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Biodegradation of acrylamide by *Enterobacter aerogenes* isolated from wastewater in Thailand

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Abstract

A widespread use of acrylamide, probably a neurotoxicant and carcinogen, in various industrial processes has led to environmental contamination. Fortunately, some microorganisms are able to derive energy from acrylamide. In the present work, we reported the isolation and characterization of a novel acrylamide-degrading bacterium from domestic wastewater in Chonburi, Thailand. The strain grew well in the presence of acrylamide as 0.5% (*W*/*V*), at pH 6.0 to 9.0 and 25°C. Identification based on biochemical characteristics and 16S rRNA gene sequence identified the strain as *Enterobacter aerogenes*. Degradation of acrylamide to acrylic acid started in the late logarithmic growth phase as a biomass-dependent pattern. Specificity of cell-free supernatant towards amides completely degraded butyramide and urea and 86% of lactamide. Moderate degradation took place in other amides with that by formamide > benzamide > acetamide > cyanoacetamide > propionamide. No degradation was detected in the reactions of *N,N*-methylene bisacrylamide, sodium azide, thioacetamide, and iodoacetamide. These results highlighted the potential of this bacterium in the cleanup of acrylamide/amide in the environment.

Key words: acrylamide; biodegradation; domestic wastewater; *Enterobacter aerogenes*

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Introduction

Acrylamide (CH_2=CHCONH$_2$) is an important monomer used as a conjugated reactive molecule in polyacrylamide production as well as a binding, thickening, or flocculating agent in industrial applications (Prabu and Thatheyus, 2007; Prasad, 1982; Wampler and Ensign, 2005). It is also a neurotoxicant, carcinogen and terratogen in animals (Cherry et al., 1956; IARC, 1994; Prabu and Thatheyus, 2007; Segerbäck et al., 1995; Tilson and Cabe, 1979). Neurotoxic effects in humans have been observed at high levels of exposure in occupational settings.

Acrylamide is oxidized to the epoxide glycidamide via an enzymatic reaction involving cytochrome P450 2E1 (Besaratinia and Pfeifer, 2004). Both acrylamide and glycidamide can form hemoglobin adducts and induce abnormalities in the daughter cells of animals and plants (Bergmark et al., 1991; Shairashi, 1978; Shanker et al., 1987). The widespread use and indiscriminate discharge of acrylamide and polyacrylamide has led to their presence in terrestrial and aquatic ecosystems (Cherry et al., 1956; Croll et al., 1974; Igisu et al., 1975; Lande et al., 1979). In spite of the general toxicity of acrylamide in monomer form, some microorganisms can use acrylamide as their sole carbon source for growth. In its catabolism, acrylamide, an aliphatic amide, is deaminated to ammonia and acrylic acid (acrylate), a process catalyzed by amidase or amidohydrolase (EC 3.5.1.4) (Nawaz et al., 1994, 1998; Shanker et al., 1990; Zabaznaya et al., 1998). The subsequent fate of acrylate is hydroxylated to β-hydroxypropionate, which is oxidized to CO_2 or reduced to propionate (Ansede et al., 1999; Wampler and Ensign, 2005). Several microorganisms are known to degrade an array of aliphatic and aromatic amides. Generally, this does not include acrylamide due to its inhibitory effect on sulfhydryl proteins and hence growth of microorganisms (Cavins and Friedman, 1968). However, there are exceptions. Acrylamide utilizing bacteria appear confined to species within the genera; *Arthobacter*, *Bacillus*, *Pseudomonas* and *Rhodococcus* (Asano et al.,

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1982; Hirrlinger et al., 1996; Nawaz et al., 1993; Shukor et al., 2009a, 2009b; Thiery et al., 1986; Wang and Lee, 2001; Yamada et al., 1979). This study reported the isolation and characterization of a novel acrylamide-utilizing microorganism, *Enterobacter aerogenes*, from domestic wastewater in Chonburi, Thailand. The potential of acrylamide and amides degradations by this microorganism was also investigated.

1 Materials and methods

1.1 Isolation of acrylamide-degrading bacterium

Domestic wastewater samples were collected close to an industrial area (n = 10) in Chonburi, Thailand. Preliminary screening for an acrylamide-degrading bacterium was by direct inoculation of 500 μL of wastewater samples into 5 mL of W-minimal medium (Kimbara et al., 1989) containing 0.5% (W/V) of acrylamide as the sole carbon. Cultures were cultivated at 25°C for 3 days with continuous shaking at 250 r/min. The positive results which showed turbidity of culture were used for further isolation by an enrichment method (7 times) in the same medium. A pure culture was isolated by spreading the turbid culture (OD_{600} ranging from 0.6 to 1.0) onto W-minimal medium agar containing 0.5% (W/V) of acrylamide and incubated at 25°C for 3 days. Growth colonies were further purified to a single colony by repeated streaking under the same conditions and maintained at 4°C for further analysis.

