ISOLATION, MOLECULAR IDENTIFICATION AND SCREENING OF FUSARIUM MYCOTOXIN DON (DEOXYNIVALENOL) DEGRADING BACTERIA IN EUROPEAN WHEAT SAMPLES

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ISOLATION, MOLECULAR IDENTIFICATION AND SCREENING OF FUSARIUM MYCOTOXIN DON (DEOXYNIVALENOL) DEGRADING BACTERIA IN EUROPEAN WHEAT SAMPLES

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CERTIFICATE

This is to certifythat the thesis entitled "**ISOLATION, MOLECULAR IDENTIFICATION AND SCREENING OF *FUSARIUM* MYCOTOXIN DON (DEOXYNIVALENOL) DEGRADING BACTERIA IN EUROPEAN WHEAT SAMPLES** " submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, Bangladesh in partial fulfillment of the requirements for the degree of masters of science (MS) in Plant Pathology, embodies the result of a piece of bona fide research work carried out by registration No. 12-04785 under my supervision and guidance. No part of the thesis has been submitted anywhere for any other degree or diploma.

I further certify that any help or source of information, as have been availed of during the course of this investigation have duly acknowledged by the author.

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ABBREVIATION

ABBREVIATION	ENGLISH NAME
DON	Deoxynivalenol
PCR	Polymerase Chain Reaction
DNA	Deoxyribonucleic Acid
HPLC	High Performance Liquid Chromatography
ppm	Parts Per Million
ANOVA	Analysis of Variance
ACN	Acetonitrile
r.p.m	Revolution per minute
et al.	Et alii
nm	Nanometer
JECFA	Joint FAO/WHO Expert Committee on Feed Additives
LB	Lysogeny Broth
MM	Mineral salt Media
PBS	Phosphate-Buffered Saline
FHB	Fusarium head blight

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ISOLATION, MOLECULAR IDENTIFICATION AND SCREENING OF FUSARIUM MYCOTOXIN DON (DEOXYNIVALENOL) DEGRADING BACTERIA IN EUROPEAN WHEAT SAMPLES

ABSTARCT

Mycotoxins are the toxic substances which are produced as a result of infection caused by fungi as their secondary metabolite. Major toxin producing fungi are *Fusarium, Penicillium, Aspergillus* and *Claviceps* which produces DON, ZEN, OTA, FB, AFB, citrinin and patulin. The experiment was carried out in Key Laboratory of Food Safety and Quality Control, Institute of Food Science and Technology,Chinese Academy of Agricultural Sciences, Beijing China under completely randomized design with three replications. The objectives of the experiment were the isolation, molecular identification and screening of the single bacterial colony which is responsible for the degradation of Fusarium mycotoxin deoxynivalenol (DON) in European wheat samples. Wheat samples were mixed using lysogeny broth (LB) media, PBS media and mineral salt (MM) media for isolation of bacteria using DONstar immunoaffinity column.Six Bacteria genera namely *Pseudomonas* spp, *Agrobacterium* sp, *Zobellella* sp, *Bacillus* sp., *Achromobacter* sp, *Advenella* sp and *Cupriavidus* sp. were isolated and identified by PCR amplifying 16SrDNA gene fragment.Maximum DON degradation was recorded by mixed bacteria in wheat samples rather than the isolated single bacteria.

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CHAPTER 1
INTRODUCTION

Wheat (*Triticum aestivum L.*) is one of most favorite food sources, reaching millions of people on a daily basis and accounts for 20% of tilth globally. The annual wheat production is 757.9 million tons and the demand are projected to rise up to 70% by 2050 due to feed the ever-growing human population (FAO, 2018; and Tilman *et al.* 2011). Although China, Russia, India, Ukraine and France are the largest producer of wheat (International Grains Council, 2009) but the production is in decreasing trend due to environmental footprint or pathological diseases. So, to meet the impending target, wheat grain production must substantially increase through evolving resistance against pathological threats is pre- requisite.

Different kind of wheat disease are found like bacterial diseases, fungal diseases, viral disease, and Nematodes disease. Fusarium blight is one amongst the devastating diseases of wheat. Wheat infected by FHB might contain mycotoxins such as deoxynivalenol (DON), additionally referred to as vomitoxin. This mycotoxin is made primarily by *F. graminearum* and *F. culmorum.* Non-ruminants are measured as the foremost sensitive to the presence of DON. In eastern Saskatchewan, Manitoba, eastern Canada and within the United States, *F. graminearum* is generally responsible for FHB. Fusarium harm kernel (FDK) are measured often found in soft white spring wheat mature underneath irrigation in southern Alberta. However, DON levels are measure typically terribly low (james *et al.*, 2003).

Fusarium head blight (FHB) was first described in England in 1884, wherever it had been considered an enormous threat to wheat and barley crops (Goswami and Kistler, 2004). International Maize and Wheat Improvement Centre (CIMMYT)

has named FHB as a significant threat to wheat production throughout the world (Goswami and Kistler, 2004). The reduction in yield will reach as high as 30–40 kg /ha one within the cases of severe poison and might occur each 3–5 years (Parry *et al.*, 1995; McMullen *et al.*, 1997). The general impact of *Fusarium* spp. in wheat production arises through the mixture of FHB and phytotoxic infection of the grain harvested from infected wheat spikes.

FHB is recognized within the field by the premature bleaching of infected spikelet's and also the production of orange, spore-bearing structures referred to as sporodochia at the bottom of the glumes. Throughout wet weather, there could also be whitish, sometimes pinkish, downy plant life growth on infected heads within the field. Unhealthy spikelet's will contain visibly affected kernels. The grading term given to visibly affected wheat seeds is fusarium damage kernels (FDK), whereas in barley, it's referred to as *Fusarium* moulds.

FDK in wheat area shrunken and usually chalky white, whereas fusarium moulds on barley is an orange or black encrustation of the seed surface, Severely infected wheat is poorly stuffed and should be blown out the back of the mix because of its low kernel weight. Grain infected once the flowering stage could also be serious enough to be harvested along with healthy grain, though most FDK can result from infections that occur throughout flowering. The fungus might eventually kill the developing seed at regarding the soft dough stage. Symptoms of fusarium moulds in barley or oats area usually typically spares, creating it tough to inform if grain has been infected.

Mycotoxins are toxic secondary metabolites made naturally by threadlike fungi similar to genus *Aspergillus* spp., *Fusarium* spp. and so on that are thought of as risky when in food for humans and feed for animals. Mycotoxins area structurally disparate cluster of fungal natural products that share the flexibility to cause damage to vertebrate animals or human health once they are contaminants of animal feed or food. The term "Mycotoxin" was first introduced in the 1906s to

explain the poisonous substance related to contaminated peanuts in animal feed and therefore the loss of turkeys in England (Turkey X disease)

Deoxynivalenol (DON) is a natural-occurring mycotoxin mainly produced by *Fusarium graminearum* (Kushiro, 2008). It is also known as vomitoxin due to his strong emetic effects after consumption, because it is transported into the brain, where it runs dopaminergic receptors. The emetic effects of this mycotoxin were firstly described in Japanese men consuming mouldy barley containing *Fusarium* fungi in 1972 (Ueno, 1985; Ueno, 1988). The objectives of the present study were:

- To isolate and molecular identification of DON degrading bacteria from European wheat samples.
- To evaluate the efficacy and screening of DON degrading bacteria in European wheat samples.

CHAPTER 2

REVIEW OF LITERATURE

Mycotoxins are secondary metabolites created by micro fungi that are capable of inflicting disease and death in humans and other animals. The virulent impact of mycotoxins on animal and human health is called as mycotoxicosis.

Deoxynivalenol (DON) is one of several mycotoxins created by certain Fusarium species that often infect corn, wheat, oats, barley, rice, and alternative grains within the field or throughout storage. Fusarium mycotoxins ar the biggest cluster of mycotoxins, which incorporates over 140 known metabolites of fungi. They're synthesized by several species of fungi, in the main by Fusarium (*F. graminearum* and *F. culmorum*). Due to the high toxicity of Fusarium mycotoxins and high incidence of the fungi species producing them, these mycotoxins belong to the foremost animal and human health endangering groups. They're abundant in cereals and their product (Yazar andOmurtag, 2008).

2.1.History of DON and currentstatus of DON in the world

More than a century past, plant pathologists in Europe and also in the United states discribed wheat head blight with infections by *Fusarium graminearum*, that produces deoxynivalenol (DON) and Nivalenol (NIV).

During world war II, consumption of over-wintered grain contaminated by *F. sporotrichioides* and related species caused alimentary toxic aleukia and deaths of hundreds of thousands of individuals within the former Soviet Union. Throughout the 1970s in Japan, *F. graminearum* caused severe epidemics of akakabi-byo (red mold disease) on green wheat and alternative grains. People who ate product containing such contaminated grains generally developed nausea, vomiting, diarrhea, hemorrhaging, anemia, and alternative symptoms of trichothecene toxicosis.

In 1972, Japanese scientists were successful in distinguishing DON and NIV in grain infected with *F. graminearum* (Desjardins, 2003). Japanese

researchers named it "Rd-toxin" (Moorooka *et al.*, 1972). Shortly thenceforth, a similar mycotoxin was isolated from maize related to vomiting in pigs and given the name vomitoxin (Vesonder *et al.*, 1973).

Deoxynivalenol (DON) may be a trichothecene mycotoxin, made primarily by *Fusarium graminearum* (Bando *et al.*, 2007). Production of DON in wheat is considered a virulence issue of *F. graminearum* (Maier *et al.*, 2006). Trichothecenes are mycotoxins made principally by members of the Fusarium genus, though alternative genera (e.g. T*richoderma, Trichothecium, Myrothecium and Stachybotrys*) also are renowned to supply these compounds. To date, 148 trichothecenes are isolated, however some are found to contaminate food and feed. the foremost frequent contaminants are deoxynivalenol (DON), additionally called vomitoxin, nivalenol (NIV), diacetoxyscirpenol (DAS), whereas T-2 poison is rarer.

