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Purification and Characterization of Thermostable Cytosine Deaminase from *Aspergillus fumigatus*

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CYTOSINE Deaminase (CDA) is a non-human enzyme converting cytosine to uracil, with significant implementation on various cancer therapeutic approaches especially prodrug mediated therapy using 5-fluorocytosine into 5-fluorouracil. However, the lower catalytic/ thermal stability and higher antigenicity of this enzyme are the main challenges for further its clinical applications, thus, screening for thermostable enzyme with higher turnover activity was the objective of this study. Among the recovered thermotolerant fungal isolates, *Aspergillus fumigatus* has been selected as potent CDA producer. Upon nitrogen starvation, the yield of intracellular CDA by *A. fumigatus* was significantly increased by about 5 folds, comparing to control. The enzyme was purified from thermotolerant *A. fumigates* into its electrophoretic homogeneity by ion-exchange and gel-filtration chromatography by 4.4 purification folds and 29.9% yield, respectively, with molecular subunit structure 48 kDa under denaturing-PAGE. The purified enzyme showed an optimum pH 7.0, optimum reaction temperature 37°C. The maximum affinity (K_m) and reaction velocity (V_{max}) of purified CDA was 0.08 mM and 400 μmol/min/mg on cytosine as substrate. At 37°C, the half-life time ($T_{1/2}$) of purified CDA was about 8 h, ensuring the structural/ catalytic thermal stability of this enzyme. Based on these preliminary results, *A. fumigatus* CDA could be a scaffold for further *in vivo* studies on cancer prodrug-mediated therapies, or gene therapy applications.

Keywords: *Aspergillus fumigatus*, Cytosine, Cytosine deaminase, 5-Fluorocytosine, Purification.

Introduction

Cytosine deaminase (E.C 3.5.4.1) is a member of aminohydrolase family catalyzing the hydrolytic deamination of cytosine, 5-fluorocytosine, and 5-methylcytosine into uracil, 5-fluorouracil, and thymine, respectively (West et al., 1982). Cytosine deaminase is a non-human enzyme (Koechlin et al., 1966), while, it was mainly reported in various bacterial and fungal cells (West et al., 1982; Esders & Lynn, 1985; Katsuragi et al., 1987). Cytosine deaminase was purified from yeasts as bakers' yeast (Katsuragi et al., 1989), bacteria as *Escherichia coli* (Katsuragi et al., 1989; Hussein & Al-Baer, 2018), *Serratia marcescens* (Sakai et al., 1975), *Salmonella typhimurium* (West et al., 1982), and higher fungi as *Aspergillus parasiticus* (Zanna et al., 2012), and Aspergillus fumigatus (Yu et al., 1991). Cytosine deaminase has received a great attention due to its implementation on various cancer therapeutic approaches especially mediating the prodrug 5-fluorocytosine into 5-fluorouracil. The enzyme catalyzes the deamination of non-toxic

compound 5-fluorocytosine into the cytotoxic compound 5-fluorouracil with selective anticancer potency and feasibility of molecular modulation. Recently, the enzyme directed prodrug cancer therapy has been received much attention due to their selectivity/ targetability to a specific type of cells with reducing potency to the side effects of traditional chemotherapeutic drugs (Ireton et al., 2002; Hamaji et al., 2007). Cytosine deaminase is one of the rapidly developing strategies of tumor gene therapy in clinical trials (Hamaji et al., 2007). Cytosine deaminase from yeasts displayed a superior catalytic properties than bacterial enzyme especially for the higher affinity to substrate but it is less thermostable, in contrast the bacterial CDA that is a thermostable but with lower affinity to its substrate (Ireton et al., 2003; Hussein & Al-Baer, 2018). Cytosine deaminase was purified and characterized from various bacterial and fungal sources, however, the lower structural stability and catalytic efficiency is the major challenge that limits this technology from worldwide applications in cancer therapy. Thus, searching for a novel CDA with higher structural, thermal stabilities, and higher affinity and catalytic efficiency towards 5-fluorocytosine is the main objective of this study.

