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Contributed Paper

Characterization of Lipolytic Producing *Proteus* and *Klebsiella* Strains Isolated from Fermented Foods

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ABSTRACT

The aim of this research was to characterize the lipolytic producing Gram-negative, facultatively anaerobic, rod-shaped bacteria isolated from fermented foods collected in Thailand. All isolates were identified based on their phenotypic characteristics and 16S rRNA gene sequence analyses. Nine isolates (Group 1) belonged to the genus *Proteus* and one in Group 2 was *Klebsiella* based on 16S rRNA gene sequence similarity. The isolates in Group 1 (NM3-7, PR10-2, PR10-3, PR11-3, PR11-4, PR12-2, NM28-1, NM42-8 and NM42-10) were closely related to *Proteus mirabilis* ATCC 29906^T with 99.78-100% similarity. Isolate NM42-1 in Group 2 was closely related to *Klebsiella ozaenae* ATCC 11296^T with 99.85% similarity. The isolates were sensitive to ampicillin, carbenicillin, cephalothin, gentamicin, imipenem, kanamycin, novobiocin, penicillin G, streptomycin, sulphonamide and tobramycin, but they were resistant to bacitracin, clindamycin, erythromycin, tetracycline and vancomycin. *Proteus* isolates showed lipolytic activity for Tween 20, Tween 40, Tween 60 or Tween 80 ranging from 0.10±0.02-0.57±0.00 U/mL, whereas *Klebsiella* isolate exhibited 0.10±0.02 -0.24±0.04 U/mL. *P. mirabilis* PR11-3 exhibited the highest activity on Tween 80 at pH 8.0 when incubated for 18 h.

Key words: antibiotic sensitivity, fermented foods, *Klebsiella, Proteus,* lipolytic activity

1. INTRODUCTION

Lipolytic enzyme, lipase (the hydrolases of glycerol esters EC 3.1.1.3) has high catalytical potential and is extensively distributed in plants, animals and microorganisms. Many strains in genera *Bacillus, Pseudomonas*, *Burkholderia*, *Acinetobacter*, *Staphylococcus*, *Aspergillus terreus* and *Fusarium heterosporum* are reported to produce lipase [1-3]. The *Enterobactericeae* in genera *Proteus* and *Klebsiella* are Gram-negative, facultatively anaerobic, rod-shaped bacteria. *Proteus* strains are widely distributed in the human gastrointestinal tract [4]. They have been reported to produce lipase as a catalyst for biodiesel synthesis, and this lipase, which is only moderately stable and methanol tolerant needs to be improved before application in industry [5]. *P. vulgaris* lipase K80 expressed in *Escherichia coli* cells has been applied to produce biodiesel from plant oil [6]. The production of biodiesel by an enzymatic catalyst such as lipases has been

used for decade, however, it is restricted in industry application due to its high cost. Many research groups have studied extensively the selection of cheap oil sources, the screening of suitable lipases, and development of lipase immobilization methods [6]. *Klebsiella* strains are applied to increase crop yields under agricultural conditions [7]. *K. pneumoniae* and *K. oxytoca* strains were reported to produce hydrolytic enzymes on Tween 20, 60 and 80 [8]. The development of an enzymatic process in vegetable oils using lipase from *K. variicola* (lipase B-22) is superior in passion fruit oil [9].

In Thailand, there are many food products and lactic acid bacteria involved in the fermentation [10]. In *Nham* (fermented pork), the changes in lipid composition and fatty acid profile during fermentation have been reported [11]. However, a lipolysis of fat in fermented foods by bacterial cultures has not been studied in Thailand. In this investigation of lipolytic bacteria from fermented meat and fermented fish, *Proteus* and *Klebsiella* strains were isolated, screened for lipase activity and identified based on their phenotypic characteristics and 16S rRNA gene sequence analyses.

