

Species Diversity of Molds in Thai Traditional Fermentation Starters (Loog-Pang)

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ABSTRACT

The results of investigation on the number of molds and mold species presented in loog-pang, a dry form of fermentation starter for production of traditional fermented products, collected in Thailand revealed that in 38 samples of loog-pang kao-mag (for alcoholic sweetened rice production) the number of mold were 2.0×10^3 - 1.6×10^6 cfu/g and 91 mold isolates were collected. The mold numbers in 19 samples of loog-pang lao (for rice wine production) were 7.0×10^3 - 1.8×10^6 cfu/g and 35 mold isolates derived. Identification of 91 mold isolates from loog-pang kao-mag showed that most isolates belonged to the genus *Amylomyces* (31) and *Rhizopus* (27). The remaining isolates were in the genus *Actinomucor* (5) *Aspergillus* (9), *Mucor* (2), *Monascus* (2) and *Penicillium* (1) while 13 isolates were *Aspergillus niger* group and 1 was unidentified isolate. Most of 35 isolates obtained from loog-pang lao were also in the genus *Rhizopus* (15) and *Amylomyces* (12). Other isolates were in the genus *Actinomucor* (5) and *Mucor* (1) while 2 were *Aspergillus niger* group. As many as 31 samples (81.6%) of loog-pang kao-mag contained *Amylomyces* sp. while *Rhizopus* sp. found only in 5 samples (13.2%). Among 19 samples of loog-pang lao *Rhizopus* sp. was found in 15 samples (79%) while *Amylomyces* sp. was in 11 samples (57.9%). Most isolates of the genus *Amylomyces* and *Rhizopus* showed relatively strong amylolytic activity.

Key words: mold, species diversity, Thai, fermentation starter, loog-pang

INTRODUCTION

“Loog-pang”, commonly known as “Chinese yeast cake” to the Western people, is a Thai term for dry form of “fermentation starter” for production of traditional fermented products from starchy raw materials, *i.e.*, *kao-mag* (alcoholic sweetened rice), *lao* (rice wine) and *num som sai chu* (vinegar) (Lotong, 1998). This type of fermentation starter has been used in many Asian countries with various local names, such as *banh men* in Vietnam, *bubod* in the Philippines, *chu* in China, *koji* in Japan, *murcha* in India, *nuruk* in

Korea, *ragi* in Indonesia and *ragi tapai* in Malaysia. Apparently, these starters are mixed cultures of amylolytic molds, fermenting yeasts and lactic acid bacteria grown on rice or other cereals. In certain localities, native herbs were added (Saono, 1982; Lotong, 1998; Tamang and Sarkar, 1995; Shrestha and Rati, 2002).

The molds species that were commonly reported found in loog-pang are *Amylomyces rouxii*, *Aspergillus oryzae*, *Asp. niger* group, *Aspergillus* spp., *Mucor* spp., *Penicillium* spp. and *Rhizopus* spp. (Chatisantien, 1977; Pichaynglura and Kulapreecha, 1977; Chaowsungket, 1978; Lotong,

1998). *Mucor indicus*, *M. circinelloides*, *R. oryzae* and *A. rouxii* were obtained from banh men (Haard *et al.*, 1999; Lee and Fujio, 1999). *Rhizopus* spp. and *Mucor* spp. were reported to present in bubod (Lotong, 1998). In murcha *M. circinelloides*, *R. chinensis* and *Rhizopus* spp. were isolated (Tamang, and Sarkar, 1995; Shrestha and Rati, 2002). *Aspergillus oryzae*, *Asp. niger* and *Rhizopus* spp. were obtained from nuruk (Kim, 1968). *A. rouxii*, *Asp. flavus*, *Asp. oryzae*, *Asp. niger*, *M. dubius*, *M. javanicus*, *M. rouxii*, *R. arrhizus*, *R. cohnii*, *R. oligosporus*, *R. oryzae* and *Fusarium* sp. were isolated from ragi (Saono, 1982; Lotong, 1998).