Materials and Methods

Isolation and screening of cytosine deaminase producing fungi

Different soil samples were collected from different localities in Monufia (30.52°N 30.99°E), Qaluobia (30.41°N 31.21°E) and Sharqia (30.7°N 31.63°E) Governorates, the collected Samples were transferred to the laboratory of microbiology at Faculty of Science, Benha University and kept for further processes. Briefly, five grams of each soil samples were suspended in 45mL sterile saline solution (0.85%) in Erlenmeyer conical flasks, shaken for 15min., one mL of the supernatant was inoculated into Potato Dextrose Agar (PDA) and Czapek's-Dox agar medium (Johnson et al., 1959). The fungal plates were incubated at 45°C for 7 days (Cooney & Emerson, 1964; El-Sayed et al., 2019b). The developed thermophilic/ thermotolerant fungal isolates were recovered and purified under the same conditions. Then a plug from the edge of each colony was transferred into 100mL Dox's broth media in 250mL Erlenmeyer conical flaks and incubated at 28°C for 7 days. The fungal pellets were collected, and their intracellular crude proteins

were extracted (El-Sayed et al., 2015 a). Briefly, 5gm of each fungal fresh weight were grinded in liquid nitrogen, dispensed in 10mL Tris buffer (pH 7.0, 50mM) with 1mM PMSF, 1mM EDTA, and 10μL β- mercaptoethanol (Borkovich & Weiss, 1987). This mixture was shaked by vortex for 5min., and then centrifuged at 10000rpm for 5min. at 4°C. The filtrate was used as a crude source for cytosine deaminase, and its activity and concentration were assessed.

Enzyme assay

The activity of cytosine deaminase was assayed as described previously (Katsuragi et al., 1987) with slight modification (El-Sayed et al., 2017, 2018, 2019d). Briefly, a mixture consists of 0.5mL of enzyme and cytosine (100mM) in Tris-HCl buffer (50mM, pH 7.0). Blanks of enzyme and substrate were prepared. The reaction mixture was incubated at 37°C for 30min., stopped by 10% TCA. Then, the concentration of released cytosine to uracil was measured spectrophotometrically at 286 nm, comparing to authentic concentrations of uracil (0.2mM to 5.3mM). One unit of CDA activity was expressed by the amount of enzyme releasing 1mM of uracil from cytosine per min under standard assay conditions.

The concentration of protein was measured according to (Lowry et al., 1951) using bovine serum albumin as a standard.

Effect of nitrogen starvation on cytosine deaminase production

To study the effect of nitrogen starvation on enzyme production, sodium nitrate was eliminated from Dox's broth media. Three treatments were made as follow; 1- Control media contain all Dox's component, 2- Temporarily starved media of sodium nitrate for 3 days, then a mended with sterilized sodium nitrate solution at the same concentration in control, and 3- Completely starved Dox's media free of sodium nitrate. All media were inoculated with the highest potent CDA producing isolates and incubated for 7 days at 28°C.

Morphological and molecular identification of the potent fungal isolate

The potent cytosine deaminase producing isolate was morphologically identified based on its features using the universal identification keys (Raper & Fennell, 1965). The identified isolate was confirmed from the sequence on internal

transcribed spacer (ITS) region (Schoch et al., 2012) the second largest kingdom of eukaryotic life, by a multinational, multilaboratory consortium. The region of the mitochondrial cytochrome c oxidase subunit 1 used as the animal barcode was excluded as a potential marker, because it is difficult to amplify in fungi, often includes large introns, and can be insufficiently variable. Three subunits from the nuclear ribosomal RNA cistron were compared together with regions of three representative protein-coding genes (largest subunit of RNA polymerase II, second largest subunit of RNA polymerase II, and minichromosome maintenance protein, with minor modification (El-Sayed et al., 2018 , 2019 c). Briefly, to extract genomic DNA, 0.2gm of fungal tissues was grinded to fine powder using liquid nitrogen then 1 ml of CTAB extraction buffer was added. The extracted DNA was used as template in addition to two pairs of primers; ITS5 and ITS4 and 2 x PCR master mixtures for PCR reaction. The PCR product was run in 2% agarose gel in 1x TE buffer with DNA marker, the produced band was purified and sequenced using the same primers pair. The obtained sequence of the current study was examined using BLAST tool of NCBI database and the phylogenetic relatedness of the obtained sequence was constructed with the MEGA 10.0 software with Maximum Likelihood Model.

Purification, subunit structure and molecular weight of cytosine deaminase

Enzyme extraction from the fungal cultures

The selected potent fungal isolate was grown on Dox's broth media, after incubation, the fungal tissues (50gm of the mycelial biomass) was collected and grinded to fine powder with liquid nitrogen to fine powder and suspended in Tris buffer (pH 7.0, 50mM) with 1mM PMSF, 1mM EDTA, and 1mM β- mercaptoethanol, shacked well for 10 min then centrifuged at 10000rpm for 10min. to remove tissue depress. The supernatant was used as a source for the crude enzyme (El-Sayed et al., 2013; El-Sayed & Shindia, 2011).

Protein concentration by dialysis membrane

The protein of crude filtrate was concentrated with 20kDa dialyzer membrane against PEG 6000 and the concentrated form was purified by ion- exchange chromatography and gel filtration technique (Kebeish & El-Sayed, 2012; Tsiatsiani & Heck, 2015).