2. MATERIALS AND METHODS

2.1 Sources and Isolation Methods

Seven food products, including 4 *Nham* (feremented pork) samples collected from Utaradit and Phitsanulok provinces, and 3 *Pla-ra* (fermented fish) samples collected from Buriram, Thailand, were used for the isolation. Bacterial strains were isolated by spread plate technique using one gram or 1 mL of the fermented food samples diluted in 99 mL of 0.1% peptone solution and then mixed by stomacher for 2 min. It was then 10-fold serially diluted with peptone solution, and 0.1 mL of each proper diluted sample was transferred to a Tryptic soy agar (TSA) plate before being spread with a glass spreader and incubated at 37°C for 48 h. The bacterial cells were counted, picked up for purification and then transferred to TSA slant.

2.2 Phenotypic and Genotypic Characterization

The morphological, cultural, physiological and biochemical characteristics including Gram staining, cell morphology, colonial appearance, catalase, oxidase, nitrate reduction, MR-VP, indole production, citrate utilization; hydrolysis of L-arginine, gelatin, starch and urea; and blood hemolysis of the isolates were determined [12]. Growth in 4, 6, 8 % NaCl (w/v), at pH 4, 5, 6, 8 and 9, at 40 and 45°C were performed. Acid production from carbohydrates was performed [13]. The isolates were examined for antibiotic susceptibility by disc diffusion assay on Mueller-Hinton agar (Difco, Detroit, MI) against commonly used 24 antibiotics based on the standard protocol [14].

The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using the primers 20F (5'-AGTTTGATCCTGGCTC -3'), 1530R (5'-AAGGAGGTGATCCAGCC-3'), 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). The amplified 16S rRNA gene sequence was analyzed by Macrogen, Korea. Sequence alignment was employed using the BLAST software from the Gen Bank. Multiple alignments of the sequences were performed with a program CLUSTAL_X (version 1.83) [15]. Gaps and ambiguous bases were eliminated prior to construction of a phylogenetic tree. A phylogenetic tree was constructed by the neighbor-joining method [16] with the program MEGA version 5.05 [17]. The confidence values of individual branches in the phylogenetic tree were determined by using bootstrap analysis [18] based on 1000 replications.

2.3 Determination of Lipolytic Activity

All of the isolates were determined for lipolytic activity on agar plate. The medium consisted of peptone 1%, $CaCl_2$ $2H_2O$ 0.01%, agar 2% and 1% of Tween 20, Tween 40, Tween 60 or Tween 80 [19]. They were incubated at 37°C for 48 h. The lipolytic activity of the isolates was detected by the appearance of an

opaque zone around the colonies. The isolates that showed lipolytic activity with different substrates on agar medium were cultivated and 0.1% seed cultures were inoculated in 50 ml nutrient broth (NB) (250 mL) with 1% Tween as substrates. They were then incubated at 37°C on a rotary shaker (200 rpm) for 24 h. The fermentation broth was collected and centrifuged at 10,000 rpm at 4 °C for 10 min, and the supernatant (crude enzyme) was used for enzyme assay. Lipase activity was determined by a spectrophotometric assay with *ρ*-nitrophenyl palmitate (*ρ*-NPP) as a substrate. The reaction mixture consisted of 135 μL of 0.4% Triton X, 0.1% gum arabic in 50 mM Tris-HCl buffer (pH 7) and 15 μL of the substrate. The mixture was added with 50 μL of crude enzyme and incubated at 37°C for 1 h. The colour change of activity was then measured at 405 nm [20].

The effect of different substrates including 1% of Tween 20 (Tw20), Tween 40 (Tw40), Tween 60 (Tw60) or Tween 80 (Tw80) in NB (pH 7.4) of the isolates on lipase activity were determined, and they were incubated at 37°C on a rotary shaker (200 rpm) for 24 h. The temperature effect on lipase activity of the selected isolate was determined when cultivated in NB with 1% Tween 80 and incubated on a rotary shaker (200 rpm) for 24 h. Effect of the initial pH at 5, 6, 7, 8 and 9 and the incubation time (0, 6, 12, 18, 24, 30, 36, 42, and 48 h) on lipase production was determined on NB broth with 1% Tween 80 incubated at 30°C on a rotary shaker (200 rpm). Lipase activity was determined by a spectrophotometric assay with ρ-nitrophenyl palmitate (*ρ*-NPP) as a substrate as mentioned above [20].