Among microorganisms found in these starter cakes only some species play the important roles in production of fermented products (Lotong, 1998; Haard *et al.*, 1999). Though there are some reports on microorganisms in loog-pang and their possible roles in product formation but only few isolates of microorganism were collected and maintained properly. Due to the limited knowledge of preparation process of loog-pang some key microorganisms are lost resulted in lower quality of loog-pang. Therefore, to control the quality of fermentation products, isolation, selection and conservation of the key microorganisms in loog-pang which could be used as pure cultures for production of the fermented products is gaining attention. Our group reported the study of yeast diversity in loog-pang (Limtong *et al.*, 2002). This present work reports the enumeration, isolation and identification of molds from loog-pang and investigation of amylolytic activity of molds isolates.

MATERIALS AND METHODS

Enumeration of molds in loog-pang

Enumeration of molds in loog-pang was performed by standard plate count using potato dextrose agar (PDA; 20% potato, 2% dextrose and 1.5% agar) and spread plate technique. Number of mold colonies was counted after incubation for 2

days at $30\pm2^\circ\text{C}$ and the average numbers from triple plates were reported.

Isolation of mold

To obtain as many mold genera as possible isolation was carried out by 4 protocols as follows: (1) spread plate technique on PDA (2) filtration of mold pellets from enrichment acidified YM (0.3% yeast extract, 0.3% malt extract and 0.5% glucose) broth and streaked on PDA plate (3) filtration of mold pellets from acidified YM broth containing 30% glucose and streaked on PDA plate and (4) isolation from sticky rice that covered with mold mycelium collected during preparation of fermented products.

Identification of molds

Mold isolates were identified based on morphological characteristics according to the monographs written by Ingold (1978), Samson and Pitt (1989), Alexopoulos *et al.* (1996), Hanlin (1998a) and Hanlin (1998b).

Investigation of amylolytic activity

Mold inoculum was 72 h culture grown on starch agar (4% soluble starch, 0.5% yeast extract and 1.5% agar) plate after point inoculation. The culture on agar was cut using cock borer (diameter 0.4 cm), placed at the center of new starch agar plate and incubated at $30\pm2^\circ\text{C}$ for 3 days. The culture plate was flooded with Lugol's iodine solution (2 g iodine, 2 g ammonium sulfate and 300 ml deionized water) for 1 min and diameter of clear zone and colony were measured. The amylolytic activity was expressed as the ratio of clear zone diameter to colony diameter and the results of triple plates were reported.

RESULTS AND DISCUSSION

Enumeration of molds in loog-pang

A total of 38 samples of loog-pang kao-mag and 19 samples of loog-pang lao were collected

from several provinces in Thailand. Most of the samples were obtained from central and northeastern regions of Thailand. The total count of mold in loog-pang kao-mag was in the range of

2.0×10^3 - 1.6×10^6 cfu/g while in loog-pang lao was 7.0×10^3 - 1.8×10^6 cfu/g. However, most samples of both types of loog-pang contained 10^4 - 10^5 cfu/g (Tables 1 and 2).

Table 1 Enumeration, isolation and identification of molds in loog-pang kao-mag and amylolytic activity of the isolates.

