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# Plasmid-mediated Detoxification of Mycotoxin Zearalenone in *Pseudomonas* Sp. ZEA-1

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**Abstract:** The *Pseudomonas* sp. Strain ZEA-1 was isolated from rhizosphere of corn plant by an enrichment technique showed capability of utilizing zearalenone as the sole source of carbon. The bacterium rapidly utilized zearalenone beyond 200 μg/ml and showed prolific growth in a minimal medium containing 100 μg/ml zearalenone source. The course of ZEA degradation as well as the formation of its metabolites was observed by UV Spectrophotometer and thin layer chromatography analysis. Toxicity of biotransformation products of zearalenone was tested in a toxicity assay with the shrimp *Artemia salina*. Residual toxicity of these metabolites against *A.salinia* was reduced. The wild type strain ZEA-1 transferred zearalenone degradation property to *E.coli* strain. The transconjugant harboured a plasmid of the same molecular size (approximately 120 Kb) as that of donor strain, while cured strain was plasmidless and did not utilize zearalenone.

**Key words:** Zearalenone, biotransformation, *E. coli*, *Pseudomonas* sp. *Artemia salin*

## INTRODUCTION

Zearalenone (ZEA) is a Mycotoxin produced mainly by fungi belonging to the genus *Fusarium* in foods and feeds [22]. Zearalenone is a resorcyclic acid lactone, chemically described as 6-[10-hydroxy-6-0x0-trans-1-undecenyl]-B-resorcylic acid lactone (Fig. 1).

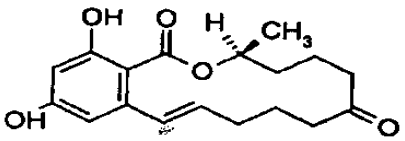


Fig 1: Structure of zearalenone mycotoxin used in this study

It is frequently implicated in reproductive disorders of farm animals and occasionally in hyperestrogenic syndromes in humans [17]. When fed to animals, ZEA exhibits potent estrogenicity in livestock, especially in female swine, and causes severe reproductive problem [19]. Regarding carcinogenicity, ZEA was reported to have carcinogenicity effect in mice [1]. Furthermore, ZEA was also reported to bind to human estrogen receptors [17] and to stimulate the growth of the human breast cancer cell lines [15]. The biotransformation for ZEA in animals involves the formation of two metabolites α–zearalenoland β–zearalenol which are subsequently conjugated with glucuronic acid. Due to economic losses engendered by ZEA and its impact on human and animal health, several strategies for detoxifying contaminated foods and feeds have been described in the literature including physical, chemical and biological process [28].

Several studies on the degradation and biotransformation of zearalenone by various microorganisms have been published. El-sharkawy *et al.* [7] investigated the transformation of ZEA by several species of microorganisms. The metabolites formed included α-zearalenol and β-zearalenol and other polar metabolite, zearalenone-4-O-sulphate. When ZEA was incubated with *Saccharomyces* strains [2], rumen and Pig microflora in vitro, it was also reduced to α-zearalenol and β-zearalenol [12 ,13]. In addition, the products of the metabolism of ZEA by the tested microorganisms were more toxic or as toxic as the parent compound. The ability of a mixed culture of bacteria to degrade completely ZEA from culture media was also reported by Meharaj *et al.* [16], HPLC and ElISA analysis of culture extracts revealed no ZEA or ZEA-like products. Takahashi-Ando *et al.*[25] identified and characterized a lactonohydolase enzyme in fungus *Clonostachys rosea* which convert ZEA to a less oestrogenic compound. Recently, ZEA was found to be completely degraded by several *Rhizopus* isolates including *R.stolonifer*, *R.oryzae* and *R. microsporus* strain [27], but further studies are needed for identification of ZEA-degrading enzymes in *Rhizopus* isolates. Molnar *et al.* [20] described a new yeast strain, *Trichosporon mycotoxinivorans*, able to degrade ZEA

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to carbon oxide and other non-toxic metabolites, neither α– and β –zearalenol were detected. Recently, Mokoena *et al.* [21] reported that lactic acid bacteria fermentation can significantly reduce the concentration of ZEA in maize by 68-75% in fourth days of fermentation. However, such a reduction may not significantly alter the possible toxic effects of the toxin.

Many xenobiotic degradation genes present in soil bacteria have been shown to reside on plasmid, a common location for other degradation genes. Degradation genes have cloned and identified in fungus, *Clonostachys rosea* [25]. for zearalenone transformation .However, no genes involved in the breakdown of zearalenone born on bacterial plasmid have been reported.