3. Results and Discussion

3.1 Phenotypic and Genotypic Characterization

Four strains (NM3-7, NM42-8, NM42-1 and NM42-10) were isolated from *Nham* (NM) collected from Utaradit while one strain NM28-1 was from *Nham* collected from Phitsanulok province. Five strains (PR10-2, PR10-3, PR11-3, PR11-4 and PR12-2) were isolated from *Pla-ra* (PR) collected from Buriram province. The total bacterial cell count in *Nham* ranged from 2.87 x 10^8-1.2 x 10^9 and in *Pla-ra* ranged from 2.33 x 10^7-1.43 x 10^8 CFU/g. All isolates were Gram-negative, facultatively anaerobic rod-shaped bacteria. Their colonies were round, regular, flat/smooth or convex and creamy-white after incubation at 37 °C on TSA agar plate for 2 days. They were divided into two groups based on their phenotypic characteristics and 16S rRNA gene sequence analyses. All isolates showed positive reaction for catalase, gelatin, starch and Tween 80; nitrate reduction, Voges-Proskauer (VP) reaction, urease, citrate utilization and ornithine decaboxylase, growth at 40 °C, 6% NaCl and at pH 6-9, but negative for L-arginine hydrolysis, blood hemolysis, indole formation and no growth at 45°C. All produced acid from D-galactose, D-glucose, glycerol, D-ribose and D-xylose but did not produce acid from D-cellobiose, fructose, inulin, D-mannitol, D-mannose, D-melibiose, D-melezitose, α-methyl-D-glucoside, raffinose, rhamnose or salicin. Their differential phenotypic characteristics are described below and in Table 1.

Group 1 contained isolates NM3-7, PR10-2, PR10-3, PR11-3, PR11-4, PR12-2, NM28-1, NM42-8 and NM42-10. These isolates grew in 4-8% NaCl, at 40°C, and pH 5-9 but did not grow at pH 4. All isolates formed hydrogen sulphide but did not produce oxidase. They did not hydrolyse esculin or lysine decarboxylase. Variable characteristics are seen in the MR test and sucrose (Table 1). The phylogenetic tree based on 16S rRNA gene sequence similarity showed that they were located within the lineage of the genus *Proteus* (Figure 1). The strains PR11-4; PR10-2 and PR10-3; NM3-7 and NM28-1; and PR11-3, PR12-2 NM42-8 and NM42-10, were closely related to *P. mirabilis* ATCC 29906^T with 99.78; 99.85; 99.93 and 100% similarity, respectively. Therefore, they were identified as *P. mirabilis* [21-22].