Gel filtration chromatography

Briefly, 0.5gm of Sephadex was dissolved in 50 mL of Tris-HCl buffer (pH 7.0, 50mM) stored at 4°C overnight. Then it was poured into column (2x40cm), and the column was equilibrated by the same buffer, with flow rate 1mL /3.5min. 2mL of concentrated protein was applied to the column, fractions containing enzyme were collected, the activity and protein contents of the eluted fractions were determined as above. The most active and fractions were collected based its activity and concentrated to 2 mL by dialysis membrane prior to the subsequent purification step (El-Sayed & Shindia, 2011).

Ion-exchange chromatography

The column of DEAE-Sepharose (2x30 cm) was pre-equilibrated by Tris-HCl buffer (pH 7.0, 50mM), and 2mL of the enzyme loaded on it with flow rate of 1mL/ 2min. Gradient concentrations of NaCl (50-250mM) in the same buffer was used for enzyme elution. The activity and protein content of each fraction was determined. The most active fractions were collected for the next biochemical characterization (El-Sayed and Shindia, 2011; El-Sayed *et al.*, 2015b)

SDS-PAGE analysis

Cytosine deaminase molecular structure of the selected potent fungal isolate was checked by SDS-PAGE following the method of Lockwood & Coombs (1991). After the sample was boiled in the buffer, running in electrophoresis unit, staining with Coomassie Brilliant Blue, and destaining, the molecular weights of the appeared bands were calculated against a protein marker.

Characterization of cytosine deaminase

Biochemical characterization of cytosine deaminase was conducted according to (El-Sayed & Shindia, 2011; Zanna et al., 2012; Hussein & Al-Baer, 2018) with slight modification.

Effect of reaction temperature on cytosine deaminase activity

The optimum temperature for the enzyme was determined by incubating the reaction mixture at 30°C, 37°C ,45°C ,50°C and 55°C and measuring activity by standard assay method.

Effect of reaction pH on cytosine deaminase activity

The optimum pH for cytosine deaminase was evaluated by measuring enzyme activity at

varying pH ranged from pH 3-10. Using different buffer systems, citrate-phosphate buffer (50mM) for pH range of 3-5, and Tris-HCl buffer (50mM) for pH range of 6-10.

Fungal deposition

The ITS sequence of the thermotolerant isolate, *Aspergillus fumigatus,* as potent CDA producer, was deposited to the genbank with accession number # MW337215.1.

Statistical analyses

All the experiments were conducted in triplicate and the results was expressed as mean ± STDEV and the data was analyzed using One-way ANOVA followed by Dunnett's multiple comparisons test was performed using GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com"

Results

Isolation and screening of cytosine deaminase

Twenty-six fungal isolates were isolated from the different soil samples and their potency to grow on L-cytosine as sole nitrogen source was determined using modified Czapek's-Dox agar media (El-Sayed et al., 2018). The recovered fungal isolates displayed an obvious morphological growth with plausible fluctuations on modified Czapek's-Dox media with cytosine. After cultural incubation, the intracellular proteins were extracted, and the enzyme activity and its concentration were determined by the standard assay (Table 1). Among the tested isolates, the CDA productivity of *A. fumigatus* 1 was reported (77.96μmol/mg/min), while the lowest cytosine deaminase productivity were recorded for Penicillium notatum (48.56μmol/mg/min). Similar screening paradigm for enzymes production by fungi were reported (El-Sayed et al., 2013, 2015a, 2019a, b).

Effect of nitrogen starvation on cytosine deaminase production

To study the effect of nitrogen (N) starvation and re-supply of sodium nitrate as nitrogen source on the formation and production of cytosine deaminase from the selected potent fungi (*A. fumigatus* 1, *A. ochraceous* 2, *A. flavus* 1 and *A. parasiticus*. Fungal isolates were first grown in control media, starved media for three days and re-supplied again with nitrogen source,

and totally starved media for 7 days, after the incubation period (7 days) the nitrogen starvation results in pigmentation change (Fig. 1 B) and also the production of cytosine deaminase was varied in each media with different isolates but there is no significant difference between control media and media starved for three days while, a significant difference appeared in totally starved media (Fig. 1 A).