DON is additionally called vomitoxin, as a result of it causes instinctive reflex, particularly once consumed by pigs. it's toxicologically relevant, and its synthesis is excited by humans (Miller, 1995), with high incidence in winter cereals, AS wheat and wheat products consumed by humans (Miller, 1995; Calori-Domingues *et al.*, 2007). The world Health Organization (WHO) considers DON as a neurotoxin with a teratogenic nature and immunological disorder characteristics, and, as trichothecenes normally, it's been related to chronic and fatal intoxication of humans and animals through consumption of contaminated food (Rotter *et al.*, 1996)

DON has been detected in cereal grains full-grown in Canada, the US (Benett *et al.* 1983), the UK (Gilbert *et al.* 1984) and Japan (Yoshizawa 1983). In Argentina, Quiroga *et al.* (1995) and Rizzo *et al.* (1997) recorded the natural incidence of this mycotoxin from the wheat (*Triticum aestivum L.*) cropping. The analysis of the presently offered European dataset showed that in *F. graminearum*, the predominant genotype was 15-acetyldeoxynivalenol (15-ADON) (82.9%), followed by 3-acetyldeoxynivalenol (3-ADON) (13.6%), and nivalenol (NIV) (3.5%). In *F. culmorum*, the prevalent genotype was 3-ADON

(59.9%), whereas the NIV genotype accounted for the remaining 40.1%. Both, geographical and temporal patterns of trichothecene genotypes distribution were known (Matias Pasquali *et al.* 2016)

Deoxynivalenol (DON) belongs to at least one of the most important cluster of mycotoxins, the kind B-trichothecene mycotoxin that are created notably by moulds belonging to the Fusarium genus. the primary isolation of DON occurred in 1972 in Japanese barley attracted by *Fusarium graminearum*. Also isolates from *F. graminearum* infected maize, inflicting vomiting in pig, DON was additionally nicknamed "vomitoxin". Deoxynivalenol (DON), usually called as vomitoxin, could be a mycotoxin that will be created in wheat and barley grain infected by Fusarium blight (FHB) or scab. DON, the foremost prominent type B trichothecene will principally be found on maize, oats, barley and wheat. Wheat is very susceptible to Fusarium infection and infrequently shows high levels of DON contamination. DON poses a major threat to each humans and animals, because it will interfere with general eukaryotic cell perform by inhibiting protein synthesis (Rotter, 1996). DON is primarily placed within the grains on that Fusarium is growing here most of the mycelium is anticipated on the external surfaces of the kernels. Contamination of grain with DON reduces grain value, produce a food safety risk and incorporates a negative impact on international food and feed trade (Wu, 2004).

2.2. Deoxynivalenol structure, chemical properties and hazards

Deoxynivalenol (DON) could be a natural-occurring plant toxin in the main created *Graminearum* (Kushiro, 2008) it's additionally referred to as vomitoxin because of his strong curative effects once consumption, as a result of it's transported into the brain, wherever it runs dopaminergic receptors. The curative effects of this plant toxin were first delineated in Japanese men consuming moldy barley containing Fusarium fungi in 1972 (Ueno, 1985; Ueno, 1988). DON is the most effective glorious and most common contaminant of grains and their subsequent products. Chemically DON could be a member of the trichothecenes family of mycotoxins.

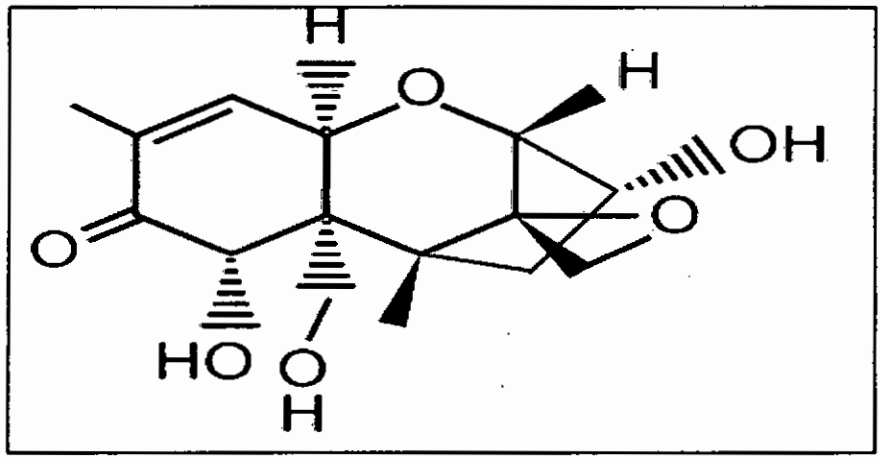

Fig. 1. Chemical structure of deoxynivalenol (DON)

Structurally, it's a polar compound, that belong to the type B trichothecenes and its chemical name is 12,13-epoxy-3α,7α,15-trihydroxytrichothec-9-en-8-on (Nagy *et al.*, 2005). In its molecule it contains 3 free hydroxy group teams (-OH), that are related to its toxicity. From its chemical structure its physical and chemical properties shown in (Table 1).

One of the foremost vital physicochemical property of DON is its ability to resist high temperatures, that is the risk of its prevalence in food (Hughes *et al.*, 1999). Various studies have documented that DON was heat-stable. DON is extremely stable underneath temperature among the interval from 170°C to 350°C, with no reduction of DON concentration once 30 min at 170°C. However, DON levels are reduced in cooked pasta and noodles because of leaching into the cooking water (Manthey *et al.*, 2004; Sugita-Konishi *et al.*, 2006), as a result of DON is soluble, however no reduction of its concentration was discovered throughout cooking DON-contaminated food in oil. Some proof indicates that DON levels could also be reduced throughout the process, chiefly boiling in water, Chinese noodles containing Kansui: a commercial preparation of potassium and sodium carbonate and phosphate salts (Kushiro, 2008).

Table 1. Physio-chemical properties of Deoxynivalenol (DON)

Property	Information
Name	Deoxynivalenol (DON), vomitoxin
IUPAC	(3α,7α)-3,7,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one
Molecular formula	$C_{15}H_{20}O_6$
Average mass	296.316 g/mol
Monoisotopic mass	296.125977 g/mol
Physical state	Colourless fine needles
Boiling point (°C)	543.9 ± 50.0°C
Melting point (°C)	151 -153°C
Flash point (°C)	206.9± 2.5
Vapour pressure (Torr)	4.26×10^{-14}, 25°C
Soluble	Polar organic solvents (*e.g* aqueous methanol, ethanol, chloroform, acetonitrile and ethyl acetate) and water

2.3. Hazards

2.3.1. Deoxynivalenol in human and animal health

Mycotoxins are secondary metabolites of moulds that exert harmful effects on animals and humans. The harmful impact of mycotoxins on animal and human health is referred to as mycotoxicosis.

In 1982, the Food and Drug Administration (FDA) issued an informative to federal and state officers recommending a "level of concern" for DON of 2.0 µg/g (2 ppm) for wheat getting into the edge method and 4.0 µg/g for wheat and wheat edge by-products used in animal feed. New pointers stressed maximums for DON in bran, flour, and germ intended for human consumption at 1.0 µg/g. (Jones and Mirocha, 1999)

Tanaka *et al.* (1986) reported that 60% of DON in naturally contaminated wheat remained when the flour edge method, and no considerable loss of poisons occurred as a results of baking method.

Potential impact of DON on human health could occur when activity of contaminated foods from oats, barley, wheat, corn or alternative grains. DON was detected also in buckwheat, sorghum, popcorn and alternative foods for human consumption, equivalent to flour, bread, noodles, brew and malt. Danger ensuing from this is often that the poisonous substance still remains in foods and feeds when basic culinary treatment. DON doesn't represent a major threat to public health. in a very few cases short nausea and vomiting have been recorded (Perkowski *et al.,* 1990).

Alternative effects include diarrhoea, abdominal pain, headache, giddiness and fever. The Netherlands, that ranged from 26 to 41 mg/L (0.088 to 0.14 μM). within the case of German beers this figure goes higher than 200 ng/ml (0.675 μM) (Schothorst and Jekel, 2003).

In animal, Dogs and cats are restricted to 5 ppm and of grains and grain byproducts and therefore the grains don't seem to be to exceed 40% of the die. In animals and livestock, vomitoxin causes a refusal to feed and lack of weight gain once fed higher than suggested levels. Restrictions are set at 10 ppm for poultry and ruminating beef and feedlot cattle older than four months. Ingredients might not exceed 50% of the animal's diet. Kine(cow) feed limits are set at 2 ppm. (Table 2) maximum DON limit in different country in food and feed.

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TABLE 2. Maximum limit of DON (Food and feed) in different country

Country	Commodity	Limit (µl/kg)
Austria	pig feed	500
	feed for fatting-poultry	1500
	feed for breeding-poultry and laying hens	1000
	feed for fatting-bovine animals	1000
Brazil	cereals and processed products thereof intended for direct human consumption or as an ingredient in foodstuffs	1000
	cereals to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs	2000
	maize and processed products	1000
Canada	domestic uncleaned soft wheat	2000
	soft wheat flour (adult food)	1200
	soft wheat flour (infant food)	600
	feed for cattle and poultry	5000
	feed for swine, calves, dairy cattle	1000
Belarus	Barley	1000
	Wheat	700
	Barley	1000
	Wheat	700
Armenia	Wheat	700
	Barley	1000
China	wheat and wheat flour, maize and maize flour	1000
Cuba	imported cereals	300
	all feeds	300
European Union (EU)	cereal products as consumed and other cereal products at retail stage	500
	flour used as raw material in food products	750

(Agriculture Organization of the United Nations (FAO), 2002/2003)

The wheat infected with *Fusarium graminearum* and its associated mycotoxins (DON) can be affectively control with the help of degrading bacteria.

2.4. DON impact on wheat

Wheat (*Triticum aestivum L.*) is one of the most important staple foods worldwide, accounting for 20% of ploughland worldwide. Annual international production is calculable at 500 million tons, and therefore the main produced area unit Russia, Ukraine, the united states, China, India and France (International Grains Council, 2009)

Fusarium sp. could be a phytopathogen that produces mycotoxins and causes Fusarium head blight (FHB). Among Fusarium species, *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* (Scw.) Petch] frequently contaminates wheat and is related to trichothecene production. Trichothecenes area unit potent inhibitors of eukaryotic protein synthesis, interfering at the initiation, elongation, and termination stages. Deoxynivalenol (DON; 12,13-epoxy-3a,7a,15 trihydoxytrichotecec-9-en-8one) could be a B type trichothecene, classified by the International Agency for analysis on Cancer in cluster three as "not distinctive on its carcinogenicity to humans" (IARC, 1993).

In plantations, wheat may be contaminated by varied diseases because of weather, soil type and crop condition one amongst the known diseases that unremarkably have an effect on this cereal is Fusarium blight, triggered by infection of Fusarium fungi, that not solely cause diseases in plants however conjointly turn out toxic substances called mycotoxins through their secondary metabolism (Calori-Domingues *et al.*, 2007).