Sample code	Mold count (cfu/g)	Mold isolation			Identification	Amylase activity ^{1/}
		No. of isolate	Method / incubation (day)	Mold isolate code		
1	5.0×10^4	2	PDA / 2 days	MKM001	<i>Amylomyces</i> sp.	1.00
			PDA / 2 days	MKM002	<i>Rhizopus</i> sp.	1.20
2	6.0×10^3	3	PDA / 2 days	MKM003	<i>Amylomyces</i> sp.	0.83
			KM / 2 days	MKM004	<i>Rhizopus</i> sp.	1.00
			KM / 3 days	MKM005	<i>Aspergillus</i> sp.	1.00
5	4.2×10^4	1	PDA / 2 days	MKM006	<i>Amylomyces</i> sp.	1.04
6	6.0×10^3	3	YM ₃₀ / 2 days	MKM007	<i>Rhizopus</i> sp.	1.13
			PDA / 2 days	MKM008	<i>Amylomyces</i> sp.	1.03
			KM / 5 days	MKM009	<i>Aspergillus niger</i> group	0
13	1.5×10^5	1	PDA / 3 days	MKM010	<i>Rhizopus</i> sp.	1.11
15	1.2×10^5	3	YM ₃₀ / 2 days	MKM011	<i>Amylomyces</i> sp.	1.05
			PDA / 2 days	MKM012	<i>Rhizopus</i> sp.	0
			KM / 3 days	MKM013	<i>Aspergillus niger</i> group	0
18	8.0×10^4	3	KM / 3 days	MKM014	<i>Aspergillus niger</i> group	0
			PDA / 2 days	MKM015	<i>Amylomyces</i> sp.	1.02
			PDA / 2 days	MKM016	<i>Rhizopus</i> sp.	0
19	2.5×10^4	2	KM / 3 days	MKM017	<i>Aspergillus niger</i> group	0
			PDA / 2 days	MKM018	<i>Amylomyces</i> sp.	1.00
20	6.0×10^4	2	YM ₃₀ / 3 days	MKM019	<i>Rhizopus</i> sp.	1.00
			KM / 3 days	MKM020	<i>Amylomyces</i> sp.	1.17
21	1.9×10^4	2	YM ₃₀ / 3 days	MKM021	<i>Aspergillus niger</i> group	1.00
			PDA / 3 days	MKM022	<i>Amylomyces</i> sp.	1.00
23	2.0×10^3	3	PDA / 3 days	MKM023	<i>Rhizopus</i> sp.	0.53
			PDA / 3 days	MKM024	<i>Aspergillus niger</i> group	1.00
			PDA / 3 days	MKM025	<i>Amylomyces</i> sp.	0
24	6.0×10^4	2	PDA / 3 days	MKM026	<i>Amylomyces</i> sp.	1.08
			YM ₃₀ / 3 days	MKM027	<i>Aspergillus niger</i> group	1.00
25	1.6×10^5	2	PDA / 2 days	MKM028	<i>Rhizopus</i> sp.	1.10
			PDA / 2 days	MKM029	<i>Amylomyces</i> sp.	0.97
27	1.8×10^5	2	PDA / 2 days	MKM030	<i>Rhizopus</i> sp.	0.97
			PDA / 2 days	MKM031	<i>Amylomyces</i> sp.	1.00
31	1.0×10^5	2	PDA / 2 days	MKM032	<i>Rhizopus</i> sp.	1.10
			PDA / 2 days	MKM033	<i>Amylomyces</i> sp.	1.20
34	7.0×10^4	3	PDA / 2 days	MKM034	<i>Amylomyces</i> sp.	1.00
			KM / 7 days	MKM035	<i>Rhizopus</i> sp.	1.08
			KM / 7 days	MKM098	<i>Monascus</i> sp.	1.00
37	1.0×10^4	2	PDA / 2 days	MKM036	<i>Amylomyces</i> sp.	1.00
			KM / 3 days	MKM037	<i>Aspergillus</i> sp.	1.00
59	1.8×10^5	1	PDA / 2 days	MKM038	<i>Amylomyces</i> sp.	1.00
60	2.2×10^5	2	PDA / 2 days	MKM039	<i>Rhizopus</i> sp.	1.20
			YM (pH3.5) / 2 days	MKM040	<i>Actinomucor</i> sp.	1.01
64	8.0×10^3	4	PDA / 2 days	MKM041	unidentified	0
			PDA / 2 days	MKM042	<i>Amylomyces</i> sp.	1.00
			PDA / 2 days	MKM043	<i>Aspergillus</i> sp.	1.00

Table 1 (continued).