TABLE 1. Screening of cytosine deaminase producing fungi

| Fungal isolate	Specific activity (μmol/ mg/ min.)
Aspergillus carneus	68.03
Aspergillus flavus 1	105.95
Penicillium sp. 1	77.89
Aspergillus ochraceous 1	74.78
Aspergillus terreus	68.44
Aspergillus flavipes	72.07
Aspergillus nidulans	66.10
Aspergillus fumigatus 1	77.96
Aspergillus ochraceous 2	92.08
Penicillium notatum	48.56
Fusarium oxysporum	72.57
Penicillium expansum	85.25
Aspergillus sp. 1	59.79
Penicillium sp. 2	51.77
Fusarium sp.	50.46
Aspergillus awamori	84.25
Aspergillus niger 1	95.62
Aspergillus carneus	71.35
Aspergillus flavus 2	97.59
Aspergillus parasiticus	95.62
Penicillium sp. 3	63.82
Aspergillus fumigatus 2	53.26
Aspergillus flavus 3	56.19
Aspergillus sp. 2	64.68
Aspergillus flavus 4	66.31
Aspergillus niger 2	121.38
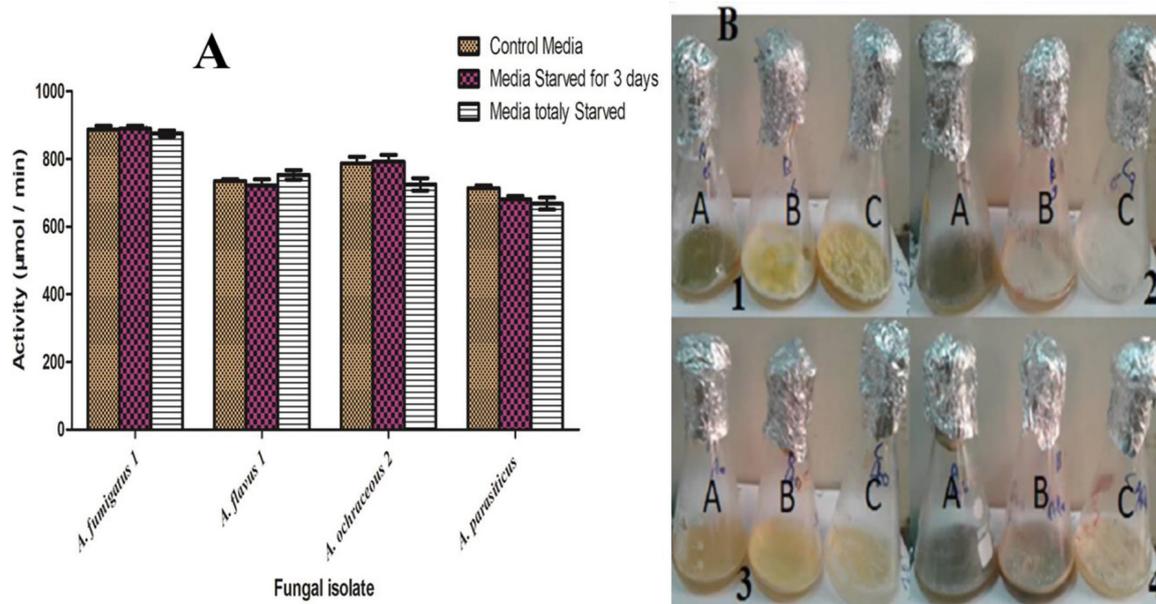
Fig. 1. Productivity of CDA by the fungal isolates in response to nitrogen starvation [The fungal cultures were starved for three days, then amended with cytosine, and the intracellular proteins were extracted, and the enzymatic activity were determined, as described in Materials and Methods. A, The activity of CDA by the fungal isolates and B, Mycelial pigmentation of the fugal isolates *A. parasiticus* (1), *A. flavus* (2), *A. ochraceous* (3), and *A. fumigatus* (4) - "A- control, B- starved for 3 days, and C- totally starved]

Morphological and molecular identification of the potent fungal isolate

The morphological features of the potent fungal isolate producing cytosine deaminase were observed on PDA and Dox's media according to the morphological identification keys. The morphology of this fungal isolate is presented in (Fig. 2 A), its typical with that of *Aspergillus fumigatus*. This identification was confirmed by the sequence of ITS region using genomic DNA as PCR templet as shown in material and methods. The PCR product was separated on 2% agarose gel at expected size 550 to 600bp (Fig. 2 B). After sequencing of PCR product it was non-redundantly searched on gene bank database using BLAST tool. The alignment and phylogenetic analysis for our isolated showed 99% similarity with *Aspergillus fumigatus* (Fig 2 C).

