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Research Article

Cytotoxicity of L-Methioninase Purified from Clinical Isolates of *Pseudomonas* Species in Cancer Cell Lines

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Abstract

Background: L-methioninase is an enzyme that was found in *Pseudomonas* spp. It changes L-methionine into α-ketobutyrate, ammonia, and methanethiol. It has been thoroughly investigated for possible antibacterial and anticancer activities. ***Objective***: The goal is to find out how well L-methioninase kills colon CaCo-2 and liver HepG2 cancer cells. ***Methods***: The enzyme was taken from 33 different types of Pseudomonas, and their ability to make L-methioninase was tested on M9 media that had been changed. An MTT assay was used to evaluate the cytotoxic activity of HepG2 and CaCo2 cell lines. ***Results***: Only 15 isolates were able to make L-methioninase. The best isolate had a specific activity of 1.4 µg/U protein. The enzyme's cytotoxicity showed that it stopped the growth of the HepG-2 cell line with an IC50 of 67.44 µg/ml, compared to an IC50 of 140.0 µg/ml for the crude enzyme, and it stopped the growth of the CaCo-2 cell line with an IC50 of 20.57 µg/ml, compared to 154.3 µg/ml for the crude enzyme. ***Conclusions***: Isolation of L-methioninase from microbial sources can be an efficient source to produce this cytotoxic agent.

Keywords: Anticancer activity, Cytotoxicity, L-methioninase, *Pseudomonas* Spp.

السمية الخلوية ل L-Methioninase المنقى من العزلات السريرية لأنواع من بكتريا Pseudomonas

الخلاصة

الخلفية: يتم عزل إنزيم L-methioninase من بكتريا *Pseudomonas* spp. ويحفز تحويل L-methionine إلى ألفا-كيتوبوتيرات، والأمونيا، والميثانيثيول، وقد تم فحصه بدقة بحثًا عن أنشطة محتملة مضادة للبكتيريا والسرطان. **الهدف:** تقييم التأثير السام للخلايا لـ L-methioninase على خطوط الخلايا السرطانية في القولون CaCo-2 والكبد HEp-G2. **الطرق:** تمت تنقية الإنزيم من 33 عزلة من عزلات *Pseudomonas*، وتم فحص قدرتها على إنتاج إنزيم L-methioninase باستخدام وسط M9 المعدل، وتم استخدام مقايسة MTT لتقييم النشاط السام للخلايا على خطوط خلايا HepG2 و-CaCo 2. **النتائج**: 15 عزلة فقط كانت قادرة على إنتاجL-methioninase ، العزلة الأكثر كفاءة كان لها نشاط نوعي قدره 1.4 ميكروجرام U\بروتين، وتشير السمية الخلوية للإنزيم إلى أنه يثبط نمو خط خلايا HepG-2 مع IC50 من 67.44 ميكروجرام/مل مقارنة بـ IC50 البالغ 140.0 ميكروجرام/مل للإنزيم الخام؛ و IC50قدره 20.57 ميكروجرام/مل ضد خط خلايا CaCo-2 مقارنة بـ 154.3 ميكروجرام/مل للإنزيم الخام. **الاستنتاجات**: عزل L-methioninaseمن المصادر الميكروبية يمكن أن يكون مصدرا فعالا لإنتاج هذا العامل كقاتل للخلايا السرطانية.

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INTRODUCTION

Pseudomonas is a huge genus of bacteria whose members have evolved to exist in a wide range of biological habitats, including humans, rivers, soils, plants, animals, reptiles, insects, and nematodes, among others [1]. One of the most prevalent species of pseudomonas is *Pseudomonas aeruginosa*, an opportunistic pathogen with multi-drug resistance (MDR). It can cause acute or persistent infection in immunocompromised people suffering from cancer, cystic fibrosis, sepsis, trauma, burns, and ventilator-associated pneumonia (VAP), including COVID-19-related cases [2]. Methionine-gamma-lyase, or L-methioninase, is one of the most fascinating enzymes. Methionine gamma-lyase (MGL) is not found in