The purpose of this study was to investigate the role of plasmid in the zearalenone detoxification/transformation

## MATERIALS AND METHODS

**Chemicals:** Mycotoxin, Zearalenone, α-zearalenol and β-zearalenol were purchased from Sigma Chemical Company (St. Louis, MO). All chemicals were at least of analytical-reagent.

Table 1: Bacterial strains and plasmids used in this study

| Strains | Plasmids and comment | Phenotype | Source |
|---|---|---|---|
| *Pseudomonas* sp.. ZEA-1 | Wild type – ZEA - degrading, pZEA-1 | $ZEA^+$ | This study |
| *Pseudomonas* sp. ZEA-1M | pZEA-1-Cured derivative of pZEA-1 | $ZEA^-$ $Nal^r$ | This study |
| *Escherichia coli* AD256 | Recipient strain (plasmidless,$ZEA^-$) | RecA56srl C300: : Tn10; $Tet^r$ | Ghai and Das 1989 |
| *Escherichia coli* AD256 Transconjugant | Transconjugants between ZEA-1 and *E.coli* AD256,pZEA-1, | $ZEA^+$ $Tet^r$ | This study |

**Bacterial strains, plasmids, and media:** Bacterial strain and plasmids were shown in Table 1. *Escherichia coli* utilized was AD256 (RecA56 srl C300: : Tn10; $Tet^r$) [8]. Characterization of natural soil isolate *Pseudomonas* ZEA-1 has been described previously [5]. *E. coli* strains harboring plasmid were grown on L-broth or on L-agar plates. with appropriate antibiotic selection (tetracycline 15 μg/ml). Selection minimal salt medium (MSM) utilizing 100 μg/ml zearalenone as a sole carbon source contained the following per Liter: $(NH4)_2$ $SO_4$, 0.5; $MgSO_4.7H_2O$ 0.2; $CaCl_2$, 0.05; $Na_2HPO_4$, 2.44, and $KH_2PO_4$, 1.52g. The pH was adjusted to 6.8 and the medium was sterilized prior to the addition of organic substrates

**Toxicity of zearalenone:** Zearalenone tolerance level for *Pseudomonas*, its transconjugant, cured mutant, and recipient were determined in nutrient broth. The concentration levels of zearalenone ranging from 5-700 μg/ml and an initial inocula of approximately 4 x $10^6$ CFU /ml. After 24 hours of incubation at 28 $^oC$, bacteria growth was observed by plating from the broth culture and counting CFU.

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**Growth kinetics:** Growth at the expense of zearalenone was verified by demonstrating an increase in bacterial growth concomitant with a decrease in zearalenone concentration. Several colonies of strain ZEA-1 grown on LB plates for 3 days were resuspended in mineral salts medium ($A_{600}$, 0.8) and the suspension was used as an inoculum (0.5 ml). Replicate batch cultures were grown in 250-ml Erlenmeyer flasks containing 50 ml of mineral medium and zearalenone (100 μg/ml). Incubation was performed at 30 $^oC$ with rotary shaking (150 rpm). Uninoculated flasks and flasks without zearalenone served as controls. Optical density (O D $_{600}$) and other analysis were measured at 24 h intervals over 120 h. UV spectroscopy was used to detect the disappearance of substrate and the accumulation of degradation products. Aqueous 1-ml samples were serially diluted and scanned from 200 to 600 nm with a Perkin-elmer lambda 2 spectrophotometer to detect changes in absorbance between controls and samples. The zearalenone concentration was determined as previously described [6, 25]. Growth in the presence of other mycotoxins that are structurally related to zearalenone (α–zearalenone and β-zearalenone) was screened using liquid mineral medium containing 100 μg substrate/ml.