1.2 Identification of bacterium

Bacterial identification was based on "API Skills Bacterial Identification Method" and via 16S rRNA gene sequence (Weisburg et al., 1991). Polymerase chain reaction (PCR) amplification of 16S rRNA gene was done with primers designed from the conserve regions at the base positions 22 to 41 and 1066 to 1085 in 16S rRNA gene of *Escherichia coli*, respectively (Precigou et al., 2004). Chromosomal DNA (100 ng) prepared with a GF-1 Nucleic acid extraction kit (Vivantis, Malaysia) was used as a DNA template for PCR reaction. PCR was conducted initially at 95°C for 10 min followed by 30 cycles at 95°C for 30 sec, 60°C for 45 sec and 72°C for 90 sec. Final extension was performed at 72°C for 7 min. The expected PCR product (about 1.1 kb) was purified with a GF-1 Gel DNA recovery kit (Vivantis, Malaysia). The obtained 16S rRNA fragment was then ligated into T/A cloning vector (RBC, Taiwan) according to manufacturer's instructions. After transformation to *E. coli* DH5α, plasmids were extracted with a GF-1 Plasmid DNA extraction kit (Vivantis, Malaysia) and used as the template for sequencing by the dideoxynucleotide method (Sanger et al., 1977). The similarity of nucleotide sequence was determined using BLAST (National Center for Biotechnology Information databases). The phylogenetic analysis was performed using Clustal W-multiple sequence alignment software package at site http://align.genome.jp/. The sequence data have been submitted to the GenBank databases under accession no. GU903317.

1.3 Characterization of the bacterium

Throughout the study, the general procedure was to add 5 mL bacteria culture into 250 mL of W-minimum medium (Kimbara et al., 1989) containing 0.5% (W/V) of acrylamide at 25°C with continuous shaking at 250 r/min for 24 hr. Temperature was varied between 20 and 40°C at intervals of 5°C, pH from 1 to 14 and acrylamide concentration from 0.5%–2.5% (W/V) at 0.5% intervals. Samples were withdrawn at specific time points for bacterial plate counts and expressed in colony-forming units (CFU)/mL. Residual acrylamide levels were measured by high-performance liquid chromatography (HPLC) analysis. Rate of acrylamide consumption was calculated to the ratio between residual acrylamide in the culture medium and cell number expressed as mg-acrylamide/cell. Experiments were done in triplicates and results reported as average ± SD.

1.4 Monitoring of acrylamide degradation

Residual acrylamide in degraded culture media was measured after filtration of aliquots through a nylon membrane filter (0.2 μm pore, Whatman, England) in a HPLC (Waters, Island) with UV spectrophotometric detection at 254 nm. A 50 μL injection loop was used to deliver 20 μL of filtrated aliquot samples onto an analytical μBondapack C_{18} (reverse phase) column, 3.9 mm × 300 mm (10 μm 125 Å), and a Nova-Pak C_{18} (4 μm 60 Å) guard pak insert. A 25 mmol/L sodium phosphate buffer (pH 7.0) was used as mobile phase and separation was performed at room temperature with a flow rate of 1 mL/min. Sample concentration was determined by comparing integrated peak areas to acrylamide and acrylic acid standards. Percentage of mass balance was calculated by comparison of individual peak areas. Degradation was equated to the ratio between acrylic acid and residual acrylamide. At the same time, suitable serial dilutions of the other aliquots were carried out for bacterial plate count. Acidity of NH_4^+ produced from the reaction was measured by the phenate method (APHA et al., 1985). Measurements were made in triplicates and average ± SD reported.