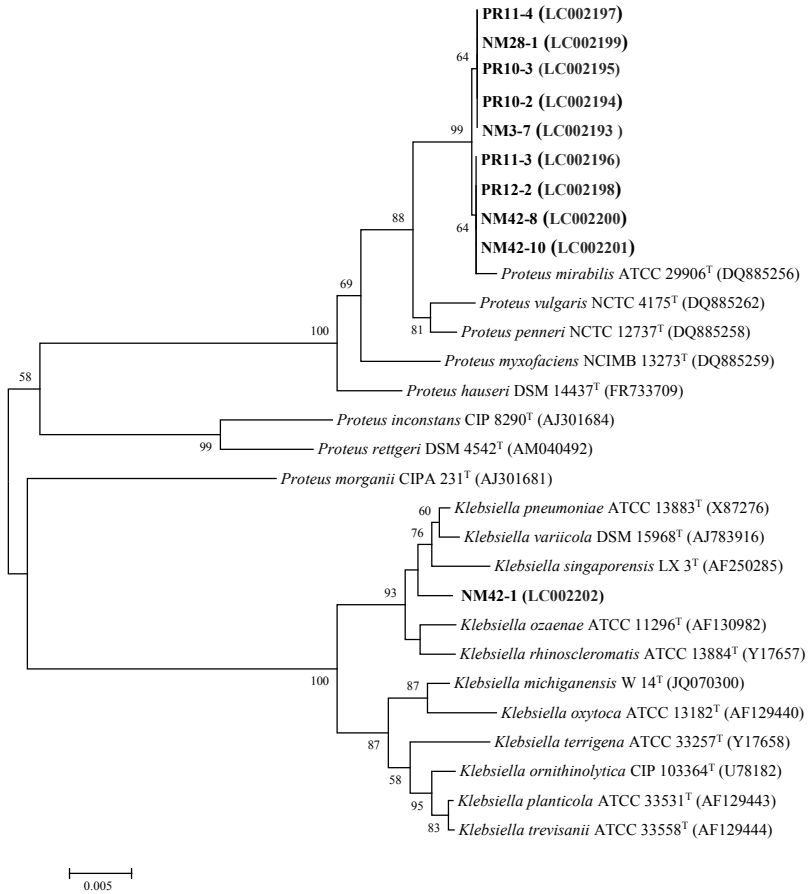


Figure 1. Phylogenetic tree constructed using the neighbor-joining method showing the position of *Proteus* and *Klebsiella* isolates and related species based on 16S rRNA gene sequences.

Group 2 contained isolate NM42-1. The isolate hydrolysed esculin and produced lysine decarboxylase. It grew at pH 4 and 40°C but showed no growth in 8% NaCl. The isolate produced acid from L-arabinose, lactose, D-maltose, and D-sorbitol (Table 1). The phylogenetic tree based on 16S rRNA gene sequence similarity showed that isolate NM42-1 was located within the lineage of the genus *Klebsiella* (Figure 1). This isolate was closely related to *K. ozaenae* ATCC 11296^T (99.85%), *K. pneumoniae* ATCC 13883^T (99.57%), *K. rhinoscleromatis* ATCC 13884^T (99.42%), *K. variicola* DSM 15968^T (99.28%) and *K. singaporensis* LX 3^T (99.06%), respectively. However, the isolate was located between two clusters of the above 5 species and was separated from them [23]. Therefore, it was represented as a novel species of the genus *Klebsiella* and further study on DNA-DNA hybridization is thus required.

The results of antibiotic sensitivity indicated that the majority of the *Proteus* strains showed antibiotic resistance to bacitracin, clindamycin, erythromycin, tetracycline, and vancomycin but they were sensitive to carbenicillin, imipenem, novobiocin, penicillin G, streptomycin, sulphonamide, ampicillin, cephalothin, gentamicin, kanamycin and tobramycin (Table 2). *P. mirabilis* was sensitive to all tested aminoglycosides, acylureidopenicillins, some cephalosporins, carbapenems, aztreonam, quinolones, sulfamethoxazole, co-trimoxazole,

Table 1. Differential phenotypic characteristics of Group 1 and Group 2 isolates.

Characteristics	1	2
No. of isolates	**9**	**1**
Growth at pH 4	-	+
Growth in 8%NaCl	+	-
Oxidase	-	+
MR	- (+3)	-
H_2S production	+	-
Esculin hydrolysis	-	+
Lysine decarboxylase	-	+
Acid from:		
L-Arabinose	-	+
Lactose	-	+
D-Maltose	-	+
D-Sorbitol	-	+
Sucrose	+ (-4)	-
D-Trehalose	+	-

+, positive reaction; -, negative reaction.

and all beta-lactams, but was resistant to penicillin G, oxacillin, all tested macrolides, lincosamides, streptogramins, glycopeptides, rifampicin and fusidic acid [24]. Species-specific differences in natural susceptibility were seen with tetracyclines, several beta-lactams, chloramphenicol and nitrufurantoin [24].

In this study, *P. mirabilis* isolates in Group 1 were different from the isolate in Group 2 based on their swarming motility, growth at pH 4, in 8% NaCl, hydrogen sulphide formation, esculin hydrolysis, oxidase, lysine decarboxylase, and acid production from L-arabinose, lactose, maltose, sorbitol, sucrose and D-trehalose (Table 1). The isolate NM42-1 did not show blood hemolysis; however, the other *Klebsiella* strains isolated from foods and clinical cases showed a different haemolytic pattern [25]. *P. mirabilis* was susceptible to most antibiotics apart from tetracycline (30 μg) and also was resistant to carbenicllin (100 μg) (Table 2).

Table 2. Zone diameter (mm) of antimicrobial disc susceptibility test of isolates.