**Analysis of zearalenone degradation products by TLC:** Zearalenone metabolites were isolated from growing cultures of strain ZEA-1 in minimal medium amended with zearalenone (100 µg/ml) as a sole source of carbon and energy. Colonies of strain ZEA-1 grown on LB plates for 5 days at 30 $^0$C were resuspended in mineral medium ($A_{600,}$ 0.8) and used as an inoculum (1%) for 0ne-liter Erlenmeyer flasks containing 200 ml of medium. On the basis of previous time course experiments in which metabolites formation was monitored by UV Spectrophotometer scanning at 200 to 600 nm, culture were incubated for 120 h at 30 $^o$C with shaking (150 rpm). After this period, the cultures were centrifuged at 10,000 rpm for 20 min at 4 $^o$C. Supernatants were extracted three times with ethyl acetate and combined organic fractions were evaporated. The dried residue was dissolved in ethyl acetate and loaded onto silica gel TLC plates (60 $F_{254}$, 0.25 nm layer, 20 x 20, Merk, Dermstadt, Germany ). After development with ethyl acetate, the spots were visualized under UV light, the corresponding silica layer was scraped off and extracted three times with ethyl acetate. Combined extracts were Vacuum evaporated and the dry residue weighed. Zearalenone, α-zearalenol and β-zearalenol were used as standards.

**Plasmid DNA Isolation** : The method described by Kado and Liu ( 1981 ) was used to isolate plasmid DNA (pZEA-1) from *Pseudomonas* sp. Strain ZEA-1. DNA preparation was characterized by agarose ( 0.8%) gel electrophoresis [23].

**Plasmid Curing:** The plasmid curing was performed as described previously. In brief, the culture was grown in the presence of a curing agent at the specified concentration for 24 h at 30 $^o$C and then plated on nutrient agar plates to obtain isolated colonies. The isolated colonies were then replica plated on nutrient agar and MSM agar containing zearalenone 100 µg/ml. The colonies that failed to grow on MSM agar plates were considered as putative cured derivatives was confirmed by agarose gel electrophoresis of the plasmid DNA preparation of respective cultures. The curing agent used was Mitomycin C (10-20 µg/ml) and the incubation was carried out at 30 $^o$C. The percentage curing efficiency was expressed as number of colonies with cured phenotype per 100 colonies tested. Stability of the cured strains was monitored by periodic cycling of cells under non-selective condition and re-testing for the ability to grow on zearalenone. One of these cured strains ($ZEA^-$) that had lost the ability to degrade zearalenone designed as *Pseudomonas* sp. Strain ZEA-1M.

**Conjugation:** To further establish the role of the plasmid of *Pseudomonas* sp. Strain ZEA-1 in zearalenone degradation, a mating experiment was performed as described previously [18] with modification. Mating experiments were done in the absence of a helper strain *Pseudomonas* sp. Strain ZEA-1 ($ZEA^+$, $Nal^r$) as the donor and *Escherichia coli* AD256 (RecA56 srl C300: : Tn10; $Tet^r$) (Ghai and Das 1989) as the recipient. Sample of 2 ml of an overnight culture of *Pseudomonas* sp. Strain ZEA-1 (donor) and *E.coli* (recipient) were centrifuged at 10,000 xg for 1 min at 4 oC, washed in a solution containing 0.85 % sodium chloride and 0.01 % Tween 20, and resuspended in 0.1ml of sterile LB broth. Cell suspension were placed on LB agar plates and incubated at 30 $^o$C overnight. Dilution of the mating mixture were plated on LB agar with zearalenone (300 µg/ml) and tetracycline (15 µg/ml) and incubated at 37 $^o$C overnight. The transconjugants were tested for acquisition of zearalenone degradation in MSM medium. The transfer frequency was calculated as the ratio of the number of transconjugants obtained per recipient cell.

## RESULTS AND DISCUSSION

The bacterium strain used in this study was isolated from the soil sample of rhizosphere of corn plant undergone enrichment process [5]. The enrichment was carried out in the shaken culture of the MSM medium. Pure culture was obtained by inoculation to nutrient plates from enrichment culture. Isolates were investigated with respect to their degrading capacity of zearalenone. The isolate ZEA-1 was selected and identified as *Pseudomonas* sp. at the Cairo Mircen center, Egypt. The strain was designated as *Pseudomonas* sp. ZEA-1.

**Toxicity of zearalenone:** Figure (2 ) shows tolerance level of zearalenone for *Pseudomonas* sp. Strain ZEA-1 (pZEA-1, $ZEA^+$), *Pseudomonas* sp.strain ZEA-1M mutant (plasmidless, $ZEA^-$), *E.coli* AD256 (pZEA-1, $ZEA^+$), and *E.coli* AD256 (plasmidless, $ZEA^-$) in nutrient broth. The original strain tolerate up to 700 µg /ml of zearalenone, however, the growth was prolific up to 200 µg /ml beyond 500 µg /ml, the toxin started showing inhibitory effects.