1.5 Amide degradation by cell-free supernatant

A sample of late-exponential culture (250 mL) was collected to obtain cell-free supernatant, then washed twice with a precooled 0.85% (W/V) NaCl. Cell pellets were resuspended with 50 mmol/L sodium phosphate buffer (pH 7.0) containing 1 mmol/L DTT, 75 μg/mL PMSF, and 1 mmol/L EDTA. Then, cells were disrupted on ice by discontinuous ultrasonication with 20% pulse and a 2 min stop interval for 10 min. Cell debris was removed by centrifugation at 10,000 ×g for 15 min at 4°C and the supernatant was concentrated by ultrafiltration with MW cut off 30 kDa (Amicon, Island) before analysis. Protein concentration was determined spectrophotometrically according to Bradford (1976) using Bio-Rad assay reagent (Hercules, USA) and bovine serum albumin as the standard. Reaction was done at 25°C by mixing cell-free supernatant (1 mg protein) with substrate (25 mmol/L) for

1 hr in 25 mmol/L of sodium phosphate buffer (pH 7.0). Degradation was monitored by HPLC. A negative control was prepared from supernatant without cell disruption. Substrates used in this study were acetamide, benzamide, butyramide, cyanoacetamide, formamide, iodoacetamide, lactamide, *N*, *N*-methylene bisacrylamide, propionamide, sodium azide, thioacetamide and urea.

1.6 Immobilization of bacterium

Cells were immobilized by the sodium alginate-$CaCl_2$ method (Prabu and Thatheyus, 2007). Briefly, cell pellets of concentrated culture ($OD_{600}\approx1.0$) were harvested by centrifugation at 10,000 $\times g$ for 10 min, washed and resuspended in an equal volume of 0.1% (*W*/*V*) of NaCl. Then, 3.5% (*W*/*V*) of sodium alginate was added slowly and mixed thoroughly without forming air bubbles in the slurry. The slurry containing the cells was extruded as drops through a sterile tube (2 mm diameter) into a 4% solution (*W*/*V*) of $CaCl_2$. For complete gelation, beads were maintained in the solution at 4°C for 1 hr. After washing with sterile distilled water, beads (10 g) were used to monitor acrylamide degradation with the bacteria-free beads serving as a control.

2 Results and discussion

2.1 Isolation and identification of acrylamide-degrading bacterium

To obtain the acrylamide-utilizing bacteria, a medium supplemented with 0.5% (*W*/*V*) acrylamide was used as the sole energy source in an enrichment culture. Forty-one isolate of bacteria were obtained and tested for acrylamide degradation. One isolate showed high growth rate and the ability to degrade acrylamide and was selected for this study. The strain was found to be a rod shape gram negative bacterium (Fig. 1) and gave negative results in reactions with cytochrome-oxidase, arginine dihydrolase, urease, tryptophane deaminase and gelatinase. Positive results were found in reactions with β-galactosidase, lysine decarboxylase and ornithine decarboxylase and utilization of trisodium citrate. The strain could produce Voges Proskauer acetoin but not with H_2S and indol. In addition, the strain could assimilate glucose, manitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose. The 16S rRNA gene sequence was consistently 99% similar to that for *Enterobacter aerogenes* including *E. aerogenes* C1111 (accession no. AB244467.1) and *E. aerogenes* NCTC10006T (accession no. AJ251468.1). The 98% identities matched well with *E. aerogenes* JCM1235 (accession no. NR_024643.1), *E. aerogenes* An19-2 (accession no. AB244456.1), *E. aerogenes* An10-1 (accession no. AB244445.1) and *E. aerogenes* An2-1 (accession no. AB244438.1). Construction of a phylogenetic tree using the neighbor-joining method (Fig. 2) showed a close relation between the isolate and *E. aerogenes* in support of the similarity in biochemical characteristics. Hence, this strain was identified as *Enterobacter aerogenes*, a normal flora found in the human gastrointestinal tract (Loiwal et al., 1999; Sanders and Sanders, 1997). Different strains of *E. aerogenes* have been reported to degrade xenobiotics (Doten and Ornston, 1987; Langlois et al., 1970; Lee et al., 1992; Liao et al., 2009; Pamela et al., 1976) and fats or petroleum-hydrocarbons (Čpinyte et al., 2009; Erhan and Kleiman, 1997; Ojo, 2006). The production of hydrogen by this bacterium has also been documented (Fabiano and Perego, 2002; Haruhiko et al., 1998; Mahyudin et al., 1997; Nandi and Sengupta, 1998; Palazzi et al., 2000; Tanisho and Ishiwata, 1994). However, no report has been found concerning acrylamide degradation. Thus, to the best of our knowledge, this is believed to be the first report describing the degradation of acrylamide by this species.

