Characterization of lipase from *Trichoderma viride* and its role in the management of pancreatic exocrine insufficiency

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Abstract: Lipases of *Trichoderma viride* were precipitated from the cultural medium with different methods. Acetone precipitation at 60% saturation point was the best one. The enzyme was partially purified by gel filtration chromatography using sephadex G-75. The enzyme was found to be of 25.2 KDa. The maximal activity has been obtained at high temperature (60°C) but the enzyme tolerates temperature up to 45°C. Also, it is characterized by its high stability and activity at the acidic pH values. The maximum activity has been maintained at pH 3 (49.1 unit/ml), with 80% residual activity. Also, it was stable with enhanced activity in the presence of bile salts (124% residual activity was maintained even after 45 min. incubation with 6 mM sodium cholate). Also, lipase enzyme showed nearly 100% residual activity in presence of trypsine enzyme. The enzyme has been tested for its ability in the management of pancreatic exocrine insufficiency using L-arginine induced acute pancreatitis mice models. The enzyme has the ability to decrease the weight loss and enhance fat digestion in this case. The total lipid level in serum after fat diet was enhanced from 837.99 mg/dl, in case of acute pancreatitis group without enzyme supplying, to 949.7 mg/dl, in case of acute pancreatitis group supplied with the enzyme, and a total weight gain of +0.21 gm body weight has been reported after the enzyme supplying for only 4 days.
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1.Introduction

Lipases (triacylglycerol acylhydrolases; EC 3.1.1.3) are one of the most important classes of hydrolytic enzymes that catalyse the hydrolysis and synthesis of esters. Hydrolysis of a triacylglycerols by lipases can yield di- and monoacylglycerols, glycerol and free fatty acids **(Brabcova *et al*., 2010)**.

Lipases are ubiquitous enzymes which are found in most organisms from the microbial, plant and animal kingdom **(Reis *et al*., 2009)**. Microbial lipases are commercially significant because of their advantages over lipases from other origins. Microbial lipases characterized by their stability in organic solvents, broad substrate specificity, their high enantio selectivity beside their wider availability, relative ease of purification and lower production cost than plant and animal lipases **(Pollero *et al*., 2001 and Aravindan *et al*., 2007)**. Fungi are potent sources of lipases. Studies on fungal lipases have been started as early as 1950 since then, many workers have exploited as a valuable source of lipase due to the following properties; thermal stability, pH stability, substrate specificity and activity in organic solvents. Fungal lipases have benefit over bacterial ones due to the fact that present day technology favors the use of batch fermentation and low cost extraction methods. In this regard, a good number of fungi have been screened for lipase production. The chief producers of commercial lipases are *Aspergillus niger, A. terreus. A. carneus, Candida cylidrocea, Rhizopus arrhizus, R. delemar, R. japonicus, R. niveus, R. oryzea Candida rugosa, Candida antarctica, Thermomyces lanuginosus* and *Rhizomucor miehei* **(Ghosh *et al*., 1996; Adnan, 1998 and Sharma *et al*., 2001)**.

Pancreatic exocrine insufficiency is a major consequence of diseases leading to a loss of pancreatic parenchyma (e.g. chronic pancreatitis, cystic fibrosis), obstruction of the main pancreatic duct (e.g. pancreatic and ampullary tumors), decreased pancreatic stimulation (e.g. celiac disease), or acid-mediated inactivation of pancreatic enzymes (e.g. Zollinger-Ellison syndrome). Abdominal cramps, fatty stools associated with steatorrhea and weight loss are the main symptoms in patients with pancreatic exocrine insufficiency as a result of malfat digestion. The aim of pancreatic enzyme substitution therapy in this case is not only to relieve maldigestion-related symptoms, but mainly to achieve a normal nutritional status (Domínguez-Muñoz, 2011). The mainstay of treatment in pancreatic steatorrhoea has been replacement of endogenous pancreatic enzymes by supplements from hog pancreas (Pancreatin - Paynes and Byrne). This has often proved unsatisfactory in the long term as many patients require 20 or 30 tablets per meal **(Griffin *et al*., 1989)**. Enzyme replacement with

pancreatic enzyme supplements is associated with the following inherent limitations:

1- Pancreatic enzymes are susceptible to being destroyed by gastric acid and pepsin. A pH sensitive enteric coating designed to dissolve above a pH of 5.5 to 6.0 has been applied to various forms of pancreatic enzymes in an attempt to protect them through the hostile environment of the stomach. The enteric coated supplements usually remained intact in the stomach but often failed to dissolve in the small intestine due to hyperacidity in the duodenum where, most patients with pancreatic insufficiency have reduced bicarbonate secretion **(Griffin *et al.*, 1989)**.

2- The activity of pancreatic enzymes is dependent on the existence of specific pH levels in the small intestine that may be lacking in those with impaired health **(Rachman, 1997)**.

Therefore, lipases derived from certain fungi have been suggested as a potential alternative treatment. A theoretical advantage of fungal lipases is their greater resistance against acid; hence their protection is not needed for their passage through the stomach **(Turki *et al.*, 2010)**. Moreover, fungal lipases show high activity over a wide range of pH values.

2. Material and methods:

I- Production and purification process:-

1:- production of lipase and preparation of cell-free filtrate:-

Two liters of lipase production medium (g/l:- $MgSo_4.7H_2o$ 0.5, KH_2Po_4 1, $NaNo_3$ 3, Peptone 30, $CaCl_2.2H_2o$ 0.5, Peanut oil 0.2% v/v, pH= 5) were prepared and distributed in 250 ml conical flasks (50 ml in each flask) and autoclaved at 121.5 °C for 20 minutes. After cooling each flask was inoculated with 4 fungal discs (0.8 cm, 4 days old culture). All flasks were incubated at 25°C for 6 days.

At the end of the incubation period, the supernatant was harvested by filtration by using Whatman filter paper no.1 and used as a crude enzyme extract.

2:-Precipitation of the enzyme from supernatant:-

Three precipitation methods have been carried out previously on a small scale to choose the best method for the enzyme precipitation.

2-1: Ammonium sulfate precipitation:-

Ten ml of cold filtrate was put on 50 ml beaker placed in ice bath then placed over a stirrer. The amount of the solid ammonium sulfate which was calculated to bring the concentration from zero % to 20 % was added to the filtrate. After the dissolving, the filtrate was kept to stand for (5-30 min). The pellets were collected by centrifugation at 6000 rpm for 15 min at 4°C. The pellets were dissolved in 5 ml phosphate buffer (0.5 M, pH 7.0). The supernatant was treated again with the ammonium sulfate by the same method to achieve the following saturation points (40, 50, 60, 70, 80 and 100 %).

To determine the amount of the ammonium sulfate which must be added to achieve any given concentration, the chart of **(Gomori, 1955)** as mentioned by **(Dixon and Webb, 1964)** has been applied.

2-2:-Ethanol and Acetone precipitation (Chykin, 1966):-

Ten ml of cold filtrate was put on 250 ml beaker placed in ice bath then placed over a stirrer.

Cold Ethanol or Acetone was added to the filtrate with the amount which was calculated previously to bring the concentration to 20 % saturation. After standing for (10-15 min), the pellets were collected as mentioned above. The supernatant was used for achieving the (40, 60, 80 and 90 %) saturation points by the same method.

The lipase activity and the protein amount were assayed in each fraction according to the **licia *et al.*, 2006** and **Bradford, 1976** methods respectively, for each fraction and also for the crude filtrate.

3:-Gel filtration chromatography (Wilson and Walker, 1995):-

The resulting pellets, obtained from 1600 ml of filtrate, were dissolved in a small volume of phosphate buffer 0.05M pH 7, and dialyzed against sucrose to concentrate it to a smaller volume.

Column baked with Sephadex G-75 (sigma) with bed of 2.2 × 45 cm was used for gel filtration process. The column was connected to the buffer reservoir and the flow rate of the buffer was maintained at a constant rate of approximately 40 ml /h. After the loading of sample, the protein band was allowed to pass through the gel by running the column. Eighty fractions each of 5 ml were collected and separately tested for both the protein content (at 280 nm) and for the lipase activity. Fractions that have the highest lipase activity were collected together and this enzyme solution was stored at the refrigerator and used for the further characterization and application study.