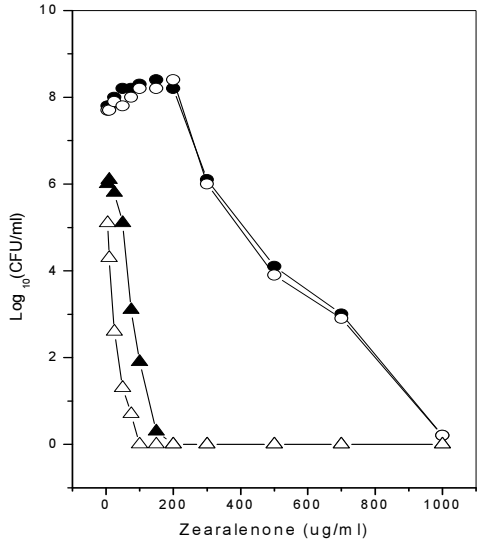


Fig. 2: Tolerance levels of isolated *Pseudomonas* sp. ZEA-1 (● ), Its transconugants *E.coli* AD256 (pZEA-1) (○), Cured derivatives of *Pseudomonas* sp. ZEA-1 (▲), and *E.coli* recipient (Δ) in nutrient broth amended with different Zearalenone.

Although the cured strain tolerated up to 50 µg /ml of zearalenone, 10 µg /ml zearalenone was inhibitory. This results indicate that the ability to degrade zearalenone offer the wild-type strain a selective advantages. Furthermore, the recipient *E.coli* AD256 ($Tet^r$, $ZEA^-$) strain was not able to degrade zearalenone and this strain tolerated zearalenone up to 25 µg /ml in nutrient broth. However, following conjugation, the recipient strain acquired, from *Pseudomonas putida* ZEA-1, zearelenone resistance property and showed zearalenone tolerance up to 700 µg /ml in nutrient broth. The transconjugant strain also acquired the ability to grow in presence of zearalenone (100 µg /ml) as the sole source of carbon and energy and this showing zearalenone degradation activity

**Growth of *Pseudomonas* sp. Strain ZEA-1**: Examination of the growth curves of strain ZEA-1 indicated that after a short lag of approximately 12 hr, this isolate was able to grow in a minimal medium containing Zearalenone as the sole carbon and energy sources. Growth of *Pseudomonas* sp strain ZEA-1 in minimal medium amended with zearalenone corresponded for the most part, to the loss of zearalenone from the medium, particularly during 12 hr to 36 hr of incubation (early-log and-early stationary phase) (Fig. 3). On further incubation, zearalenone was virtually undetectable in growth medium. No growth occurred if zearalenone was omitted from the medium. In addition, strain ZEA-1 was able to assimilate other zearalenone derivatives such as α-zearalenol and β-zearalenol (Fig 4).

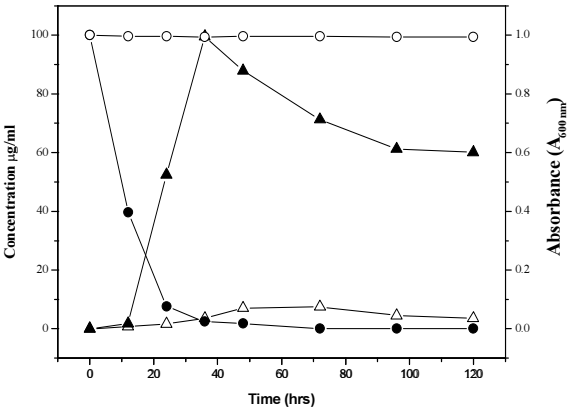


Fig. 3: Utilization of Zearalenone by *Pseudomonas* sp. Strain ZEA-1 in liquid mineral medium with zearalenone as the sole source of carbon and energy at 30 °C and 150 rpm. Growth is shown as increase in the optical density (▲) and in control without carbon source (Δ). Zearalenone concentrations were determined by HPLC analysis of organic extracts from culture (●) and uninoculated control (○).