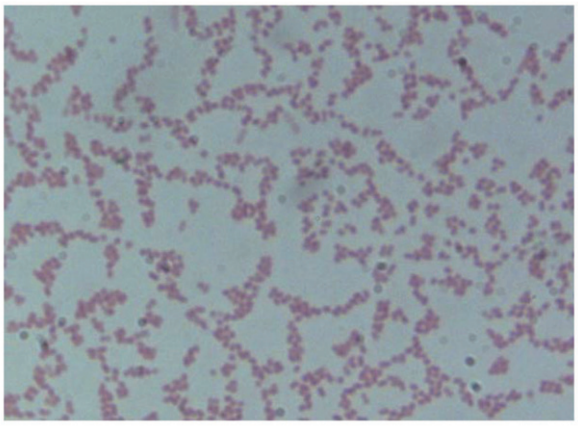

Fig. 1 *Enterobacter aerogenes* isolated from domestic wastewater in Thailand.

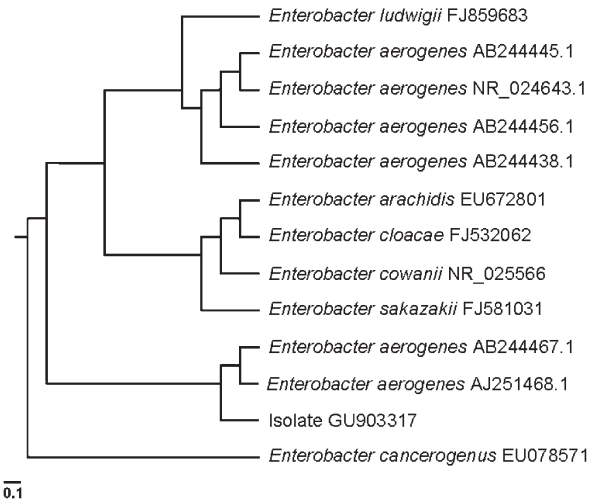

Fig. 2 Phylogenetic tree of *Enterobacter* sp. homologues to the isolate. The phylogenetic tree was constructed by the neighbor-joining method with Clustal W-multiple sequence alignment software package at site http://align.genome.jp/. Species names are followed by the accession numbers of their 16S rRNA gene sequences. The scale represents the number of nucleotide substitutions per site.

2.2 Characterization of acrylamide-degrading bacterium

Effective physical parameters for growth of *E. aerogenes* were examined in a medium containing 0.5% (*W*/*V*) of acrylamide. Growth of *E. aerogenes* increased rapidly and reached a stationary phase in 6 hr and persisted for 30 hr

