

## SOME NOTES ON "RAGITAPE" AN INOCULUM FOR "TAPE" FERMENTATION.

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### RINGKASAN

Ragi tape di Indonesia sudah banjak dikenal. Ragitape merupakan suatu jang terdiri atas tjam-puran tepung beras dengan beberapa matjam rempah<sup>2</sup>. Mikrobia dominant jang aktif melakukan sac-chari fikasi ialah *Mucor* sp., *Rhizopus* sp., dan *Chlamydomucor* sp.

Rempah<sup>2</sup> jang berperanan penting dalam menghambat kegiatan mikrobia jang tidak dikehendaki ialah bawang putih (*Allium sativum* Linn.), lengkras (*Alpinia galanga* Sw.), djahe (*Zingiber officinale* Rosc.) dan kapulaga (*Amomum cardamum* Willd)

Dari hasil penelitian bahwa bawang putih mempunyai daja hambat mikrobia jang selektif. Bawang putih tidak menghambat pertumbuhan mikrobia jang amilolitik, tetapi menghambat mikro-bia jang dapat tumbuh dengan tjepat seperti *Rhizopus oryzae*, *Aspergillus niger* dan *Bacillus subtilis*. Pengaruh dari rempah<sup>2</sup> lainnja serta kemungkinan komposisi jang tepat dari ramuan ragi tape djuga dibitjarakan.

### SUMMARY

A preparation called "ragi tape" (pronounced as ra-ge ta-pa) is used for "tape" fermentation in Indonesia. The ingredients of ragi consist of riceflour and aromatic spices. The predominant amy-lytic molds of ragi are *Mucor* sp., *Rhizopus* sp., and *Chlamydomucor* sp.

The spices which inhibited the growth of undesired microorganisms are garlic (*Allium sativum* Linn.), lengkuas (*Alpinia galanga* Sw.), ginger (*Zingiber officinale* Rosc.) and kapulaga (*Amomum cardamum* Willd.).

It was observed that garlic has a selective inhibition character. Garlic in general did not inhibit the growth of amylytic molds but inhibited significantly the fast growing *Rhizopus oryzae*, *As-pergillus niger* and *Bacillus subtilis*. The effect of other spices is discussed in this paper also.

### INTRODUCTION

"Tape" is an Indonesian fermented food made from starchy materials, e.g. cassava and glutinous rice. It is sweet and alcoholic, and has a slightly sour taste. This fermented food can be consumed fresh or as deep fried tape. It can be made also into pudding, pie or dessert.

A common practice of tape preparation is as follows : cassava or glutinous rice is steamed and then allowed to cool, after cooling it is then inoculated with powdered "ra-gi". The inoculated material should be placed in leaves layered bamboo container or ena-gi<sup>1</sup>. The inoculated material should be placed in leaves layered bamboo container or ena-gi<sup>1</sup> pan, and incubated at room temperature (28°C to 30°C) for 2 to 3 days.

Ragi is made from a mixture of rice flour and aromatic spices. This mixture al-lowed the growth of amylytic molds and alcoholic forming yeast. Humidity should be controlled during this incubation period. The spices used in ragi are composed of aromatic spices, e.g. garlic (*Allium sativum* Linn.), ginger (*Zingiber officinale* Rosc.), lengkuas (*Al-pinia galanga* Sw.), djeruk nipis (*Citrus aurantium* subsp. *aurantifolia* var *fusca*), pepper, sugar and others (3).

The active microorganisms in ragi have been identified as *Mucor rouxii*, *Chlamydomucor oryzae*, *Rhizopus oryzae*, *Saccharomyces cerevisiae* and *Candida javanica* (3).

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Since ragi is made by various small holders with different methods, it is hard to obtain ragi from the market which ensured constant product of fermentation. The quality of ragi depends on the strains of amylolytic and alcoholic forming microorganisms in the preparation. The composition of the ragi ingredients and the environmental conditions during incubation seem to be the critical factors which affect the quality of ragi.

The objective of this study is to determine the function of the spices in ragi preparation.

## EXPERIMENTAL MATERIALS AND METHODS.

Ragi was collected from several places in Java, especially from Surakarta, Bandung, Bondowoso, Banjumas, and Bogor.

The molds of ragi were not directly isolated from ragi, but they were isolated from the fermented product (tape). The molds were isolated by placing aseptically a small piece of the inner part of tape on mungo sprout extract plate agar.

