

# MYCOFLORA OF "RAGI" AND SOME OTHER TRADITIONAL FERMENTED FOODS OF INDONESIA \*)

by

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## SUMMARY

Studies had been conducted on the mycoflora of samples of "ragi", "tapé ketéla" or "peuyem", "tapé ketan hitam", "oncom hitam", and "oncom mérah" or "oncom beureum" from various places in West Java. The organisms that had been found in "ragi" include *Candida* sp., *C. guilliermondii*, *C. humicola*, *C. japonica*, *C. parapsilosis*, *C. pelliculosa*, *C. solani*, *Fusarium* sp., *Mucor circinelloides*, *M. javanicus*, *M. rouxii*, and *Rhizopus oryzae*; in "tapé ketéla" - *Candida* sp., *C. guilliermondii*, *C. intermedia*, *C. japonica*, *C. mycoderma*, *C. parapsilosis*, *C. parapsilosis* var. *intermedia*, *C. pelliculosa*, *C. solani*, *M. circinelloides*, and *M. javanicus*; *C. intermedia* seems to be more prevalent in samples from the Bogor - Cianjur - Sukabumi region and *C. pelliculosa* in those from the Bandung - Garut - Karawang area; in "tapé ketan hitam" - *Candida* sp., and *C. pelliculosa*; in "oncom hitam" - *C. mesenterica*, *C. parapsilosis*, *C. parapsilosis* var. *intermedia*, *C. pelliculosa*, *C. reukaufii*, *C. solani*, *Mucor* sp., *M. javanicus*, *R. oligosporus*, and *R. oryzae*; *C. mesenterica* and *C. parapsilosis* were more often found than the others; in "oncom mérah" - *C. guilliermondii*, *C. humicola*, *C. mesenterica*, *C. parapsilosis*, *C. pelliculosa*, *C. reukaufii*, *C. solani*, *C. tropicalis*, *C. utilis*, *Cryptococcus laurentii*, *M. inaequisporus*, *M. javanicus*, *Neurospora* sp., *N. sitophila*, and *R. oryzae*. Most of the yeasts were isolated from the samples from Bogor - Cianjur - Sukabumi area.

## INTRODUCTION

It is estimated that not less than 15 different traditional fermented products are known in Indonesia (vide Appendix I). Only a number of them are, however, widely consumed, viz. various kinds of "tépé", "tapé", and "oncom". Many of these foods are prepared with the help of a starter called "ragi", a small, slightly flattened dry ball consisted of rice powder, spices, and microorganisms.

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The majority of "tapé", "oncom", and "ragi" are manufactured on home industry scale utilizing primitive techniques and under impure culture condition (vide Appendix II). Consequently, it is possible to find a wide variety of microorganisms in such products. Yet little has been known about their microflora. The available information is mostly concerned with the mycoflora and even this is far from adequate (OCHSE, 1931; HEYNE, 1950; BOEDIJN, 1958; DWIDJOSEPUTRO, 1961; RAMLAN et al., 1964; HESSELTINE, 1965; KO SWAN DJIEN, 1965, 1972; DWIDJOSEPUTRO and WOLF, 1970; SOEDARSONO, 1972).

This study deals with the mycoflora, viz. yeasts and molds, of "ragi" and the most widely consumed traditional fermented foods of West Java, i.e. "tapé ketéla" or "peuyeum", "tapé ketan hitam", "oncom hitam" (black "oncom"), and "oncom mérah" or "oncom beureum" (red "oncom").

