CHAPTER 4

DISCUSSION AND CONCLUSION

4.1) DISCUSSION

4.1.1) Screening and characterization of a myrosinase-producing fungus

By a plate method, *Rhizopus* sp., *Mucor* sp., *Aspergillus* sp. and a gram negative rod bacterium were isolated from decayed mustard seed meal samples as glucosinolate-degrading microorganisms. During the screening process, sinigrin was an only carbon source available in the agar medium. Strains capable of growing in this selective medium were distinguished as sinigrin-degrading strains. *Brassica* crops and other glucosinolate containing materials were stated as sources of glucosinolate-degrading microorganisms such as *Enterobacter cloaceae* no. 406, *Aspergillus clavatus* II-9 and *Fusarium oxysporum* @146 [34, 43]. Likewise, *Streptomyces* sp., *Bacillus* sp. and *Staphylococcus* sp. have been screened from petrochemically contaminated soil samples by the method of the same concept [24].

Among three fungal isolates, the *Aspergillus* exhibited a potential in degrading sinigrin in liquid culture, whereas, sinigrin-degrading capabilities of the *Rhizopus* and *Mucor* were relatively low. From most reports, glucosinolate-degrading fungi were the genus *Aspergillus,* including *A. sydowi* IFO4284 [102-104], *A. sydowi* QM31c [14], *A. niger* AKU3302 [100, 107] and *A. clavatus* II-9 [42, 43]. However, *Fusarium oxysporum* @146 was an only non-*Aspergillus* previously established as glucosinolate-degrading strain [43]. It should be mentioned that the sinigrin-degrading property of *Rhizopus* sp. and *Mucor* sp. was firstly reported here. Information from

physical morphology, growth characteristics and scanning electron micrographs confirmed that *Aspergillus* sp. NR-4201 was a member of *flavus* group [117-119]. Fungi of the *flavus* group comprise with several species such as *A. flavus*, *A. parasiticus* and *A. oryzae*. Some varieties of *A. flavus* and *A. parasiticus* have been stated as aflatoxin-producing strains [117, 119]. In case of *Aspergillus* sp. NR-4201, no aflatoxin was detected in cultured filtrates after growth in mustard extract medium for 4 days (data not shown). This was the first evidence describing glucosinolate-degrading property of the *Aspergillus flavus* group.

In addition to the preliminary assessment of sinigrin degradation in liquid culture, the presence of myrosinase activity in fungal mycelium of the *Aspergillus* indicated role of this enzyme in sinigrin-degrading process. Whereas, the degradation of sinigrin by *Rhizopus* and *Mucor* did not involve with myrosinase, since enzyme activity was not detected in either cell-free extracts or cultured filtrates. These suggestions were supported by the evidence in sinigrin-barium agar plate. Opaque zone was formed surrounding colonies of the *Aspergillus* in sinigrin-barium agar plate (5 mM sinigrin, 5 mM ammonium chloride, 2.5 mM barium chloride and 1.5 g/l agar in deionized water) [120]. The formation of this opaque zone indicated the action of myrosinase that released sulfate from sinigrin into the agar medium, which then formed the insoluble barium sulfate salt. Meanwhile, such formation was not produced by the *Rhizopus* and *Mucor*. Degradation of sinigrin by *Rhizopus* and *Mucor* resembled that of *F. oxysporum* @ 146, which was also not associated with myrosinase [43].

Myrosinase activity of the *Aspergillus* expressed exclusively when the strain was cultured in the medium containing glucosinolate (sinigrin). This indicated an

inducible feature of this enzyme which resembled other myrosinase-producing fungi such as *A. sydowi* IFO4284 [102, 103] and QM31c [14], *A. niger* AKU3302 [100, 107] and *A. clavatus* II-9 [43]. At lower concentrations of sinigrin (0.5-2.5 mM), enzyme activity was expressed proportionally. However, expression of the enzyme was not inducible at higher concentrations of sinigrin (2.5-5.0 mM).

