methylisocitrate lyase, 2.18), which can catalyze the tricarboxylate to succinate. Other such enzyme activities included the proteome data of isomerase as A0A0G8B4P9 (peptidyl-prolyl cis-trans isomerase, 3.58) on isomerization of the NO2-site of substances, the protein synthesis protein A0A6M5HVT2 (4-hydroxy-tetrahydrodipicolinate synthase, 5.33) substituting the ferrous ion of the compound, and the cell metabolism protein V5YV29 (maltodextrin-binding protein, 6.89) on binding maltose for sugar transport.

Lastly, the result of the transcriptome and proteome expressed the related hydrolase and transferase gene functions from *S. marcescens* during the FB1 degrading. Further investigation can focus on a specific enzymes activity utilized during FB1 degradation with the transcriptome and proteome data of *S. marcescens*.

# CONCLUSIONS AND RECOMMENDATION

## Conclusions

The main focus of this thesis was to screen a fumonisin-degrading bacterial strain and determine the potential of bacterial enzymes for playing a key role in FB1 degradation.

The initial stage of this study screened for FB1 reducing bacteria. The FB1 reducing bacteria were in approximately 5.3% of the natural source samples. The reduction rates of these isolates ranged from 7.72-31.34% after 24 h of incubation. The highest FB1 reduction rate (31.34%) was exhibited by bacterial isolate 302-2, followed by isolate 329-2 at 26.48%.

The active component was determined for FB1 reduction from culture supernatant, cell suspension, and cell-free extract parts. The percent reduction ranged from 0-30.29%. The highest reduction occurred with treatment by isolate 329-2 cell-free extract at a rate of 30.29%, followed by 25.80% using the cell suspension of 302-2 and 22.13% using the cell suspension of S2. The isolate 329-2 was chosen for further study.

Various techniques including morphological, molecular (16s rRNA gene), protein (MALDI-TOF/TOF MS), and biochemical identification (VITEX-2) were applied for the identification of the potential bacterial isolate 329-2. The results conclude that bacterial isolate 329-2 was identified as *S. marcescens.*

The *in vitro* application of the fumonisin-reducing bacteria from *S. marcescens* 329-2 was investigated. The cell-free extract of *S. marcescens* 329-2 had the highest reduction rate at 37.00%, followed by the culture supernatant at 31.30% and the cell suspension at 13.40%.

The potential of *S. marcescens* 329-2 as an antagonist to fumonisin-producing *Fusarium* sp. was evaluated. This study noted that the mycelia of all isolates of fumonisin-producing *Fusarium* sp. show slightly inhibition zone between *Fusarium* sp. and *S. marcescens* 329-2 on the dual culture test.

The high throughput technology in the form of the transcriptome was used to elucidate the relevant genes and the proteome for determining the relevant enzymes during the degradation. Various enzymes were involved in the upregulation during FB1 reduction. The enzymes most related to FB1 degradation were hydrolases and transferases groups. It assumes that the hydrolase enzyme may be involved in FB1 conversion to HFB1 and tricarballylic acids, and the transferase enzyme may react with one end of fumonisin (-$CHNH_2CH_3$).

## Recommendation

Additional studies on DNA recombination and gene expression techniques are recommended to confirm the candidate FB1 degradation enzyme.

# LITERATURE CITED


Afolabi, C. G., Ojiambo, P. S., Ekpo, E. J. A., Menkir, A., & Bandyopadhyay, R. (2007). Evaluation of maize inbred iines for resistance to Fusarium ear rot and fumonisin accumulation in grain in tropical Africa. *Plant Disease, 91*(3), 279-286. doi:10.1094/pdis-91-3-0279

Anklam, E., Stroka, J., & Boenke, A. (2002). Acceptance of analytical methods for implementation of EU legislation with a focus on mycotoxins. *Food Control, 13*(3), 173-183. doi: 10.1016/S0956-7135(01)00098-6

Ayalew, A. (2010). Mycotoxins and surface and internal fungi of maize from Ethiopia. *African Journal of Food, Agriculture, Nutrition and Development, 10*(9). doi:10.4314/ajfand.v10i9.62890

Benedetti, R., Nazzi, F., Locci, R., & Firrao, G. (2006). Degradation of fumonisin B1 by a bacterial strain isolated from soil. *Biodegradation, 17*(1), 31-38. doi:10.1007/s10532-005-2797-y

BIOMIN. (2021). BIOMIN world mycotoxin survey 2020. https://www.biomin.net /downloads/2020-biomin-world-mycotoxin-survey-report/#c41851