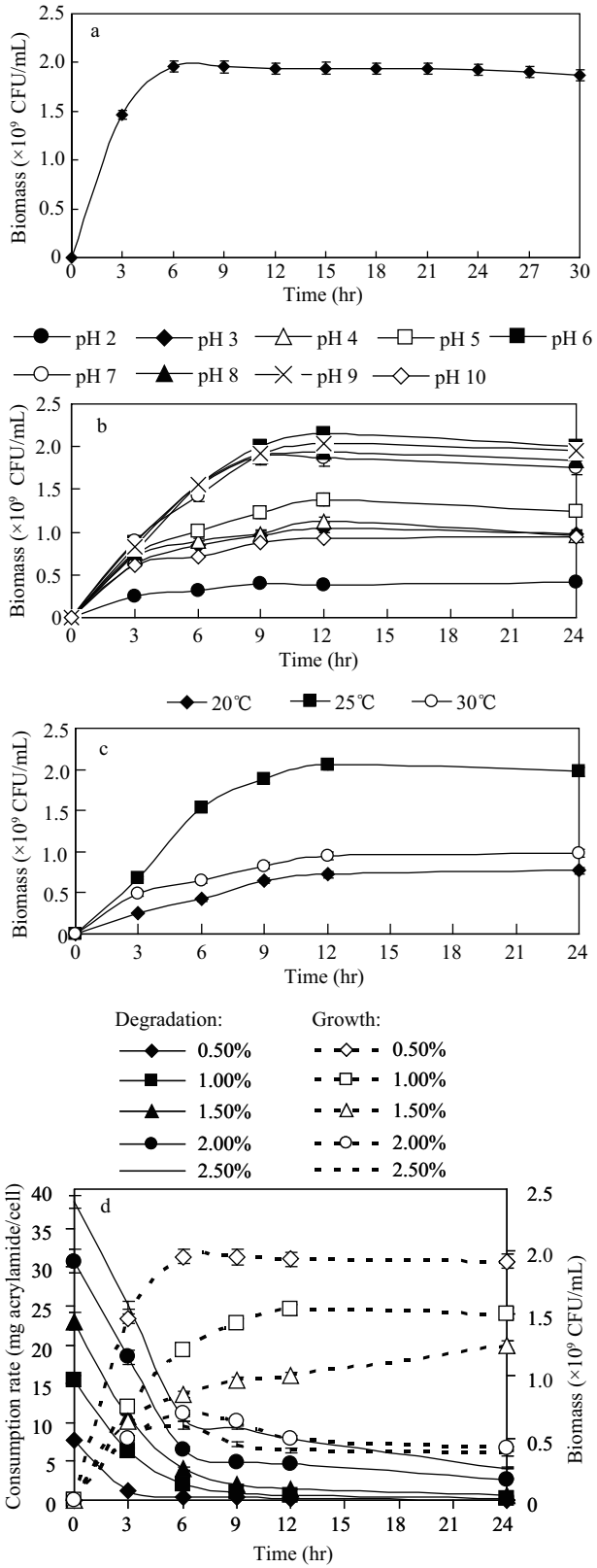

Fig. 3 Growth curve of *E. aerogenes* in a W-minimum medium (pH 7.0) containing 0.5% (*W*/*V*) of acrylamide as a sole carbon source (a), effects of initial pH (b), incubation temperature (c), and growth and degradation of *E. aerogenes* in relation to acrylamide (d). Error bars represent standard deviations for three replications.

(Fig. 3a). The bacterium grew in pH from 2 to 10 but productivity was the highest between 6 and 9 (Fig. 3b). This is similar to previously documented acrylamide-degrading bacteria. For example, Egorova et al. (2004) reported optimum pH for growth of *Pseudonocardia thermophilia* as 7.2 while for *Rhodococcus* sp. and *Pseudomonas* sp. MCI3434 it was 7.0 (Komeda et al., 2004; Nawaz et al., 1998). *Pseudomonas* sp. DRY J7 grew over a relatively wide pH range from 6.0 to 8.5 (Shukor et al., 2009b) while for *Bacillus cereus* DRY135 growth was restricted to 6.8–7.0 (Shukor et al., 2009a). Growth of *E. aerogenes* decreased gradually between < 6.0 and > 9.0 and ceased at pH 1.0 and pH 11.0 (data not shown). This is probably due to lower stability of plasma membrane, inhibition of membrane enzymes and transport proteins (Booth, 1985).

Information on temperature optimum for microbial growth would be useful for bioremediation purposes (King et al., 1992). Although generally it is not practical to change temperature in bioremediation applications, screening for indigenous microbes would match optimum temperatures of microbes with bioremediation site temperatures (Shukor et al., 2009b). Most of the formerly studied acrylamide/aliphatic amides-degrading bacteria are mesophiles with optimum temperature for growth between 25 and 40°C (Ciskanik et al., 1995; Kotlova et al., 1999; Nawaz et al., 1994; Prabu and Thatheyus, 2007; Shukor et al., 2009a, 2009b; Wang and Lee, 2001). Optimum incubation temperature of *E. aerogenes* was within this range (Fig. 3c). A dramatic drop in growth was found at temperatures lower or higher than 25°C. These results matched well with the growth characteristics of *E. aerogenes* at neutral pH and mesophilic temperatures (Gupta et al., 2006).

Acrylamide consumption and growth of *E. aerogenes* was rapid over the initial 6 hr followed by a stationary phase (Fig. 3d). The strain grew well at an acrylamide concentration of 0.5% (*W*/*V*) and was tolerant of concentrations to 1.5% (*W*/*V*). Growth was suppressed at higher concentrations, presumably due to an inhibitory effect of acrylamide on thiol groups of proteins (Cavins and Friedman, 1968). Earlier studies reported bacterial growth was inhibited at around 0.05% (*W*/*V*) (Shukor et al., 2009a, 2009b; Wang and Lee, 2001) well below that by *E. aerogenes*, certainly a unique characteristic that merits further investigation.

2.3 Degradation of acrylamide in bacterial culture

Degradation of acrylamide by *E. aerogenes* was estimated by the production of acrylic acid and NH_4^+ in the culture medium during 72 hr of cultivation. *E. aerogenes* degraded acrylamide (initial concentration of 7681 mg/L) to acrylic acid within 15 min of cultivation (Fig. 4), and continued to do so in a time-dependent manner for 6 hr when maximum production was 741 mg/L. This indicated degradation was due mainly to biological processes. This was followed by a decline in the amount of acrylic acid and the presence of low polarity compounds (Fig. 4c). Thereafter, acrylamide was utilized and acrylic acid produced and gradually catabolized to other products. Similar results were obtained with NH_4^+, a byproduct during acrylamide degradation. However, after 24 hr of cultivation, the amount of NH_4^+ increased while *E. aerogenes* biomass