II-Characterization of the partially purified enzyme:-

Lipase activity was determined with paranitrophenyl- palmitate (pNPP) as the method described by **Licia *et al.*, 2006** (modified method).

Absorbance was read at 410 nm using T 60U spectrometer against blank. One unit of lipase is defined as the amount of the enzyme which librates 1 µmole pNP per minute under the assay conditions.

(II-1):-Effect of temperature:-

(a)-On enzyme activity: The reaction mixture (1 ml substrate emulsion + 1.9 phosphate buffer (pH7.0, 0.1 M) + 0.1 ml partially purified enzyme) was incubated

at different temperature values (15,30,45, 60, and 75 °C) for 1 h, then the assay was completed as the standard assay method.

(b)- On enzyme stability: The partially purified enzyme solution was incubated at different temperature values (15, 30, 45, 60, and 75°C) for 15, 30 and 60 min, and after each period the solution was immediately cooled to 4 °C. The activity was determined as the standard method.

(II-2):-Effect of pH:-

(a)-On enzyme activity: Different pH values (3, 4, 5, 6, 7, 8, 9, 10, and 11) have been applied using Tris-buffer (0.1M) as the reaction buffer. In this test the activity was calculated by the aid of different paranitrophenol standard curves that are prepared at different pH values (because the molar absorptivity of pNP is changed according to the pH value of the solution).

(b)- On enzyme stability: The partially purified enzyme solution (0.1 ml) has been incubated with 0.2 ml of the Tris buffer (0.1 M) of different pH values for 15, 30 and 60 min. At the end of the incubation period the substrate emulsion and the assay buffer (phosphate buffer 0.1 M pH 7) were added immediately to complete the assay under the standard conditions.

(II-3):-Stability of the partially purified enzyme in the presence of bile salts:-

Fifty µl of the partially purified enzyme solution were incubated with 50µl of a bile salt solution (Sodium Cholate, C24H39Nao4) of different concentrations (2, 4 and 6 mM) for different periods of time (zero time, 15 and 45 min). The control was the enzyme without any bile salt addition. At the end of the incubation period the assay was completed as usual immediately to determine the enzyme activity. The residual activity was calculating as following:

Residual activity = (activity of treatment / activity of control) × 100.

(II-4):- Stability of the partially purified enzyme in the presence of proteolytic enzymes (Trypsine enzyme):-

Enzyme solution (0.1 ml) was incubated with 10, 50, 100 µl of the trypsine enzyme solution (0.25%, 1X) at a basic pH (Tris buffer, 0.1 M, pH 8) for 30 min at room temperature. At the end of the incubation period the assay was completed as usual immediately to determine the enzyme activity and the residual activity. The enzyme activity was compared with the activity of the control (without trypsine addition) and with the activity of the zero time incubation period.

(II-5):-Determination the enzyme molecular weight by Polyacrylamide Gel Electrophoresis (SDS-PAGE) (laemmli, 1970):

The enzyme molecular weight was determined by one dimensional Sodium Dodecyle Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) (12% separating gel and 4% stacking gel) according to **laemmli, (1970)** in minigel apparatus (Bio-rad) according to the method of **Hames, (1990)**.

III- Management of pancreatic exocrine insufficiency:-

(III-1) Animals:-

Twenty seven Swiss male mice weighting about 25-30 g were purchased from VACSERA (Helwan-Cairo- Egypt). All animals were kept at a constant temperature of 25 °C with a 12 h light- dark cycle. The animals were maintained on a fixed dietary intake and were allowed free access to water for 5 days for adaptation then they were divided into 3 groups each group of 9 mice.

(III-2) Induction of Pancreatic Exocrine Insufficiency by the induction of acute pancreatitis (Naito, *et al,* 2003 and Dawra and Saluja, 2012):-

(a)- Solutions:-

1- Saline solution (0.15 M NaCl)

2- L-arginine hydrochloride solution (0.8 %),

(prepared freshly before the injection):-

(800 g L-arginine hydrochloride was dissolved in 8 ml saline solution then the pH was adjusted to 7 with 1N NaoH and the volume was made up to 10 ml with the saline solution).