Blackwell, B. A., Gilliam, J., Savard, M., Miller, J., & Duvick, J. (1999). Oxidative deamination of hydrolyzed fumonisin B1 (AP1) by cultures of *Exophiala spinifera*. *Natural Toxins, 7*, 31-38. doi:10.1002/(SICI)1522-7189(199902)7:1<31::AID-NT36>3.0.CO;2-W

BRENDA. (2021). The comprehensive enzyme information system. Retrieved from https://www.brenda-enzymes.org/index.php

Cole, R. J., & Cox, R. H. (1981). 20 - Fusarium toxins. In *handbook of toxic fungal metabolites* (pp. 893-910). San Diego: Academic Press. doi:10.1016/B978-0-12-179760-7.50025-5

Collins, T. F., Sprando, R. L., Black, T. N., Olejnik, N., Eppley, R. M., Shackelford, M. E., Howard, P. C., Rorie, J. I., Bryant, M., & Ruggles, D. I. (2006). Effects of aminopentol on in utero development in rats. *Food and Chemical Toxicology, 44*(2), 161-169. doi:10.1016/j.fct.2005.06.009

Dalie, D. K. D., Deschamp, A. M., Atanasova-Penichon, V., & Richard-Forget, F. (2010). Potential of *Pediococcus pentosaceus* (L006) isolated from maize leaf to suppress fumonisin-producing fungal growth. *Journal of Food Protection, 73*(6), 1129-1137. doi: 10.4315/0362-028x-73.6.1129

Davies, J. A., Anderson, G. K., Beveridge, T. J., & Clark, H. C. (1983). Chemical mechanism of the gram stain and synthesis of a new electron-opaque marker for electron microscopy which replaces the iodine mordant of the stain. *Journal of Bacteriology, 156*(2), 837-845. doi:10.1128/JB.156.2.837-845.1983

Deepthi, B. V., Poornachandra Rao, K., Chennapa, G., Naik, M. K., Chandrashekara, K. T., & Sreenivasa, M. Y. (2016). Antifungal attributes of *Lactobacillus plantarum* MYS6 against fumonisin producing *Fusarium proliferatum* associated with poultry feeds. *PLOS ONE, 11*(6), e0155122. doi:10.1371/journal.pone.0155122

Devlin, T. M. (2002). *Textbook of biochemistry*. New York: John Wiley.

Dingle, T. C., & Butler-Wu, S. M. (2013). Maldi-tof mass spectrometry for microorganism identification. *Clinics in Laboratory Medicine, 33*(3), 589-609. doi:10.1016/j.cll.2013.03.001

Duncan, K. E., & Howard, R. J. (2010). Biology of maize kernel infection by *Fusarium*

*verticillioides*. *Molecular Plant-Microbe Interactions, 23*(1), 6-16. doi: 10.1094/MPMI-23-1-0006

Duvick, J., Rood, T., Maddox, J., & Gilliam, J. (1998). Detoxification of mycotoxins in planta as a strategy for improving grain quality and disease resistance: identification of fumonisin-degrading microbes from maize. In K. Kohmoto & O. C. Yoder (Eds.), *Molecular genetics of host-specific toxins in plant disease: proceedings of the 3rd Tottori international symposium on host-specific toxins, Daisen, Tottori, Japan, August 24–29, 1997* (pp. 369-381). Dordrecht: Springer Netherlands. doi:10.1007/978-94-011-5218-1_41

EFSA. (2018). Risks for animal health related to the presence of fumonisins, their modified forms and hidden forms in feed. *EFSA Journal, 16*(5), e05242. doi:10.2903/j.efsa.2018.5242

European Commission. (2006). Commission recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding (2006/576/EC). *Official Journal of the European Union, L229*, 7-9.

Food and Agriculture Organization of the United Nations. (2004). *Food and nutrition paper:81 Worldwide regulations for mycotoxins in food and feed in 2003*. Rome: FAO.

Goesaert, H., Courtin, C. M., & Delcour, J. A. (2008). 11 - Use of enzymes in the production of cereal-based functional foods and food ingredients. In E. K. Arendt & F. Dal Bello (Eds.), *Gluten-Free Cereal Products and Beverages* (pp. 237-265). San Diego: Academic Press.