There were some interesting points on two-step culture of the *Aspergillus*, in comparison to other microbial strains [43, 45]. Firstly, non-induced cells of the *Aspergillus* exhibited highly consuming-rate for glucose, whereas, that for sinigrin was very low. This was similar to non-induced cells of *Lactobacillus agilis* R16 [45]. Secondly, consuming-rates for sinigrin and glucose of induced cells of the *Aspergillus* were considerably equalable. These were quite different from *Lactobacillus agilis* R16 [45], *Aspergillus clavatus* II-9 [43] and *Fusarium oxysporum* @146 [43]. Induced cell of the *Lactobacillus* and *Fusarium* exhausted glucose firstly and then sinigrin, whereas, the *A. clavatus* consumed sinigrin prior to glucose. Thirdly, the occurrence of myrosinase activity in cell-free extracts of the *Aspergillus* resembled those of *A. clavatus* [43], *Aspergillus niger* AKU3302 [100, 107], *Enterobacter cloacae* no. 406 [34} and *Paracolobactrum aerogenoides* [33]. This implied that the *in vivo* glucosinolate degradation of these microbes was an intracellular process. This study demonstrated firstly that the presence of myrosinase activity in fungal mycelium of the *Aspergillus* was closely related to its degradative potential for the glucosinolate sinigrin. Opposingly, enzyme activity was not detected in cell-free extracts or cultured filtrates of the *Lactobacillus* [45] and *Fusarium* [43]. Fourthly, sinigrin degradation by intact cells of the *Aspergillus* led to the production of allylcyanide, which resembled that of *A. clavatus* [43]. By contrast, allylisothiocyanate was produced by the

Lactobacillus [45], whereas, none of product was produced by the *Fusarium* [43]. Lastly, very little amounts of allylcyanide were detected in cell-free extract of the *Aspergillus* (data not shown). This suggested that the cells be metabolically active to eliminate the toxic allylcyanide.

During the incubation of whloe cells of the *Aspergillus* in mustard extract medium, it was interesting that glucose was produced in cultured filtrates. This was also demonstrated in one-step culture. In such case, endogeneous glucose should not be a product of glucosinolate hydrolysis, since the amounts of glucosinolates were not changed during the glucose production period. The production of allylcyanide by fungal cells, was, however, related to sinigrin degradation. This indicated that sinigrin was a main, or probably an only glucosinolate contained in brown mustard seeds. Sinigrin served as a sole preferred carbon source for the fungus. A success of one-step cultivation of the *Aspergillus* in mustard extract medium revealed information for enzyme production at lab-scale level.

4.1.2) Enzyme production in liquid culture

It was noteworthy that myrosinase production from the *Aspergillus* by a one-step liquid culture was firstly reported here. Other reports on myrosinase production by such microorganisms as *A. sydowi* [102, 103], *A. niger* [100, 107] and *E. cloacae* [34] were traditionally employed by a two-step culture. In such cases, materials containing glucosinolates such as mustard seed meals were later introduced in the induction medium [14, 100, 102, 104, 107]. However, such induction medium did not work well in some cases *e.g.* the bacterium *E. cloacae* no. 406, in which, pure sinigrin was added to a final concentration of 0.02 % [34]. In our one-step culture, a medium derived from

brown mustard seeds (*Brassica juncea*) without any nutrient supplements was used. *Brassica* oilseeds were valuable protein sources [23, 105, 106, 121], and contained some amounts of polysaccharides [122]. This was supported by an evidence in our laboratory that mustard seeds contained significant amounts of starch or polysaccharides (unpublished results). These polysaccharides probably served as substrates for some exoglucanase enzymes that released glucose into cultured filtrates.

Cultured conditions of this one-step culture and the other two-step cultures of *A. sydowi* IFO4284 [102, 103], *A. sydowi* QM31c [14] and *A. niger* AKU3302 [100, 107], are compared in Table 4.1. One of several advantages of the one-step culture was a shorter cultivation period (2 days). The cultivation was completed within 48 h. While, the two-step culture of *A. sydowi* IFO4284 required 17 days, including 3 days for pre-culture and 14 days for enzyme production [102, 103]. In case of *A. sydowi* QM31c, the culture was employed for 6 days, 3 days for pre-culture and another 3 days for enzyme production [14]. Cultivation for myrosinase production by *A. niger* AKU3302 required 4 days. This included 2 days for pre-culture and another 2 days for enzyme production [100, 107].

The one-step culture had a higher enzyme yield, compared with the two-step culture using synthetic medium supplemented with mustard flour. By one-step culture of the *Aspergillus*, enzyme activity of 35 U was produced in 40 ml mustard extract medium. While those two step-cultures of the *A. sydowi* IFO4284 [102, 103] and *A. sydowi* QM31c [14] yielded the activity of 400 U/5 l medium and 4 U/50 ml medium, repectively. In case of *A. niger*, enzyme activity of only 4 U was obtained from 100 ml medium [107].