Sample code	Mold count (cfu/g)	Mold isolation			Identification	Amylase activity 1/
		No. of isolate	Method / incubation (day)	Mold isolate code		
65	2.2×10^5	4	KM / 3 days	MKM044	<i>Aspergillus</i> sp.	1.00
			PDA / 2 days	MKM045	<i>Mucor</i> sp.	1.00
			PDA / 3 days	MKM046	<i>Aspergillus</i> sp.	1.00
			KM / 3 days	MKM047	<i>Aspergillus niger</i> group	1.00
			KM / 7 days	MKM100	<i>Monascus</i> sp.	1.00
66	1.6×10^5	4	PDA / 2 days	MKM048	<i>Rhizopus</i> sp.	1.09
			KM / 3 days	MKM049	<i>Aspergillus</i> sp.	1.00
			PDA / 2 days	MKM050	<i>Actinomucor</i> sp.	1.05
			PDA / 2 days	MKM051	<i>Amylomyces</i> sp.	1.07
67	5.2×10^4	4	PDA / 2 days	MKM052	<i>Amylomyces</i> sp.	1.00
			KM / 3 days	MKM053	<i>Aspergillus niger</i> group	1.00
			KM / 2 days	MKM054	<i>Rhizopus</i> sp.	1.06
			KM / 4 days	MKM055	<i>Penicillium</i> sp.	1.00
68	5.0×10^4	3	KM / 2 days	MKM056	<i>Aspergillus niger</i> group	1.00
			PDA / 2 days	MKM057	<i>Rhizopus</i> sp.	1.00
			YM ₃₀ / 2 days	MKM058	<i>Aspergillus</i> sp.	1.00
69	1.2×10^5	4	PDA / 2 days	MKM059	<i>Rhizopus</i> sp.	0.89
			YM (pH3.5) / 2 days	MKM060	<i>Amylomyces</i> sp.	0.80
			KM / 3 days	MKM061	<i>Aspergillus niger</i> group	1.00
			KM / 3 days	MKM062	<i>Mucor</i> sp.	1.00
70	1.2×10^5	4	PDA / 2 days	MKM063	<i>Amylomyces</i> sp.	0.83
			PDA / 2 days	MKM064	<i>Rhizopus</i> sp.	0.86
			PDA / 2 days	MKM065	<i>Aspergillus niger</i> group	1.00
			KM / 2 days	MKM066	<i>Aspergillus</i> sp.	1.00
71	1.3×10^5	4	PDA / 2 days	MKM067	<i>Aspergillus niger</i> group	1.00
			PDA / 2 days	MKM068	<i>Amylomyces</i> sp.	1.06
			PDA / 2 days	MKM069	<i>Rhizopus</i> sp.	0.97
			YM (pH3.5) / 2 days	MKM070	<i>Aspergillus</i> sp.	1.00
74	5.0×10^4	2	PDA / 2 days	MKM071	<i>Rhizopus</i> sp.	1.00
			PDA / 2 days	MKM072	<i>Amylomyces</i> sp.	1.06
77	8.0×10^4	2	YM ₃₀ / 2 days	MKM073	<i>Amylomyces</i> sp.	1.00
			PDA / 2 days	MKM074	<i>Rhizopus</i> sp.	0.91
78	1.3×10^5	1	PDA / 2 days	MKM075	<i>Amylomyces</i> sp.	0.68
79	1.9×10^5	2	PDA / 2 days	MKM076	<i>Actinomucor</i> sp.	0.97
84	2.4×10^5	1	PDA / 2 days	MKM077	<i>Amylomyces</i> sp.	0.96
			KM / 3 days	MKM078	<i>Amylomyces</i> sp.	1.08
			PDA / 2 days	MKM079	<i>Actinomucor</i> sp.	1.00
22	1.6×10^6	2	KM / 3 days	MKM080	<i>Rhizopus</i> sp.	1.08
			PDA / 2 days	MKM081	<i>Rhizopus</i> sp.	1.14
			KM / 2 days	MKM082	<i>Amylomyces</i> sp.	1.11
39	1.0×10^4	2	PDA / 2 days	MKM083	<i>Rhizopus</i> sp.	0.94
40	6.0×10^4	1	KM / 2 days	MKM084	<i>Rhizopus</i> sp.	1.16
63	5.0×10^4	1	KM / 2 days	MKM085	<i>Rhizopus</i> sp.	1.07
72	2.0×10^5	2	KM / 2 days	MKM086	<i>Amylomyces</i> sp.	0.93
			KM / 1 days	MKM087	<i>Amylomyces</i> sp.	0.97
			PDA / 2 days	MKM088	<i>Actinomucor</i> sp.	1.00
73	1.6×10^6	3	PDA / 2 days	MKM089	<i>Rhizopus</i> sp.	1.07
			PDA / 3 days			
			PDA / 2 days			

Note: YM (pH 3.5) = Enrichment in acidified YM broth (pH 3.5).