Grenier, B., Loureiro-Bracarense, A.-P., Leslie, J. F., & Oswald, I. P. (2014). Physical and chemical methods for mycotoxin decontamination in maize. In *mycotoxin reduction in grain chains* (pp. 116-129). doi:10.1002/9781 118832790.ch9

Guo, Z., Zhang, X., Wu, J., Yu, J., Xu, M., Chen, D., Zhang, Z., Li, X., Chi, Y., & Wan, S. (2020). In vitro inhibitory effect of the bacterium *Serratia marcescens* on *Fusarium proliferatum* growth and fumonisins production. *Biological Control, 143*, 104188. doi:10.1016/j.biocontrol.2020.104188

Gupta, A. K., & Gupta, U. D. (2014). Chapter 19 - Next generation sequencing and its applications. In A. S. Verma & A. Singh (Eds.), *Animal Biotechnology* (pp. 345-367). San Diego: Academic Press. doi:10.1016/B978-0-12-416002-6.00019-5

Hafner Česen, M., Stoka, V., & Turk, B. (2016). Role of lysosomes in intracellular degradation. In R. A. Bradshaw & P. D. Stahl (Eds.), *Encyclopedia of Cell Biology* (pp. 612-620). Waltham: Academic Press.

Hartinger, D., Heinl, S., Schwartz, H. E., Grabherr, R., Schatzmayr, G., Haltrich, D., & Moll, W.-D. (2010). Enhancement of solubility in *Escherichia coli* and purification of an aminotransferase from *Sphingopyxis* sp. MTA144 for deamination of hydrolyzed fumonisin B(1). *Microbial Cell Factories, 9*, 62-62. doi:10.1186/1475-2859-9-62

Hartinger, D., Schwartz, H., Hametner, C., Schatzmayr, G., Haltrich, D., & Moll, W. D. (2011). Enzyme characteristics of aminotransferase FumI of *Sphingopyxis* sp. MTA144 for deamination of hydrolyzed fumonisin B(1). *Applied Microbiology and Biotechnology, 91*(3), 757-768. doi:10.1007/s00253-011-3248-9

Heimpel, G. E., & Mills, N. J. (2017). *Biological control*: Cambridge University Press.

doi:10.1017/9781139029117

Heinl, S., Hartinger, D., Thamhesl, M., Vekiru, E., Krska, R., Schatzmayr, G., Moll, W.-D., & Grabherr, R. (2010). Degradation of fumonisin B1 by the consecutive action of two bacterial enzymes. *Journal of Biotechnology, 145*(2), 120-129. doi:10.1016/j.jbiotec.2009.11.004

Jaiganesh, V., Eswaran, A., Balabaskar, P., & Kannan, C. (2007). Antagonistic activity of *Serratia marcescens* against *Pyricularia oryzae*. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 35*(2). doi:10.15835/nbha352219

Jin, L.-Q., Liu, Z.-Q., Zheng, Y.-G., & Shen, Y.-C. (2010). Identification and characterization of *Serratia marcescens* ZJB-09104, a nitrile-converting bacterium. *World Journal of Microbiology and Biotechnology, 26*(5), 817-823. doi:10.1007/s11274-009-0238-5

Karlovsky, P., Suman, M., Berthiller, F., De Meester, J., Eisenbrand, G., Perrin, I., Oswald, I. P., Speijers, G., Chiodini, A., Recker, T., & Dussort, P. (2016). Impact of food processing and detoxification treatments on mycotoxin contamination. *Mycotoxin Research, 32*(4), 179-205. doi:10.1007/s12550-016-0257-7

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution, 35*(6), 1547-1549. doi:10.1093 /molbev/msy096

Lane, D. J. (1991.). 16S/23S rRNA sequencing. In S. E. & G. M. (Eds.), *Nucleic acid techniques in bacterial systematics* (pp. 115-175.). New York: John Wiley & Sons, Inc.

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature Methods, 9*(4), 357-359. doi:10.1038/nmeth.1923

Leslie, J. F., & Summerell, B. A. (2006). *The Fusarium laboratory manual*: Blackwell Publishing.