(b)- Injection:-

(1) Animals were divided into 3 groups: Group 1, group +ve control and group –ve control. Each group contains 9 mice.

(2) Animals were weighted before the injection.

(3) Group 1 and group +ve control were injected with L-arginine solution in a dose of 400 mg / 100 g body weight (1.5 ml / 30 g b.w.) intra-peritoneally followed by second i.p. injection of the same dose after 1 h.

(4) Group –ve control was injected with equivalent volume of the saline solution also in double dose i.p. injection.

The animals were kept under observation and allowed free access to feed and water for 5 days then reweighted.

(III-3) The effectiveness of the produced lipase enzyme on the enhancement of fat digestion in acute pancreatitis mice models:

1- All tested mice groups have been feed on high fat containing diet: after 5 days of the injection, the diet was replaced with high fat containing diet.

2- Supplying the mice of group 1 with the lipase enzyme:

After 3 days of high fat diet, mice of group 1 were weighted then supplied with the partially purified lipase enzyme for further 4 days, where the

lipase enzyme was added to the drinking water after the high fat containing meal

The total lipase units that were supplied to the animals of this group during this study were 200 units dissolved in 40 ml phosphate buffer (0.1 M, pH7).

3- Decapitation and collection of blood serum:

After the high fat meal (and the enzyme supplying in case of group 1) and within 4h., three mice from each group were weighted and decapitated for blood collection. The blood of the three mice of each group were pooled together and left to coagulate at room temperature then centrifugated at 6000 rpm for 20 minutes for serum collection. The serum was stored at -20°C until determination the total lipid level.

This process has been carried out through 3 successive days by the same method.

4- Assessment of the lipase effectiveness:

(a) By determination the total lipid level in the serum of each group. The total lipid level was measured by the use of total lipid kit (Bio-Diagnostic).

(b) By calculating the weight loss during the study in each group.

3. Results and Discussion:-

I- Production and purification process:-

1:- production of lipase and preparation of cell-free filtrate:-

The protein content and lipase activity were estimated in the filtrate, they were 68.06 µg protein/ ml and 5.87 lipase unit/ml. This crude enzyme extract used for further purification steps.

2:- Precipitation of lipase enzyme from the supernatant:-

The best method for the enzyme precipitation was determined previously by testing different methods for the enzyme precipitation such as; ammonium sulfate, acetone and ethanol precipitation. This was carried out on a small volume of the crude enzyme extract (10 ml) prepared previously.

By calculating the specific activity and the purification fold for each fraction in each precipitation method, as shown in table (1), F3 (60 %) in the acetone precipitation was the best fraction, with lipase activity of 11.88 unit/ml and a lowest protein content (35.10 µg/ml).

(Note):- Specific activity = enzyme activity/ mg protein.

Recovery (yield) = enzyme activity of the fraction / enzyme activity of the crude ×100.

Purification = specific activity of the fraction / specific activity of the crude.

3:-Gel filtration chromatography:-

The protein content and the lipase activity were represented graphically against the fraction number as in the fig. (1).

The fractions (11:21) were collected, mixed together and tested for lipase activity and the protein content which was 3.74 units/1 ml for lipase activity and 7.27µg/ml for protein. The specific activity and the purification fold were calculated (table 2).

Table (1): Specific activity and the purification fold for each fraction in each precipitation method:

Fraction no.	Total volume (ml)	Total protein (mg)	Total activity (unit)	Specific activity	Recovery (%)	Purification fold
Crude	10.00	2.58	142.20	55.12	100	1.00
Amm. F1	5.00	0.096	5.15	53.65	3.62	0.97
Amm. F2	5.00	0.62	65.50	105.70	46.10	1.92
Amm. F3	5.00	0.27	52.50	194.40	36.92	3.53
Amm. F4	5.00	0.47	30.15	64.15	21.20	1.164
Amm. F5	5.00	0.14	0.30	2.14	0.21	0.039
Amm. F6	5.00	0.11	0.20	1.82	0.14	0.03
Amm. F7	5.00	Zero	Zero	Zero	-	-
Eth. F1	5.00	0.09	2.65	29.40	1.86	0.53
Eth. F2	5.00	0.09	3.55	39.40	2.50	0.72
Eth. F3	5.00	0.20	5.50	27.50	3.86	0.50
Eth. F4	5.00	0.75	68.50	91.30	48.17	1.66
Eth. F5	5.00	0.59	6.55	11.10	4.61	0.20
Ac.F1	5.00	0.125	3.85	30.80	2.71	0.56
Ac.F2	5.00	0.193	7.15	37.05	5.03	0.67
Ac F3	5.00	0.175	59.40	339.40	41.77	6.16
Ac.F4	5.00	0.42	23.70	56.43	16.67	1.02
Ac.F5	5.00	0.24	3.85	16.04	2.71	0.29

Amm.: Ammonium sulfate, Eth.: Ethanol, Ac.: Acetone, F: Fraction

Table (2): Lipase activity and protein content after acetone precipitation and column chromatography:

Fraction no.	Total volume (ml)	Total protein (mg)	Total activity (unit)	Specific activity	Recovery (%)	Purification fold
Crude	1600.00	108.90	9392.00	86.24	100.00	1.00
After precipitation	192.00	16.20	1824.00	113.15	19.40	1.31
After column purification	386.00	2.81	1445.00	514.24	15.50	6.00

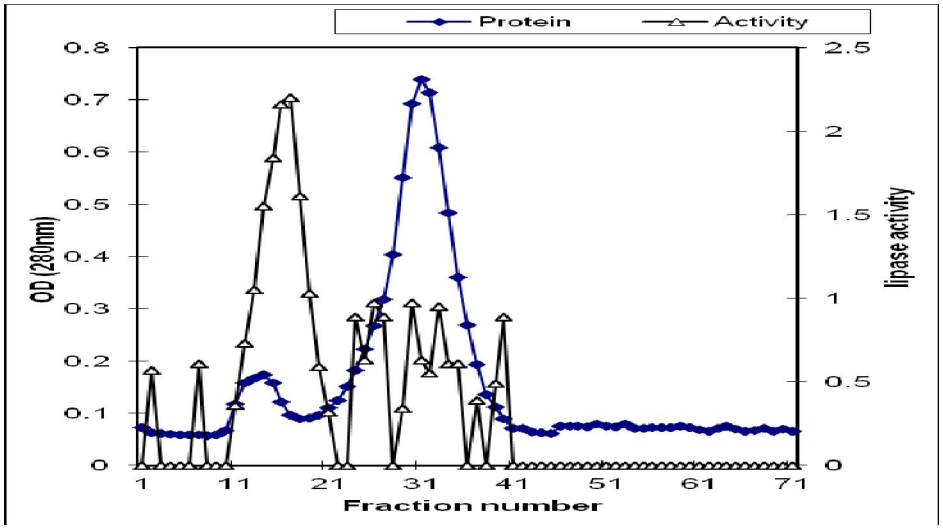

Fig. (1): Purification chart for lipase enzyme produced by *Trichoderma viride* using gel filtration chromatography

II-Characterization of the partially purified enzyme:-

(II-1):-Effect of temperature:-

(II-1-a): On enzyme activity:-

The results were as shown in table (3), where the increase in the temperature value leads to the increase in the enzyme activity until reach a maximum value at 60 °C (8.71u/ml) but at 75°C the activity has been lost completely. The results are not similar to that reported for the maximum lipase activity of *Epipactis gigantea* and other lipases which were found to be in the range of 30-35°C, but in agreement for the lipase produced by thermophilic bacteria obtained from Icelandic hot springs that had higher lipase activity at 40-60°C **(Aravindan *et al*., 2007)**.