	Isolates				
Antibiotics	**NM3-7, PR10-2, PR10-3, PR11-3, PR12-2**	**PR11-4**	**NM28-1**	**NM42-10, NM42-8**	**NM42-1**
Ampicillin (10 μg)	19.8±0.4-28.9±0.1	24.6±0.6	0	17.5±0.2-24.9±0.2	0
Amoxicilin/Clavulanic 2:1	20.5±0.6-32.6±0.3	0	28.2±0.3	26.4±0.6-29.8±0.2	30.8±0.4
Carbenicllin (100 μg)	34.7±0.4-37.4±0.4	38.8±0.1	35.1±0.1	32.2±0.3-35.2±0.4	0
Cephalothin (30 μg)	18.4±0.2-34.3±0.5	27.9±0.1	0	21.3±0.2-22.3±0.1	23.7±0.4
Novobiocin (5 μg)	0	11.7±0.1	17.0±0.5	10.5±0.1-11.2±0.2	0
Penicillin G (20 U)	18.1±0.0-31.0±0.5	29.2±0.5	0	8.3±0.3-12.8±0.4	0
Streptomycin (10 μg)	17.8±0.6-22.6±0.5	11.2±0.4	20.1±0.1	0	21.7±0.3
Sulfa/Trimetho (Bactrim)	19.3±0.1-28.2±0.4	28.2±0.5	28.3±0.4	0	21.2±0.1
Sulphonamide (300 μg)	18.8±2.4-29.5±0.7	12.4±0.1	31.9±0.1	0	0
Tetracycline (30 μg)	0	0		0	25.8±0.1

All are sensitive to amikacin (30 μg), cefotaxime (30 μg), cefoxitin (30 μg), chloramphenicol (30μg), gentamicin (10 μg), imipenem (10 μg), kanamycin (30 μg), netilmicin (30 μg), piperacillin/tazobactam and tobramycin (10 μg) but resistant to bacitracin (10 U), clindamycin (2 μg), erythromycin (15 μg) and vancomycin (30 μg). Data are means (n=2) ± standard deviation of two replicates.

3.2 Lipolytic Activity

The isolates showed lipolytic activity for Tween 20 ranged from 0.14±0.00-0.36±0.03 U/mL, Tween 40 from 0.09±0.05-0.40±0.01 U/mL, Tween 60 from 0.12±0.04-0.44±0.03 U/mL and Tween 80 from 0.10±0.02-0.57±0.00 U/mL, when they were cultivated in broth culture supplemented with Tween 20, Tween 40, Tween 60 or Tween 80 (Figure 2). The isolates showed lipolytic activity as reported in *Bacillus*, *Brevibacterium*, *Corynebacterium*, *Staphylococcus*, *Klebsiella*, and *Stenotrophomonas* strains (0.1-0.25 U/mL) (26). *P. mirabilis* PR11-3 strain (Figure 2) produced the highest lipase activity (0.57±0.00 U/mL) while the *Klebsiella* strain NM42-1 produced low lipase activity (0.10±0.02-0.24±0.04 U/mL). Further study of the *P. mirabilis* strain PR11-3 showed the highest lipase activity (0.48±0.02 U/mL) on Tween 80 at pH 8.0 after incubated for 24 h (Figure 3a). In addition, the optimum temperature was 37°C, and the incubation time for lipase production of this strain was 18 h (0.45±0.02 U/mL) as in (Figure 3b).

The Gram-negative, rod- shaped bacteria have been indicated as the spoilage of foods such as in meat and meat products. The presence of *Enterobacteriaceae* in genus *Proteus* formed hydrogen sulphide while *Pseudomonas* spp. formed dimethyl sulphide [27]. *P. mirabilis* and *P. vulgaris* are among the spoilage flora of bacon stored at high temperatures over 20 °C. The spoilage is characterized by production of large quantities of hydrogen sulphide [28]. *Klebsiella* strains are frequently isolated from various infections in animals and humans [29]. However, they were isolated from food prepared for intensive care patients [30], wheat and grass roots [31-32] and, botanical and aquatic environments [33], including soil [23]. *Klebsiella* strains isolated from various infections in animals and humans are opportunistic pathogens. Some strains produced extended-spectrum beta-lactamases (ESBLs), which made them resistant to cephalosporins, ceftazidime including aminoglycosides, quinolones and carbapenems [29, 34]. *K. pneumoniae* strain as an enteroinvasive food-borne pathogen transmitted from a hamburger was reported