Li, Y., Shan, M., Zhu, Z., Mao, X., Yan, M., Chen, Y., Zhu, Q., Li, H., & Gu, B. (2019). Application of MALDI-TOF MS to rapid identification of anaerobic bacteria. *BMC Infectious Diseases, 19*(1), 941. doi:10.1186/s12879-019-4584-0

Logrieco, A., Bottalico, A., Mulé, G., Moretti, A., & Perrone, G. (2003). Epidemiology of toxigenic fungi and their associated mycotoxins for some mediterranean crops. *European Journal of Plant Pathology, 109*(7), 645-667. doi:10.1023/A:1026033021542

Loi, M., Fanelli, F., Liuzzi, V. C., Logrieco, A. F., & Mulè, G. (2017). Mycotoxin biotransformation by native and commercial enzymes: present and future perspectives. *Toxins, 9*(4), 111. doi:10.3390/toxins9040111

Lyagin, I., & Efremenko, E. (2019). Enzymes for detoxification of various mycotoxins: origins and mechanisms of catalytic action. *Molecules, 24*(13), 2362. doi:10.3390/molecules24132362

Marasas, W. F. O. (2001). Discovery and occurrence of the fumonisins: a historical perspective. *Environmental Health Perspectives, 109*(Suppl 2), 239-243. doi: 10.1289/ehp.01109s2239

Masching, S., Naehrer, K., Schwartz-Zimmermann, H. E., Sarandan, M., Schaumberger, S., Dohnal, I., Nagl, V., & Schatzmayr, D. (2016). Gastrointestinal degradation of fumonisin B(1) by carboxylesterase FumD prevents fumonisin induced

alteration of sphingolipid metabolism in turkey and swine. *Toxins, 8*(3). doi:10.3390/toxins8030084

Mazzoni, E., Scandolara, A., Giorni, P., Pietri, A., & Battilani, P. (2011). Field control of *Fusarium* ear rot, Ostrinia nubilalis (Hübner), and fumonisins in maize kernels. *Pest Management Science, 67*(4), 458-465. doi:10.1002/ps.2084

McKee, T., & McKee, J. R. (2003). *Biochemistry*. Boston: McGraw-Hill.

Melissa, H. D., Luis E López-Jácome, Claudia A Colín-Castro, Guillermo Cerón-González, Silvestre Ortega-Peña, E Samuel Vanegas-Rodríguez, Jaime A Mondragón-Eguiluz, & Franco-Cendejas, R. (2017). Comparison of the microscan walkaway and VITEK 2 compact systems for the identification and susceptibility of clinical gram-positive and gram-negative bacteria *Investigación en Discapacidad, 6*(3), 105-114.

Merrill, A. H., Jr., Wang, E., Vales, T. R., Smith, E. R., Schroeder, J. J., Menaldino, D. S., Alexander, C., Crane, H. M., Xia, J., Liotta, D. C., Meredith, F. I., & Riley, R. T. (1996). Fumonisin toxicity and sphingolipid biosynthesis. *Advances in Experimental Medicine and Biology, 392*, 297-306. doi:10.1007/978-1-4899-1379-1_25

Montella, I. R., Schama, R., & Valle, D. (2012). The classification of esterases: an important gene family involved in insecticide resistance--a review. *Memorias do Instituto Oswaldo Cruz., 107*(4), 437-449. doi:10.1590/s0074-02762012000400001

Munkvold, G. P. (2003). Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. *European Journal of Plant Pathology*, *109*, 705–713. doi:10.1023/A:1026078324268

Munkvold, G. P., & Desjardins, A. E. (1997). Fumonisins in maize: can we reduce their occurrence? *Plant Disease, 81*(6), 556-565. doi:10.1094/pdis.1997.81.6.556

Neville, S. A., LeCordier, A., Ziochos, H., Chater, M. J., Gosbell, I. B., Maley, M. W., & van Hal, S. J. (2011). Utility of matrix-assisted laser desorption ionization–time of flight mass spectrometry following introduction for routine laboratory bacterial identification. *Journal of Clinical Microbiology, 49*(8), 2980-2984. doi:10.1128/jcm.00431-11

Ng, L. S. Y., Sim, J. H. C., Eng, L. C., Menon, S., & Tan, T. Y. (2012). Comparison of phenotypic methods and matrix-assisted laser desorption ionisation time-of-flight mass spectrometry for the identification of aero-tolerant *Actinomyces* spp. isolated from soft-tissue infections. *European Journal of Clinical Microbiology & Infectious Diseases, 31*(8), 1749-1752. doi:10.1007/s10096-011-1496-3

Niderkorn, V., Morgavi, D. P., Pujos, E., Tissandier, A., & Boudra, H. (2007). Screening of fermentative bacteria for their ability to bind and biotransform deoxynivalenol, zearalenone and fumonisins in an *in vitro* simulated corn silage model. *Food Additives & Contaminants, 24*(4), 406-415. doi:10.1080/02652030601101110

Norred, W. P., Voss, K. A., Bacon, C. W., & Riley, R. T. (1991). Effectiveness of ammonia treatment in detoxification of fumonisin-contaminated corn. *Food and Chemical Toxicology, 29*(12), 815-819. doi:10.1016/0278-6915(91)90108-J