YM₃₀ = Enrichment in acidified YM broth containing 30% glucose.

KM = Preparing alcoholic sweeten rice fermentation (kao-mag) using loog-pang kao-mag and isolated from kao-mag.

1/ = diameter of clear zone per diameter of colony

Table 2 Enumeration, isolation and identification of molds in loog-pang lao and amylolytic activity of the isolates.

Sample code	Mold count (cfu/g)	Mold isolation			Identification	Amylase activity 1/
		No. of isolate	Method / incubation (day)	Mold isolate code		
3	7.0×10 ³	1	PDA / 2 days	ML001	<i>Amylomyces</i> sp.	0.75
4	2.5×10 ⁴	1	PDA / 2 days	ML002	<i>Rhizopus</i> sp.	0.77
7	7.0×10 ⁴	1	PDA / 2 days	ML003	<i>Rhizopus</i> sp.	1.00
12	2.0×10 ⁴	2	PDA / 2 days	ML004	<i>Rhizopus</i> sp.	1.00
			L / 2 days	ML005	<i>Amylomyces</i> sp.	0.99
14	1.1×10 ⁵	2	L / 2 days	ML006	<i>Amylomyces</i> sp.	1.04
			PDA / 2 days	ML007	<i>Rhizopus</i> sp.	0.85
17	6.0×10 ⁴	2	PDA / 2 days	ML008	<i>Rhizopus</i> sp.	1.00
			L / 2 days	ML009	<i>Amylomyces</i> sp.	1.06
26	8.0×10 ⁴	2	PDA / 2 days	ML010	<i>Rhizopus</i> sp.	1.00
			PDA / 2 days	ML011	<i>Amylomyces</i> sp.	1.01
28	2.1×10 ⁴	3	PDA / 2 days	ML012	<i>Amylomyces</i> sp.	1.02
			PDA / 2 days	ML013	<i>Rhizopus</i> sp.	0.91
			L / 2 days	ML014	<i>Amylomyces</i> sp.	1.00
30	9.0×10 ⁴	2	YM (pH3.5) / 2 days	ML015	<i>Amylomyces</i> sp.	0.99
			PDA / 2 days	ML016	<i>Rhizopus</i> sp.	1.00
33	3.20×10 ⁵	2	YM ₃₀ / 2 days	ML017	<i>Aspergillus niger</i> group	1.00
			PDA / 2 days	ML018	<i>Rhizopus</i> sp.	0.83
48	7.0×10 ⁴	2	PDA / 2 days	ML019	<i>Rhizopus</i> sp.	1.00
			L / 2 days	ML020	<i>Amylomyces</i> sp.	1.00
57	8.00×10 ⁴	2	PDA / 2 days	ML021	<i>Rhizopus</i> sp.	0.64
			L / 2 days	ML022	<i>Amylomyces</i> sp.	1.02
58	8.0×10 ⁴	1	L / 2 days	ML023	<i>Amylomyces</i> sp.	1.12
81	9.0×10 ⁵	4	YM ₃₀ / 2 days	ML024	<i>Actinomucor</i> sp.	1.00
			L / 2 days	ML025	<i>Mucor</i> sp.	1.00
			L / 2 days	ML026	<i>Amylomyces</i> sp.	1.08
			YM (pH3.5) / 2 days	ML027	<i>Aspergillus niger</i> group	1.00
83	4.0×10 ⁵	1	PDA / 2 days	ML028	<i>Actinomucor</i> sp.	1.00
85	1.5×10 ⁶	2	L / 2 days	ML029	<i>Actinomucor</i> sp.	1.05
			PDA / 2 days	ML030	<i>Rhizopus</i> sp.	0.86
86	1.5×10 ⁶	2	PDA / 2 days	ML031	<i>Rhizopus</i> sp.	0.95
			YM (pH3.5) / 2 days	ML032	<i>Actinomucor</i> sp.	1.03
87	1.8×10 ⁶	2	YM (pH3.5) / 2 days	ML033	<i>Actinomucor</i> sp.	1.12
			PDA / 2 days	ML034	<i>Rhizopus</i> sp.	1.01
49	2.5×10 ⁴	1	PDA / 2 days	ML035	<i>Rhizopus</i> sp.	1.04