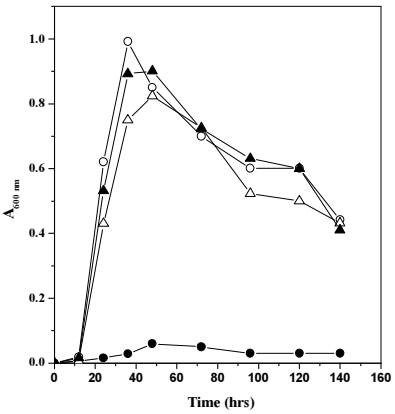


Fig. 4**:** Growth of *Pseudomonas* sp. Strain ZEA-1 on zearalenone and Zearalenone isomers. The organism was grown in minimal salt medium containing 50 µg/ml zearalenone (○), α–zearalenol (Δ), and β-zearalenol (▲), and control without carbon source (●)

**The Kinetics of ZEA Degradation:** The kinetics of Zearalenone degradation was determined by growing ZEA-1 isolate in minimal medium-containing toxin as a sole source of carbon. Growth at the expense of zearalenone was verified by demonstrating an increase in bacterial growth concomitant with a decrease in

zearalenone concentration (Fig. 3). Quantitative analysis of the ZEA in cultures broth revealed (Fig. 3) that zearalenone diminished more than half of initial concentration 12 h after culture and not ZEA toxin was detected 3 days after. No loss of Zearalenone was observed under the same condition in absence of bacterial cells (Fig. 3). The qualitative characteristics of toxin and its degradative products were also determined spectrophotometerically at 12-h intervals up to 120 h. The experiment was performed three times with comparable results, the results of one experiment are shown in figure 5. Changes in UV absorption were measured after the addition of Zearalenone-degrading bacteria into the minimal salt media containing 100 μg/ml of toxin. As shown in Fig. 5, Zearalenone, which exhibit absorbance maxima at 236 nm, was degraded with the concomitant formation of a compound with maximal absorption at 400 nm.

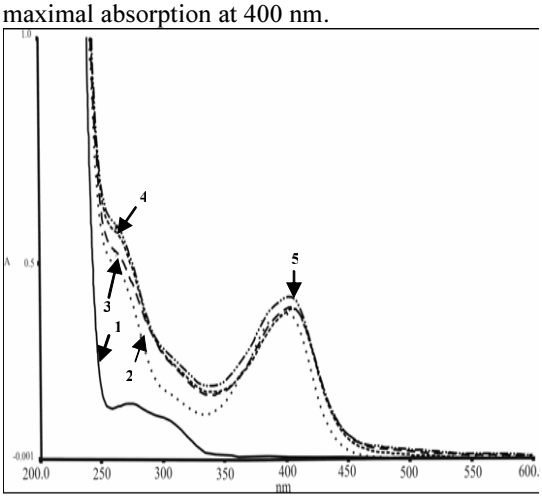


Fig. 5: UV absorption spectra between 200 and 600 nm for zearalenone and degradation products; Lane 1, Zearalenone standard, Lane 2, *Pseudomonas* sp. ZEA-1 (pZEA-1) , Lane3, *E.coli* AD256 (pZEA-1), lane 4, resting cells of ZEA-1 (pZEA-1), and lane 5 resting cells *E.coli* AD256 (pZEA-1)

In addition, the spectra profile remained the same throughout the 120 h of the study period. I reasoned that metabolites corresponding to this peak (400 nm) had accumulated as a results of zearalenone degradation by ZEA-1 strain since zearalenone degradation rapidly within 12 h post-incubation (Fig. 5).

**Analysis of ZEA degradative product by TLC:** To confirm the biotransformation of Zearalenone by Strain ZEA-1 and its derivatives strains, degradative product extracts were analyzed by TLC as described previously. Experimental samples were run alongside standards, and *Rf* values were calculated and compared. Data revealed that the conversion product had an R*f* 0.19 as compared with an $R_f$ of 0.61, for zearalenone and no other spots appeared throughout the 120 h of the study period (Fig. 6). Furthermore, α-zearalenol and β-zearalenol had an Rf 0.27 and 0.24 respectively. Controls incubation studies carried out using sterile media containing zearalenone without bacteria as a negative control or MSM media containing zearalenone with *Pseudomonas* sp.strain ZEA-1M mutant (plasmidless, $ZEA^-$) as a positive control, failed to show the formation of this metabolite ($R_f$ 0.19) or new spot than parent compound. Accordingly, the new compound represents a product of an extractable product of bacteria, since the compound was not detected in control flasks.