Purification, subunit structure and molecular qeight of cytosine deaminase

The potent fungal isolate was grown and incubated at standard conditions, then the fungal mycelia were collected and grinded to fine powder and their intracellular protein was extracted. The cytosine deaminase was purified using ion -exchange chromatography and gel-

filtration. The enzyme activity and protein was measured simultaneously for ion-exchange column, the enzyme was eluted with different NaCl concentration and the most active fraction (Fig. 3 B) was collected and concentrated then applied to gel-filtration chromatograph, and the most active fraction (Fig. 3 A) was collected and concentrated for further use. The results in (Table 2) for overall purification profile showed that the activity of cytosine deaminase was increased by 3.36 and 4.46 folds after purification by ion-exchange chromatographic and gel-filtration in compare to the crude enzyme. The yield of cytosine deaminase was increased by nearly 30%. The subunit structure of purified cytosine deaminase from *A. fumigatus* was checked on SDS-PAGE (Fig. 3 C). The purified enzyme showing a single band of molecular mass 48kDa.

Biochemical characterization of cytosine deaminase

Effects of reaction temperature on cytosine deaminase activity

The effect of reaction temperature on activity of cytosine deaminase was studied by incubating at different temperatures at 30, 37, 45, 50 and 55°C. The enzymatic activity was measured by

standard assay. From the profile of enzymatic activity (Fig. 4 A), the highest enzymatic activity was recorded at incubation temperature 37°C, with significant reduction at lower and higher incubation temperature. The activity of purified enzyme was proportionally increased with incubation temperature, till maximum value at 37°C (300.6μmol/ mg/ min.). At 50°C, the activity of CDA was reduced by about 40 %, comparing to control at 37°C. The highest activity of CDA at 37°C, ensuring the feasibility of this enzyme on various *in vivo* therapeutic applications.
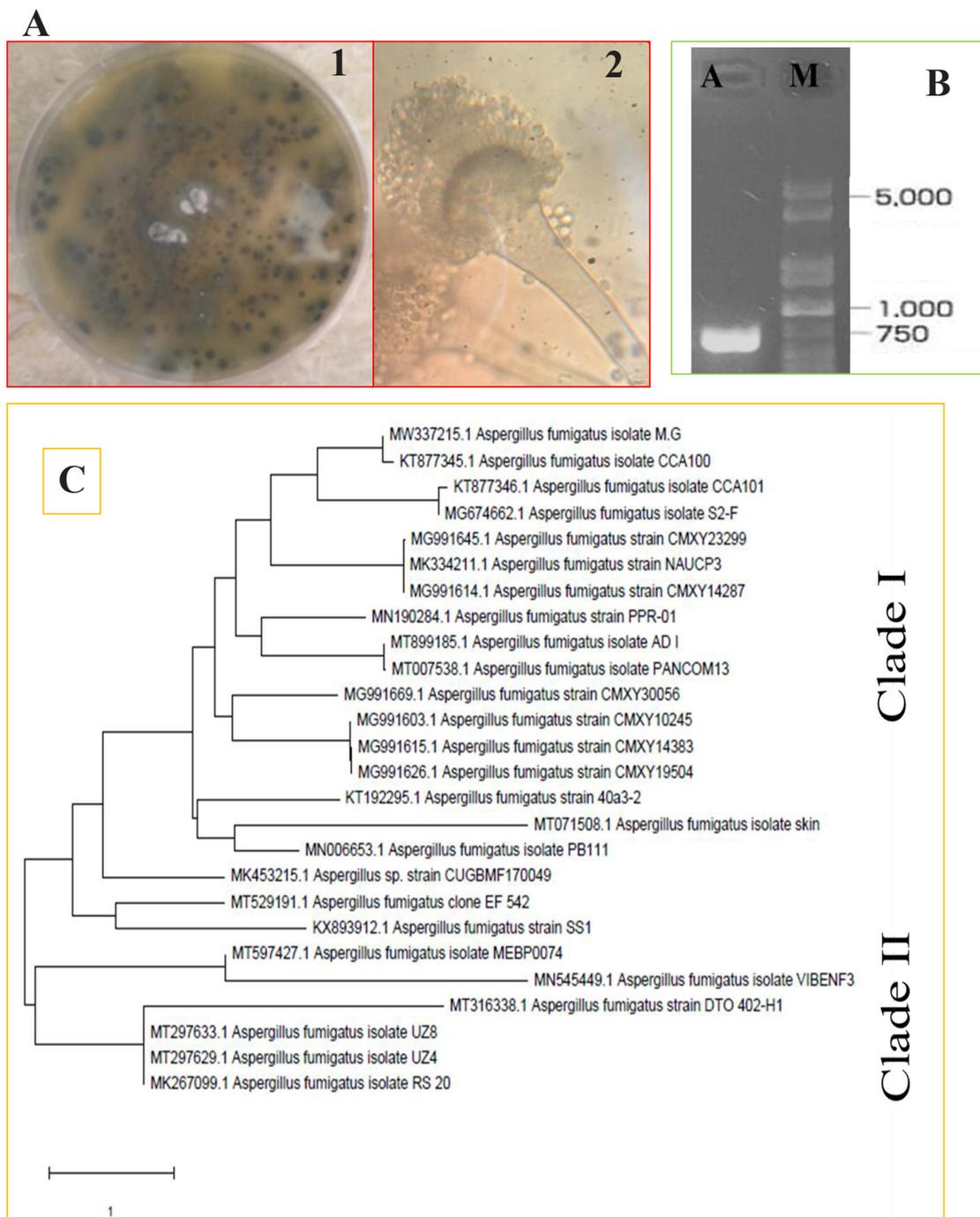