Remark: YM (pH 3.5) = Enrichment in acidified YM broth (pH 3.5).

YM₃₀ = Enrichment in acidified YM broth containing 30% glucose.

L = Preparing rice wine (lao) using loog-pang lao and isolation from fermenting mash.

1/ = diameter of clear zone per diameter of colony

The range of mold counts in loog-pang found in this study was slightly lower than that present in banh men (1.3×10^6 cfu/g) (Shrestha and Rati, 2002), bubod (1×10^3 - 1×10^7 cfu/g) (Tanimura *et al.*, 1978), murcha (2×10^5 - 1×10^7 cfu/g) (Lee and Fujio, 1999) and nuruk (1×10^7 cfu of *Aspergillus oryzae*/g, 1×10^7 cfu of *Asp. niger*/g and 1×10^6 cfu of *Rhizopus*/g) (Kim, 1968).

Isolation and identification of molds in loog-pang

Isolation of molds was carried out using 4 protocols resulted in 91 and 35 isolates from loog-pang kao-mag and loog-pang lao, respectively. The results showed that most of the isolates were obtained by simple spread plate technique. Identification following the taxonomic keys of several monographs revealed that most isolates obtained from loog-pang kao-mag were the members of genus *Amylomyces* (31) and *Rhizopus* (27). The remaining isolates belonged to genus *Actinomucor* (5), *Aspergillus* (9), *Aspergillus niger* group (13), *Mucor* (2), *Monascus* (2), *Penicillium* (1), and one unidentified isolate (Table 1). Among 35 isolates of mold obtained from loog-pang lao most of them were identified in the genus *Rhizopus* (15) and *Amylomyces* (12). The small number of isolates was in the genus *Actinomucor* (5) and *Mucor* (1), while 1 isolate was identified as *Aspergillus niger* group (Table 2).

As many as 31 samples of loog-pang kao-mag contained *Amylomyces* sp., however, only *Amylomyces* sp. was found in 4 samples, while 7 samples did not contain this genus. The other 9 samples consisted of *Amylomyces* sp. and *Rhizopus* sp. while in 12 samples *Amylomyces* sp., *Rhizopus* sp. and 1-2 isolates of the other molds genera were found. *Amylomyces* sp. and an isolate in another mold genus were presented in 6 samples. Only *Rhizopus* sp. was found in 3 samples. Two samples consisted of *Rhizopus* sp. and *Actinomucor* sp. One sample each contained *Aspergillus* sp., *Asp. niger* group together with *Rhizopus* sp. or *Monascus*

sp. and *Mucor* sp. (Table 3).

Among 19 samples of loog-pang lao 15 samples contained *Rhizopus* sp. Only *Rhizopus* sp. was found in 3 samples, *Rhizopus* sp. and *Amylomyces* sp. were in 8 samples, *Rhizopus* sp. and *Amylomyces* sp. were in 3 samples and *Rhizopus* sp. together with *Asp. niger* group were in 1 sample. The remaining 2 samples contained only *Amylomyces* sp., 1 sample contained *Actinomycetes* sp. and another sample contained *Actinomycetes* sp., *Asp. niger* group and *Mucor* sp. (Table 4).