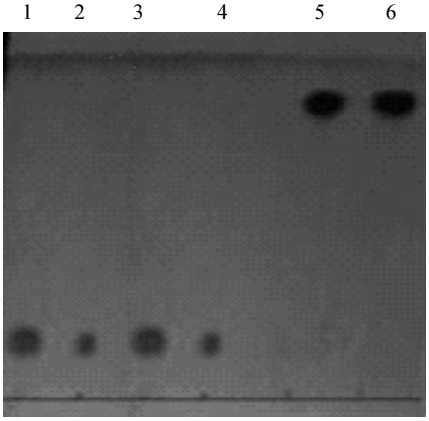


Fig. 6: TLC detection of ZEA degradation in a resting cell system and crude extract. Lane 1, ZEA incubated with resting cells of *Pseudomonas* sp. ZEA-1 (pZEA-1) , lane 2 ZEA incubated with resting cells of *E.coli* AD256 (pZEA-1), Lane 3 ZEA incubated with crude extract *Pseudomonas* sp. ZEA-1 (pZEA-1), Lane 4, ZEA incubated with crude extract of *E.coli* AD256 (pZEA-1), Lan 5, ZEA Incubated with *E.coli* AD256 ( pasmidless, $ZEA^-$), and Lan 6 ZEA standard.

Other transformation products such as α-zearalenol and β-zearalenol were not detected in TLC analysis of this study. In contrast, a variety of microorganisms including bacteria, yeast and fungi were found to be able to convert zearalenone to α-zearalenol and β-zearalenol. However, according to Karlovsky [11], this transformation can not be regarded as detoxification since the estrogenic activity of these metabolites is similar to that of ZEA.

**Detection of Plasmids and localization of Catabolic Properties:** Many bacteria contain plasmids that carry

genes functional in antibiotic resistance, virulence for animal or plant hosts, or the catabolism of diverse chemical compounds. Several catabolic pathways involved in the degradation of aromatic and heterocyclic compounds have been found to be encoded on catabolic plasmid, and biochemical and genetic studies of these plasmid have been extensively investigated [26]. This was the case for the plasmid location of the aromatic heterocyclic organic compounds degradation determinant in other bacterial strains [14], confirming the value of detecting and studying degradative properties of plasmid in the ZEA-degrading strain isolated in this study.

**Plasmid content of bacteria:** The analytical in situ lysis procedure initially described by Kado and liu [10] permitted detection of single plasmid in isolate ZEA-1; this plasmid had molecular weight of approximately 120 Kb, based on comparisons with standard plasmid of *shigella* sp (Fig. 7).

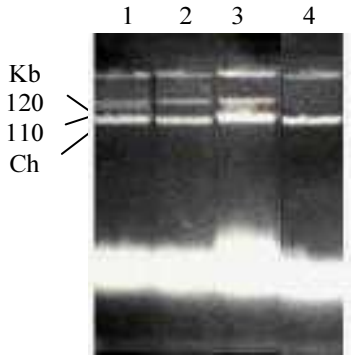


Fig. 7: Agarose gel electrophoresis of plasmid DNA purified from *Pseudomonas* sp. Strain ZEA-1. Lane 1, *Shigella* sp. Plasmids (size marker), and Lane 2, *Pseudomonas* sp strain ZEA-1. , Lane 3, *E.coli* AD256 (pZEA-1), Lan 4, Cured *Pseudomonas* sp strain ZEA-1M strain ( $ZEA^-$), and Ch. Chromosomal DNA. DNA molecules were resolved by electrophoresis in 0.7% agarose in TAE buffer.

By determining and adding together the sizes of fragments generated by digesting plasmid with various restriction enzymes analysis (data not shown). Plasmid size was confirmed to be 120 kb and designated pZEA-1.

**Isolation of cured strain ZEA-1:** The Knowledge that catabolic functions are often carried by plasmids has stimulated my research directed towards defining their role in Zearalenone degradation. This was done by curing the plasmid using mitomycin C (10 µg /ml) and the resultant ZEA-1 mutant strain was designated as ZEA-1M. All the cured strains obtained were not able to grow in minimal media-containing Zearalenone as a sole source of carbon and energy. Furthermore, the results obtained by comparing the growth of ZEA-1 and ZEA-1M on zearalenone as the sole carbon source are shown in Figure 8. With succinate, which is metabolized by ZEA-1 strain, as the sole carbon source, ZEA-1M grew as well as ZEA-1, whereas with zearalenone as the sole carbon source, the growth of the mutant was negligible, the same as the growth observed without any carbon source.