Fusarium graminearum is that the principal DON-producing fungus in grains. Corn and tiny grains equivalent to wheat, oats and barley area unit the most important crops affected. The organism survives on previous infected residue left

on the sector from the previous season, providing an inoculum source for the new crop. The organism will well in cool, dampish conditions with contamination of the crop occurring once spores (conidia) of the organism are windblown to the silks of the corn and in little grain to the anthers (male parts of the flower) that emerge outside the blossom throughout what's known as flowering. The flora penetrates the host ear or blossom and produces the disease and DON. In wheat, it seems that DON production is important for the organism to produce the disease.

2.5. Management

Promising choices for dominant Fusarium blight include chemical measures and also the development of resistant cultivars. Registered fungicides are often somewhat effective in reducing FHB (Jones, 2000; Matthies, and Buchenauer. 2000; Suty and Mauler-Machnik, 1997), however residue issues relating to the utilization of fungicides late in crop development reduce their attractiveness. Advances in developing resistant cultivars using ancient breeding (Bai *et al.* 2000) and recombinant DNA technology (Bushnell *et al.* 1998) are occurring, however all wheat cultivars presently in production stay at risk of infection. Although part effective in reducing FHB, conventional tillage of fields once gathering to bury plant residues and reduce available pathogen inoculum (Dill-Macky and Jones, 2000; Miller *et al.*1998. Yi *et al.*2001) isn't compatible with the soil conservation apply of minimum tillage.

Biological management of FHB holds considerable promise and includes the treatment of crop residues with antagonists to reduced pathogen inoculum (Bujold *et al.* 2001) and wheat heads at anthesis to reduce infection (Khan *et al.* 2001; Perondi *et al.* 1996; Stockwell *et al.*1997; Wang *et al.* 1992.). Biological management of FHB is attractive due to its potential for being environmentally effective, compatible with alternative management measures, and durable. The environment plays an essential role within the development of FHB and also the production of DON. Wet and heat conditions throughout flowering favor FHB and

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DON production. once environmental conditions area deal, multiple management practices are required to regulate this pernicious disease.

Crop rotation won't eliminate Fusarium head blight and DON accumulation, however can help reduce the severity, even in epidemic years. variety resistance, no wheat varieties are completely against Fusarium head blight, however some varieties show additional tolerance to the disease and DON accumulation. of types presently out there, Glenn, Alsen and Freyr are among the foremost tolerant.

Fungicides will help reduce Fusarium head blight and DON levels by 50% to 70% in North Dakota in most years, when using the best available fungicides and acceptable application timings. Success is greatest once fungicides are applied to moderately vulnerable to moderately resistant spring wheat varieties below severe epidemics, fungicides haven't sufficiently reduced disease or DON levels to attain a top market grade in barley or in terribly susceptible wheat and durum wheat cultivars. Seed treatments before planting could improve seed germination and seed vigor, however they'll not prevent FHB infection or DON accumulation.

2.6. Biological control strategies

The application of several of the existing chemical and physical approaches for the detoxification of agricultural foodstuffs contaminated with mycotoxins is limited due to concerns with safety issues and potential reduction in the nutritional quality of treated foodstuffs, in addition to restricted effectiveness and value repercussions (Keohl *et al.*, 2011). Additionally, the event of antifungal resistance in varied fungous pathogens, likewise as increasing public concern over risks related to chemical application, has led to a notable interest within the development of substitute nonchemical, environmentally friendly, approaches to pest and disease management. the character of FHB makes it a possible target for biocontrol as a result of heads are vulnerable to contamination during throughout and, for a brief amount, after flowering (Xu and Nicholson, 2009). Biological management has

been used as a way of managing the development of FHB in wheat (Luz *et al.*, 2003).

2.6.1. Microbes as antagonistic agents

Biological management using microbial antagonists, either severally individual basis or as a part of an integrated management approach to minimize chemical inputs, is a favored strategy to minimize mycotoxin and aflatoxin concentrations in pre- and postharvest crops (Tsitsigiannis *et al.*, 2012). Several organisms like yeasts, microorganism (bacteria) and nontoxigenic genus Aspergillus fungi are according for their ability to reduced plant toxin accumulation. Though the utilization of biological control agents (BCAs) has often been effective within the laboratory, their exploitation depends on whether or not they will faithfully management mycotoxigenic fungi in numerous locations and cultivars and reduce plant toxin levels below the legislative limits. Some studies have indicated that biological management has the potential reduced the extent of FHB infection in wheat as well as to prevent the toxins from getting into the organic food chain (Matarese *et al.*, 2012).

Microorganisms have a narrow spectrum of activity compared to most chemical fungicides. Indeed, biological management of pre- or postharvest infections is complicated and includes variety of biological, ecological and economic variables. New BCAs should not solely be effective towards the targeted plant pathogen, however additionally should be nontoxic/safe and cost-efficient (Keohl *et al.*, 2011). using a BCA oriented strategy is especially worthy within the case of the organic production of crops whereby fungicides can't be applied to manage FHB and DON. Bacterial and fungal BCAs may be directly applied to the residues or into wheat spikes so as to suppress the assembly of perithecia or impede the disease aggression (Xue *et al.*, 2014). Many bacterial and fungal species, including Gram-positive and -negative bacteria and filamentous fungi, capable of dominant the disease and mycotoxin

F. graminearum dispersion and contamination have been identified in nature (Luz *et al.*, 2003; Gilbert & Fernando, 2004). Soil/rhizobacteria are recognized as BCAs able to reduce Fusarium contamination and phytotoxic levels in in vitro assays, greenhouse experiments and field trials (Palazzini *et al.*, 2007; Shi *et al.*, 2014; Zhao *et al.*, 2014). Bacterial BCAs that are familiar to be antagonistic to FHB-causing *Fusarium sp.* Include *Bacillus* spp. (Schisler *et al.*, 2002; Zhao *et al.*, 2014), *Lysobacter enzymogenes* (Palazzini *et al.*, 2007), *Bacillus* spp. and *Streptomyces* spp. *Basillus amyloliquefaciens* FLN13, *lactobacillus plantarum* SLG17 and alternative lactobacillus species have additionally been used for biocontrol methods against *F. culmorum* and *F. graminearum* (Rosello *et al.*, 2013; Baffoni *et al.*, 2015).

Bacterial species of the genera Bacillus and Pseudomonas genus are extensively exploited in BCA methods against FHB (Yoshida *et al.*, 2012). Most antagonistic microorganism are endophytic microbes that inhabit the plant or rhizosphere while not inflicting diseases or adverse effects (Bello et al., 2002). For example, Zhao *et al.* (2014) reported that the isolation of bacillus SG6 from wheat anthers and conclusive that its presence prevents development of *F. graminearum.*

2.6.2. Natural fungicides

Metabolites created by plants, together with phenolic compounds and essential oils, are a promising substitute for synthetic fungicides as a result of plants produced an extensive variety of compounds either as a part of their development or in reaction to stress or pathogens (da Cruz Cabral *et al.*, 2013). Various plant metabolites provoke robust restrictive responses against some necessary target proteins of *F. graminearum*, e.g. cytochrome P450s and carbonic anhydrases (Cuperlovic-Culf *et al.*, 2016). Phenolic compounds derived from Spirulina are extremely efficient in inhibiting *F. graminearum* activity (Pagnussatt *et al.*, 2014).

Chemical compounds resembling p-coumaric acids and phenolic acid derivatives resembling ferulic and caffeic acids are a number of the foremost effective inhibitors of fungal infections *(F. graminearum)* and plant toxin production (Gauthier *et al.*, 2016). Furthermore, *F. graminearum* will decompose

chlorogenic acid into its constituent compounds resembling hydroxy chlorogenic, caffeic and protocatechuic acids, all of that are extraordinarily effective at curtailing the expansion of mycelia as well as production of DON (Gauthier *et al.*, 2016).

Essential oils extracted from plants contain some antimicrobial or antioxidant compounds, and that they are thought-about as a suitable alternative of natural fungicides (Bakkali *et al.*, 2008). A recent study reported that natural fungicides obtained from plant metabolites have massive potential in dominant DON accumulation (Tian *et al.*, 2016). Oil extracts from plants resembling cinnamon, clove, lemongrass, oregano and palmarosa are tested for their ability to reduced DON accumulation in *F. graminearum*-infested grain; clove essential oil was found to be the most effective (Marın *et al.*, 2004; Gurjar *et al.*, 2012).

2.6.3. Use of resistant cultivars

As far as the vulnerability of a variety to FHB and DON worries, breeding improvement in cereals, exploitation typical strategies, molecular markers or via transgenic strategies are represented in many reviews (Hollins *et al.*, 2003; Snijders, 2004; Shah *et al.*, 2017). Arthur (1891) was the first to observe variation in vulnerability to FHB among wheat cultivars.

However, at present, there's no viable and fully FHB-resistant wheat variety available; disease management depends on the utilization of the use of cultivars with partial resistance (Mesterhazy *et al.*, 2003).

2.6.4. Control by cultural methods

Fusarium graminearum has the potential to survive on wheat, maize, barley still as many wild grasses. Cultural strategies have a significantly higher impact on minimizing FHB incidence. strategies where crop rotation, tillage, sowing date and alternative integrated approaches, once used judiciously, all have the potential to drastically reduce infection. (Shahabc *et. al*, 2018).

CHAPTER 3

MATERIALS AND METHODS

3.1. Experimental site

The experiment was conducted at, Key Laboratory of Agro-products Quality and Safety Control, Institute of Food Science and Technology, CAAS (Chinese Academy of Agricultural Sciences, Beijing, China.

3.2. Experimental period: September 2017 to June 2018

3.3. Crop used: Wheat seed

3.4. Sample preparation

3.4.1. Sample collection and preparation

The original European wheat spike 8.4g (a total of 4 panicles) were divided into two subsamples. Of them 4g (2 spike) was taken to preparethe mixed bacteria solution, the remaining 4.4g was placed in the refrigerator at 4°C.

Four gram(4g) of infected panicle was cut into small pieces in 250mL conical flask. Then 50mL sterile distilled water was used to wash and were all the bacteria from the sample byshaking it in incubator shaker at 28^0c [200rpm for 24hr]. The sample was filtered with filter paper (medium size 15-20μm) and centrifuged the filtrate at 4°C, 10000 rpm, for 10minutes. The supernatant was poured, and the bacteria was suspended by adding 5ml of 1x PBS (PBS- Phosphate-buffered saline was used as buffer solution. It was a water-based salt solution containing disodium hydrogen phosphate, sodium chloride and, potassium chloride and potassium dihydrogen phosphate). The mixer was then shacked and vortex for 2 to 3 minutes for collect all cell.