The result of this work agreed well with the previous reports on the presence of *Amylomyces rouxii* and *Rhizopus* spp. together with some other mold genera such as *Aspergillus*, *Mucor* and *Penicillium* in loog-pang (Chatisantien, 1977; Pichyanglura and Kulpreecha, 1977; Chaowsungket, 1978). These mold genera were also found in various other alcoholic fermentation starters for examples in banh men, bubod, murcha and ragi (Kim, 1968; Kozaki, 1976; Tamang and Sarkar, 1995; Lotong, 1998; Lee and Fujio, 1999). However, there was no report on finding *Actinomucor* in any fermentation starters before this study.

Amylolytic activity of mold isolates obtained from loog-pang

Most isolates of *Amylomyces*, 25 of 31 isolates obtained from loog-pang kao-mag and 11 of 12 isolates from loog-pang lao revealed relatively strong amylolytic activity on starch agar, ratio of clear zone diameter and colony diameter were 1-1.2, while the remaining isolates showed low amylolytic activity and one isolate having no amylolytic activity at all (Tables 1 and 2). Among the *Rhizopus* spp. isolates most of them showed strong amylolytic activity, 19 from 27 isolates from loog-pang kao-mag and 9 of 15 isolates from loog-pang lao. The highest activity (ratio 1.2) derived from 3 isolates, *Rhizopus* sp. MKM002, *Rhizopus* sp. MKM039 and *Amylomyces* sp.

MKM033, from loog-pang kao-mag. Most of remaining isolates of various mold genera, namely *Actinomucor*, *Aspergillus*, *Monascus*, *Mucor* and *Penicillium* including *Asp. niger* group, revealed strong amylolytic activity except 3 isolates of *Asp. niger* group indicated no activity.

High amylolytic activities of *Amylomyces* sp. and *Rhizopus* spp. isolates agree well with the

fact that these mold genera play the important role in hydrolysis of starch in glutinous rice.

CONCLUSIONS

The results of this work revealed that most samples of loog-pang kao-mag (approximately 80%) comprised molds in the genus *Amylomyces*

Table 3 Summary of mold genera in loog-pang kao-mag.

Genera	Number of sample
<i>Amylomyces</i>	4
<i>Rhizopus</i>	3
<i>Amylomyces + Rhizopus</i>	9
<i>Amylomyces + Rhizopus + one or two mold genera</i>	12
<i>Amylomyces + Rhizopus + Aspergillus</i> (1)	
<i>Amylomyces + Rhizopus + Asp. niger</i> groups (4)	
<i>Amylomyces + Rhizopus + Asp. niger</i> groups + <i>Penicillium</i> (1)	
<i>Amylomyces + Rhizopus + Asp. niger</i> groups + <i>Aspergillus</i> (2)	
<i>Amylomyces + Rhizopus + Aspergillus + Actinomucor</i> (1)	
<i>Amylomyces + Rhizopus + Actinomucor</i> (1)	
<i>Amylomyces + Rhizopus + Monascus</i> (1)	
<i>Amylomyces + another genus</i>	6
<i>Amylomyces + Aspergillus</i> (1)	
<i>Amylomyces + Asp. niger</i> groups (3)	
<i>Amylomyces + Actinomucor</i> (1)	
<i>Amylomyces + unidentified mold</i> (1)	
<i>Actinomycetes + Rhizopus</i>	2
<i>Aspergillus + Asp. niger</i> groups + <i>Rhizopus</i>	1
<i>Aspergillus + Asp. niger</i> groups + <i>Monascus</i> + <i>Mucor</i>	1
Total	38

Table 4 Summary of mold genera in loog-pag lao.

Genera	Number of sample
<i>Rhizopus</i>	3
<i>Rhizopus + Amylomyces</i>	8
<i>Rhizopus + Actinomucor</i>	3
<i>Rhizopus + Asp. niger</i> group	1
<i>Actinomucor</i>	1
<i>Amylomyces</i>	2
<i>Amylomyces + Actinomucor + Asp. niger</i> group + <i>Mucor</i>	1
Total	19

while similar percentage of loog-pang lao contained *Rhizopus*. At the same time approximately 15% of loog-pang kao-mag *Rhizopus* existed and the genus *Amylomyces* were recorded in approximately 60% of loog-pang lao. Most isolates of *Amylomyces* and *Rhizopus* showed strong amylolytic activity, which indicated that these two mold genera played vigorous role in hydrolysis of starch in glutinous rice, the raw material for production of kao-mag and lao.