The mold was identified by its microscopic and macroscopic appearance. The screening test for amylolytic activity was done by using starch plate method and I-KI solution as indicator (4). The saccharifying activity was determined qualitatively and quantitatively by SELIWANOFF test (2) and by LUFF SCHOORL method (1), respectively. The alcohol formation was determined qualitatively by iodoform reaction method.

The phytoncide activity of the spice was determined by Oxford cup method (6). The spice extract was prepared by grinding a certain amount of spice, and then centrifuged. The supernatant liquid was used in the phytoncide activity test.

## RESULT AND DISCUSSION

The amylolytic molds that have been isolated from tape is presented in table 1. They were identified as *Mucor* sp. R<sub>B10</sub>, *Mucor* sp. R<sub>J2</sub>, *Mucor* sp. R<sub>B03</sub>, *Chlamydomucor* sp. R<sub>B911</sub>, and *Rhizopus* sp. R<sub>Bs4</sub>.

Among these molds *Mucor* sp. R<sub>B03</sub> and *Chlamydomucor* sp. R<sub>B911</sub> have the highest activity in saccharification ( see table 2).

From microscopic and macroscopic observations it can be seen that in general these molds are not septate ; with many gemmae (except R<sub>Bs4</sub>) ; few or no fruiting bodies ; whitish in colour.

Other microorganisms that have been observed in ragi were *Aspergillus* sp., *Penicillium* sp., *Saccharomyces* sp. and *Bacillus* sp. (personal communication, unpublished). These microorganisms were active in alcoholic and acid fermentation, and some of the molds have saccharifying activity.

Ragi is made from a mixture of rice flour and mungo sprout extract. The growth of amylolytic molds and alcoholic forming yeast. The spices used in ragi are composed of aromatic spices, e.g. garlic (*Allium sativum* Linn.), ginger (*Zingiber officinale* Rose), lemongrass (*Citrus aurantium* Sw.), black pepper (*Piper nigrum* Linn.), and others (3).

The active microorganisms in ragi have been identified as *Mucor* sp., *Chlamydomucor* sp., *Rhizopus* sp., *Saccharomyces* sp. and *Candida* sp. (3).



Table 1. Morphology of amylolytic molds on mungo sprout extract agar.

Isolates.	Part of Mold, in micron.	Genus
R <sub>B10</sub> (Bogor)	Sporangia : 8.6 - 10.4 Sporangiophore : 94.2 - 98.4 Gemmae : 14.0 - 25.2 Hyphae : 10.0 - 12.5 Rhizoid : None Colour : White	Mucor sp.
R <sub>J2</sub> (Surakarta)	Sporangia : 6.7 - 9.3 Sporangiophore : 49.2 - 93.0 Gemmae : 6.5 - 10.0 Hyphae : 6.0 - 8.0 Rhizoid : None Colour : White	Mucor sp.
R <sub>B03</sub> (Bondowoso)	Sporangia : 8.6 - 10.6 Sporangiophore : 50.7 - 120.0 Gemmae : 13.5 - 20.0 to 18.5 - 39.6 Rhizoid : None Hyphae : 12.0 - 14.5 Colour : White	Mucor sp.
R <sub>Bg11</sub> (Bandung)	Sporangia : None Sporangiophore : None Gemmae : 14.5 - 20.7 Hyphae : 11.5 - 15.0 Rhizoid : None Colour : White	Chlamydomucor sp.
R <sub>Bs4</sub>	Sporangia : 120 - 140. Sporangiophore : very long up to 1.5 cm Gemmae : None Hyphae : 7.5 - 10.4 Colour : greyish white. Rhizoid : many.	Rhizopus sp.



Table 2 Amylolytic and alcohol forming ability of amylolytic molds of tape.\*

Isolates	Saccharifying activity			Alcohol formation**)
	I	II	III	
R <sub>B10</sub>	slightly sweet	++	2.0	-
R <sub>J2</sub>	fairly sweet	+++	5.2	±
R <sub>B03</sub>	sweet	+++	8.0	±
R <sub>Bg11</sub>	very sweet	+++	10.9	-
R <sub>Bs4</sub>	slightly sweet	+	2.0	-

\* ) glutinous rice tape.