3.5. Sample Toxin Detoxification

Procedure

200µL, 100ppm of DON was suspended into 10ml sterilized tube then dried with nitrogen gas under 60^{0}C. Then 1 ml filtrated sample was added with 1 ml MM media (MM media composition and concentration was K_2HPO_4 2.5gL^{-1}, NH_4NO_3 1gL^{-1}, KH_2PO_4 1.2gL^{-1}, $MgSO_4.7H_2O$ 0.2gL^{-1}, Ca $(NO_3).4H_2O$ 0.4g L^{-1}, NaCl 0.5g L^{-1}, $Fe_2(SO_4)_3$ 0.0001gL^{-1} for 1L) so total concentration of the DON was 10ppm. The sample was shaking by incubator shaker at 200rpm at 28°C for 7 days. After 7 days of shaking, the degradation rate of DON was checked by using HPLC (High Performance Liquid Chromatography (HPLC) 2695, Water Company, USA). The remaining 4mL of the European wheat mixed bacteria sample was placed in the refrigerator at 4°C.

3.5.1. Extraction procedure for 10ppm sample solution

After 7 days of shaking 1mL suspended solution was taken and 5.25ml of Acetonitrile (ACN) was added to extract DON from the solution. All solution was suspended DON was purified by using Elute bound column (Romer labs) Then 4 ml of Elute solution was evaporated and dried with nitrogen gas at 60^{0}C water bath. 1ml mobile phase was added to re-dissolved, then stirring with vortex to mix well for 1 minute. After filter through 0.22µm membrane filtered sample was stored at 4^{0}c until analysis HPLC to detect DON degradation rate.

The degradation percentage was calculated by following equation: (Mengling *et al.* 2016)

$$\% \text{ Degradation} = 1 - \left(\frac{\text{sample peak area}}{\text{control peak area}} \right) \times 100$$

3.5.2. High performance liquid chromatography with ultraviolet detection

The column was used Agilent 6 TC-C18 (2) 250 mm x 4.6 mm (Agilent Technologies Inc. USA), the column temperature was maintained at 35 °C. The injection volume was 20µl with the flow rate was 1 mL/ min, and the mobile phase

consisted of ACN/Water (10:90 V:V). The running time was 20 min and wavelength were 220mm.

3.6. Sample enrichment culture in LB media

Procedure

100ml LB media (Lysogeny Broth (LB media) composition and concentration was Tryptone 10 g/L^1, Yeast extract 5 g/L^1, NaCl 10g/L^1 and water 1000ml) was taken in 300ml flask and sterilized it and 100µl original sample was added and shaking into incubator. After 1 days, 10% sample was taken into 10 ml sterilized tube and 160µl DON solution was added. The total concentration of the solution was 4ppm (160µl DON + 400µL sample +3440µl LB media= 4ml solution) and CK(control) 160µl DON into the LB media (160µl.DON+ 3840µL LB media) was prepared. All the sample was shaken into the incubator for 3 days at 200rpm, temp. 28^{0}C. After 3 days the degradation rate was checked by using HPLC.

3.6.1. Extraction procedure for 4ppm sample solution

After 3 days of shaking, 1ml sample was taken from incubation culture solution in 10ml clean sterilized tube and 2ml de-ionized water was added. The sample was mixed well by high speed vortex for 3 minutes and centrifuge at 10,000 rpm, at 4 °c for 6 minutes. The supernatant was suspended and filtered with 0.22-µm pore size filter. Then 1ml of extracted sample was passed through the DON Star TM R-Immunoaffinity column (Agilent technologies Inc. USA); allow dripping at a flow rate of 1-3 ml/min. After completed of extraction through the column, the column was rinsed with 5 ml of PBS at flow rate 1-3 ml/ml and the column was dried by the air flow carefully. Then elute with 3 ml of methanol to collected DON and dried the column by passing air through the column to remove all remain liquid. Then the eluted solution was evaporated and dried at 60°C under a gentle stream of nitrogen. Then, 1 ml mobile phase was added [10% acetonitrile (ACN)+ 900µL Deionized water] and mixed it and filtered by using vacuum filter. After completed the filtration bottle was placed into degassing machine for 15mint] to re-dissolved, then stirring with vortex to mixed well for 1 minute. Afterward the sample was

filtered by using 0.22μm membrane filter and stored at 4°C until analysis by HPLC to detect DON degradation rate with following formulation:

$$\% \text{ Degradation} = 1 - \left(\frac{\text{sample peak area}}{\text{control peak area}}\right) \times 100$$

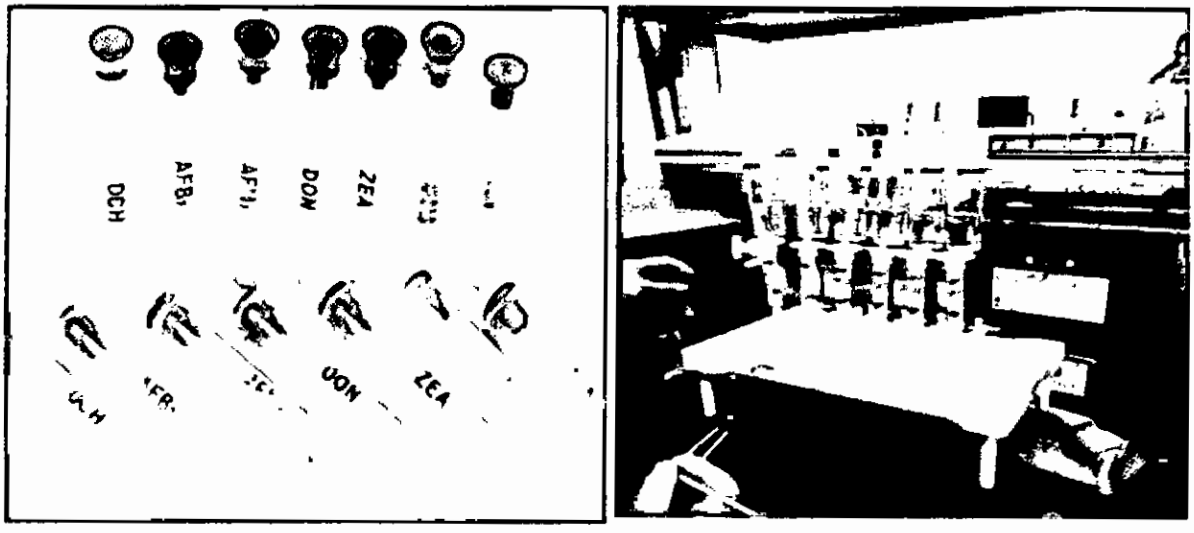

Fig. 2. DON column for Extraction.

3.7. Testing sample for best media to degrading DON

Three different media LB, PBS, MM, media was taken in 10ml sterilized tube and 10% wheat sample was taken. 160μl DON was taken from 100ppm DON solution (160μL DON+ 3440μL LB media + 400μL sample) and mixed in sterilized tube. Total concentration of the solution was 4ppm and 3 different media controls was prepared (160μl DON + 3840μL Media). The samples were shaken for 3 days into incubator at 200rpm, at 28^{0}C temperature. After 3 days the degradation rate was checked. Each treatment was replicated 3 times.

3.8. Testing sample for best time to degrading DON

The different time was checked to grow the bacteria which degraded the DON (Table.6). The degradation rate was checked by using HPLC. 10 mL sterilized tube and 10% mixed bacteria enrichment sample was taken. 160μL DON was added into the LB media and total concentration of the solution was 4ppm and also prepared CK (control) for different time. All the sample was shaking in the

incubator (at 200rpm, temp. 28^{0}C). The extraction was done and the degradation rate was checked by using HPLC.

3.9. Count the sample cell concentration in different time

Ten percent mixed bacteria enrichment wheat sample was poured in LB media. 160µl DON was taken from 100ppm DON solution. The total concentration of the sample was 4ppm and controlled also prepared. The sample was shaken in the incubator at 200rpm, at 28^{0}C temperature. The degradation rate was checked by using HPLC and the cell concentration was counted using electron microscope. 100µL sample was taken from the 1st ,3rd ,5th, 7th day shaking sample and counted the cell number using hemocytometer. The sample concentration was very high. So the sample was diluted and then used hemocytometer to count the cell concentration in different times. The equation of the cell concentration was (Amrita, 2011).

$$\textbf{Cell concentration} = \textbf{CELL NUMBER} \times 25 \times 10^4 \times \textbf{Dilution}$$

3.10. Dilution series of a sample

After the degradation percentage was checked, the dilution was done by the sample 10^{-1} to 10^{-10}. 100µl mixed bacteria dilution sample was poured 900µl LB media. 10% sample was taken from the dilution solution and 160µl mycotoxin from 100ppm DON solution was added in LB media. The total concentration of the solution was 4ppm and control treatment was also prepared similarly. The sample was shacked in incubator for 3 days at 200rpm, at 28^{0}C temperature. After 3 days the degradation percentage was checked by using HPLC.

As the result of 10^{-6} was good. 100µl sample was collected from 10^{-6} dilution. The sample was cultured on LB ager plate at 28°C for 3days. The single colony was collected randomly after 3 days in the plate from 10^{-6}dilution. The single colony was collected in 10ml sterilized tube containing 4ml fresh LB medium. The

final concentration of the sample was 4ppm (µg/ml) the sample was shaken in incubation shaken at 200rpm, 28°C for 72hr. The extraction was done after 3 days and the degradation percentage was checked by using HPLC.

3.10.1. High performance liquid chromatography with ultraviolet detection

The used Column was Agilent with 6 TC-C18 (2) 250 mm x 4.6 mm and the column temperature was maintained at 35 °C. The injection volume was 20µl with the flow rate was 1 mL/ min, and the mobile phase consisted of ACN/Water (10:90 V: V). The running time was 20 min and wavelength were 220 m.

The mixed bacteria enrichment wheat sample was diluted again using LB media (10^{-1} to 10^{-10}). 10% sample was collected from each dilution and 160µl mycotoxin from 100ppm DON solution in the LB media. So total concentration of the sample was 4ppm and the control sample was also prepared similarly. The sample was shaken in the incubator for 3 days shaking. the degradation percentage was checked after 3days by using HPLC.

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3.10.2. Isolation of single bacteria

Procedure

As 10^{-7} dilution sample had the good results. 20 LB agar plate (Tryptone 10 g/L^{1}, Yeast extract 5 g/L^{1}, NaCl 10g/L^{1}, agar 15g and water 1000ml) was used to culture the sample and randomly 200 single bacteria were picked up. The single bacteria were purified on LB ager media by streaking method.