### MATERIALS AND METHODS

The study had been made on 14 samples of "ragi" from: Bogor - 1 sample, Cianjur - 2 samples, Sukabumi - 1 sample, Garut - 3 samples, Bandung - 4 samples, and Karawang - 3 samples. One sample from Garut (sample Garut no. 2) is "ragi" specially used for preparing "oncom mérah" and actually is an old "oncom mérah"; 11 samples of "tapé ketéla" from: Bogor - 1 sample, Cianjur - 2 samples, Sukabumi - 2 samples, Garut - 2 samples, Bandung - 2 samples, and Karawang - 2 samples; 1 sample of "tapé ketan hitam" from Bandung; 9 samples of "oncom hitam" from: Bogor - 2 samples, Sukabumi - 4 samples, Garut - 2 and Jakarta - 1 sample; 17 samples of "oncom mérah" from: Bogor - 2 samples, Cianjur - 4 samples, Sukabumi - 2 samples, Garut - 2 samples, Bandung - 3 samples, Karawang - 2 samples, and Jakarta - 2 samples.

Isolations of the microorganisms were immediately carried out as soon as the samples reached the laboratory in Bogor, which usually required 1-3 days after sampling. Isolations of molds were conducted by plating aqueous suspension of the samples in sterile distilled water (1 : 9 w/v) on malt extract agar, while the yeasts were isolated by plating the suspension on malt extract agar acidified to pH 4.5 with sterile lactic acid. The plates were then incubated at room temperature (ca. 27°C) for 3-7 days. Representative colonies were subcultured on malt extract agar slants for further studies. Isolations of bacteria were also made by plating the suspension on nutrient agar with subsequent subculturing of representative colonies on nutrient agar slants, but the results will be dealt elsewhere.

The identification of the molds was based on morphological characteristics of the colony grown on malt extract agar, potato dextrose agar, and mung bean (*Phaseolus radiatus*) seedlings agar (100 g fresh, clean, 3–4 days old seedlings were boiled in 1 l tap water for 1½ hours. The broth was then filtered through a piece of thick cloth. The filtrate was brought to 1 l with tap water. 60 g of saccharose and 2% agar were added, melted, dispensed into the appropriate containers or tubes, and sterilized at 121°C for 15 minutes). The species of *Mucor* were identified by following the keys of GILMAN (1945), BOEDIJN (1958), and DWIDJOSEPUTRO and WOLF (1970); those of *Rhizopus* followed the keys of BOEDIJN (1958), INUI et al. (1965), ZYCHA et al. (1969), and DWIDJOSEPUTRO and WOLF (1970); the species of *Neurospora* followed DWIDJOSEPUTRO (1961).

Morphological and physiological properties as outlined by LODDER and KREGER VAN RIJ (1952) were used for the identification of the yeast species. These properties include: characteristics of the vegetative reproduction on malt extract agar and corn meal agar; shape and size of cells from culture in malt extract broth; ascospore formation by culture grown on malt extract agar, Gorodkova agar, potato agar, yeast water agar, vegetable juice agar, carrot plugs, potato plugs, beet plugs, and gypsum block; pellicle formation on malt extract broth; fermentation and assimilation of glucose, galactose, saccharose, maltose, lactose, and raffinose; assimilation of nitrate; utilization of ethanol as sole carbon source; splitting of arbutin; production of starch-like compounds; reaction in litmus milk.

## RESULTS AND DISCUSSION

### "Ragi"

Among the products under study, "ragi" had received more attention than the others. Various names have been given to this product, viz. "ragi" (OCHSE, 1931; HEYNE, 1950; HESSELTINE, 1965; KO SWAN DJIEN, 1972), "ragi beras" (KO SWAN DJIEN, 1965), and "ragi tapé" (DWIDJOSEPUTRO and WOLF, 1970; SOEDARSONO, 1972), all of which refer to the same product mentioned as "ragi" in Appendixes I and II. In addition, other names related to "ragi" have also been used by DWIDJOSEPUTRO and WOLF (1970), such as "ragi roti", "ragi tempé", and "ragi kécap". These products are "ragi" for special purposes as indicated by the adjective. Thus "ragi roti" is "ragi" for "roti" (bread) making, "ragi tempé" – for "tempé" preparation, and "ragi kécap" – for "kécap" (soy sauce) preparation.