Table 4.1 Comparison of myrosinase production by *Aspergillus* sp. NR-4201, *A. sydowi* IFO4284 [102, 103], *A. sydowi* QM31c [14] and *A. niger* AKU3302 [100, 107].

Item	NR-4201	IFO4284	QM31c	AKU3302
Incubation temperature (°C)	30	29	25	29
pH of cultured medium	6.5	6.5	6.3	7.0
Cultivation period (days)	2	17	6	4
Cofactor supplement	none	none	none	ascorbic acid
Inorganic salt supplement	none	$MgSO_4$	$MnSO_4$, $MgSO_4$ $FeSO_4$, $ZnSO_4$ $CaCl_2$, $CoCl_2$	$CoCl_2$
Enzyme production (U/cultured medium)	35U/40 ml	400U/5 l	32U/330 ml 4U/50 ml	4U/100 ml

Ohtsuru *et al.* reported that supplement with 0.1 % L-ascorbic acid in cultured medium, myrosinase production was significantly increased by *A. niger* AKU3302 [107]. Significant elevation of the enzyme was shown by replacing 0.2 % sucrose with 0.1 % glucose [107]. In our case of the *Aspergillus*, enzyme level was, however, declined proportionally if L-ascorbic acid or glucose supplements were increased stepwisely. Cobalt chloride was stated as an essential cofactor for myrosinase production by *A. niger* AKU3302 [107]. Whereas, magnesium sulfate at final concentration of 0.03 % was traditionally supplemented for the myrosinase production

by *A. sydowi* IFO4284 [102, 103]. Magnesium sufate, manganese sulfate, zinc sufate, ferrous sulfate, calcium chloride and cobalt chloride were usually used as cofactors for *A. sydowi* QM31c [14]. In case of *Aspergillus* sp. NR-4201, it required no metal ion.

Crude enzyme of the *Aspergillus* was quite stable at 4 °C. Addition of 0.02 % thiomerosal totally destroyed the activity. This indicated that sulfhydryl residues were important for myrosinase activity. The *Aspergillus* enzyme exhibited considerable stability, comparing to other microbial myrosinases from *A. niger* [107] and *E. cloacae* no. 406 [34]. In the case of *A. niger* AKU3302 enzyme, 1 mM L-ascorbic acid and 10 mM mercaptoethanol supplements could preserve 80 % of enzyme activity for 2 days at 20 °C [107]. However, complete destruction of enzyme activity of the *Aspergillus* was shown with the presence of 1 mM mercaptoethanol.

4.1.2) Enzyme purification

The *Aspergillus* myrosinase purification was begun by ammonium sulfate fractionation between 40 and 60 % saturation. However, enzyme activity was mostly obtained at 50-60 % saturation. In case of the *A. sydowi* QM31c, the enzyme was obtained between 40 and 80 % saturation [14]. Whereas, the *A. sydowi* IFO4284 enzyme was recovered at a concentration of ammonium sulfate up to 90 % saturation [102, 103]. Most plant myrosinases were precipitated at varying concentrations of ammonium sulfate, ranging from 40 to 90 % saturation [9, 13, 20, 54, 82, 83, 85, 88].

It was shown that chromatography on the DEAE Sephadex-25 column was effective in eliminating protein impurities. However, enzyme activity was decreased about 50 % in each purification step. A specific activity of 65.9 U/mg protein of the highly purified enzyme was comparatively high when compared with 10.07 U/mg

protein of the *A. sydowi* IFO4284 enzyme [102] and 1.916 U/mg protein of *A. niger* AKU3302 enzyme [100]. However, an improved purification of the *A. sydowi* IFO4284 myrosinase exhibited a very high specific activity of 111 U/mg protein [103].

Most *Brassica* myrosinases have been established as oligomeric proteins with varying molecular weights from 125 to over 150 kDa [4]. While, the *Aspergillus* enzyme of this study was a monomeric protein, exhibiting a molecular weight of about 94 kDa by SDS-PAGE, and of about 90 kDa by gel-filtration chromatography. Molecular masses of the *A. sydowi*, *A. niger* and *E. cloacae* myrosinases were reported to be 120, 90 and 61 kDa, respectively [90]. However, there was no report stating that which myrosinases had isoenzymes.