Fig.2. Morphological and molecular identification of the potent cytosine deaminase producing fungi, growing on PDA at 28°C for 7 d *A. fumigatus*, cultural view (A1), microscopical view (A2), (B) PCR amplicons using fungal DNA as template for PCR with the primers ITS4 and ITS5, (c) Molecular phylogenetic analyses of *A. fumigatus* by Maximum Likelihood Model of MEGA 10.0 software
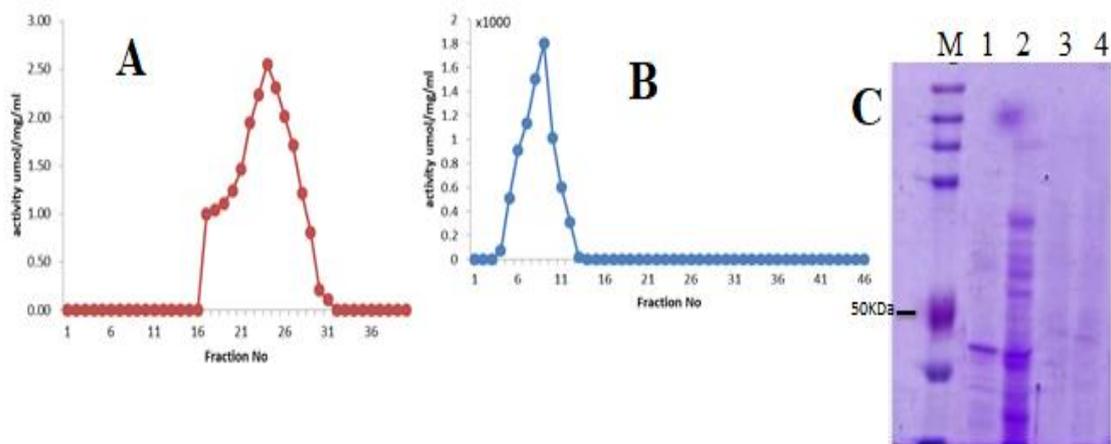
Fig . 3. Purification and subunit structure of cytosine deaminase from *Aspergillus fumigatus*. Gel-filtration (A) and ion-exchange purification (B) and (C) SDS-PAGE showing the overall steps for CDA purification [M, protein Marker, lane 2 crude enzyme, lane 3 & 4 is gel-filtration column, lane 1 is ion-exchange column]

TABLE 2. Overall purification profile of Cytosine deaminase from *Aspergillus fumigatus*

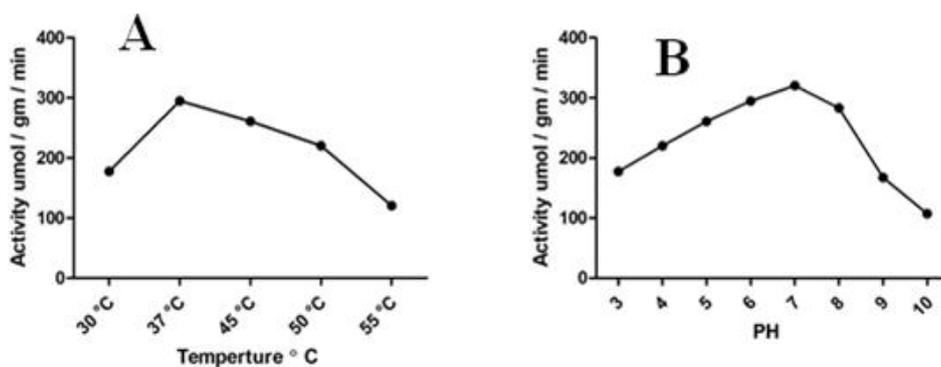| Purification fold	Recovery (%)	Specific activity (μmol/mg/min)	Total protein (mg)	Total activity (U)	Volume (mL)	Step
1.00	100.00	35.10	752.15	26401.94	39	Crude Enzyme
1.49	10.25	52.23	51.84	2707.30	4.5	dialysis
3.36	23.36	117.83	56.85	6698.33	8.5	DEAE - Sepharose
4.46	29.97	156.62	50.51	7911.55	10.5	Sephadex G200

