DISCUSSION

The protein extraction from Candida yeast cells was low when followed the method of Gunsalus (1955), actually the total amount of soluble protein should be high. It seemed that using of Shechter's technique was better for protein preparation of the yeast cells. Adequate amount of acetone dried powder was obtained from each strains and enough for loading on disc gel electrophoresis. It was a convenient method to perform in a simple laboratory when microblender instrument was lacking. However, it was not the best method since the percentage of broken cell yielded was low when observed the cells under the microscope.

It was possible to separate a mixture of soluble protein in solution based on their different rates of migration in the electric field at a given pH (Davis, 1964). The migratory distance depended on charges and sizes of the protein molecule. The matching bands of protein between a pair of gel electrophoretic columns could be considered as similar kinds of protein in different isolates.

Gel electrophoresis permits high resolution analysis of extremely small samples of complex mixtures of protein. It is also used for detecting mutant forms of hemoglobin and other proteins. Gel electrophoresis was

accepted as an accurate method for studying the protein of interspecies relationship of many plants (Brewbaker, 1968) and fungal species such as Candida species by Shechter in 1972.

There were many reports showed the application of gel electrophoresis in the classification of many microorganisms such as some myxomycetes in the order Physarales (Franke and Berry; 1972), the group of dermatophytes (Shechter et. al., 1968), Candida species (Shechter et. al., 1972) and dematiaceous fungi (Beneke et. al., 1975).

Chang (1962) used disc electrophoresis technique to examine the component of soluble proteins which were presented in different species of Neurospora. By this method he found particular proteins and recommended that the synthesis and metabolic significance of proteins could be investigated by gel electrophoresis.

According to figure 12 the number of matching bands of soluble protein gel of interspecies between pair of C. albicans and C. utilis was fewer than the pair of C. albicans and C. krusei. Since the soluble proteins reflected the physiological difference of the cells rather than morphological one, so the result showed that C. krusei had six common bands when comparing with C. albicans which equal to the number

of matching bands that had been reported by Shechter in 1972 was closer to C. albicans the pathogenic species than C. utilis was.

The result on sugar fermentation (Table I) showed that C. albicans fermented glucose and maltose which produced acid and gas but none of C. albicans fermented lactose, the result were supported by fermentation chart (Rippon, 1974). The outstanding was the result of utilizing sucrose which showed variation among intraspecies of C. albicans. There were seven strains of sputum isolates and each strain from urine and unknown location No. 2 showed active fermentation of sucrose. These isolates of C. albicans seemed to isolate from the systemic infection areas. On the other hand, the superficial pathogen, the strains from vaginal group and various locations, showed only slightly activity on sucrose fermentation.

In accordance with the nature of isolates, it was reasonable to consider that the strains which gave active fermentation of sucrose would be more adaptive than the other ones. There were only two strains, one isolated from vagina and the other one from skin, which showed negative reaction in utilized sucrose. This might be suggested that these two strains were saprobes when they were collected.

The results of comparison the matching bands of soluble proteins by gel electrophoresis (Figure 13), twenty-three

isolates of C albicans showed some significance of intra-specific relationship among their pathogens.

Shechter (1972) had done disc electrophoresis of six strains of C. albicans and only two strains of them showed some variation. He concluded that at least six matching bands of protein between strains were enough to recognize as C. albicans species.

In figure 12, there were nine common bands among intraspecies of twenty-one strains and only two strains showed disconnected line at Rf 6.82 and 6.30, 9.05 which isolated from urine and unknown area No. 2

It seemed that urine's and unknown's isolates possessed six common bands which linked through C. albicans species. This might accept these six Rf values for the specific characteristic of C. albicans

The gel columns of twenty-three strains were rearranged in order to show the relationship of common bands and the variation of band among C. albicans isolates (Figure 13). The diagram showed some significance of these C. albicans isolates which could be separated into two groups. The first group was forteen strains which showed nine common bands and no variation of protein bands among intraspecies of these organisms, the lines were connected through the bands.

The second group was nine isolates, showed nine matching bands and some variations in between bands of protein.

Hasenclever (1964) arranged C. albicans into two groups which based on their antigenic properties, A and B. The group A antigen possessed antigenicity that was not present in the B group. However, the group A strain still contained same part of those antigenicity that associated with in the B group. The first report of Hasenclever and Mitchell (1961) was presented that group A strain possessed much more polysaccharide and protein antigens than the group B. This evidence was supported by the result in figure 13. The gel protein column of intraspecies of C. albicans were arranged into two groups. One group showed lower number of bands and constant in number of matching bands among the forteen isolates. The other group showed higher number of bands which consisted of the common bands and some variation of proteins.

This might be considered that the first group of C. albicans was group B and the second group was group A, which due to their number of matching bands and variations.

Hasenclever (1964) reported 68% of incident candidosis found in patients belonged to A group and 32% to B group, the report had been done in the United States.

In accordance with this experiment, the result was different from previous report, Hasenclever (1964). It might conclude that there was 55.56% from the vaginal strains (Figure 9), 71.4% from the sputum strains (Figure 10) and 57.1% from the various location strains (Figure 11) were considered to be the antigenic B group. This equal to 61.35% in total C. albicans group B and 38.65% C. albicans group A caused disease in Thai patients.

The distribution of the fungus C. albicans B group seemed to be dominant over A group in the tropical country. This might suggest that group B strains prefered tropical environment than A group.

According to the study of Hasenclever and Mitchell (1961), there was not enough information to indicate that one antigenic group was more pathogenic for human than the other. Their experiment had been done in animal inoculation and the result showed similarity of the pathogenicity between two groups of antigenic A and B.

This experiment supported Hasenclever (1961) that no significant relationship between the ability of fungal infection at specific lesions to the soluble proteins of organism. Both group A and B seemed to have similar ability to infect at a certain lesion of the body.

The study on isoenzymes of some oxidoreductase in the Candida species as a basis of species identification after electrophoresis had been shown by Bercher (1967). He reported some significance of isoenzyme pattern which could be used in the identification of Candida five species including C. albicans.

The amylase activity showed only a single band with low concentration of enzyme in most strains, which was opposite to the idea that there should be found some activity patterns of amylase, since the organism could grow on polysaccharide substrate (Nickerson, 1953).

There were many reports refered to some kinds of fungi that fungal amylase was an inducible enzyme when starch was a substrate. Since the Sabouraud medium has dextrose as a carbon source, therefore, low concentration of amylase was produced. It is interesting to study further on amylase activity of this fungus when various concentration of starch are used.