3.11. PCR reaction to detect bacteria

Sterilized toothpick was used to dipped bacteria into the PCR tube (upstream primer 27F AGAGTTTGATCCTGGCTCAG 1µL(10µM), Downstream primer 1492R TACGGTTACCTTGTTACGACTTPCR 1µl (10µM), ddH_2O (deionized water) 9.50µl, GoTaq 12.5µl, 1µl of lysed bacterial liquid) was taken to each tube. 20µL Lysis Buffer was added. The sample was dipped into 80°C hot water bath for 15 min. 24µL of PCR reaction solution was taken and centrifuge at low speed.

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3.12. PCR amplification

PCR (Japan TAKARA Company) reaction program was done in Pre-denaturation 94°C for 5 minutes, Denaturation 94°C for 30 seconds, Annealing 55°C for 30seconds, Extension 72°C for 1 minutes and 72°Cextension for 7 minutes. That procedure was continued for 35 cycles, at 4°C preservation. Sample was sent the company for 16SrDNA detection. The genera were identified using the blast software.

3.13. Purification of 10^{-7} sample

10^{-7}sample was rechecked. 100µL sample (10%) was taken from 10^{-7} original sample and 160µL was added from 100ppm DON solutionthe sample was shaken in the incubator for 3 days at 200rpm speed and 28°C temperature. The degradation percentage was checked after 3 days by HPLC.

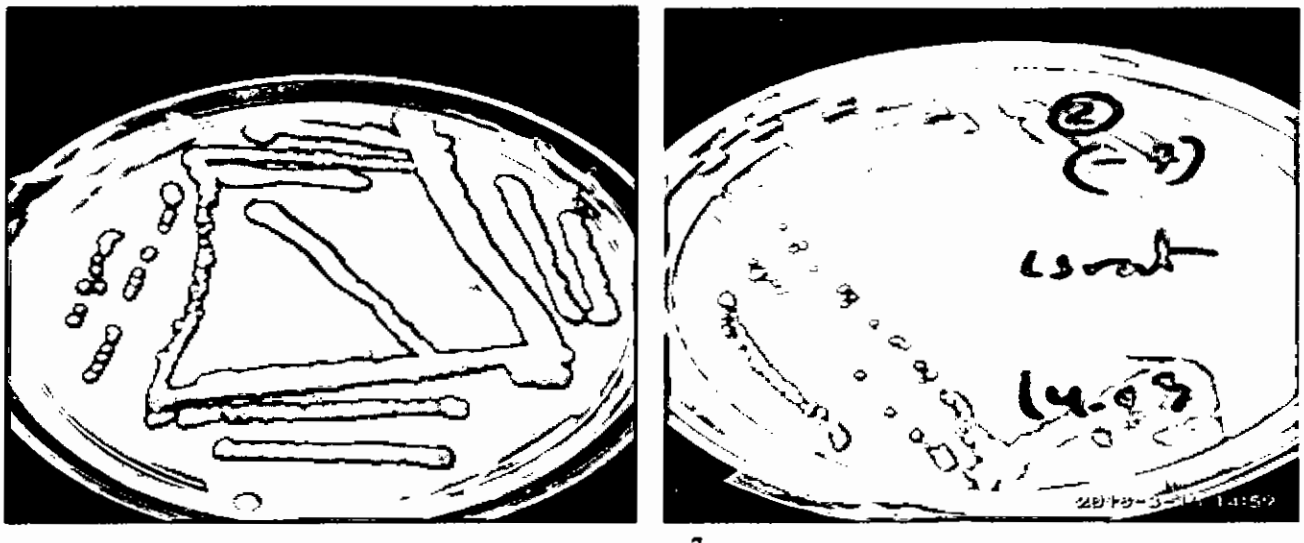

Fig. 3.Mixed bacterium on LB media (10^{-7} dilution sample)

3.14. Bacteria preservation procedure

Sterilized glycerol 30% and distilled water 70% was mixed and used for preservation. Biological Safety Cabinets was used to take 1 mL sample and 1 ml glycerol solution. The sample was kept at –80°C temperature for long time in future use.

3.15. Statistical analysis of data

The recorded data was compiled and tabulated for statistical analysis. Analysis of variance was done with the help of MSTAT-C software. The treatment means were separated by Tukey Honestly Significance Difference Test (HSD) when necessary.

CHAPTER 4

RESULTSAND DISCUSSION

An experiment was carried out in Key Laboratory of Agro-products Quality and Safety Control, Institute of Food Science and Technology, CAAS (Chinese Academy of Agricultural Sciences, Beijing, China to isolate, molecular identification and screening of deoxynivalenol (DON) degrading bacteria in European wheat sample.

Collected the sample from the lab and prepared the sample. After sample preparation adding toxin DON in two different concentration, extracted the sample and checked the degradation rate by using HPLC. After getting the good degradation percentage culture the bacteria in to the LB agar media to isolate and checked the PCR detection to know the genera. Evaluate the bacterium to detoxifying DON were done in the three different media.

Mycotoxins are secondary metabolites created by micro fungi that are capable of inflicting disease and death in humans and other animals. The virulent impact of mycotoxins on animal and human health is called as mycotoxicosis.

Deoxynivalenol (DON) is one of several mycotoxins created by certain Fusarium species that often infect corn, wheat, oats, barley, rice, and alternative grains within the field or throughout storage. Fusarium mycotoxins ar the biggest cluster of mycotoxins, which incorporates over 140 known metabolites of fungi. They're synthesized by several species of fungi, in the main by Fusarium (*F. graminearum* and *F. culmorum*). Due to the high toxicity of Fusarium mycotoxins and high incidence of the fungi species producing them, these mycotoxins belong to the foremost animal and human health endangering groups. They're abundant in cereals and their product (Yazar andOmurtag, 2008).

DON Degradation in two different media

The result showed that 90.98% of the sample was degraded by mixed bacteria in 10ppm concentration of DON solution in MM media (Table 3) and the degradation percentage was 72.61% in 4ppm concentration (Table 4). Using LB media the degradation percentage was found 41.2% on an average at 4ppm concentration. (Table 5). In the MM media toxin detoxification percentage was higher than the LB media.

In this experiment, three culture media were used. LB media, MM media and PBS media were evaluated, and results showed. LB media had a positive impact on the activity of the bacteria and these results showed some acquaintance with Sambrook *et al.* (1989) in which both found that LB media was an excellent media for the culturing of *E. coli* as LB media has peptone and yeast extract which was suitable for the growth of bacteria. Mineral salt media was suitable media for *E. coli* during the cloning, DNA production and recombinant proteins.

For the extraction of DON, DON Star Immunoaffinity column was used and it was found that DONStar proved to be the best extraction column for DON. These results had significant relationship with Gilbert *et al.* (2010) in which they expressed that Immunoaffinity column was the best tool for accurate extraction of mycotoxins and they had gained popularity worldwide in the field of mycotoxicology. Due to its high sensitivity to toxin and having related antibodies they can bind the toxin with itself achieving accurate result both for simple and complex mycotoxin samples.

Analysis of best time of sample to degrading DON

The sample degradation percentage was checked by using HPLC of different media in different times. Every 18hr, 36hr, 54hr, 72hr checked the cell growth of the mixed bacteria which degrade the DON. The analysis of the different time showed that best time to degrade DON was 54hr.

Table 3. Degradation percentage of DON at 10ppm in European wheat sample using MM media in 10ppm concentration

Sample	Media	Degradation %	Mean	Concentration
European wheat sample	MM	98.96	90.98	10pmm
	MM	88.50		10ppm
	MM	85.50		10pmm

Table 4. Degradation percentage of DON at 10ppm in European wheat sample using MM media in 4ppm concentration

Sample	Media	Degradation %	Mean	Concentration
European wheat sample	MM	70.63	72.61	4pmm
	MM	66.70		4pmm
	MM	80.50		4ppm

Table 5. Degradation of DON at 4ppm in European wheat sample using LB media

Sample	Media	degradation	Mean	Concentration
European wheat sample	LB	38.90	41.3	4ppm
	LB	43.50		4ppm
	LB	41.50		4pmm

Table 6. Different time to percent DON degradation.

Time (hrs.)	Mean Degradation (%)
18	9.73
36	27.01
54	37.24
72	36.10

Analysis of cell concentration

Hemocytometer was used to count the cell concentration determining accordingly in the 7days using mixed bacteria sample on LB media (Table 7). Spectrophotometer was used to count the CFU of the sample, the cell concentration was 3×10^8 cfu/mL. The sample maintained the pH 7. Miller *et al.* (1998) confirmed that pH of 6.5 and temperature of 28°C was optimum for the efficient growth of bacteria. Alexander (1999) found that biodegradation via bacteria normally occurred at neutral pH (pH=7). Tyagi (1991) and Tano-Debrah *et al.* (1999) also determined the pH range of 6.5 to 8.0 for the active degradation in a biological system as most of bacteria grow at neutral pH.

Analysis to best media

Analysis of statistical data was used Tukey HSD All-Pairwise Comparisons in bacteria 3 different media to analysis the best media to grow the bacteria which degrade DON. MM media was showed 73.16% degradation in 4ppm DON concentration to grow the bacteria. (Table 8). Fig 5. showed that the MM media was significantly different between the LB media, but PBS media was same effect in MM and LB media. Analysis of variance showed that MM media had significance behavior in responses to degradation of DON as compare to control and other media.