mammals and has been the subject of more research recently due to its numerous potential medical uses, including as an antibiotic and in cancer treatment [3]. MGL (EC4.4.1.11) is an enzyme that needs pyridoxal phosphate (PLP) to work. It changes L-methionine directly into α-ketobutyrate, ammonia, and methanethiol [4]. A necessary amino acid for humans, methionine is involved in many aspects of mammalian metabolism, including the creation of protein, glutamine, and polyamines [5]. Multiple cancer cells were found to have an elevated need for plasma methionine in order to synthesize proteins and control DNA expression [6]. There are reports that bacterial enzymes have limited substrate specificity, high immunogenicity, and potentially harmful effects on the liver and kidney [7]. Current efforts in cancer research focus on developing novel chemotherapy regimens that make use of the distinct distinction between cancerous and normal cells, as is the case with MGL [4,8]. One particular feature of several cancers is methionine deficiency. Methionine was specifically required by cancer cells for their proliferation, and when MGL deprived these cells of it, the cancer cells were unable to develop because they lacked a method to make up for the methionine that was lost. Since cancer cells are more dependent on methionine in order to regulate the high synthesis of proteins and their DNA expression, enzyme therapy was therefore thought to be an effective way of destroying cancer cells using MGL [9].

METHODS

Sample collection

Thirty-three clinical samples collected from patient with various sources such as burns, sputum, ear infections, and UTIs patients whom attending to AL-Kadhimiya Teaching Hospital and Medical City in Baghdad, Iraq.

Screening for methioninolytic activity

Modified M9 media was utilized to determine the MGL productivities of *Pseudomonas aeruginosa* bacterial isolates using the qualitative fast assay plate technique. Prior to autoclaving, the medium's pH was finally brought to 7.0 and as an indicator, phenol red 0.007% was used [10]. The M9 plates were cultivated for 48 hours at 37°C for bacterial isolates.

In vitro cytotoxicity in HepG2 CaCo2 cell lines

An in vitro technique was used to look at how L-methioninase affected HepG2 and Caco-2 cancer cell lines. The cell line is maintained according to the Freshney method [11]. The MTT ready-to-use kit was used to test the cytotoxic effects of different concentrations (25, 50, 100, 200, and 400 µg/mL) of L-methioninase enzyme, both pure and crude. 96 flat-well micro-titer plates were used to grow tumor cells ($1x10^4$–$1x10^6$ cells/ml), with 200 µL of culture media added to each well to make the full volume. The plates were incubated overnight with 5% CO_2 at 37 ºC. Following the removal of the media, double serial dilutions of the required MGL concentrations (25, 50, 100, 200, and 400 µg/mL) were placed in the wells. For every concentration, triplicates of the controls—cells cultured in serum-free medium—were employed. Ten microliters of MTT solution were added to each well after the chosen exposure time of 24 hours at 37°C and 5% CO_2. The mixture was then incubated for another four hours under the same conditions. After the incubation, each well's medium was carefully removed, and 100 µL of solubilization solution was added. This was left for five minutes. By measuring using an ELISA reader at a wavelength of 575 nm. To find the chemical concentration required to cause a 50% loss in cell viability for each line of cells, a statistical analysis of the optical density data was conducted [11].

Statistical analysis

To determine the significance of the difference between groups, a one-way analysis of variance (ANOVA) and Duncan *post hoc* tests are used at $p<0.05$ to consider statistical significance. To conduct statistical significance tests and express information as mean±SD, GraphPad Prism version 6 was utilized (Graph Pad Software Inc., La J).

RESULTS

We used modified M9 media to find out how well l-methioninase worked in bacteria isolates using a qualitative rapid assay plate method. We found out how much L-methioninase was being made by seeing if a pink colony could grow on minimal salt media with phenol red (Figure 1).

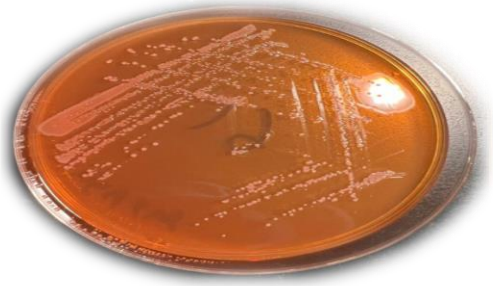

Figure 1: The screening results of pink colony of Pseudomonas aeruginosa for positive producers of L-methioninase on modified M9 media supplied with L-methionine at 37°C for 48 hrs.