LITERATURE CITED

Alexopoulos, C.J., C.W. Mins and M. Blackwell. 1996. **Introductory Mycology**. 4th ed. John Wiley & Sons, New York, Chichester, Brisbane, Toronto and Singapore. 868 p.

Chaowsungket, M. 1978. **Selection of yeast and mould strains for rice wine production**. M.S. Thesis, Kasetsart University, Bangkok.

Chatisantien, C. 1977. **Selection of mould and yeast strains in loog-pang kao-mag fermentation**. M.S. Thesis, Kasetsart University, Bangkok.

Haard, N.F., S.A. Odunfa, C.H. Lee, R.Q. Ramerez, A.L. Quinones and C.W. Radarte. 1999. **Fermented Cereals. A Global Perspective**. FAO Agricultural Services Bulletin. No.138. Food and Agriculture Organization of the United Nations. Rome.

Hanlin, R.T. 1998a. **Combined Keys to Illustrated Genera of Ascomycetes. Vol. I and II**. APS Press, The American Phytopathological Society, St. Paul, Minnesota. 133 p.

Hanlin, R.T. 1998b. **Illustrated Genera of Ascomycetes. Vol II**. APS Press, The American Phytopathological Society, St. Paul, Minnesota. 258 p.

Ingold, C.T. 1978. **The Biological of *Mucor* and Its Allies**. The Camelot Press Ltd., Southampton. 59 p.

Kim, C.J. 1968. Microbiological and enzymological studies on Takju brewing. **J. Korean Agricul. Chem. Soc.** 60: 69-99.

Kozaki, M. 1976. Fermented foods and related microorganisms in Southeast Asia. **Proc. Jap. Assoc. Mycotoxicol.** 2: 1-9.

Pichayanglura, S. and S. Kulapreecha. 1977. **Survey of mycelial molds in loogpang from various sources in Thailand**. Symposium on indigenous fermented foods, Bangkok, Thailand.

Lee, A.C. and Y. Fujio. 1999. Microflora of banh men, a fermentation starter from Vietnam. **World J. Microbiol. Biotechnol.** 15(1): 57-62.

Limtong, S., S. Sintara, P. Suwanarit and N. Lotong. 2002. Yeast diversity in Thai traditional fermentation starter (loog-pang). **Kasetsart J. (Nat. Sci.)** 36: 149-158.

Lotong, N. 1998. Koji, pp. 658-695. In J.B Wood (ed.). **Microbiology of Fermented Food**. Vol. 2. 2nd ed. Blackie Academic and Professional. London.

Samson, R.A. and J.I. Pitt. 1989. **Modern Concepts in *Penicillium* and *Aspergillus* Classification**. Plenum Press, New York and London. Published in cooperation with NATO Scientific Affairs Division. 478 p.

Saono, J.K.D. 1982. Microflora of ragi, its compositions and as a source of industrial yeasts, pp. 241-250. In S. Saono, F.G. Winarmo and D. Karjadi (eds.). **Traditional Food Fermentation as Industrial Resources in ASCA Countries**, The Indonesian Institute of Sciences (LIPI), Jakarta.

Shrestha, H. K. and E.R. Rati. 2002. Microbiological profiles of murcha starters and physicochemical characteristics of pokò, a rice based traditional fermented food product of Nepal. **Food Biotechnology** 16(1): 1-15.

Tanimura, W., P.C. Sanches and M. Kozoki. 1978. The fermented food in the Philippines (Part 1) Tapuy (Rice wine). **J. Agric. Soc. (Japan)** 22: 118-133.

Tamang, J.P. and P.K. Sarkar. 1995. Microflora of murcha: an amylolytic fermentation starter. **Microbios** 81(327): 115-122.