4.1.4) Physico-chemical properties of the purified *Aspergillus* myrosinase

There were some similar properties between the *Aspergillus* myrosinase and the other two myrosinases from *A. sydowi* IFO4284 [102-104] and *A. niger* AKU3302 [100] (Table 4.2). Firstly, their pH optima were nearly neutral, while, those of other plant enzymes were acidic ranging from 5.5-6.5 [4]. Secondly, their temperature optima were relatively low (25-37 °C). By contrast, higher temperatures of about 55-65 °C were optimum for most plant enzymes [4]. Thirdly, the stability of all microbial enzymes were temperature dependent. The purified enzyme of this study was quite stable at 4 °C, comparing to other fungal enzymes. Lastly, all these fungal myrosinases recognized sinigrin as well as pNPG as their hydrolyzable substrates, which have been usually demonstrated in plant enzymes [4].

The activity of *A. sydowi* myrosinase was activated by Cu^{+}, Cu^{2+}, Mn^{2+} and Co^{2+}, and inhibited by Hg^{2+} and Sn^{2+} [102-104]. On the contrasy, the inhibitory effect

Table 4.2 Comparison of physico-chemical properties of the purified myrosinases from *Aspergillus* sp. NR-4201, *A. sydowi* IFO4284 [102-104], *A. niger* AKU3302 [100] and *Sinapis alba* [88, 91].

Item	NR-4201	IFO4284	AKU3302	*S. alba*
Molecular mass (kDa)	94	120	90	135
Number of subunit	1	n.f.	n.f.	2
Optimum pH	7.4	7.0	6.2	6.5
Optimum temperature (°C)	28	25	34	60
pH stability	6-8	6-9	7.8	4-9
Thermostability (°C)	25	45	45	60
K_m for sinigrin (mM)	0.645	3.6	3.3	0.17
K_m for pNPG (mM)	2.8	0.1	1.5	n.f.
Ascorbic acid	no effect	no effect	no effect	activate
Metal activate	none	Co^{2+}	Co^{2+}, Cu^{+} Cu^{2+} Mn^{2+}	n.f.

n.f. : no information

of Co^{2+} and stimulatory effect of Hg^{2+} and Fe^{2+} towards the activity of *A. niger* myrosinase have been reported [100, 107]. In the case of the *Aspergillus* enzyme, no metal ion was found to activate enzyme activity. By contrast, its activity was strongly inhibited by a variety of metal ions such as Ag^{+}, Al^{3+}, Co^{2+}, Fe^{3+}, Hg^{+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, Sn^{2+} and Zn^{2+}. The inactivation of these fungal myrosinases by such heavy metal

as Hg^{+}, Hg^{2+} and Sn^{2+}, and the amino acid L-cycteine indicated sulfhydryl residue(s) played role on myrosinase activity.

L-Ascorbic acid showed neither activation nor inhibition effect on enzyme activity of the *Aspergillus* myrosinase. This result agreed with those reports for *A. sydowi* and *A. niger* myrosinases [100, 102-104, 107]. While L-ascorbic acid was stated as an activator for most myrosinases from plant sources [90-92, 96].

4.1.5) Substrate specificity and kinetic constants

The *S. sydowi* IFO4284 myrosinase exhibited a wide specificity towards a variety of β-glucoside substrates such as cellobiose, amygdalin, salicin, arbutin, β-methylglucoside and β-phenylglucoside [104]. While the *A. niger* AKU3302 myrosinase case, the activity of sinigrin hydrolysis was inhibitied by salicin, arbituin and amygdalin [100]. The lack of activity towards different β-glucosides and p-nitrophenyl-β-D-glycosides was demonstrated by the *Aspergillus* myrosinase of this study. Similar to the *S. alba* myrosinase, the *Aspergillus* enzyme only recognized the β-D-glucose moiety that formed β-glycosidic linkage with specific leaving groups such as p-nitrophenyl (of pNPG) and N-hydroxysulfate (of glucosinolates) [46, 97].