Early microbiological studies were mainly conducted on "ragi" and had been cited by OCHSE (1931), HEYNE (1950), and reviewed by KO SWAN DJIEN (1965). The organisms reported to be found in "ragi" include *Mucor dubius*, *M. javanicus*, *Chlamydomucor oryzae*, *Rhizopus oryzae*, *Saccharomyces vordermanii*, *Willia indica* (*Torula indica*), and *Candida javanica* (*Monilia javanica*). Later studies showed that *C. javanica* (*Monilia javanica*) are similar to *Hansenula anomala* while *S. vordermanii* similar to *S. cerevisiae* (HESSELTINE, 1965). The limited number of species reported seems to be due to the stage of mycology at that time, because recent studies on the mycoflora of "ragi" had yielded a number of species not reported before, viz. *C. parapsilosis*, *C. melinii*, *C. lactosa*, *C. solani*, *H. subpelliculosa*, *H. malanga* (DWIDJOSEPUTRO and WOLF, 1970), *Endomycopsis chodati*, *E. fibuliger* (KO SWAN DJIEN, 1972), *M. rouxii*, *R. stolonifer* (KO SWAN DJIEN, 1965; DWIDJOSEPUTRO and WOLF, 1970), *Aspergillus niger* (KO SWAN DJIEN, 1965), *A. oryzae*, *R. arrhizus*, *R. oligosporus*, *A. flavus* (DWIDJOSEPUTRO and WOLF, 1970). Moreover unidentified species of *Penicillium* had been found by KO SWAN DJIEN (1965) and SOEDARSONO (1972), of *Mucor* and *Rhizopus* by KO SWAN DJIEN (1972) and SOEDARSONO (1972), of *Aspergillus*, *Chlamydomucor*, and *Saccharomyces* by SOEDARSONO (1972) in various samples of "ragi". Evidently the finding of more species of yeast and mold can be expected from a product such as "ragi", a fact that has been corroborated by results of this study.

Out of 14 samples of "ragi" from different places in West Java 6 species of yeast and 2 of mold, so far not reported, had been isolated, and identified as: *C. guilliermondii* (Fig. 1 - a), *C. humicola* (Fig. 1 - i), *Candida* sp., *M. circinelloides*, and *Fusarium* sp. (Table I). While the yeasts are more or less uniformly distributed in most of the samples giving no apparent relationships between the kind of species and the origin of the sample, the mold species tend to be found more often and with more varieties in samples from the Bandung - Garut - Karawang area rather than in those from the Bogor - Cianjur - Sukabumi region. This might be due to the fact that they are two regionally separated trading and cultural regions in West Java.

TABLE I. MYCOFLORA OF "RAGI"

Origin	Yeast							Fungus					
	<i>Candida</i> sp.	<i>C. guilliermondii</i>	<i>C. humicola</i>	<i>C. intermedia</i>	<i>C. japonica</i>	<i>C. parapsilosis</i>	<i>C. pelliculosa</i>	<i>C. solani</i>	<i>Fusarium</i> sp.	<i>Mucor circinelloides</i>	<i>M. javanicus</i>	<i>M. rouxii</i>	<i>Rhizopus oryzae</i>
Bogor								+					
Cianjur no. 1				+								+	
Cianjur no. 2							+						
Sukabumi	+												
Garut no. 1		+	+				+						
Garut no. 2										+			
Garut no. 3					+				+				
Bandung no. 1	+			+		+					+		
Bandung no. 2		+									+		
Bandung no. 3											+		
Bandung no. 4													+
Karawang no. 1	+								+				+
Karawang no. 2	+									+			
Karawang no. 3	+												+

+ : present

*"Tapé ketéla" and "tapé ketan hitam"*

Although "tapé" has been known for a long time in Indonesia, little studies had been done on its microflora. This is probably due to the fact that "ragi" is always used as a starter for "tapé" preparation, hence it is assumed that the microflora of these two products are similar. In general this is true. However, the possibility of the introduction of organisms other than those found in "ragi" during the process of "tapé" preparation cannot be discounted.

