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**DIES ON THE PRODUCTION OF PHYTASE
BY
SOME SOIL FUNGI**

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of

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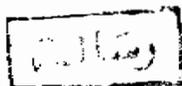


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This thesis has not been submitted
for any degree at this or any other uni-
versity. The references mentioned hereafter,
show how far I have availed myself to the
work of the others.

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PREFACE

Efforts to utilize microorganisms for the synthesis of enzymes dates back for several decades. Mycological production of phytases was of prime interest of some investigators in the last few decades.

In view of this trend, the present work was carried out aiming to obtain fungal species not recorded before as a phytase producers extra and/or intracellularly.

The work was commenced by screening 24 fungal species belonging to nine genera, previously isolated from Egyptian soils, to test their potentialities as phytase producers. Two organisms viz Aspergillus flavipes and Macrophomina phaseolina were recorded as a potential producers of extra and intracellular phytase production respectively, and selected for the present study.

The most important nutritional and environmental factors which govern phytase biosynthesis was investigated.

Examination of the phytase-productivity of the two employed organisms in presence of certain compounds selected on the basis of their possible effect on phytase production was ascertained. These compounds were: natural products and by-product, vitamins, amino acids, sea water and metal ions.

The work was extended to purify A. flavipes extracellular phytase and M. phaseolina intracellular enzyme by the following steps :

- 1) Dialysis of cell-free culture filtrate or cell-free extract.
- 2) Precipitation of the enzyme from the cell-free dialysate.
- 3) Gel filtration through sephadex G-75.
- 4) Column chromatography on DEAE-cellulose chromatography.

The general physiological properties of the two purified enzymes were also studied.

PART I

SECTION A : INTRODUCTION

SECTION B : MATERIALS AND
METHODS

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PART I

SECTION A : INTRODUCTION

INTRODUCTION

Phytate- hydrolyzing enzymes are commonly known as phytases. These enzymes have been found in wheat bran (Nagai and Funahashi, 1962), in various plant seeds (Mayer, 1956; Mayer et al., 1961 and Peers, 1953), in plant storage organs (Hsu et al., 1966), in tobacco leaves (Shaw, 1966) and have been obtained from different bacterial species (Greaves et al., 1967; Powar and Jagannathan, 1967 & 1982; Applebury et al., 1970; Cosgrove et al., 1970 and Irving and Cosgrove, 1971 a & b).

Production of phytases by fungi dates back for few decades ago. Different species of Aspergillus were the most studied organisms in this respect, and the potency of producing phytase is restricted to A. awamori (Tsukamoto , 1969), A. ficuum (Nelson et al., 1968 and 1971 ; Shieh and Ware 1968; Shieh et al., 1969 and Howson and Davis, 1983), A. oryzae (Nelson et al., 1968 and Wang et al., 1980) and A. terreus (Kobayashi et al., 1968; Yamada et al., 1968 a & b; Yamamoto et al., 1972 and 1973.)

Moreover, the phytase producing capacity of mycorrhizal fungi i.e. Rhizopogon luteolus, Boletus luteus, B. granulatus and Coenococcum graniform was recorded by Theodorou (1968) and (1971).

Many investigators reported that phytase production by fungi has been affected by the prevailing nutritional and environmental conditions.

For optimal phytase production by A. terreus, Yamada et al. (1968 a) reported that the initial pH of the medium should be adjusted to 5.5. Moreover, Shieh and Ware (1968) confirmed that the maximal synthesis of phytase has been obtained by A. ficuum at initial acidic pH value.

Phytase-producing organisms could utilize different carbon sources for the enzyme biosynthesis. Shieh and Ware (1968) found that corn starch and glucose are good sources of carbon for phytase production by A. ficuum. Moreover, Howson and Davis (1983) studied the production of phytate-hydrolyzing enzymes by some fungi and reported that glucose is a suitable source of carbon for phytase synthesis.

The effect of different nitrogen sources on phytase biosynthesis was studied by Kobayashi et al. (1968), Yamada et al. (1968 a) and Howson and Davis (1983). They found that inorganic NH_4^+ salts was the best favourable sources of nitrogen for the enzyme biosynthesis either extra or intracellularly.

With regard to phosphorous sources, Oota et al. (1968) successfully used phytin as a sole source of phosphorus

for phytase production. Moreover, Howson and Davis (1983) reported that phytate can be utilized by A. ficuum NRRL 3135, for the production of phytase, as the only source of phosphorus. They added that the inorganic phosphorus produced an inhibitory effect on the enzyme formation. Contrary to these results, are the findings of Shieh and Ware (1968) who found that low levels of inorganic phosphate support the phytase producing capacity of the same strain of A. ficuum.

Ratios of carbon to phosphorus (C/P ratio) appear to be the controlling factor in phytase biosynthesis. Shieh and Ware (1968) and Oota et al. (1968) showed that the content of phytase increased with increasing C/P ratio at low levels of phosphorus in the medium.

Employment of microorganisms for the synthesis of different products depends to great extent on the cost of the fermentation operation. Utilization of raw materials offered cheap media for the commercial applications. Certain authors reported that rice bran is a suitable medium for phytase production by fungi (Yamada et al., 1965, 1968 and Kobayashi et al., 1968). Shieh and Ware (1968) produced phytase by different soil microorganisms on medium, containing malt and yeast extracts. Moreover, Howson and Davis (1983) showed that different fungi utilize rapeseed

meal for extracellular phytase production. The promoting effect of natural products and by-product on the production of enzymes other than phytases has been previously recorded by different investigators (Elwan et al., 1978 a & b; Naguib et al., 1979; El Moussallamy, 1982 and Ghonemay, 1983).

Concerning the role of vitamins on phytase production, Yamamoto et al.(1973) recorded that A. terreus could produce phytase only when mycelium was in contact with inositol.

With regard to the effect of mineral nutrition on different enzymes biosynthesis, many workers confirmed the dependance of microbial enzyme production on the type and concentration of trace elements included in the production media (Taha et al., 1968; Chaudhary et al., 1969; Applebury et al., 1970; Onishi, 1972; Naguib et al., 1979; Gabr and Mansour, 1982).

Sea water was recorded as a most appropriate natural mineral supplement for the synthesis of certain microbial products (Jann et al., 1953 and Taha et al., 1968). Sea water is a buffered solution and contain probably all the chemical elements although only about 50 have so far been detected (Jann et al., 1953).

Purification of phytases from different sources was of prime interest of many investigators. Different methods were included in this respect but the combination-al precipitation procedures, gel filtration and ion-exchange chromatography are the subject of choice by most investigators.

Nagai and Funahashi (1962) purified the phytase from wheat bran more than 1500-fold by Ca-phosphate gel, $(\text{NH}_4)_2\text{SO}_4$, acetone, methanol, diethyl amino ethyl (DEAE)-cellulose and carboxymethyl cellulose treatments.

Powar and Jagannathan (1967) obtained from culture filtrates of Bacillus subtilis a purified enzyme with 50-fold by precipitation with alcohol and acetone and adsorption on Ca-phosphate gel.

Phytase from A. terreus was purified about 520-fold by Yamada et al. (1968 a) using $(\text{NH}_4)_2\text{SO}_4$ and acetone precipitation and chromatography through sephadex G-50 and sephadex G-200 columns.

Shieh et al. (1969) differentiated two types of phosphatases produced in the broth of A. ficuum by heat denaturation at acid and alkaline pH levels. They are separated into two distinct fractions on sephadex G-100 followed by carboxymethyl cellulose column chromatography.