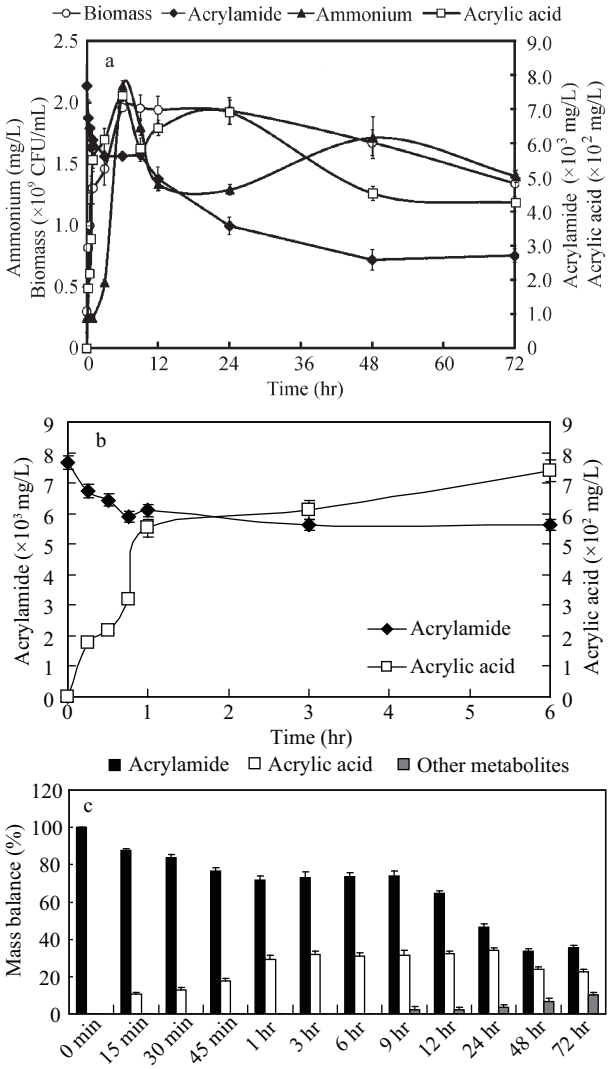

Fig. 4 (a) Monitoring of acrylamide degradation in culture medium of *E. aerogenes* for 72 hr. Amounts of decreased acrylamide, cell biomass and production of acrylic acid and NH_4^+ found in culture medium were detected. Cultivations were performed in W-minimum medium (pH 7.0) containing 0.5% (*W*/*V*) acrylamide at 25°C with continuous shaking at 250 r/min. (b) Expansion of the time points within 6 hr. (c) Percentages of mass balance of acrylamide, acrylic acid and other metabolites estimated from the peak area of HPLC chromatogram. All results expressed as the average of three determinations with the standard deviation.

was reduced. Thus, the first step of acrylamide degradation by *E. aerogenes* occurs faster than cell growth. Perhaps the strain used acrylamide as an initial carbon source with the catabolism of acrylic acid commencing in the late logarithmic phase of growth in a biomass-dependent fashion. This is consistent with the quorum sensing theory that proposes once cell densities have reached a threshold level the expression of inducible genes encoding the enzymes relevant to the metabolism are completed (Swift et al., 1996). The quick disappearance of acrylic acid was probably due to its toxic effect on microorganisms and the possibility of biodegradative potential of acrylic acid as a carbon source since subsequent metabolites were detected in the culture medium (Fig. 4c). Growth of *E. aerogenes* was closely correlated with acrylamide consumption and acrylic acid production and subsequent consumption. Cultures inoculated with an initial biomass of 1.36×10^9 CFU/mL partially consumed 2059 mg/L of acrylamide within 3 hr and produced acrylic acid and NH_4^+. Biomass was continuously produced at a constant rate when acrylic acid was utilized. After 9 hr, amounts of acrylamide decreased again, resulting in an immediate growth spurt correlated with acrylamide degradation and acrylate production/degradation. Thus, acrylate is believed to be the stoichiometric product of acrylamide degradation and acrylate degradation appears to be rate-limiting reaction for acrylamide-dependent growth (Wampler and Ensign, 2005). The amount of NH_4^+ detected was highest (approximate 1.8 mg NH_3-N/L) at 9 hr after which it gradually declined. After 24 hr cultivation, the NH_4^+ content increased again while cell biomass slightly decreased. In accord with an earlier suggestion by Swift et al. (1996), it is suggested that with a decline in cells, metabolism also decreases allowing for higher waste production.