Novogene. (2020). Prokaryotic RNA Sequencing. https://en.novogene.com/services/research-services/transcriptome-sequencing/prokaryotic-rna-

sequencing/#demoresult

Okamoto, H., Sato, M., Sato, Z., & Isaka, M. (1998). Biocontrol of *Phytophthora capsici* by *Serratia marcescens* F-1-1 and analysis of biocontrol mechanisms using transposon-insertion mutants. *Annals of the Phytopathological Society of Japan, 64*(4), 287-293. doi:10.3186/jjphytopath.64.287

Othman, M. A., El-Zamik, F. I., Hegazy, M. I., & Salama, A. S. A. (2019). Isolation and identification of egyptian strains of *Serratia marcescens* producing antibacterial and antioxidant prodigiosin pigment. *Zagazig Journal of Agricultural Research, 46*(5), 1573-1582. doi:10.21608/zjar.2019.48175

Park, D. L., López-García, R., Trujillo-Preciado, S., & Price, R. L. (1996). Reduction of risks associated with fumonisin contamination in corn. *Advances in Experimental Medicine and Biology, 392*, 335-344. doi:10.1007/978-1-4899-1379-1_29

Park, D. L., Rua, S. M., Jr., Mirocha, C. J., Abd-Alla, E. S., & Weng, C. Y. (1992). Mutagenic potentials of fumonisin contaminated corn following ammonia decontamination procedure. *Mycopathologia, 117*(1-2), 105-108. doi:10.1007/bf00497285

Patel, J. B. (2001). 16S rRNA gene sequencing for bacterial pathogen identification in the clinical laboratory. *The Journal of Molecular Diagnostics, 6*(4), 313-321. doi:10.1054/modi.2001.29158

Pereira, P., Nesci, A., Castillo, C., & Etcheverry, M. (2010). Impact of bacterial biological control agents on fumonisin B1 content and *Fusarium verticillioides* infection of field-grown maize. *Biological Control, 53*(3), 258-266. doi:10.1016/j.biocontrol.2010.02.001

Pincus, D. H. (2006). Microbial identification using the bioMérieux Vitek®2 system. . In *Encyclopedia of Rapid Microbiological Methods*. Bethesda: Parenteral Drug Association.

Pohland, A. E. (1993). Mycotoxins in review. *Food Additives & Contaminants, 10*(1), 17-28. doi:10.1080/02652039309374126

Presello, D. A., Botta, G., Iglesias, J., & Eyhérabide, G. H. (2008). Effect of disease severity on yield and grain fumonisin concentration of maize hybrids inoculated with *Fusarium verticillioides*. *Crop Protection, 27*(3), 572-576. doi:10.1016/j.cropro.2007.08.015

Press, C. M., Loper, J. E., & Kloepper, J. W. (2001). Role of iron in rhizobacteria-mediated induced systemic resistance of cucumber. *Phytopathology, 91*(6), 593-598. doi:10.1094/phyto.2001.91.6.593

Pujol, R., Torres, M., Sanchis, V., & Canela, R. (1999). Fate of fumonisin B1 in corn kernel steeping water containing SO2. *Journal of Agricultural and Food Chemistry, 47*(1), 276-278. doi:10.1021/jf9805045

Rafii, F. (2014). Serratia. In C. A. Batt & M. L. Tortorello (Eds.), *Encyclopedia of food microbiology (Second Edition)* (pp. 371-375). Oxford: Academic Press.

Rheeder, J. P., Marasas, W. F. O., & Vismer, H. F. (2002). Production of fumonisin analogs by *Fusarium* species. *Applied and Environmental Microbiology, 68*(5), 2101-2105. doi:10.1128/aem.68.5.2101-2105.2002

Rödel, J., Mellmann, A., Stein, C., Alexi, M., Kipp, F., Edel, B., Dawczynski, K., Brandt, C., Seidel, L., Pfister, W., Löffler, B., & Straube, E. (2019). Use of MALDI-TOF mass spectrometry to detect nosocomial outbreaks of *Serratia*

*marcescens* and *Citrobacter freundii*. *European Journal of Clinical Microbiology & Infectious Diseases, 38*(3), 581-591. doi:10.1007/s10096-018-03462-2

Sauget, M., Valot, B., Bertrand, X., & Hocquet, D. (2017). Can MALDI-TOF mass spectrometry reasonably type bacteria? *Trends in Microbiology, 25*(6), 447-455. doi:10.1016/j.tim.2016.12.006

Scarpino, V., Reyneri, A., Vanara, F., Scopel, C., Causin, R., & Blandino, M. (2015). Relationship between European corn borer injury, *Fusarium proliferatum* and *F. subglutinans* infection and moniliformin contamination in maize. *Field Crops Research, 183*, 69-78. doi:10.1016/j.fcr.2015.07.014

Schmale, D. G., & Munkvold, G. P. (2009). *Mycotoxins in crops: A threat to human and domestic animal health.* Paper presented at the The Plant Health Instructor.