(II-1-b): On enzyme stability:-

The results of table (4) show the effect of temperature on enzyme stability where, the enzyme remains stable even for long periods (60 min) up to 45°C (retains nearly 75.9 - 94 % of its activity), but began to lose its activity completely at temperature 60 °C and above this value. The present study revealed that lipase of *T. viride* is more sensitive to temperature than lipase of *Fusarium oxysporum* which loses its activity completely at 90°C (**Donia, 2010)**.

(II-2):-Effect of pH:-

(II-2-a): On enzyme activity:-

The obtained results were shown in table (5), where the enzyme showed activity over a wide range of pH values, but the maximum activity has been observed at acidic medium. The enzyme shows very high activity (49.1unit/ml) at pH 3.0 and this value decreased with the increase in the pH value until pH 6.0 at and above which the enzyme showed reduced activity with a small fluctuation in the activity values to be lost completely at pH 11. That pH activity profile is resemble to that reported for some microbial lipases which showed maximal activity at low pH values, where the extracellular lipase produced by *Aspergillus niger, Chromobacterium viscosum* and *Rhizopus* species are particularly active at acidic pH **(Aravindan *et al*., 2007 and Marcrae *et al*., 1985)**. *Aspergillus niger* NCIM 1207 showed maximum activity at pH 2.5 **(Licia *et al*., 2006)**.

(II-2-b): On enzyme stability:-

The results of table (6) show that, the highest stability has been observed at alkaline pH values. However at acidic medium the enzyme retained a considerable stability (nearly 80%) if compared with the control value (pH 7.0). That supports the data published by **Aravindan *et al*., 2007** where, some lipases are stable over a wide range of pH values.

Table (3): Effect of different temperature values on the enzyme activity:-

Temperature (°C)	15	30*	45	60	75
Enzyme activity (units/ml)	0.72	1.09	4.52	8.71	zero

* Control

Table (4): Effect of different temperature values on the enzyme stability:-

Temperature (°C)	Enzyme activity (units/ml)		
	15 min.	30 min.	60 min.
15	o.54	0.4	o.41
30	0.55	0.49	0.49
45	0.51	0.47	0.47
60	Zero	Zero	Zero
75	Zero	Zero	Zero

Table (5): Effect of different pH values on the enzyme activity:-

pH value	3.0	4.0	5.0	6.0	7.0*	8.0	9.0	10.0	11.0
Enzyme activity (units/ ml)	49.1	25	8.17	2.89	1.03	3.9	3.6	2.1	zero

* Control pH

Table (6): Effect of different pH values on the enzyme stability:-

pH value	Enzyme activity (units/ ml)		
	15 min.	30 min.	60 min.
3.0	1.7	1.2	1.2
4.0	1.18	1.06	1
5.0	1.3	1.13	0.96
6.0	1.23	1.16	0.96
7.0*	1.5	1.5	1.49
8.0	2.55	2.15	1.8
9.0	2.7	2.8	2
10.0	3.5	3.15	2.24
11.0	2.9	2.8	2.8

* Control pH value

II-3:- Stability of the partially purified enzyme in the presence of bile salts:-

The results were shown in table (7), the enzyme retains its activity in the presence of bile salt at the tested values. On the other hand, the activity of the enzyme increases with the increase in the incubation period for each concentration if compared with the control value (without addition of bile salt). The stability was also observed in the case of lipase enzyme produced by *Rhizopus japonicus* NR400 which was not affected by the addition of bile salts **(Hasan *et al*., 2009)**.

II-4:- Stability of the partially purified enzyme in the presence of proteolytic enzymes (Trypsine enzyme):-

The obtained results were shown in table (8).The enzyme showed high stability in the presence of trypsine enzyme even in the presence of high concentration. The stability of lipase enzyme in the presence of the proteolytic enzymes gives it an advantage for the medical applications, where Proteolytic inactivation of lipase was a possible cause of the uneven results obtained with enzyme substitution in pancreatic insufficiency **(Pap and Varro, 1984)**. Hence, that lipase enzyme has been proved in vitro to possess several biochemical properties that are needed for its application as a digestive aid. The enzyme was found to be fairly stable and still active at pH 3.0 in the presence of bile salts and in presence of Trypsine enzyme.