In this study, K_m value for sinigrin of the purified *Aspergillus* myrosinase assessed by the spectrophotometric assay (0.180 mM) was about 3.6 times less than that determined by the coupled-enzyme assay (0.65 mM). It was generally accepted that the K_m value assessed at higher substrate concentrations was more reliable. Regarding the affinity of the *Aspergillus* enzyme to glucosinolate substrate, the K_m value for sinigrin (0.65 mM) was approximately five times less than those values of the *A. sydowi* (3.6 mM) and *A. niger* (3.4 mM) enzymes [100, 102, 104]. In particular

with pNPG, the K_m value of 2.8 mM was relatively large when compared with those values of 0.1 and 1.5 mM of the *A. sydowi* and *A. niger* enzymes [100, 102, 104], respectively. It was considered that the affinity of plant myrosinases to glucosinolate substrates was about twenty times higher than that for pNPG [102]. Alternatively, the affinity of microbial myrosinases for pNPG was higher than for glucosinolates [102]. In the case of the *Aspergillus* enzyme, the K_m value with sinigrin was 4.3 times less than for pNPG. This indicated that affinity of the *Aspergillus* myrosinase for glucosinolate, like sinigrin, was considered between those plant enzymes [4] and the two fungal enzymes previously reported [100, 102, 104].

4.1.6) Enzyme inhibition

In the inhibition study of the purified *Aspergillus* myrosinase, sinigrin acted as a competitive inhibitor for pNPG-hydrolyzing activity. Similar inhibitory effect was also demonstrated in the *A. sydowi* IFO4284 and *A. niger* AKU3302 enzymes [100, 102, 103]. In such cases, the K_i for sinigrin were reported to be the same (3.8 mM). Conversely, the activity of sinigrin hydrolysis was inhibited competitively by pNPG. This suggested that sinigrin and pNPG occupy the same catalytic site of the *Aspergillus* myrosinase, resembled the *A. sydowi* and *A. niger* enzymes [100, 102, 103]. It was interesting, however, that D-glucose displayed as a non-competitive inhibitor for sinigrin-hydrolyzing activity of the *Aspergillus* enzyme. This was quite different from the *A. sydowi* myrosinase, in which, D-glucose was stated as a competitive inhibitor with a calculated K_i value of 2.2 mM [103, 104].

4.1.7) Analysis of glucosinolate breakdown products

In the purified *Aspergillus* enzyme-catalyzed sinigrin hydrolysis, only the allylisothiocyanate was produced at a wide pH ranging from 5 to 9. Whereas, no liberation of allylcyanide was shown. It has been hypothesized that the mechanism of glucosinolate degradation is processed by two steps [9, 11, 17]. The first step is an enzymatic cleavage of the β-thioglucoside linkage to liberate D-glucose and an unstable thiohydroximate-O-sulfonate intermediate. In the second step, the intermediate undergoes non-enzymatic rearrangement to become any reaction products, depending on such factors as substrates, pH or the availability of ferrous ion and epithiospecifier protein [10, 11, 13, 15, 17, 64, 66, 105]. Regarding the effect of pH, the rearrangement of the intermediate to become nitrile or isothiocyanate is a key switching factor. Nitrile is normally produced at an acidic pH, while the isothiocyanate is achieved at neutral [10, 11, 13, 64, 66]. The hypothesis agreed with the results of enzyme from *S. alba*. In respect to the *Aspergillus* myrosinase, it was absolutely inactive at pH 4.0. The enzyme was slightly active at pH 5.0 and allowed some degradation of sinigrin, but none of allylcyanide was detected.

However, there was an evidence from benzylglucosinolate degradation by the *Lepidium sativum* myrosinase that nitrile was produced predominantly at a pH greater than 7.0 [10, 11]. In examination of cell-free extracts of the *Aspergillus*, pH values of 6.2-6.5 were revealed. This implied that pH may be not a key switching factor for the degradative mechanism of glucosinolate (sinigrin) *in vivo*.

It was surprising that none of allylcyanide was formed in the incubation mixtures of sinigrin and disrupted fungal cells of the *Aspergillus*. In this case, allylisothiocyanate was an only main product detected. Clarification of how the intact fungal

cells produced only allylcyanide during glucosinolate (sinigrin) consumption was uncertain. Glucosinolate degradation by the isolated *Aspergillus* myrosinase could not represent the degradative mechanism *via* intact fungal cells. The *in vivo* mechanism for glucosinolate degradation may be more complicated than those mechanisms proposed for purified plant enzymes. Some unknown factors might influence as well.