Shetty, P. H., & Bhat, R. V. (1999). A physical method for segregation of fumonisin-contaminated maize. *Food Chemistry, 66*(3), 371-374. doi:10.1016/S0308-8146(99)00052-7

Shibamoto, T., & Bjeldanes, L. F. (1993). *Chapter 6 - Fungal toxins occurring in foods* (S. L. Taylor Ed.). San Diego: Academic Press.

Singh, R. P., & Jha, P. N. (2016). The multifarious PGPR *Serratia marcescens* CDP-13 augments induced systemic resistance and enhanced salinity tolerance of wheat (*Triticum aestivum* L.). *PLOS ONE, 11*(6), e0155026. doi:10.1371/journal.pone.0155026

Someya, N., Kataoka, N., Komagata, T., Hirayae, K., Hibi, T., & Akutsu, K. (2000). Biological control of cyclamen soilborne diseases by *Serratia marcescens* strain B2. *Plant Disease, 84*(3), 334-340. doi:10.1094/pdis.2000.84.3.334

Starkl, V., & Nahrer, K. (2015). *Fumonisin compendium*. Austria: BIOMIN Holding GmbH.

Sydenham, E. W., Shephard, G. S., Thiel, P. G., Marasas, W. F. O., & Stockenstrom, S. (1991). Fumonisin contamination of commercial corn-based human foodstuffs. *Journal of Agricultural and Food Chemistry, 39*(11), 2014-2018. doi:10.1021/jf00011a028

Timperio, A. M., Gorrasi, S., Zolla, L., & Fenice, M. (2017). Evaluation of MALDI-TOF mass spectrometry and MALDI biotyper in comparison to 16S rDNA sequencing for the identification of bacteria isolated from Arctic sea water. *PLOS ONE, 12*(7), e0181860. doi:10.1371/journal.pone.0181860

Tola, M., Kebede, B., & Yildiz, F. (2016). Occurrence, importance and control of mycotoxins: A review. *Cogent Food & Agriculture, 2*(1). doi:10.1080/23311932.2016.1191103

Turner, S., Pryer, K. M., Miao, V. P., & Palmer, J. D. (1999). Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. *Journal of Eukaryotic Microbiology, 46*(4), 327-338. doi:10.1111/j.1550-7408.1999.tb04612.x

Vanhoutte, I., Audenaert, K., & De Gelder, L. (2016). Biodegradation of Mycotoxins: Tales from Known and Unexplored Worlds. *Frontiers in Microbiology, 7*, 561. doi:10.3389/fmicb.2016.00561

Voss, K. A., & Riley, R. T. (2013). Fumonisin toxicity and mechanism of action: overview and current perspectives. *Food Safety, 1*(1), 49-69. doi:10.14252/foodsafetyfscj.2013006

Wang, J. Q., Yang, F., Yang, P. L., Liu, J., & Lv, Z. H. (2018). Microbial reduction of zearalenone by a new isolated *Lysinibacillus* sp. ZJ-2016-1. *World Mycotoxin Journal, 11*(4), 571-578. doi:10.3920/wmj2017.2264

Woese, C. R. (1987). Bacterial evolution. *Microbiological reviews, 51*(2), 221-271. doi:10.1128/mr.51.2.221-271.1987

Zhao, Z., Zhang, Y., Gong, A., Liu, N., Chen, S., Zhao, X., Li, X., Chen, L., Zhou, C., & Wang, J. (2019). Biodegradation of mycotoxin fumonisin B1 by a novel bacterial consortium SAAS79. *Applied Microbiology and Biotechnology, 103*(17), 7129-7140. doi:10.1007/s00253-019-09979-6

Zhu, Y., Hassan, Y. I., Lepp, D., Shao, S., & Zhou, T. (2017). Strategies and methodologies for developing microbial detoxification systems to mitigate mycotoxins. *Toxins, 9*(4). doi:10.3390/toxins9040130

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