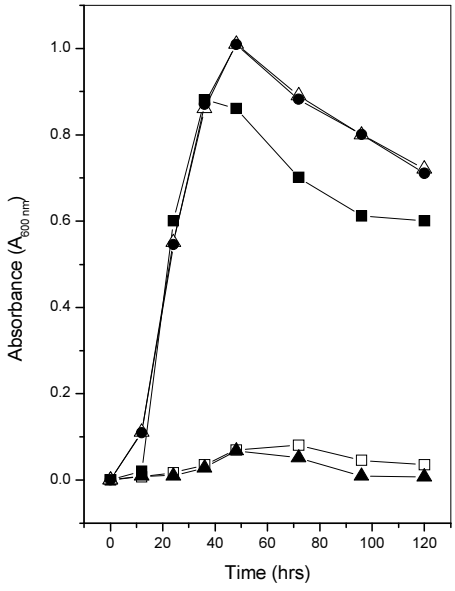


Fig. 8: Growth of ZEA-1 and ZEA-1M strains on zearalenone as the sole carbon source. These strains were grown in 50 ml MSM medium supplemented with 100 µg/ml zearalenone (ZEA-1, ■; ZEA-1M ▲), succinate 10 mg/ml (ZEA-1, Λ; ZEA-1M, Δ) (positive control) or without any carbon source (ZEA-1, □) (negative control).

Therefore, the absence of significant growth of ZEA-1M on zearalenone indicate that pZEA-1 plasmid is necessary for growth of ZEA-1 strain on the toxin. This indicate that the toxin degradation function of the strain ZEA-1 being presumably located on the plasmid.

**Transfer of zearalenone degradation activity:** To confirm that plasmid is necessary for zearalenone metabolism, an experiment was designated to determine if the zearalenone degradation genes were located on a plasmid and were self-transmissible. Mating

experimentwere were done in absence of helper plasmid with *Pseudomonas* sp.strain ZEA-1 ($Nal^r$, $ZEA^+$) as the donor and *Escherichia coli* AD256 (RecA56 srl C300: : Tn10; $Tet^r$) as recipient as described above. Eight colonies ($Tetr^+$) from mating mixture were analyzed for zearalenone degradation ability, and three colonies were able to degrade zearalenone in MSM liquid medium. These colonies did not grow on plates containing nalidixic acid (20 µg/ml), indicating that they were no *Pseudomonas* sp. Strain ZEA-1 that had acquired resistance to tetracycline. Mating experiments with ZEA-1 strain as the donor yield zearalenone degrading *E.coli* transconjugants at frequency $3.8 \times 10^{-3}$ per recipient. One of these transconjugant designated *E.coli* (pZEA-1). Accordingly, the recipient *E.coli* strain was not able to degrade zearalenone, and this strain tolerated zearalenone up to 15 µg/ml in nutrient broth. However, following conjugation, the recipient strain acquired, from *Pseudomonas* ZEA strain, zearalenone resistance property and showed zearalenone tolerance up to 500 µg/ml in nutrient broth (Fig. 2). The transconjugant strain also acquired the ability to grow in the presence of zearalenone (100 µg/ml) as the sole source of carbon and energy and completely converted ZEA ($R_f$ 0.61) into a product with an Rf value identical with that of compound ($R_f$ 0.19) produced by the original strain ZEA-1. ( fig. 6 ). On the other hand, the results of the plasmid isolation studies indicated that the *E.coli* transconjugant ($Tet^r$) that acquired zearalenone degradation ability gained a plasmid of approximately (120 kb ) as the size of donor strain ZEA-1 plasmid.