Among the 33 bacterial isolates, 15 showed a positive result: *P. aeruginosa* A6 was possibly the most extensive producer of L-methioninase, with a specific activity of 1.4 µg/U, and was selected for further assays. We tested l-methioninase's cytotoxic effect on HepG2 and CaCo2 cell lines in a lab setting. The concentrations used ranged from 25 to 400 µg/ml. Applying varying concentrations of MGL to tumor and normal cell lines allowed for the measurement of cell viability in this test. When cell viability was assessed at each time point (24 hours) using the MTT

colorimetric test, the best values for the most significant IC50 values were chosen [12].

Table 1: Cytotoxicity effect of L-methioninase on HepG2 and HdFn cells after 24-hour incubation at 37ºC

L-methioninase (µg/ml)	Viable HepG2 cells	Viable HdFn cells	*p*-value
25	85.03±1.98	95.83±2.23	0.0033
50	82.98±10.47	95.06±0.37	0.1165
100	75.81±3.64	91.89±1.00	0.0018
200	61.92±2.02	82.44±7.22	0.0090
400	54.78±2.43	69.83±2.84	0.0022

Values are presented as mean±SD.

After treating cells with crude L-methioninase, the increase in L-methioninase enzyme concentration lowers cell viability. A 400µg/ml of crude l-methioninase (54.78±2.43%) drop in HepG2 cell viability (%) was seen (Table 1), and it had the strongest cytotoxic effect, with an IC50 value of 140 µg/ml (Figure 2A).

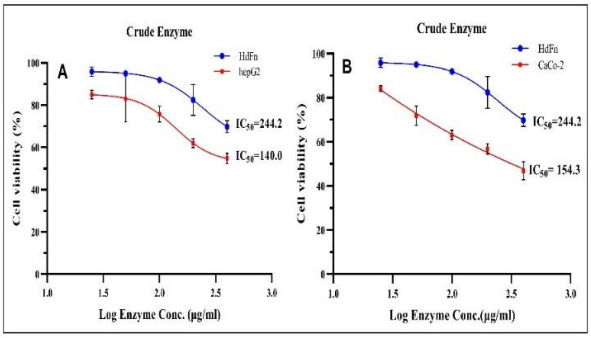

Figure 2: The cytotoxic effect of (A) Crude L-methioninase on liver HepG-2 and HdFn cells, (B) Crude L-methioninase on CaCo2 and normal cells HdFn, after 24-hour incubation period at 37 ºC.

At 400 µg/ml (46.84±4.11), crude L-methioninase was also found to lower the viability (%) of CaCo2 cells (Table 2). It also showed very strong cytotoxicity, with an IC50 value of 154.3 µg/ml compared to 244.2 µg/ml for the effect of crude L-methioninase on the HdFn normal cell line (Figure 2B).

Table 2: Cytotoxicity effect of L-methioninase on CaCo2 and HdFn cells after 24-hour incubation at 37ºC

L-methioninase (µg/ml)	Viable CaCo2 cells	Viable HdFn cells	*p*-value
25	84.18±1.38	95.83±2.23	0.0015
50	71.87±4.39	95.06±0.37	0.0008
100	63.07±2.12	91.89±1.00	<0.0001
200	56.75±2.38	82.44±7.22	0.0043
400	46.87±4.11	69.83±2.84	0.0013

Values are presented as mean±SD.

When cancer cells were treated with purified L-methioninase, the enzyme had the most cytotoxic effects (IC50 = 67.44 µg/ml) and cell viability dropped to 400 µg/ml for the HepG2 cancer cell line (Figure 3A).

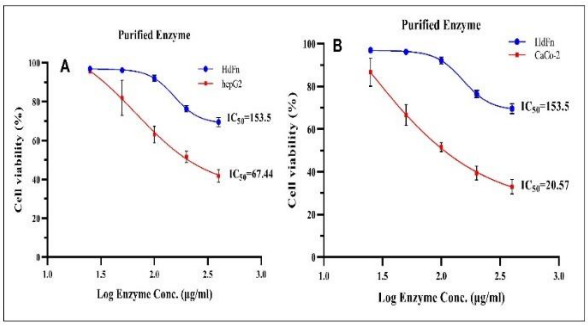

Figure 3: The cytotoxic effect of (A) Purified L-methioninase on liver HepG-2 and HdFn cells, (B) Purified L-methioninase on CaCo2 and normal HdFn cell, after 24 hours incubated at 37 ºC.