\*\* ) Iodoform reaction test.

I = organoleptic test.

II = Seliwanoff test.

III = % sugar.

The growth of amylolytic molds were affected by several kind of spices such as garlic, ginger and others (see table 3 and 4). Among the spices, garlic had a selective inhibition effect. Garlic did not inhibit the growth of amylolytic molds, but greatly inhibited the growth of *Aspergillus niger* and *Bacillus subtilis*. The fast growing *Rhizopus oryzae*, however, was only slightly inhibited by garlic. From these facts it is easy to be understood that the addition of garlic in ragi preparation will reduce undesirable microorganisms that will affect the growth of amylolytic molds (see table 5).

Table 3 The inhibition effect of garlic and ginger on the growth of amylolytic molds and other microorganisms.\*)

Amount of extract in ml	Kind of Microorganisms					R. oryzae	A. niger	S. cerevisiae	B. subtilis
	R <sub>B10</sub>	R <sub>J2</sub>	R <sub>B03</sub>	R <sub>Bg11</sub>	R <sub>Bs4</sub>				
Garlic**)									
0.0	—	—	—	—	—	—	—	—	—
0.1	—	—	—	—	—	+	+	—	—
0.2	—	—	—	—	—	+	+	—	—
0.4	—	—	—	—	—	+	+	—	—
0.6	—	—	—	—	—	+	+	—	—
Ginger***)									
0.0	—	—	—	—	—	—	—	—	—
0.1	++	++	+	+	++	+	+	—	—
0.2	++	++	+	+	++	+	+	—	—
0.4	++	++	+	+	++	+	+	—	—
0.6	++	++	+	+	++	+	+	—	—

\*) cup method.

\*\*\*) extract of garlic was prepared from 2 g of garlic per 10 ml of water.

\*\*\*\*) extract of ginger was prepared from 5 g of ginger per 10 ml of water.

Notes :

- + inhibition zone 2 - 3 mm.
- ++ inhibition zone 3 - 5 mm.
- +++ inhibition zone more than 5 mm.
- no inhibition.

Table 4 The inhibition effect of several spices on the growth of amylolytic molds and other microorganisms

Kind and amount of extract in ml.	Kind of Microorganisms					R. oryzae	A. niger	S. cerevisiae	B. subtilis
	R <sub>B10</sub>	R <sub>J2</sub>	R <sub>B03</sub>	R <sub>Bg11</sub>	R <sub>Bs4</sub>				
Kapulaga1) 0.2	—	—	—	—	—	—	—	—	—
Lengkuas2) 0.2	++	++	++	++	++	++	++	—	—
Clove 3) 0.2	+	+	+	+	+	+	+	—	—
Pepper 4) 0.2	—	—	—	—	—	—	—	—	—

(continued)



- 1) 4 g of kapulaga per 10 ml of water.
- 2) 5 g of lengkuas per 10 ml of water.
- 3) 1 g of clove per 10 ml of water.
- 4) 1 g of pepper per 10 ml of water.

\*) showed stimulation of growth..

Notes : same as table 3.

Table 5 Ragi tape preparation.

Ragi	Composition of ragi	Contamination in %	Taste of tape*)	% sugar in tape
I	25R:2A:13X	0	sweet	5.5
II	25R:1A:14X	0	very sweet	8.6
III	25R:3A:12W	10	sweet	3.0
IV	25R:2A:13W	25	sweet, slightly sour	2.8
Market	?	—	sweet, alcoholic and slightly sour	5.0

\*) glutinous rice tape.

Notes :

1. A is an extract of 5 g garlic per 25 ml of water.
2. X is a solution consisted of dextrose 15 g, mungo sprout extract 100 ml, and the pH was adjusted to 4.5 - 5 with 2 NHCl.
3. W is water.
4. R is rice flour.

Ginger did not inhibit *Mucor* sp. R<sub>911</sub>, *Saccharomyces cerevisiae* and *Bacillus subtilis*. Pepper, however, stimulated the growth of all microorganisms especially *Bacillus subtilis*.

From these examinations it seems, that certain spices act as agents that inhibit the development of certain microorganisms. Thus the rice flour-spices mixture in ragi functioned as a selective medium for amylolytic, alcoholic and acid forming microorganisms. By knowing the exact chemical constituents of the spices it is then possible to obtain a constant quality of ragi.

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