Table (7): Stability of the partially purified enzyme at different concentrations of bile salts:-

Bile salt conc. (mM)	Enzyme activity (units/ml)			Residual activity (%)		
	Zero time	15 min.	45 min.	Zero time	15 min.	45 min.
Control	1.85	1.85	1.85	100	100	100
2.0	1.5	1.7	2.2	81.1	91.9	118.9
4.0	1.7	2.12	2.2	91.9	114.6	118.9
6.0	1.6	2.2	2.3	86.5	118.9	124.3

Table (8): Stability of the partially purified enzyme in the presence of proteolytic enzymes (Trypsine enzyme):-

Trypsine volume (µl)	Enzyme activity (units/ml)	Residual activity (%)
Control (zero)	1.8	100
10.0	2.1	116
50.0	1.9	105
100.0	1.9	105

(II-5):- Determination of the enzyme molecular weight by Polyacrylamide Gel Electrophoresis (SDS-PAGE):

The SDS-PAGE was analyzed by (Quantity 1) software and showed a single band separated in the purified enzyme lane of molecular weight 25.2 KDa and that band hase been observed in all lanes (precipitated enzyme - precipitated enzyme after dialysis – after column chromatography) as in fig. (2). Similar observations were reported for *Penicillum expansum* DSM 1994 and *Pe. Roqueforti* IAM 7268, the produced lipase was of molecular weight of 25 KDa (**Stöcklein *et al.*, 1993 and Sharma *et al.*, 2001).**

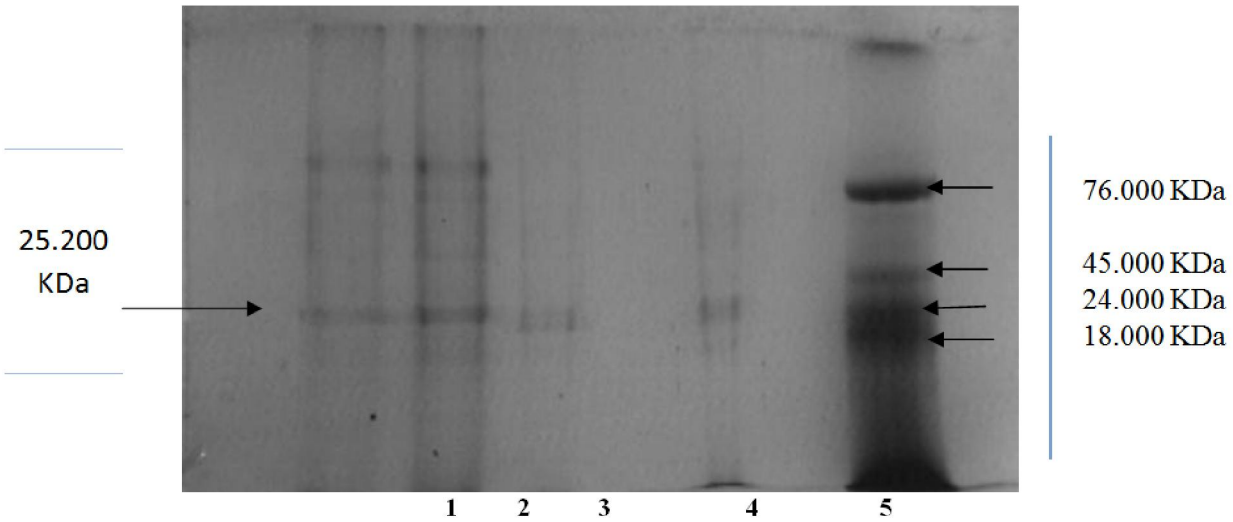

Fig. (2): Result of SDS-PAGE for determination of the enzyme molecular weight.
Lane 1: Precipitated enzyme at F3 with Acetone before dialysis
Lane 2: Precipitated enzyme at F3 with Acetone after dialysis
Lane 3,4: Collected fractions of lipase activity after column chromatography
Lane 5: Protein molecular weight markers

(III) The effectiveness of the produced lipase enzyme on enhancement of fat digestion in acute pancreatitis mice models:

Mice of group 1, and +ve control group were injected with L-arginine to induce acute pancreatitis. They were weighted before the injection and after the injection (after 5 days of carbohydrate diet and after another 5 days of high fat diet) and the results were as shown in table (9). From the results it was obvious that the weight loss in the first 5 days after the injection was - 2.1 g and increased after the second 5 days to – 2.56 g. That represents an evidence of acute pancreatitis (bad digestion), especially when compared with the weight loss in the – ve control group (injected with saline only) which show weight loss of -0.65 g only after the 10 days as shown in table (10).