Table 7. Cell concentration of different bacteria of different time of incubation

Sample	Culture media	Temperature (°C)	pH	Incubation period(day)	Cell concentration(ml)
European wheat sample	LB	28	7	1	3×10^8
	LB	28	7	3	1.6×10^8
	LB	28	7	5	2.8×10^8
	LB	28	7	7	1.8×10^8

Table 8. Analysis of Variance Best Media to Grow the Bacteria to Degradation of DON

Source of Variation	Degree of Freedom	Sum of Squares	Mean Sum of Squares	F-Value	P-Value
Replication	3	234.15	117.07		
Media	3	8110.58	2703.53	13.08	0.0048*
Error	6	1240.53	206.75		
Total	11	9585.26			

* 0.05% level of Significant

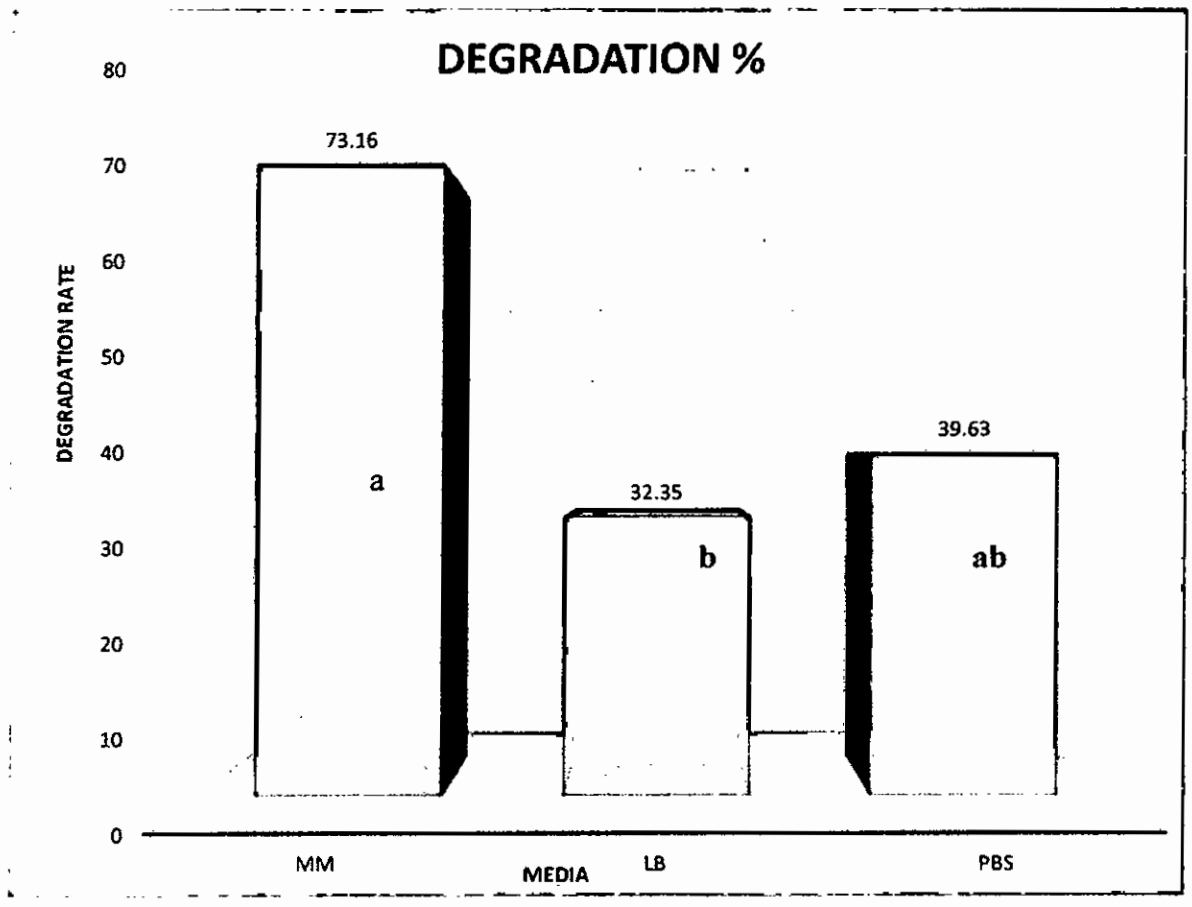

Fig. 4. Percentage of degradation of DON in different growth media

Analysis of dilution series and molecular Identification of mixed sample

The dilution was done because of finding the degradation single bacteria. The dilution series of the sample 10^{-7}, showed that 78.43% degradation of DON (Table 9). Culture the sample to getting the single bacteria which were responsible for degradation the DON. Randomly selected the 200 single bacteria and the PCR was done. After the PCR, blast software was used. The result of the blast software showed that lot of bacterium were found. Table 11 showed single bacterium were identified *Pseudomonas* spp, *Agrobacterium* sp, *Zobellella* sp, *Bacillus* sp., *Achromobacter* sp, *Advenella* sp, *Cupriavidus* sp. All of these bacteria showed the lowest rate of DON degradation when analysis. Fig.6 showed the dilution result of mixed bacteria which degrade the DON while using LB media.

Zhou and gang, 2008; He *et al.*, 2010, Awad *et al.*, 2010 and Karlovsky, 2011 reported that 'Several microorganisms was found from various sources such as soils, animal guts, and plants which had the ability to degrade DON' and Shima *et al.*, 1997 found that '*Agrobacterium–Rhizobium* strain E3-39 was obtained from soil samples by an enrichment culture procedure'. Völk *et al.* 2004 reported that 'The strain E3-39 could oxidize the 3-OH group of DON to generate 3-keto-4-deoxynivalenol (3-keto-DON), which exhibited a remarkably decreased immunosuppressive toxicity relative to DON. Among 1,285 microbial mixed cultures obtained from farmland soils, cereal grains, insects and other sources, screened one mixed culture from spontaneously infected minimal medium with protein and glucose (MMGP), able to transform DON into 3-keto-DON'.

In the present study six different bacterial genera were isolated and identified based on molecular analysis (Table 11) But their combined efficacy to degrade DON was higher in compare to single use of the bacteria to degrade DON.

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Table 9. DON degradation rate of 10^{-7}dilution bacteria in LB media

Sample	% Degradation
10^{-7}	70.48
10^{-7}(recheck)	39.05

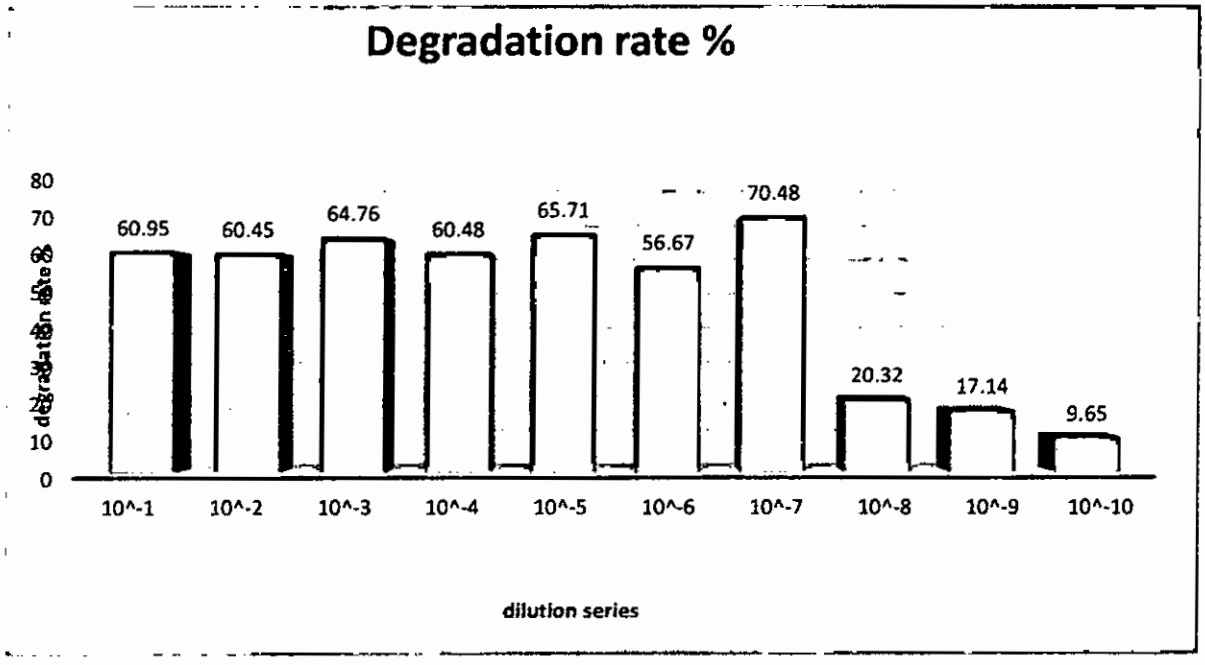

Fig. 5. Degradation of DON using dilution series 10^{-1}-10^{-10} on LB media

Table 10. Showed degradation rate of randomly selected single bacteria from 10^{-7}culture plate

Sample	Concentration of DON	Retention time	Peak area	Degradation %	Bacterial isolation
Standard	4ppm	10.96	97.13	-	
C_K	4ppm	11.025	28.57	-	
10^{-7}-1	4ppm	11.027	28.39	6%	*Pseudomonas* spp
10^{-7}-2	4ppm	11.024	27.45	7%	*Zobellella sp*
10^{-7}-3	4ppm	11.024	33.31	1%	*Bacillus sp*
10^{-7}-4	4ppm	11.024	33.31	1%	*Cupriavidus sp*
10^{-7}-5	4ppm	11.028	33.78	3%	*Achromobacter* sp
10^{-7}-6	4ppm	11.027	33.05	4%	*Agrobacterium* sp
10^{-7}-7	4ppm	11.027	33.78	3%	*Advenella* sp

Table 11. DNA sequence of identified bacteria by using colony PCR of 16SrDNA amplification (sample 10^{-7})

Serial number	DNA Sequence	Name of genera
1	GTCCCCCCGAAGGTTAGACTAGCTACTTCTGGAGCACCCACTCCCATGGTGTGACG GGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGACATTCTGATTCACGATTA CTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGGACTACGATCG GTTTTATGGGATTAGCTCCACCTCGCGGCTTGGCAACCCTTTGTACCGACCATTGTA GCACGTGTGTAGCCCTGGCCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTT CCTCCGGTTTGTCACCGGCAGTCTCCTTAGAGTGCCCACCCGAGGTGCTGGTAACTA AGGACAAGGGTTGCGCTCGTTACGGGACTTAACCCAACATCTCACGACACGAGCTG ACGACAGCCATGCAGCACCTGTGTCTGAGTTCCCGAAGGCACCAATCCATCTCTGG AAAGTTCTCAGCATGTCAAGGCCAGGTAAGGTTCTTCGCGTTGCTTCGAATTAAAC CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCG GCCGTACTCCCCAGGCGGTCAACTTAATGCGTTAGCTGCGCCACTAAAATCTCAAG GATTCCAACGGCTAGTTGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCT GTTTGCTCCCCACGCTTTCGCACCTCAGTGTCAGTATCAGTCCAGGTGGTCGCCTTC GCCACTGGTGTTCCTTCCTATATCTACGCATTTCACCGCTACACAGGAAATTCCACC ACCCTCTACCGTACTCTAGCTCGCCAGTTTTGGATGCAGTTCCCAGGTTGAGCCCGG GGCTTTCACATCCAACTTAACGAACCACCTACGCGCGCTTTACGCCCAGTAATTCCG ATTAACGCTTGCACCCTTCGTATTACCGCGGCTGCTGGCACGAAGTTAGCCGGTGCT TATTCTGTTGGTAACGTCAAAACAGCAGGGTATTAGCCAACAGCCCTTCCTCCCAA CTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCA GGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCG TGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCTT GGTGAGCCATTACCTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCA AGGCCCGAAGGTCCCCTGCTTTCTCCCGTAGGACGTACGCGGTATTAGCGTTCCTTT CGGAACGTTATCCCCCACTACCAGGCAGATTCCTAGGCATTACTCACCCGTCCGCC GCTAAATCA	*Pseudomonas* sp.