2.4 Degradations of acrylamide and amides by cell-free supernatant

Acrylamide and amide were monitored over time in a cell-free supernatant of *E. aerogenes*. One milligram protein of the supernatant was reacted with 25 mmol/L acrylamide for 1 hr and acrylamide, amide and their metabolites followed by HPLC analyses. Low amounts of acrylic acid and many suspected metabolites with low polarity were found in the reaction (Fig. 5). No degradation was found in the reaction of supernatant prepared by omitting the cell disruption step (data not shown). Of these, it seems the cell-free supernatant of *E. aerogenes* contains the relevant enzymes that provide a quick catabolism of acrylamide and subsequently acrylic acid but validation requires further investigation.

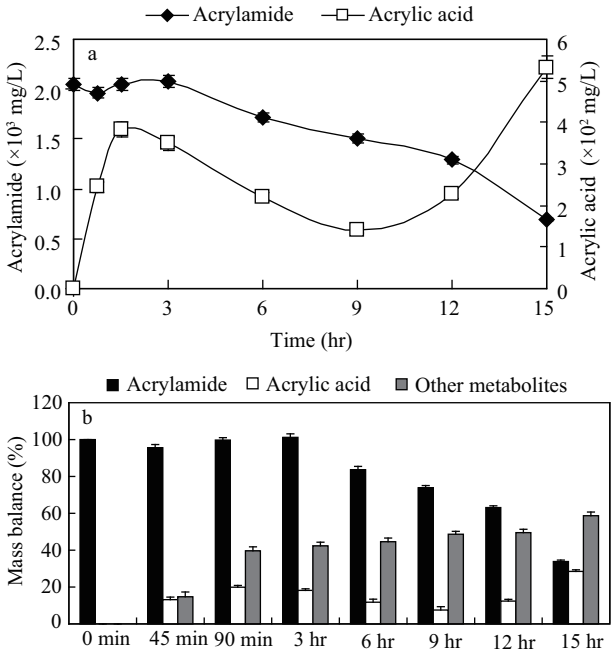

Fig. 5 Amount of acrylamide and acrylic acid in the reaction mixture of 1.0 mg/mL protein of cell-free supernatant from *E. aerogenes* (a) and the percentages of mass balance of acrylamide, acrylic acid and other metabolites estimated from the peak area of HPLC chromatogram (b). All results expressed as the average of three determinations with the standard deviation.

Table 1 Amide degradation by cell-free supernatant of *E. aerogenes*

Amides	Degradation rate* (%, conversion ± SD)
Acrylamide (control)	94.44 ± 0.07
Acetamide	49.57 ± 0.01
Benzamide	54.82 + 0.04
Butyramide	100 ± 0.01
Cyanoacetamide	48.81 ± 0.04
Formamide	55.27± 0.08
Iodoacetamide	ND
Lactamide	86.77 ± 0.02
N,N-methylene bisacrylamide	ND
Propionamide	30.20 ± 0.09
Sodium azide	ND
Thioacetamide	ND
Urea	100 ± 0.01

* Conversion ± SD: the ratio between the produces compound and the substrate presents in the reaction with standard deviation of three replications.
ND: no degradation.

As shown in Table 1, complete degradations were detected in the reactions of butyramide and urea while 86.77% degradation was obtained with lactamide. Half degradation was found in the presence of acetamide, benzamide, cyanoacetamide and formamide whereas propionamide gave 30.20% degradation. No degradation was detected in the reactions of iodoacetamide, *N,N*-methylene bisacrylamide, sodium azide and thioacetamide. The results suggest there was an enzyme in cell-free supernatant of *E. aerogenes* capable to degrade amides, preferentially short-chain aliphatic amides. This is similar with the results described by Shanker et al. (1990) in which cell-free extracts prepared from *Pseudomonas* sp. C-3 grown on acrylamide possessed an enzyme active on formamide, acrylamide, and acetamide. Also, crude extract of *P. acidovorans* promoted hydrolysis of acetanilide, phenylacetamide and acetamide (Alt and Krisch, 1975). Normally the enzyme present in cell-free extracts of acrylamide grown cells exhibited a non-linear acrylamide hydrolysis. This may be due to the presence of an enzyme inhibitory electrophilic vinyl group in the molecule or its metabolite (Shanker et al., 1990). Hence, specificities toward amides, especially short-chain aliphatic amides of *E. aerogenes* should be applied for degradation of other amides and needs in-depth investigation.