**Presence of zearalenone metabolizing enzyme:** To determine if pZEA-1 contained a gene(s) that encoded zearalenone degradation/transformation, resting cells and cell extract from *Pseudomonas* sp. Strain ZEA-1 (pZEA-1, $ZEA^+$), *Pseudomonas* sp.strain ZEA-1M mutant (plasmidless, $ZEA^-$), *E.coli* AD256 (pZEA-1, $ZEA^+$), and *E.coli* AD256 (plasmidless, $ZEA^-$) were tested for their ability to degrade/transform zearalenone. UV spectrophotometer and TLC analysis was done on supernatant from whole resting cells and cell extracts incubated with zearalenone (100 µg/ml) as described above. At the end of the experiment, zearalenone metabolites compound with $R_f$ 0.19 with *Pseudomonas* sp. Strain ZEA-1 (pZEA-1, $ZEA^+$) and *E.coli* AD256 (pZEA-1, $ZEA^+$) were detected whereas, with *Pseudomonas* sp.strain ZEA-1M mutant (plasmidless, $ZEA^-$) , and *E.coli* AD256 (plasmidless, $ZEA^-$) zearalenone were recovered and no transformed compound was detected. (Fig. 6). These results indicated that the transconjugant *E.coli* strain acquired the ZEA gene(s) encoding enzyme(s) involved in the transformation of zearalenone. Takahashi-Ando *et al.* (2002) identified and characterized a lactonohydolase enzyme in fungus *Clonostachys rosea* which convert ZEA to a less estrogenic compound. Similar results were obtained with cell extract from *Pseudomonas* sp. Strain ZEA-1 (pZEA-1, $ZEA^+$), *Pseudomonas* sp. strain ZEA-1M mutant (plasmidless, $ZEA^-$), *E.coli* AD256 (pZEA-1, $ZEA^+$), and *E.coli* AD256 (plasmidless, $ZEA^-$ ) were tested for their ability to degrade/transform zearalenone**.** The results in Figure (6) show that only crude extract from *Pseudomonas* sp. Strain ZEA-1 (pZEA-1, $ZEA^+$) and *E.coli* AD256 (pZEA-1, $ZEA^+$) was capable of transforming a zearalenone into a product with $R_f$ 0.19. Further more, An average of 96% of ZEA was recovered after incubation with heat-treated crude protein extract (1 mg/ml protein ) whereas, DNase1-treated crude extract degraded 90% of ZEA in solution (data not shown) ,suggesting that removal of Zearalenone by *E.coli* (pZEA-1) or *Pseudomonas* sp (pZEA-1) is not due to nonspecific binding with Bacterial plasmid DNA but possibly an enzymatic [25] . (details of such studies are in progress )

**Toxicity Bioassay:** Bioassay methods for detecting mycotoxin have strong appeal because of the broad range of chemical structure they encompass. The use of brine shrimp larvae as a screening system for mycotoxins has the advantages that brine shrimp eggs are commercially available. Figure 9 gives the zearalenone and its degradative products toxicity to *A. salina* larvae. The concentration of pure zearalenone in wells that proved lethal to 50% of *A. salina* larvae was about 50 µg/ml. Little or no toxicity was observed at concentration 5 µg/ml. However, the increasing concentration of ZEA (100 µg/ml) exhibited a high toxicity for *A. salina larvae* , the mortality rate of which was 97%. On the other hand, as in the 24 h exposure, no mortality was recorded in zearalenone degradative products extracts at all concentration levels studied ( Fig. 9). Based on the results obtained in the bioassay with mycotoxin standard and those provided by ZEA degradative product, I believe new compound produced by bacterial degradation of zearalenone that less toxic than parent compound and this strain could be candidate for the detoxification of respective mycotoxins.

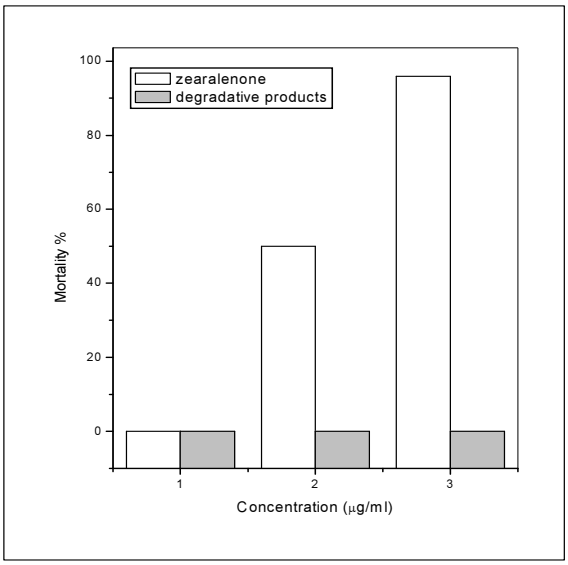


Fig. 9: Percent mortality of brine shrimp larvae after 24 h exposure to different concentrations of zearalenone and its degradative products extracts.1, (5 μg/ml); 2, (50 μg/ml); 3, (100 μg/ml)

Molnar *et al.* [20] described a new yeast strain, *Trichosporon mycotoxinivorans*, able to degrade ZEA to carbon oxide and other non-toxic metabolites, neither α– and β –zearalenol were detected, authors suggested that yeast strain can be used for detoxification of such mycotoxins.

Finally, The demonstration that enzyme(s) of zearalenone catabolic pathway could covert substrate to one product has specific UV spectra (400 nm) and $R_f$ Rf value 0.19 immediately suggested a method for identifying recombinant bacteria carrying genes encoding those enzyme(s). such studies are in progress.

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