While the purified L-methioninase manifested significantly the most efficient cytotoxic activity with an IC50 of 20.57 µg/ml at 400 µg/ml on the CaCo2 cancer cell line (Figure 3B), the purified L-methioninase, on the other hand, had an IC50 value of 153.5 µg/ml when tested on the HdFn normal cell line (Table 3).

DISCUSSION

Mammals do not contain L-methioninase. There are reports that bacterial enzymes have a limited substrate specificity, a high immunogenicity, and potentially harmful effects on the liver and kidney [7].

Table 3: Cytotoxicity effect of L-methioninase on HepG2, CaCo2 and HdFn cells after 24-hour incubation at 37°C

L-methioninase (µg/ml)	Viable HepG2 cells	Viable CaCo2 cells	Viable HdFn cells	*p*-value (ANOVA)
25	85.03±1.98[a]	84.18±1.38[b]	95.83±2.23[a,b]	0.0005
50	82.98±10.47	71.87±4.39[b]	95.06 ±0.37[b]	0.0158
100	75.81±3.64[a]	63.07±2.12[a,b]	91.89± 1.00[a,b]	<0.0001
200	61.92 ±2.02[a]	56.75±2.38[b]	82.44 ±7.22[a,b]	0.0010
400	54.78+2.43[a]	46.87±4.1[b]	69.83±2.84[a,b]	0.0003

Values are presented as mean±SD. Values with identical superscripts (a,b) are significantly different ($p<0.05$) based on *post hoc* analysis.

They also have an effective anticancer agent that has been proven to be active against several cancer cell lines, including those from the lung, glioblastoma, kidney, breast, and colon [13]. From these isolates from *P. aeruginosa*, the most efficient isolate producing l-methioninase was selected. The current study corresponds with Sundar and Nellaiah (2013), who stated that MGL-producing isolates were recognized as pink colonies or growth forms for *Serratia marcescens* species, which are produced when MGL reacts with L-methionine to produce ammonia [14]. According to El Sayed *et al.* (2012), L-methioninase's *in vitro* anticancer efficacy was also reported; they reported that L-methioninase has

demonstrated notable efficacy against multiple cancer cell lines, including those from the breast, lung, colon, neck, and head; testicular germ cell cancer; acute myeloid leukemia; and glioblastoma. Numerous cancer cells were shown to have an elevated plasma methionine requirement for DNA expression regulation and protein synthesis [15]. It might be possible to effectively treat cancer by using l-methioninase to stop the growth and spread of cancer cells that depend on l-methionine [6]. when methionine-dependent (MET-dp) cancer cells in vitro undergo methionine degradation, the enzyme l-methioninase inhibits mitosis and causes arrest of the cell cycle, principally in the cell cycle's late S/G2 phase, which is followed by apoptosis [16]. As reported previously above, l-methioninase purified from *P. aeruginosa* proved to have a concentration-dependent impact that was effective against the cancer cell lines CaCo2 and hepG2. Our findings support the earlier research by Salim et al. 2020, who showed the potent anticancer action of l-methioninase on cancer cells within IC50 values of 14.12 µg/ml for the purified L-methioninase from *Trichoderma harzianum* [17], where l-methioninase from *Aspergillus fumigatus* possessed an IC50 value of 243 ± 4.87 µg/ml (0.486 U/ml) against Hep-G2 [18]. Also, Abdelraof *et al*. (2019) indicated that Streptomyces DMMMH60 was found to produce MGL, a potent anticancer agent with high efficiency. Colon carcinoma (HCT-116) was the most sensitive cell type, with an IC50 of 0.69 µg/ml. Hepatocellular carcinoma (HepG2) was next, with an IC50 of 5.46 µg/ml, and intestinal carcinoma (CACO) was third, with an IC50 of 11.3 µg/ml [4].

Conclusion

Pseudomonas aeruginosa was one of the most efficient sources of L-methioninase production. Also, L-methioninase wasn't cytotoxic to normal cell lines, but it was cytotoxic to both Hep-G2 and CaCo-2 cell lines.

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Conflict of interests

No conflict of interests was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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