2.5 Degradation of acrylamide by immobilized cells

Successful bioremediation depends on the introduction of specific microorganisms capable of degrading undesirable substances. However, indigenous predators, parasites and toxicants are known to severely restrict biodegradation (Mallory et al., 1983; Murakami and Alexander, 1989). Immobilization of bacterial cells has been reported to increase degradation capacity of microbes since the enzyme activities within the cells continue for long periods (Buchnolz et al., 2005). In this study, cells of *E. aerogenes* were immobilized by the sodium alginate-$CaCl_2$ method. Particles of about 3 mm diameter and white circle beads were obtained. After 6 hr cultivation, immobilized cells of *E. aerogenes* degraded acrylamide to acrylic acid. Then, degradation rate increased gradually to a maximum 170 mg/L (25% conversion) at 36 hr (Fig. 6a). Thereafter production of acrylic acid decreased continuously while other metabolites were detected in increasing concentrations in the culture medium in a time-dependent manner (Fig. 6b). Although immobilized cells offer several advantages over freely suspended cells (Dwyer et al., 1986), acrylamide degradation by the former was slower than the latter. This is similar to the more rapid degradation of phenol by non-immobilized cells of methanogenic consortium than by immobilized cells (Dwyer et al., 1986). It is difficult to conclude whether the slow disappearance of acrylamide/acrylic acid observed was due to its toxic inhibitory on microorganisms, the lack of an essential cofactor for its consumption, an inappropriate immobilization process or to difficulties in substrate-cell contact (Chibata, 1978; Shanker et al., 1990). Comparison of the acrylamide degradation by immobilized and free cells of *E. aerogenes* indicates that the concentration of acrylamide influences its biodegradation. Moreover, immobilized cell beads of *E. aerogenes* seem to be partially broken during cultivation (data not shown). One possibility describing this problem might be because alginate is also endothermic into the cells making insufficient stability or mass transfer limitation (Kierstan and Coughlan, 1985) or calcium ions and other mono- (K^+-, Na^+-) or divalent cations that can exchange with Ca^{2+}. To solve this problem in the future, alginate beads including either glutaraldehyde or polyetheneimine co-immobilized with the cells might be tried for the bead preparation (Buchnolz et al., 2005; Yang et al., 1994)

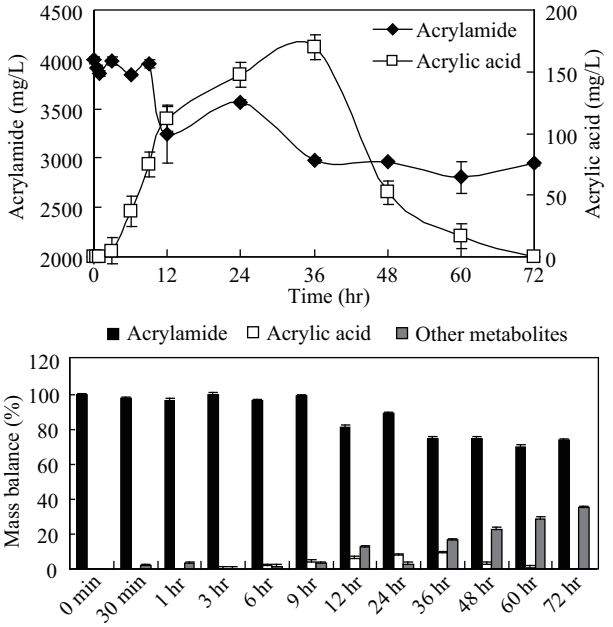

Fig. 6 The amount of decreased acrylamide and produced acrylic acid found in medium containing immobilized cells of *E. aerogenes* (a) and the percentages of mass balance of acrylamide, acrylic acid and other metabolites estimated from the peak area of HPLC chromatogram (b). All results expressed as the average of three determinations with the standard deviations.

Since the distribution of acrylamide-degrading bacteria is limited to a few genera, to the best of our knowledge this is believed to be the first report describing the degradation of acrylamide by *E. aerogenes*. A rapid degradation rate of acrylamide and some amides and a preferred growth at tropical advocates suggest the potential of this bacterium

for local bioremediation of either acrylamide or other amides.

3 Conclusions

Enterobacter aerogenes is a novel acrylamide-degrading bacterium isolated from domestic wastewater in Thailand. The strain grew well in 0.5% (*W*/*V*) acrylamide, at pH 6.0 to 9.0 at 25°C. Free and immobilized strain cells degraded acrylamide to acrylic acid and further to lower polarity compounds. Cell-free supernatant of the strain preferentially degraded short-chain aliphatic amides but no degradation was found in acrylamide derivatives.

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