Fig. 4. Effect of temperature (A) and pH (B) on the activity of purified cytosine deaminase from *Aspergillus fumigatus*

Effects of reaction pH on cytosine deaminase activity

The reaction pH effects on the activity of cytosine deaminase was studied. The enzyme showed the maximum activity at pH range 6.0–7.0 (Fig. 4 B). While, a great inhibition of the activity was showed at acidic pH (3.0–4.0) and alkaline pH (9.0-10). The significant reduction on the enzymatic activity at higher acidic and alkaline pH, might be due to the denaturation, unfolding of enzyme tertiary structure, halting the formation of enzyme-substrate complex (El-Sayed et al., 2014, 2015a).

Kinetics of cytosine deaminase towards cytosine

The affinity of purified CDA from *A. fumigatus*

towards cytosine has been evaluated. Different concentrations of cytosine were used, the activity of CDA was assessed by the standard assay (Table 3). The purified *A. fumigatus* CDA had a higher affinity to cytosine substrate (K_m 0.08mM), comparing to *Escherichia coli* CDA (K_m 0.25-0.46mM) (Porter, 2000; Fuchita et al., 2009; Kohila et al, 2012). As well as, the purified CDA had a higher maximum velocity (V_{max} 400µmol/ mg/ min.). From the kinetics, the higher affinity of purified CDA authenticate the catalytic efficiency of this enzyme for further *in vivo* therapeutic implementations.

Discussion

Cytosine deaminase is one of the most important enzymes for gene therapy applications, especially with the non-human identity of this enzyme (Koechlin et al., 1966; Koon et al., 2008; Tudzynski, 2014). This enzyme has been frequently used for various prodrugs mediated therapy (Katsuragi et al., 1986), however, the enzyme turnover number, catalytic efficiency and structural stability are the main challnges that limits the further *in vivo* applications of this enzyme. Thus, purification and characterization of thermostable CDA from fungi was main objective of this work. Among the recovered thermotolerant fungi, *Aspergillus fumigatus* was the potent CDA producer (77.96µmol/ mg/ min.), that being significantly higher than *Escherichia coli* CDA activity (0.7µmol/ mg/ min.) (Hussein & Al-Baer, 2018), *Chromobacterium violaceum* (0.86µmol/mg/ min.) (Kim & Yu, 1998) and *Aspergillus parasiticus* (77.00µmol/ mg/ min.) (Zanna et al., 2012). Similar reports for fungal CDA production were reported from *Aspergillus fumigatus* (Yu et al., 1991) and *A. parasiticus* (Zanna et al., 2012). The productivity of CDA was strongly increased upon starvation of the fungal isolates for 3 days, then amendment with the cytosine. Similar results reporting the activation

of amino acids transporters of the fungal plasma membrane upon starvation of sulfur and nitrogen (El-Sayed et al., 2013, 2014, 2015b). The enzyme was purified from the thermostolerant isolate *A. fumigatus* by ion-exchange and gel-filtration chromatography (El-Sayed & Shindia, 2011; El-Sayed et al., 2015a, 2016). By the last purification step, CDA was purified by 4.4 folds and 29.9% yield, respectively. Similar purification protocols has been reported for CDA from different fungal isolates such as *Aspergillus fumigatus* IFO 5840 with 150 fold and 0.75% yield (Yu et al., 1991), *Escherichia coli* with 12.85 fold and 30.85% yield (Hussein & Al-Baer, 2018), *Chromobacterium violaceum* YK 391 with 264.7 fold and 14.3% yield (Kim & Yu, 1998), and *A. parasiticus* with 384.1 fold and 12.4% yield (Zanna et al., 2012). From the denaturing-PAGE, the purified *A. fumigatus* CDA showed a single proteineous band of molecular mass 48kDa. Consistently, the apparent molecular mass of *E. coli* was 48kDa (Hussein & Al-Baer, 2018), yeasts was 34kDa (Ipata et al., 1971) or 41kDa (Katsuragi et al., 1989), and a 3kDa for bacterial CDA (Kim et al., 1987). CDA from *A. fumigatus* displayed its highest activity at temperature of 37°C that coincident with those reported by Kim & Yu (1998). In partially consistent, the enzyme from different bacterial isolates showing an optimum temperature range from 40°C to 50°C (West et al., 1982; Zanna et al., 2012; Hussein & Al-Baer, 2018). The optimum pH for *A. fumigatus* CDA was 7.0 that being consistent with those reported by West et al. (1982), Yu et al. (1991), Zanna et al. (2012), and partially consistent with those reported by Hussein & Al-Baer (2018) with optimum pH at 7.5 to 9.0. From the kinetic parameter, the K_m values for cytosine deaminase was 2.01mM for cytosine as substate, that relatively consistent with those reported for Baker's yeast (3.1mM) (Katsuragi et al., 1989), *Chromobacterium violaceum* (1.55mM), *A. fumigatus* (2mM) (Mahan et al., 2004).

