CHAPTER 1

INTRODUCTION

Enzyme is used quite extensively in the industry and will be employed more and more as enzyme technology develops. They are produced by living cells of all types - human, plant and microorganism cells - under specific conditions. They consist mainly of protein. Their molecular weights vary from 10,000 to several millions and they function as catalysts promoting specific reactions. The material undergoing the reaction is called the substrate. Enzymes show a pronounced degree of specificity for the substrates on which they act and under a particular set of conditions, one enzyme catalyzes only one kind of reaction. This is one way in which enzyme differ from other types of catalyst. The enzyme which hydrolyze starch molecules to give such diverse products as dextrins and progressively smaller polymers composed of glucose units is known as amylases. Microbial amylases have been shown to be produced by mold such as Rhizopus, Aspergillus, by bacteria such as Bacillus, Pseudomonas and by yeast such as Endomyces, Saccharomyces and Endomycopsis (2, 34). The microbial amylases which are commonly used in the starch industry include α-amylase, and glucoamylase.

Amylases find use in the manufacture of grain alcohol, in brewing (in place of malt), in the production of starch syrups, maltose syrups and glucose, in baby food preparations, in clarification of fruit juice for jelly manufacture, in desizing of textiles, in baking industry and for pharmaceutical purpose such as digestive preparation. (2, 29, 34).

It was reported in 1974 in the Annual Statement of Foreign Trade of Thailand, Department of Customs that the imported malt and amylase hydrolyzed products, such as glucose syrup, dextrin and soluble starch, value at 178 million bahts. There is still no commercial production of amylase in our country. The commercial pure enzymes have never been used in starch conversion concerned industry, only the locally produced starter called "Look Pang" made by their own conventional method were used in manufacturing of grain alcohol, in baking field, in production of maltose syrup (Bae Sae), and vinegar.

"Look Pang" of known or unknown composition usually contains mixed culture of molds, bacteria and yeasts (27). These microorgainsms produce amylases which are responsible in starch conversion. In the starch conversion concerned industry, the reaction performed by these microorganisms from "Look Pang" is difficult to control. The conversion efficiency of fermentations are varied, due to sources of "Look Pang", kinds of active microorganisms, other contaminants and quantity of "Look Pang" used.

Previously, the author isolated several kinds of microorganisms responsible for amylolytic activity in samples of "Look Pang". They were : seven isolates of yeasts, one isolate of bacteria and fifteen isolates of molds. These microorganisms belong to three groups, Endomycopsis spp., Rhizopus spp., and Aspergillus niger group. It was found that Endomycopsis sp. and Aspergillus group gave highest yield of amylase. It should be noted that one single sample of "Look Pang" contained several kinds of microorganisms, and only 6 out of 10 samples of "Look Pang" gave high amylolytic activity (27).

Purpose of Study

A few investigators worked on enzymatic activity of "Look Pang" though it was known for hundreds of years. The aim of the study is for screening of amylolytic potent strain with reliable quality, and to give the idea of the industrial amylase production in Thailand by using these native potent isolates which have been adapted to local environment and substrates.

Scope of Work

In the present paper the study was divided into 3 parts:-

Part I. Samples of "Look Pang" were determined for amylase activity to get the best one which yield high amylolytic activity and to prove that "Look Pang" is not only the starter containing seed culture but also the source of enzyme.

Part II. The amylolytic producing capacity of single and mixed cultures, isolated from each "Look Pang" was comparatively studied to obtain the knowledge of future development of "Look Pang"

Part III. The identification of the most potent strain on amylolytic activity will be carried out. Some of its physical and chemical properties of amylase produced will also be studied.

Literature Review

The locally produced starter of Indonesian called "Ragi" (Hesseltine, 1965) and a commercial starter of Chinese yeast from Taiwan name "lévure chinoise" (Went and Geerligs, 1896) seem to be similar to Thai "Look Pang" (11, 31). Ragi is made of rice flour with spices and finally it contains yeast, bacteria and fungi. It is used to saccharify starch in the manufacture of Indonesian native fermented food such as arak, tapé ketan, brem and tapé ketella which raw materials are molasses, well-cooked rice, glutinous rice and cooked cassava respectively. Lévure chinoise contains pure mixed culture of two mucoraceous fungi and a yeast.

According to Went and Geerligs, ragi is prepared from rice flour to which sugar cane and rhizome of Alpinia galanga are added. These two materials are cut into small pieces and mixed with rice flour, dried in the sun, and then ground into

pulp with the addition of the Citrus limonellus juice. After 3 days the large plant parts are removed, the liquid is decanted, and round balls are made from the rest of the material. The old ragi is inoculated to the balls which laid between rice straw and dried in the sun. One of the yeast from ragi was Hansenula amonala. This strain existed in the Dutch Yeast Collection and in the [1]ARS Culture Collection as [2]NRRL Y-6703. A second yeast isolated from ragi was Saccharomyces cerevisiae. The fungi in ragi were Rhizopus oryzae and Chlamydomucor oryzae.

Stithnimarnkarn (1949) mentioned the composition and procedure in making Thai "Look Pang" which was used in the production of alcohol and whisky from rice grain (26). "Look Pang" was made of uncooked ground rice, and many kinds of spices, such as Allium sativum, Myristica fragrans, Piper chaba, Piper nigrum, Alpinia galanga and etc. All ground ingredients were mixed with water and round balls were made. The balls inoculated with the old "Look Pang", incubated for 72 hours at room temperature (28°-34°C) and sun dried.

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[1]ARS - Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois, U.S.A.

[2]NRRL - Northern Regional Research Laboratory, Illinois, U.S.A.

In the preceding paper (Sukhumavasi, 1973) many strains of amylase producing microorganisms were isolated from "Look Pang" of different sources. These microorganisms belong to three groups, Rhizopus sp., Endomycopsis sp., and Aspergillus niger group (27). Amylases were reported to be produced by bacteria, yeasts and molds including basidiomycetes, (23, 10, 19, 13). In 1964, Robyt and French studied the purification and action pattern of an amylase produced by Bacillus polymyxa and found that it was β-amylase resembled to that produced by higher plants (23).

Pazur and Ando (1959) purified the amyloglucosidase produced by Aspergillus niger and studied the reaction mechanism on oligosaccarides (19). It was shown that this amyloglucosidase hydrolyzed the oligosaccarides from the non-reducing end. In 1969, Lineback et al found that this Aspergillus niger strain produced two forms of glucoamylase which had the same physical and chemical properties, except electrophoretic mobility and isoelectric point (14).

Bauman and Lineback (1970) studied the properties of amylase produced by Aspergillus phoenicis and also found that it produced two forms of glucoamylase (4). Morita et al (1966) performed the experiment on amylase of Aspergillus oryzae and found that it produced 4 fractions of glucoamylase of the same physical and chemical properties except sedimentation rate (16).

The properties of the glucoamylase from Rhizopus delemar were studied by Pazur and Okada (1967)(20). They found that the action pattern of glucoamylase of Rhizopus delemar on starch and malto-oligosaccharides was the same to glucoamylase of Aspergillus niger including the same molecular weight except electrophoretic property. However, the glucoamylase from Rhizopus delemar had higher catalytic reversion reaction than glucoamylase from Aspergillus niger.

Coniophora cerebella of Basidiomycetes group (King, 1967) also produced glucoamylase as well as the yeast, Endomyces sp. IFO 0111 (Hattori, 1960) and Endomycopsis fibuligera IFO 0108 (Fukumoto et al, 1960).