This study reported firstly on the finding of minor compound generated from sinigrin hydrolysis. Information from separating feature by carbowax, (5 %)-diphenyl-(95 %)-diphenylpolysiloxane and polydimethylsiloxane gas chromatography indicated that this compound was more polar than the allylisothiocyanate. GC-MS analyses revealed that total mass and spectral pattern did not resemble other possible reaction products of sinigrin *e.g.* allylcyanide, allylthiocyanate and 1-cyanoepithiopropane but allylisothiocyanate itself. Kawakishi *et al.* [123] reported that allylisothiocyanate was not stable in aqueous solution in which the amounts of 75 % was decomposed within 10 days. Diallyldithiocarbamate, diallyltetrasulfide and diallylpentasulfide were formed as major decomposing products [123, 124]. In addition, elemental sulfur and allylthiocyanate were found as minors. However, those decomposing compounds were not likely to correspond with mass spectrum of the minor compound of this study. The similarity between MS pattern of the minor compound and that of allylisothiocyanate suggested that the compound be an isomer of allylisothiocyanate (CH_2=CH-CH_2-NCS). The possible structure is CH_3-CH=CH-NCS. However, the absolute structure should be confirmed further by NMR technique.

This study demonstrated that cell-free extracts of the *Aspergillus* showed the capability to produce allylisothiocyanate from brown mustard seeds (*Brassica juncea*).

This indicated further application of the enzyme in the production of mustard volatile oil.

4.2) CONCLUSION

Three fungal isolates, *Rhizopus*, *Mucor* and *Aspergillus*, and a gram negative rod bacterium were isolated from decayed mustard seed meals by growth in sinigrin agar plate. All microbial strains were assessed for glucosinolate degradative potential in liquid culture by using sinigrin as a model substrate. Sinigrin-degrading activity was shown by the three fungal strains but not by the bacterium. Among three fungi, only the *Aspergillus* exhibited high potential to degrade sinigrin. The capability to degrade sinigrin by the *Aspergillus* was closely related to the presence of intracellular myrosinase activity within the mycelium. The *Aspergillus* also exhibited high ability to degrade glucosinolates which were contained in brown mustard seeds (*Brassica juncea*).

To produce myrosinase, cultivation of *Aspergillus* sp. was performed by a one-step liquid culture. Optimum condition for enzyme production was achieved in mustard extract medium with glucosinolate concentration of 5.5 mM, operating at pH 6.5 and temperature of 30 °C for 48 h, under reciprocal shaking of 150 rpm. Neither metal ions nor L-ascorbic acid was required for enzyme production. Cell-free extracts obtained after fungal cell disruption of a 40-ml culture contained 35 U of myrosinase activity.

Purification of the *Aspergillus* myrosinase was employed by ammonium sulfate fractionation, and chromatography on DEAE Sephadex A-25 columns (two times) and Sephadex G-100 column, stepwisely. The final enzyme preparation

exhibited one major band on SDS-PAGE, corresponded to a molecular weight of 94 kDa. A native mass of 90 kDa was estimated by Sephadex G-200 chromatography, suggested that the enzyme occured as a monomeric protein. A specific activity of 65 U/mg protein of the highly purified enzyme was obtained.

Optimum activity of the enzyme was shown at pH 7.4 and at temperature of 28-29 °C. The enzyme was stable at temperature up to 25 °C and at pH between 6 and 8. Enzyme activity was substantially inhibited by Ag^{+}, Al^{3+}, Co^{2+}, Fe^{3+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, Sn^{2+}, Zn^{2+} and L-cysteine, and no stimulation by metal ions was appeared. L-ascorbic acid showed neither activation nor inhibition effect on enzyme activity. No hydrolyzing activity was shown towards a series of glycosides such as p-nitrophenyl-β-D-arabinoside, p-nitrophenyl-β-D-galactoside, p-nitrophenyl-β-D-xyloside, p-nitrophenyl-β-D maltoside, cellobiose, starch, digitonin, stevioside, amikacin and gentamicin. However, strong hydrolyzing activity was demonstrated towards the glucosinolate sinigrin as well as p-nitrophenyl-β-D-glucoside (pNPG). K_m values with sinigrin and pNPG were calculated to be 0.65 and 2.8 mM, respectively. Sinigrin acted as a competitive inhibitor for pNPG hydrolysis and *vice versa*. In addition, D-glucose acted as a non-competitive inhibitor for the activity of sinigrin hydrolysis.

Sinigrin hydrolysis by the purified enzyme at pH between 5 and 9, yielded allylisothiocyanate as a major product. Whereas, no liberation of allylcyanide was observed in the reaction mixtures. Furthermore, a minor compound was detected as the amount 10 % of total hydrolytic products from sinigrin. GC-MS analysis revealed that this minor compound was a possible isomer of allylisothiocyanate.