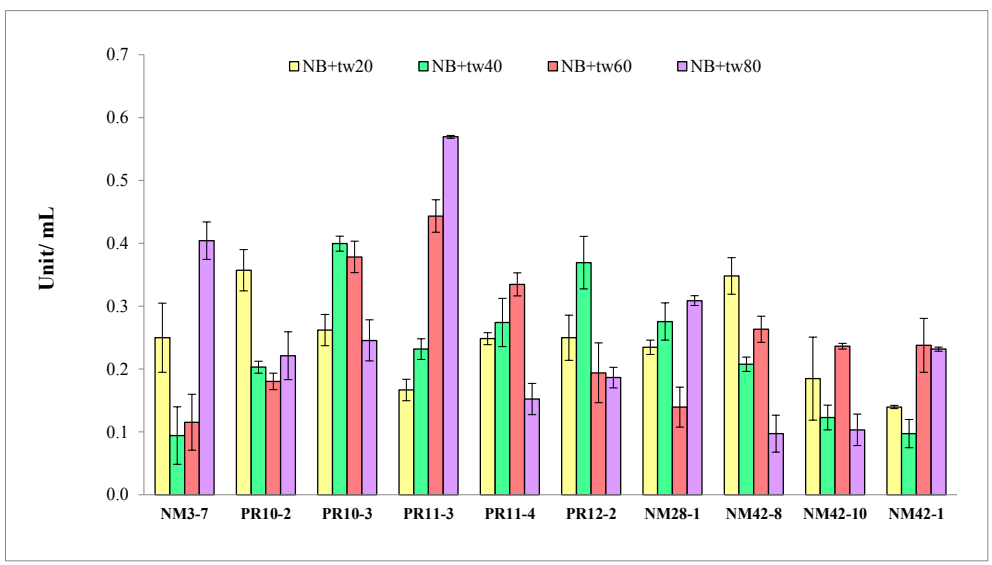
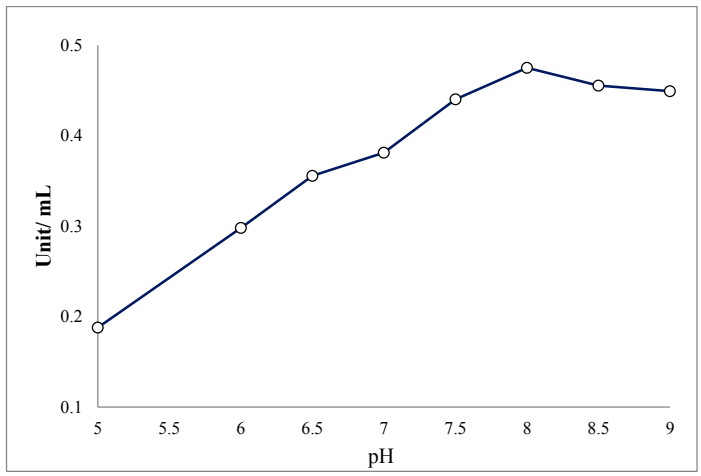


Figure 2. Lipolytic activity in nutrient broth (NB) with Tween 20, Tween 40, Tween 60 or Tween 80 (U/mL) of *Proteus* and *Klebsiella* isolates.



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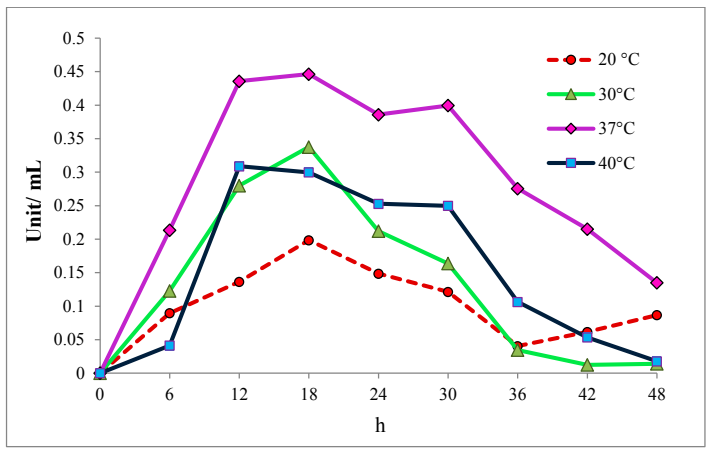

b

Figure 3. Effect of initial pH on lipolytic activity at 37°C, 24 h (a) and effects of temperature and incubation time on lipolytic activity of isolate PR11-3 cultivated in NB with 1% Tween 80, pH 8.0 (b).