2	AGCTATCTACTTCTGGAGCACCCACTCCCATGGTGTGACGGGCGGTGTGT ACAAGGCCCGGGAACGTATTCACCGCAACATTCTGATTTGCGATTACTAG CGATTCCGACTTCACGGAGTCGAGTTGCAGACTCCGATCCGGACTACGAC GCGCTTTTTGGGATTCGCTTACCATCGCTGGTTCGCCGCCCTCTGTACGC GCCATTGTAGCACGTGTGTAGCCCTACCCGTAAGGGCCATGATGACTTGA CGTCGTCCCCACCTTCCTCCGGTTTATCACCGGCAGTCTCCTTTGAGTTC CCGACCGAATCGCTGGCAACAAAGGACAAGGGTTGCGCTCGTTGCGGGAC TTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGT ATGTAAGTTCCCGAAGGCACCCATCCATCTCTGGAAAGTTCTTACTATGT CAAGGGTAGGTAAGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCC ACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCC GTACTCCCCAGGCGGTCAACTTAACGCGTTAGCTCCGGAACCCGCGCTCA AATGGCACAGACTCCAAGTTGACATCGTTTACAGCGTGGACTACCAGGGT ATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTTG GCCAGGGGGCCGCCTTCGCCACTGGTATTCCTTCCGATCTCTACGCATTT CACCGCTACACCGGAAATTCTACCCCCCTCTCCAAGACTCTAGCCTGCCA GTTCCAAATGCAGTTCCCAGGTTGAGCCCGGGGCTTTCACATCTGGCTTA ACAGACCGCCTGCGTGCGCTTTACGCCCAGTCATTCCGATTAACGCTTGC ACCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCCGGTGCTTCTTC TGTGGGTAACGTCACAGCAAGTGGGTATTCGCCACTCACCTTTCCTCCCC ACTGAAAGTGCTTTACAACCCGAAGGCCTTCTTCACACACGCGGCATGGC TGCATCAGGGTTTCCCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGT AGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGGTCATCCTCTCAGA CCAGCTAGAGATCGTCGCCTTGGTGAGCCATTACCTCACCAACCAGCTAA TCTCACTTGGGCTCATCCAATCGCGCAAGGCCCGAAGGTCCCCTGCTTTC CCCCGTAGGGCGTATGCGGTATTAGCCGTCGTTTCCAACGGTTATCCCCC TCGACCGGGCAGATACCCAAGCCTTACTCACCCGTCCGCCGCTCGTCAGC AAAGTAGCAAGCTACCCTCTGTTACCGCTCGAC	*Zobellella* sp

3	CCAACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATT CACCGCAGCATGCTGATCTGCGATTACTAGCGATTCCAACTTCATGCACT CGAGTTGCAGAGTGCAATCCGAACTGAGATGGCTTTTGGAGATTAGCTCG ACATCGCTGTCTCGCTGCCCACTGTCACCACCATTGTAGCACGTGTGTAG CCCAGCCCGTAAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCTC GGCTTATCACCGGCAGTCCCCTTAGAGTGCCCAACTAAATGCTGGCAACT AAGGGCGAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACA CGAGCTGACGACAGCCATGCAGCACCTGTTCTGGGGCCAGCCTAACTGAA GGACAATGTCTCCACTGCCCAAACCCCGAATGTCAAGAGCTGGTAAGGTT CTGCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCC CCGTCAATTCCTTTGAGTTTTAATCTTGCGACCGTACTCCCCAGGCGGAA TGTTTAATGCGTTAGCTGCGCCACCGAACAGTATACTGCCCGACGGCTAA CATTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTC CCCACGCTTTCGCACCTCAGCGTCAGTAATGGACCAGTAAGCCGCCTTCG CCACTGGTGTTCCTCCGAATATCTACGAATTTCACCTCTACACTCGGAAT TCCACTTACCTCTTCCATACTCAAGATACCCAGTATCAAAGGCAGTTCCA GAGTTGAGCTCTGGGATTTCACCCCTGACTTAAATATCCGCCTACGTGCG CTTTACGCCCAGTAATTCCGAACAACGCTAGCCCCCTTCGTATTACCGCG GCTGCTGGCACGAAGTTAGCCGGGGCTTCTTCTCCGGATACCGTCATTAT CTTCTCCGGTGAAAGAGCTTTACAACCCTAAGGCCTTCATCACTCACGCG GCATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCCCACTGCTGC CTCCCGTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCTGATCATCC TCTCAGACCAGCTATGGATCGTCGCCTTGGTAGGCCTTTACCCCACCAAC TAGCTAATCCAACGCGGGCCAATCCTTCCCCGATAAATCTTTCCCCCGTA GGGCGTATGCGGTATTAATTCCAGTTTCCCGGA	*Agrobacterium* sp.

4	ATCGCCCCCCTTGCGGTTAGGCTAACTACTTCTGGTAAACCCACTCCCAT GGTGTGACGGGCGGTGTGTACAAGACCCGGGAACGTATTCACCGCGACAT GCTGATCCGCGATTACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGA CTGCGATCCGGACTACGATCGGGTTTCTGGGATTGGCTCCCCCTCGCGGG TTGGCGACCCTCTGTCCCGACCATTGTATGACGTGTGAAGCCCTACCCAT AAGGGCCATGAGGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACC GGCAGTCTCATTAGAGTGCCCTTTCGTAGCAACTAATGACAAGGGTTGCG CTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCC ATGCAGCACCTGTGTTCCGGTTCTCTTGCGAGCACTTCCAAATCTCTTCG GAATTCCAGACATGTCAAGGGTAGGTAAGGTTTTTCGCGTTGCATCGAAT TAATCCACATCATCCACCGCTTGTGCGGGTCCCCGTCAATTCCTTTGAGT TTTAATCTTGCGACCGTACTCCCCAGGCGGTCAACTTCACGCGTTAGCTG CGCTACTAAGGCCCGAAGGCCCCAACAGCTAGTTGACATCGTTTAGGGCG TGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGTGCATG AGCGTCAGTGTTATCCCAGGAGGCTGCCTTCGCCATCGGTGTTCCTCCGC ATATCTACGCATTTCACTGCTACACGCGGAATTCCACCTCCCTCTGACAC ACTCTAGCCCGGTAGTTAAAAATGCAGTTCCAAAGTTGAGCTCTGGGATT TCACATCTTTCTTTCCGAACCGCCTGCGCACGCTTTACGCCCAGTAATTC CGATTAACGCTTGCACCCTACGTATTACCGCGGCTGCTGGCACGTAGTTA GCCGGTGCTTATTCTGCAGGTACCGTCAGTTTCACGGGGTATTAACCCAT GACGTTTCTTTCCTGCCAAAAGTGCTTTACAACCCGAAGGCCTTCATCGC ACACGCGGGATGGCTGGATCAGGGTTTCCCCCATTGTCCAAAATTCCCCA CTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTG GTCGTCCTCTCAAACCAGCTACGGATCGTCGCCTTGGTGAGCCGTTACCC CACCAACTAGCTAATCCGATATCGGCCGCTCTAATAGTGCAAGGTCTTGC GATCCCCTGCTTTCCCCCGTAGGGCGTATGCGGTATTAGCTACGCTTTCG CGTAGTTATCCCCCGCTACTAGGCACGTTCCGATACATTACTCACCCGTT CGCCACTCGCCACCAGACCGAAGTCCGTGCTGCCGTTCGACT	*Achromobacter* sp.

5	ATCGCCCTCCTTGCGGTTAGGCTACTACTTCTGGCAAACCCACTCCCATGGTGTGAC GGGCGGTGTGTACAAGACCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATT ACTAGCGATTCCAGCTTCACGTAGTCGAGTTGCAGACTACGATCCGGACTACGATG CGTTTTCTGGGATTGGCTCCCCCTCGCGGGTTGGCAACCCTCTGTACGCACCATTGT ATGACGTGTGAAGCCCTACCCATAAGGGCCATGAGGACTTGACGTCATCCCCACCT TCCTCCGGTTTGTCACCGGCAGTCTCTCTAGAGTGCTCTTGCGTAGCAACTAAAGAC AAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGAC AGCCATGCAGCACCTGTGTCCACTTTCCCTTTCGGGCACCTGATGCATCTCTGCTTC GTTAGTGGCATGTCAAGGGTAGGTAAGGTTTTTCGCGTTGCATCGAATTAATCCAC ATCATCCACCGCTTGTGCGGGTCCCCGTCAATTCCTTTGAGTTTTAATCTTGCGACC GTACTCCCCAGGCGGTCAACTTCACGCGTTAGCTACGTTACTGAAGAAATGAATCC CCAACAACTAGTTGACATCGTTTAGGGCGTGGACTACCAGGGTATCTAATCCTGTTT GCTCCCCACGCTTTCGTGCATGAGCGTCAGTGACGTCCCAGGGGGCTGCCTTCGCC ATCGGTATTCCTCCACATCTCTACGCATTTCACTGCTACACGTGGAATTCTACCCCC CTCTGACATACTCTAGCCTTGCAGTCACAAGCGCCATTCCCAAGTTGAGCTCGGGG ATTTCACGCCTGTCTTACAAAACCGCCTGCGCACGCTTTACGCCCAGTAATTCCGAT TAACGCTCGCACCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGGTGCTTA TTCTTCCGGTACCGTCATCCCCCCGCCGTATTAGGGCAGAGGATTTCTTTCCGGACA AAAGTGCTTTACAACCCGAAGGCCTTCTTCACACACGCGGCATTGCTGGATCAGGG TTGCCCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGT CTCAGTCCCAGTGTGGCTGATCGTCCTCTCAGACCAGCTACTGATCGTCGCCTTGGT GGGCCTTTACCCCACCAACTAGCTAATCAGACATCGGCCGCTCCTATCGCGCGAGC CTTGCGGTCCCCCGCTTTCACCCTCAGGTCGTATGCGGTATTAGCTAATCTTTCGAC TAGTTATCCCCCACGACAGGGCACGTTCCGATGTATTACTCACCCGTT CGCCACTCGCCACCAGGAT	*Cupriavidus* sp.

6	AAAGGTTACCCCACCGACTTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTG TGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGA TTCCAGCTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGAGAACGGTTTTAT GAGATTAGCTCCACCTCGCGGTCTTGCAGCTCTTTGTACCGTCCATTGTAGCACGTG TGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGT TTGTCACCGGCAGTCACCTTAGAGTGCCCAACTTAATGATGGCAACTAAGATCAAG GGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAAC CATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCTCTAGGGTTTTCA GAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCT CCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGCCTTGCGGCCGTACT CCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAAGGGCGGAAACCCTCTA ACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCT CCCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTG GTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTT CTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCA CATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACG CTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGT TAGGTACCGTCAAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAG AGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCG TCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAG TCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCC GTTACCTCACCAACTAGCTAATGCGACGCGGGTCCATCCATAAGTGACAGCCGAAG CCGCCTTTCAATTTCGAACCATGCGGTTCAAAATGTTATCCGGTATTAGCCCCGGTT TCCCGGAGTTATCCCAGTCTTATGGGCAGGTTACCCACGTGTTACTCACCCGTCCGC CGCTAACTTCATAAGAGCAAGCTCT	*Bacillus* sp.

7	TCGCCCCCCTTGCGGTTAGGCTAACTACTTCTGGTAAACCCACTCCCATGGTGTGAC GGGCGGTGTGTACAAGACCCGGGAACGTATTCACCGCGACATGCTGATCCGCGATT ACTAGCGATTCCGACTTCATGCAGGCGAGTTGCAGCCTGCAATCCGGACTACGATC GGGTTTATGAGATTAGCTCCACCTTGCGGTTTGGCAACCCTCTGTCCCGACCATTGT ATGACGTGTGAAGCCCTACCCATAAGGGCCATGAGGACTTGACGTCATCCCCACCT TCCTCCGGTTTGTCACCGGCAGTCTCATTAGAGTGCTCAACTAAATGTAGCAACTAA TGACAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGA CGACAGCCATGCAGCACCTGTGTTCCGGTTCTCTTGCGAGCACTCCTAAATCTCTTC AGGATTCCAGACATGTCAAGGGTAGGTAAGGTTTTTCGCGTTGCATCGAATTAATC CACATCATCCACCGCTTGTGCGGGTCCCCGTCAATTCCTTTGAGTTTTAATCTTGCG ACCGTACTCCCCAGGCGGTCAACTTCACGCGTTAGCTGCGCTACTAAGCCCCGAAG GGCCCAACAGCTAGTTGACATCGTTTAGGGCGTGGACTACCAGGGTATCTAATCCT GTTTGCTCCCCACGCTTTCGTGCATGAGCGTCAGTATTATCCCAGGGGGCTGCCTTC GCCATCGGTGTTCCTCCACATATCTACGCATTTCACTGCTACACGTGGAATTCCACC CCCCTCTGACATACTCTAGTTCGGTAGTTAAAAATGCAGTTCCAAGGTTGAGCCCTG GGATTTCACATCTTTCTTTCCGAACCGCCTGCGCACGCTTTACGCCCAGTAATTCCG ATTAACGCTTGCACCCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGGTGCT TATTCTTCAGGTACCGTCATCAGTTCCGGGTATTATCCGAAACCTTTTCTTCCCTGAC AAAAGTGCTTTACAACCCGAAGGCCTTCATCGCACACGCGGGATGGCTGGATCAGG GTTTCCCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTG TCTCAGTCCCAGTGTGGCTGGTCGTCCTCTCAAACCAGCTACGGATCGTCGCCTTGG TAGGCCTTTACCCCACCAACTAGCTAATCCGATATCGGCCGCTCCAATAGTGAGAG GTCCTAAGATCCCCCCCTTTCCCCCGTAGGGCGTATGCGGTATTAGCCACGCTTTCG CGTAGTTATCCCCCGCTACTGGGCACGTTCCGATACATTACTCACCCGTTCGCCACT CGCCGGCAAAAGTAGCAAGCTACTTCCCGCTGCCGTTCGACTG	*Advenella* sp.

Mechanism of DON degradation

Mycotoxins are toxic secondary metabolites that are produced by toxigenic molds and may contaminate different cereal grains (Goswami *et al.*2004). Deoxynivalenol (DON), also known as vomitoxin, is mainly produced by the *Fusarium graminearum* species that can infect crops and because devastating diseases called *Fusarium* head blight (FHB) or scab (Alexander *et al*, 2011). DON is an inhibitor of protein, DNA, and RNA synthesis at the molecular level, and exerts toxic potential to plants, animals, and humans (Pestka *et al* 2010) The functions of DON in interactions of *F. graminearum* and other organisms have been studied before. As a crucial secondary metabolite in development of *F. graminearum*, DON is beneficial for *Fusarium* to deal with a complex environment and compete with other organisms (Audenaert *et al.* 2013). *Fusarium* may utilize DON to disrupt plant defense system in the infection process. DON is regarded as a virulence factor when infecting plants, and it can facilitate disease spread in infected plant tissues. Genetic studies have proved that DON non-producing *Fusarium* mutants showed less virulence on crops than wild-type strains (Bai *et al.* 2002). On the other hand, plants usually have the self-defense mechanisms to cope with mycotoxins, such as conjugating them with endogenous metabolites to fewer toxic products. An important detoxification process reducing the toxicity of DON in plants is glycosylation catalyzed by a special kind of glycosyltransferases (Poppenberge *et al*, 2003), and a common detoxification product of DON is deoxynivalenol-3-glucoside (D3G). This conjugated mycotoxin was originally termed as a masked mycotoxin, because its structure has been changed and it may escape routine detection by conventional analytical methods (Berthiller 2007)

The biological detoxification of mycotoxins including deoxynivalenol (DON), represents a very promising approach to address the challenging problems of cereal grain contamination. The recent discovery of Devosia mutants 17-2-E-8, a bacterium isolates capable of transforming DON to the non-toxic stereoisomer 3-epi-deoxynivalenol, along with earlier reports of bacteria; species capable of

oxidizing DON to 3-keto-DON, has generated interest in the possible mechanism and enzyme(s) involved. It was previously shown that DON epimerization proceeds through a two-step biocatalysis. Carer *et al* (2017) described that the identification of the first enzymatic step in this pathway. The enzyme, a dehydrogenase responsible for the selective oxidation of DON at the C3 position, was shown to readily convert of DON at the C3 position was shown to readily convert DON to 3-keto-DON, a less toxic intermediate in the DON epimerization pathway. Furthermore, this study provides insights into the PQQ dependence of the enzyme. This enzyme may be part of feasible strategy for DON mitigation within the near future.

Management of DON production by Trichoderma strains as a biological control - based strategy has drawn great attention recently. Tians *et al* (2016) evaluated eight selected Trichoderma strains for their antagonistic activities on *F. graminearum* by dual culture on potato dextrose agar (PDA) medium. As potential antagonists, Trichoderma strains showed prominent inhibitory effects on mycelial growth and mycotoxins production of *F. graminearum.* In addition, the modified mycotoxins deoxynivalenol-3-glucosides(D3G), which was once regarded as a detoxification product of DON in plant defense, was detected when Trichoderma were confronted with *F. graminearum.* The occurrence of D3G in *F. graminearum* and Trichoderma interaction was reported for the first time, and these findings provide evidence that Trichoderma strains possess a self-protection mechanism as plants to detoxify DON into D3G when competing with *F. graminearum.*

The most frequently encountered *Fusarium* mycotoxins in FHB in Europe has proved to be deoxynivalenol and zearalenone produced by *F. graminearum* and *F. culmorum,* respectively. Several Fusarium species are widespread pathogens on small-grain cereals around the world, including all infected plants still standing in the fields, or in stored grain. Mycotoxins in wheat and barley, which constitute almost two-thirds of the world production of small-grain cereals and almost 80% of the European small-grain production, are causing great concern, because of the extent of infection and contamination of food products. (Bottalico, 1998).

Mycotoxin contamination occurs widely in feedstuff of plants origin, especially in cereals, fruits, hazelnuts, almonds, seeds, fodder and other agricultural feed or food intended for animal or human consumption (Guan *et al.*, 2011). Deoxynivalenol (DON) is the most prevalent trichothecene mycotoxin secreted from fusarium species in the world. This compound can infect monocotyledon-based crops such as maize, wheat, and barley (Rasmussen *et al.*, 2003; Yazar and Omurtag, 2008).

Human exposure to DON occurs primarily from oral consumption of food prepared from DON-contaminated grains or occupational inhalation exposure while handling DON-contaminated crops or grains. Human foods and animal feeds have high incidences of DON contamination based on survey data worldwide (Streit *et al.*, 2013; Schatzmayr and Streit, 2013; Pereira *et al.*, 2014).

Many countries have already established limits for DON levels in the European Union (EU), the limits for DON in wheat are 0.2mg Kg^{-1} for baby food, 0.5mg Kg^{-1} for bread and pastries, 0.75mg Kg^{-1} for pasta, and 1.25mg Kg^{-1} for unprocessed cereals (Cheli *et al.*, 2014). Thus, to avoid health risks, DON levels must be estimated before the cereals are processed and incorporated to humans. Moreover, mycotoxins lead to huge economic losses annually including loss of human and animal life.

CHAPTER 5

Summary and conclusion

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The experiment was conducted at, Key Laboratory of Agro-products Quality and Safety Control in the Institute of Food Science and Technology, CAAS (Chinese Academy of Agricultural Sciences, Beijing, China to isolate, identification and screening DON degrading bacteria in European wheat samples.

In this experiment, after the sample preparation, 3 media MM, LB, PBS were used which helps to increase the bacteria growth. PBS actually a buffer media which enhanced the bacteria growth. MM media found 93% degradation and LB media showed 41.2% degradation of DON.

DON concentration was maintaining 4ppm. In the 10ppm concentration bacterial growth was slow. The climate condition was also responsible for growth the bacterium. pH was maintaining in 7. Most of the bacteria were grown well in pH 6.6 or 7. In the incubation period most of the time the temperature was maintained 28°C which washelped to bacterium growth rapidly.

In the dilution series of 10^{-6}, 10^{-7}, was found 50.63 and 70.48% degradation percentage was found respectively. So, in this experiment used 10^{-7} sample to identify the single bacteria which degraded the DON.

The blast softwareUsed,After the PCR was done. The result of the blast software showed that single bacterium were *Pseudomonas* spp,*Agrobacterium* sp,*Zobellella* sp, *Bacillus* sp., *Achromobacter* Sp., *Advenella* sp., and *Cupriavidus* sp.All of these bacteria were degrading DON in 6, 4, 7, 1, 4, 3 and 1%, respectively which was very low.

So that in this experiment it has been concluded that in mixed bacteria culture degradation rate has higher than the single bacteria culture. The findings of the present study emphasized the combined application of bacteria to degrade DON rather than their single application.

CHAPTER 7

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