TABLE II. MYCOFLORA OF "TAPE KETELA"/"PEUJEUM" AND "TAPE KETAN HITAM"

Origin	Yeast								Fungus		
	<i>Candida</i> sp.	<i>C. guilliermondii</i>	<i>C. intermedia</i>	<i>C. japonica</i>	<i>C. mycoderma</i>	<i>C. parapsilosis</i>	<i>C. parapsilosis</i> var. <i>intermedia</i>	<i>C. pelliculosa</i>	<i>C. solani</i>	<i>Mucor circinelloides</i>	<i>M. javanicus</i>
Bogor			+			+					
Cianjur no. 1			+			+					+
Cianjur no. 2							+				+
Sukabumi no. 1			+								
Sukabumi no. 2		+	+								
Garut no. 1					+						+
Garut no. 2	+							+		+	
Bandung no. 1								+			
Bandung no. 2				+				+			
Karawang no. 1	+										
Karawang no. 2								+	+		
Tapé ketan hitam from Bandung	+							+			

+ : Present

Existing literature on the microflora of "tapé" are mainly concerned with "tapé ketéla" and they list the following organisms: *Chlamydomucor oryzae*, *R. oryzae*, *Willia indica* (HEYNE, 1950), *R. chlamydosporus* (RAMLAN et al., 1964), *Mucor* sp., *Chlamydomucor* sp., and *Rhizopus* sp. (SOEDARSONO, 1972).

The majority of "tapé" sold in West Java is "tapé ketéla". "Tapé ketan" is, on the contrary, sold in more limited quantities. Thus from a total of 12 samples of the material under study, 11 were "tapé ketéla" and only 1 sample was "tapé ketan hitam" (Table II).

All the samples studied yielded yeasts of which *C. intermedia* seems to be more prevalent in samples from the Bogor - Cianjur - Sukabumi region and *C. pelliculosa* in those from the Bandung - Garut - Karawang area. Other yeast species which had been detected include *C. guilliermondii*, *C. japonica*, *C. mycoderma* (Fig. 1 - f), *C. parapsilosis* (Fig. 1 - g), *C. parapsilosis* var. *intermedia* (Fig. 1 - h), and *C. solani* (Fig. 1 - k). Only 2 species of mold had been found, both of which have not been reported in the existing literature, viz. *M. circinelloides* and *M. javanicus*. The latter appeared in more samples than the former.

#### "Oncom hitam"

Literature on the microbiology of the "oncom hitam" is almost non-existent. OCHSE (1931) and HEYNE (1950) cited the name "oncom bodas" (white "oncom") or "oncom Bogor" for another product besides "oncom mérah" or "oncom Bandung". The former has a white or ashen grey color due to the growth of *R. oryzae*. This seems to be the same product as "oncom hitam". The difference in color (black or white) is probably a matter of subjective interpretation, since both refer to the color of the mold colony. As to the difference of the raw materials of "oncom hitam" and "oncom mérah" OCHSE (1931) wrote that the former contains rice meal which is introduced together with the *R. oryzae* as an inoculant, while corn meal is mixed with the *Monilia sitophila* (*Neurospora sitophila*) for "oncom mérah" preparation. No further information was given on the quantity of both meals. At present, as shown in Appendix II, the principal difference of the raw materials of the two products is the absence of the waste of "tahoo" in "oncom hitam".