[35]. *K. aerogenes* was found to be responsible for early blowing and poor cheese quality in other white-brined cheeses [36].

In this study, *P. mirabilis* and *Klebsiella* strains were distributed in fermented products, and they could exhibit lipolytic activity that may involve the odor of the products. However, the isolates that belonged *Enterobacteriaceae* acted as a hygienic index of foods. Their presence in meat and fish foods should receive particular attention because their presence indicates a public health hazard and can serve as a warning for the possible occurrence of food borne intoxication.

4. Conclusion

Nine isolates in Group 1 identified as *Proteus mirabilis* and one isolate in Group 2 identified as *Klebsiella* sp. showed antibiotic resistance to bacitracin, clindamycin, erythromycin,

tetracycline, and vancomycin but they were sensitive to carbenicillin, imipenem, novobiocin, penicillin G, streptomycin, sulphonamide, ampicillin, cephalothin, gentamicin, kanamycin and tobramycin. *Proteus* isolates showed lipolytic activity for Tween 20, Tween 40, Tween 60 and Tween 80 ranging from 0.10±0.02-0.57±0.00 U/mL whereas *Klebsiella* isolate exhibited 0.10±0.02 -0.24±0.04 U/mL. *P. mirabilis* PR11-3 exhibited the highest activity on Tween 80 at pH 8.0 when incubated for 18 h.

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References

[1] Walavalkar G.S. and Bapat, *Indian J. Exp. Biol.,* 2001; **40:** 1280-1284.

[2] Mrozik A., Hubert-Kocurek K. and Łabużek. S., *Postępy Mikrobiol.,* 2006; **45(1):** 19-26.

[3] Gayathri V.R., Perumal P., Mathew L. P. and Prakash, B., *Int. J. Sci. Technol.,* 2013; **2:** 502-509.

[4] ShuiLian B., Lin L., ShuZe T., ShouYi C., Yang W. X., Wu Z.W. and Lei S., *Modern Food Sci. Technol.,* 2009; **25 (6):** 690-695.

[5] Korman T.P., Sahachartsiri B., Charbonneau D.M., Huang G.L., Beauregard M. and Bow J.U., *Biotechnol. Biofuel,* 2013; **6:** 70. DOI:10.1186/1754-6834-6-70.

[6] Yoon S.-A., Han J.Y. and Kim H.K., *Korean. J. Microbiol. Biotechnol.ol.*, 2011; **39(3)**: 238-244.

[7] Riggs P.J., Chelius M.K., Iniguez A.L., Kaeppler S.M. and Triplett E.W., *Aust. J. Plant Physiol.,* 2001; **28 (9):** 829-836. DOI:10.1071/PP01045.

[8] Sekowska A., Gospodarek E., Janickca G., Jachna-Sawicka K. and Sawicki M., *Medycyna Doświadczalna i Mikrobiologia.,* 2006; **58(2):** 135-141.

[9] Willerding A.L., da R. Carvalho Neto F.G.M., da Gama A.M., Carioca C.R.F., de Oliveira L.A. *Química Nova,* 2012; **35 (9):** 1782-1786. DOI:10.1590/s0100-40422012000900015.

[10] Tanasupawat S. and Komagata K., Foods in Southeast Asia; in Nga B.H , Tan H. M. and Suzuki K., eds., *Microbial Diversity in Asia: Technology and Prospects,* World Scientific Publishing Co. Pte. Ltd, Singapore, 2001: 43-59.

[11] Visessanguan W., Benjakul S., Riebroy S., Yarchai M. and Tapingkae W., *Food Chem.*, 2006; **94:** 580-588.