The effect of the supplied enzyme in the enhancement of lipid digestion in mice of acute pancreatitis of group 1 can be determined by observing:

(a) The reduction in the value of the weight loss after the lipase supplying in group 1 as observed in table (11). After the high fat diet, the mean value of the weight loss in this group was -0.92 g but after supplying this group with the lipase enzyme for 4 days, there was no further increase in the weight loss value but there was a weight gain with a value of +o.2 g. Thus the total weight loss in this group at the end of the study was (-0.75 g) less than the total weight loss in the + ve control group which show total weight loss

(-0.97), as shown in table (12), which represents an evidence that there was an enhancement in the digestion in the group 1 after the lipase supplying.

(b) The increase in the level of total lipid in the serum of group 1 (if compared with the + ve control group) after the lipase supplying as shown in table (13). That was another evidence of the enhancement in the lipid digestion after lipase supplying.

Table (9): The body weight of mice in group 1 and group + ve control before and after the l-Arginine injection:

Body wt.(g) Group 1 and +ve control before injection	Body wt.(g) Group 1 and +ve control after the injection(5days)	Body wt.(g) Group 1 and +ve control after further 5 days of lipid diet
Mean=29.3±2.26	Mean=27.07±2.85	Mean=26.23 ±2.9

The L-arginine injection induced pancreatitis and led to weight loss with - 3.07 g (- 2.23 g after the first 5 days and - 0.84 g after the second 5 days)

Table (10): The body weight of mice in group – ve control before and after the saline injection:

Body wt.(g) before the injection	Body wt.(g) after the injection (10 days)
Mean=28.31±1.9	Mean=27.66±1.25

There was a weight loss with - 0.65 g only

Table (11): The body weight of mice in group 1 during the lipid diet and the lipase supplying:

Body wt.(g) after carbohydrate diet	Body wt.(g) after lipid diet	Body wt.(g) after lipid diet and lipase supplying
Mean=25.91±2.6	Mean=25 ±2.8	Mean=25.2±2.74

Total weight loss -0.71 g, (- 0.91g after lipid diet and + 0.2 g after lipid diet and lipase supplying)

Table (12): The body weight of mice in group + ve control during the lipid diet:

Body wt.(g) after carbohydrate diet	Body wt.(g) after lipid diet
Mean=28.23±2.75	Mean=27.26±2.8

Total weight loss - 0.97 g

Table (13): Serum total lipids

Group	Total lipid level in serum [mean of 3 successive decapitations through 3 days] (mg/dl)
Group 1	860.23 ±126.9
Group + ve control	807.06 ± 107.8
Group – ve control	1001.73 ±142.06

Group 1: mice injected with L-arginine to induce acute pancreatitis and supplied with the lipase enzyme.
Group + ve control: mice injected with L-arginine to induce acute pancreatitis but without the lipase enzyme supplying.
Group – ve control (healthy group): mice injected with saline only.

4. Conclusion:

Lipase enzyme obtained from *Trichoderma viride* has been proved to be stable and active under certain important physiological conditions resemble to those of human gasterointestinal system. It can resist the acidic conditions thus cannot be deactivated in the stomach. Also, doesn't deactivated by trypsin enzyme and bile salts. The application of this enzyme in the management of pancreatic exocrine insufficiency in acute pancreatitis mice models has improved the fat digestion and decreased the weight loss. Further studies will be required to get the enzyme in highly purified form to get highly effective responses in fat digestion. Also, the role in controlling pancreatic exocrine insufficiency could be better understood by further investigations and extending the experiment duration for long periods.

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