TABLE 3. Table 3. Kinetic parameters of cytosine deaminase from *Aspergillus fumigatus*

Organism	K_m (mM)	V_{max} (µmol/ mg/ min.)	K_{cat} (s^{-1})	K_{cat}/K_m ($mM^{-1}S^{-1}$)	References
A. fumigatus	0.08	400	0.0996	1.25	This study
Escherichia coli	0.25			12.67	(Kohila et al., 2012)
Escherichia coli	0.46		49.68	106.85	(Fuchita et al., 2009)
Salmonella typhimurium	0.74	47.16			(West et al., 1982)
Escherichia coli	0.20		185	925	(Porter, 2000)
Baker's yeast	1.17	1.70E−04	170	1.45E+05	(Stolworthy et al., 2008)

Conclusion

Thermotolerant *Aspergillus fumigatus* was reported as the potent CDA producer. The enzyme was purified from *A. fumigatus* displaying a relative thermal stability at 37°C, pH range 6.0-7.0. The enzyme had a lower K_m and higher V_{max} values for cytosine comparing to other sources. The higher activity and thermal stability of the purified CDA pave the way for various studies of therapeutic applications of this enzyme in prodrug mediating and gene-therapies.

Conflict of interests: The authors declare no conflict of interest.

Authors contribution: A.S.A.E. designed the research plane. M.G.A performed the experiments. A.A.S, R.M.E. and N.E contributed in data analysis. A.S.A.E. and M.G.A writing-original draft preparation. M.M.A revised the manuscript and approved the final form of manuscript. A.S.A.E wrote and edits the manuscript. All authors have read and approved the final manuscript.

Ethical approval: Not applicable.

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تنقية و توصيف أنزيم السيتوزين دى أمينيز الثابت حراريا من الأسبراحلس فيوميجاتس

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إنزيم السيتوزين دى أمينيز هو إنزيم غير بشري (لايوجد فى خلايا الثديات) يقوم بتحويل السيتوزين إلى
اليوراسيل ، مع استخدامه الواسع في مختلف الأساليب العلاجية للسرطان وخاصة العلاج بوساطة العقاقير
الأولية باستخدام 5-فلوروسيتوزين الذى يتحول الى 5-فلوروبوراسيل. ومع ذلك ، فإن انخفاض النشاط
التحفيزي و الثبات الحراري لهذا الإنزيم يمثلان اهم التحديات الرئيسية اثناء تطبيقات الأنزيم العلاجيه
، وبالتالي ، كان الهدف من هذه الدراسة هو البحث عن إنزيم ثابت حراريا وله نشاط عالى. من بين
العزلات الفطرية المعزوله والثابته حراريا كان فطر الأسبراجلس فيوميجاتس هو أفضل العزلات أنتاجا
للإنزيم وبدراسه تجويع النتيروجين على أنتاج الإنزيم وجد ان أنتاج الإنزيم قد ازداد بمقدار (5) أضعاف
مقارنه بالكنترول. تم تنقيه الإنزيم من فطر الأسبراجلس فيوميجاتس بأستخدام تقنيه كروماتوجرافى
الفصل الأيونى وفلتره الجل بمقدار(4.4) أضعاف وانتاجيه (29.9%) على
التوالى. وتم حساب الوزن الجزيئ (Vmax)و (Km) للإتزيم ليكون (48) كيلو دالتون. وقد
أظهر الإنزيم المنقى انه يتميز ب تساوى 0.08 مللى مول و 400 ميكرو مول /دقيقه/مللى جرام تجاه
السيتوزين. وأظهر الإنزيم المنقى ان احسن درجه حموضه هى (7) وان أفضل درجه حراره هى (37)
درجه سيليزيه وان فتره عمر النصف للإنزيم المنقى هى (8) ساعات مما يضمن الثبات الحرارى لكلا
من التركيب والنشاط لهذا الإتزيم. وأعتمادا على هذة النتائج فان انزيم السيتوزين دى أمينيز من فطر
الأسبراجلس فيوميجاتس يمكن أن يكون نواه للتطبيق فى علاجات السرطانات المعتمده على عقاقير اوليه
أو تستخدم تقنيه العلاج بالجينات.