[12] Barrow G.I. and Feltham R.K.A., *Cowan and Steel's Manual for the Identification of Medical Bacteria,* 3rd Edn., Cambridge University Press, Cambridge, 1993.

[13] Tanasupawat S., Okada S. and Komagata K., *J. Gen. Appl. Microbiol.,* 1998; **44:** 193-200. DOI:10.2323/jgam.44.193.

[14] Bauer A.W., Kirby W.M., Sherris J. C. and Turck M., *Am. J. Clin. Pathol.,* 1966**; 45:** 493-496.

[15] Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F. and Higgins D.G., *Nucleic Acids. Res.,* 1997; **25:** 4876-4882.

[16] Saitou N. and Nei M., *Mol. Biol. Evol.,* 1987; **4:** 406-425.

[17] Tamura K., Peterson D., Peterson N., Stecher G., Nei M. and Kumar S. *Mol. Biol. Evol.,* 2011; **28:** 2731-2739. DOI:10.1093/molbev/msr121.

[18] Felsenstein J., *Evolution,* 1985; **39:** 783-791.

[19] Sierra G., *Antonie van Leeuwenhoek,* 1957; **23:** 15-22. DOI:10.1007/BF02545855.

[20] Arora P.K., *Ann. Microbiol.,* 2013; **63:** 913-922. DOI:10.1007/s13213-012-0544-2.

[21] Janda J.M., Abbott S.L., Khashe S. and Probert W., *J. Clin. Microbiol.,* 2001; **39(4):** 1231-1234. DOI: 10.1128/JCM.39.4.1231-1234.2001.

[22] Giammanco G.M., Grimont P.A.D., Grimont F., Lefevre M., Giammanco G. and Sarina Pignato S., *Int. J. Syst. Evol. Microbiol.,* 2011; **61:** 1638-1644. DOI:10.1099/ijs.0.021964-0.

[23] Li X., Zhang D., Chen F., Ma J., Dong Y. and Zhang L., *Int. J. Syst. Evol. Microbiol.*, 2004; **54:** 2131-2136. DOI:10.1099/ijs.0.02690-0.

[24] Stock I., *J. Chemotherapy*, 2003; **15(1):** 12-26.

[25] Singh B.R. and Sharma V.D., *Indian J. Anim. Sci.,* 1999; **69:** 762-765.

[26] Feng W., Wang X-Q., Zhou W., Liu G-Y. and Wan Y-J., *J. Insect Sci.*, 2011; **11:** 135. DOI:10.1673/031.011.13501.

[27] Borch E., Kant-Muemans M-L. and Blixt Y. *Int. J. Food Microbiol.*, 1996; **33:** 103-120. DOI:10.1016/0168-1605(96)01135-X.

[28] Varnam A.H. and Sutherland J.P., *Meat and Meat Products Technology, Chemistry and Microbiology*, Chapman and Hall, London, 1995.

[29] Brissea S. and van Duijkeren E., *Vet. Microbiol.*, 2005; **105:** 307-312. DOI:10.1016/j.vetmic.2004.11.010.

[30] Casewell M. and Phillips I., *J. Clin. Pathol.,* 1978; **31(9):** 845-849. DOI:10.1136/jcp.31.9.845.

[31] Cakmaki M.L., Evans H.J. and Seidler R.J., *Plant Soil*, 1981; **61**: 53-64. DOI:10.1007/BF02277362.

[32] Haahtela K, Laakso T. and Korhonen T.K., *Appl. Environ. Microbiol.*, 1986; **52:** 1074-1079.

[33] Bagley S.T., Seidler R.J. and Brennerw D.J., *Curr. Microbiol.,* 1981; **6:** 105-109. DOI:10.1007/BF01569013.

[34] Podschun R. and Ullmann U., *Clin. Microbiol. Rev.,* 1998; **11:** 589- 603. DOI:10.1099/00222615-21-2-133.

[35] Sabota J.M., Hoppes W.L., Ziegler J.R., DuPont H., Mathewson J. and Rutecki G.W., *Am. J. Gastroenterol.,* 1998; **93(1):** 118-119. DOI:10.1016/S0002-9270(97)00036-1.

[36] Abo-Elnaga I.G., *Milchwissenchaft,* 1971; **26:** 747-750.