As shown by Table III, six species of yeast and four of mold had been found in 9 samples of "oncom hitam". Among the yeasts, *C. mesenterica* (Fig. 1 - e) and *C. parapsilosis* existed in more samples than the other species, which include *C. parapsilosis* var. *intermedia*, *C. pelliculosa*,

TABLE III. MYCOFLORA OF "ONCOM HITAM"

Origin	Yeast						Fungus			
	<i>Candida mesenterica</i>	<i>C. parapsilosis</i>	<i>C. parapsilosis</i> var. <i>intermedia</i>	<i>C. pelliculosa</i>	<i>C. reukaufii</i>	<i>C. solani</i>	<i>Mucor</i> sp.	<i>M. javanicus</i>	<i>Rhizopus oligosporus</i>	<i>R. oryzae</i>
Bogor no. 1	+				+					
Bogor no. 2	+	+				+			+	
Sukabumi no. 1		+								
Sukabumi no. 2	+									+
Sukabumi no. 3		+								
Sukabumi no. 4	+					+				
Garut no. 1		+			+					
Garut no. 2			+	+			+			
Jakarta		+						+		

+ : present

*C. reukaufii* (Fig. 1 - j), and *C. solani*. All the molds were Mucoraceous and consisted of *M. javanicus*, *Mucor* sp., *R. oligosporus*, and *R. oryzae*. It is interesting to note that the last 2 organisms are also considered to be the principal molds in "tépé kedelé" (OCHSE, 1931; HEYNE, 1950; HESSELTINE, 1965; KO SWAN DJIEN, 1965). Therefore "oncom hitam" could probably be considered as another variety of tépé.

#### "Oncom mérah"

As in the case of "oncom hitam", little had been known about the microbiology of "oncom mérah". The distinctive character of this product is its saffron pink color due to the abundance of conidia of its principal mold, *N. sitophila*. It is surprising that no other organisms had been reported in the literature, considering the nature of the product and its wide consumption.

In this study ten species of yeast and four of mold, other than *N. sitophila* or *Neurospora* sp., had been found in 17 samples of "oncom mérah" (Table IV).

TABLE IV. MYCOFLORA OF "ONCOM MERAH"

Origin	Yeast										Fungus			
	<i>Candida guilliermondii</i>	<i>C. humicola</i>	<i>C. mesenterica</i>	<i>C. parapsilosis</i>	<i>C. pelliculosa</i>	<i>C. reukaufii</i>	<i>C. solani</i>	<i>C. tropicalis</i>	<i>C. utilis</i>	<i>Cryptococcus laurentii</i>	<i>Mucor inaequisporus</i>	<i>M. javanicus</i>	<i>Neurospora</i> sp.	<i>N. sitophila</i>
Bogor no. 1		+							+					
Bogor no. 2		+		+				+		+			+	
Cianjur no. 1													+	
Cianjur no. 2	+												+	
Cianjur no. 3				+				+				+		
Cianjur no. 4			+	+										
Sukabumi no. 1		+					+				+	+		
Sukabumi no. 2	+			+	+			+						
Garut no. 1			+	+		+								
Garut no. 2						+					+			
Bandung no. 1	+												+	
Bandung no. 2												+		
Bandung no. 3														
Karawang no. 1													+	
Karawang no. 2											+			+
Jakarta no. 1													+	
Jakarta no. 2													+	

+ : present

The majority of the yeasts were isolated from samples from the Bogor - Cianjur - Sukabumi area, and from only one sample from Bandung - Garut - Karawang region. Similar explanation as for the case of "ragi" could perhaps be used for this case. Moreover, this might also be the reason for the wellknown superior quality of "oncom mérah" from Bandung region as compared to that from other places in West Java.

As might be expected the predominant organism found was *N. sitophila* or *Neurospora* sp. Other Mucoraceous molds and yeast species so far not reported were, however, detected, viz. *M. inaequisporus*, *M. javanicus*, *R. oryzae*, *C. guilliermondii*, *C. humicola*, *C. mesenterica*, *C. parapsilosis*, *C. pelliculosa*, *C. reukaufii*, *C. solani*, *C. tropicalis* (Fig. 1 - 1), *C. utilis* (Fig. 1 - m), and *Cryptococcus laurentii* (Fig. 1 - n).

It is not unlikely that there is a dependency of *N. sitophila* or *Neurospora* sp. on the waste of "tahoo", but further studies are required for such a conclusion.

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