# Characterization of Extra- and Intracellular Phytases from Bacteria Proliferating during Tempe Production.

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## ABSTRACT

*Bacteria species Streptococcus faecium and Streptococcus dysgalactiae, proliferating during tempe (fermented soybeans) production produced both extra- and intracellular phytases. The enzymes were isolated from growth media and the cultured bacteria and partially purified by acetone fractionation. Extracellular phytase activities were higher than that of the intracellular enzymes. The extra- and intracellular phytases of S. faecium and S. dysgalactiae acted over a nerrow pH range at 4.1 – 4.3 and the maximum activity at 45°, 45°, 45°, and 40°C, respectively. Among the estimated $K_m$ values, the value of 1.0 x $10^{-4}$ M for the intracellular phytase of S. dysgalactiae was the lowest, indicating strong affinity of the enzyme for the substrate. $V_{max}$ values were 0.005 and 0.007 mmole $P_i$ liberated/min/ml enzyme for the extra- and intracellular phytases of S. faecium, while the extra- and intracellular phytases of S. dysgalactiae had $V_{max}$ values of 0.003 and 0.003 mmole $P_i$ liberated/min/ml enzyme, respectively. The activation energy for hydrolysis of phytic acid by extra- and intracellular phytases of S. faecium and S. dysgalactiae were calculated by the Arrhenius equation to be 3,400; 3,600; 14,600 and 7,100 cal/mole, respectively.*

*Key words: characterization, Streptococcus sp., phytase, tempe.*

## INTRODUCTION

Tempe is a traditional Indonesian fermented food made from soaked and cooked soybeans inoculated with a mould, usually of the genus *Rhizopus*. Soaking of dry soybeans usually forms an integral part of processing method for tempe production. Eventually, microorganism such as bacteria proliferating during soaking of soybeans has been expected to produce either extra- or intracellular phytases that are able to reduce phytic acid content of beans as suggested by Jaffe (1981).

Phytase, or myo-inositol hexaphosphate phosphohydrolase (EC 3.1.1.8 or 3.1.3.26) (IUB, 1979), which can hydrolyze phytic acid into myo-inositol and orthophosphate ($P_i$), has been isolated from several different microorganisms including bacteria (Greaves *et al.*, 1967), yeast (Nayini and Markakis, 1984) and moulds (Wang *et al.*, 1980; Sutardi and Buckle, 1988), and also some legume seeds (Lolas and Markakis, 1977; Sutardi and Buckle, 1986).

The phytase enzyme of bacteria the most common microorganisms contaminating during tempe production were isolated, purified and characterized as part of a study to establish whether phytate reduction during tempe production is the result of physical treatments such as soaking, boiling and steaming or due to hydrolysis by phytases produced by bacteria contaminating during tempe production e.i. during soaking of raw soybeans for overnight.

## MATERIALS AND METHODS

### Organisms

Bacteria proliferating during the soaking of soybeans were isolated and identified as *Streptococcus faecium* and *Streptococcus dysgalactiae*. Pure cultures of the bacteria were transferred to deMan Rogosa Sharpe (MRS) agar slants, incubated for 18 h at 30°C and maintained for further experimens.

### Cultivation of organisms

A loop of pure bacterial culture was inoculated into 10 ml MRS broth and incubated for 24 h at 30°C, then transferred into 10 ml of MRS broth, 0.5 ml antifoaming agent was added to a 300 ml Erlenmeyer flask, and incubated in an orbital shaker incubator for 24 h at 30°C and 200 rpm. The culture was transferred into 500 ml MRS broth, 2.5 ml antifoaming agent added in a 2 l Erlenmeyer flask, and incubated in an orbital shaker incubator for 24 h at 30°C and 200 rpm. Cells at each step were enumerated by a spread plate method using MRS agar. Cultivations were carried out in triplicate on at least two different culture preparations.

### Harvesting of organism

Pure culture of bacteria were harvested by centrifugation for 30 min at 4°C and 15,000 x g. Supernatants were collected and used as the source of extracellular phytase, while precipitates were used as the source of intracellular phytase.

### Crude enzyme preparation

Extracellular enzyme supernatants obtained from centrifugation of growth media were concentrated by freeze drying (- 35°C, 3.75 kPa), thawed, and dialysed for 48 h against 0.01 M tris-maleat buffer, pH 6.5, at 4°C, then subjected to acetone fractionation.

Intracellular phytase was prepared from the precipitate obtained from centrifugation. It was washed with double distilled water and then centrifuged for 15 min at 4°C and 15,000 x g. Precipitate was suspended with 20 ml 2% $CaCl_2$ per 5 g cells (wet weight) in 50 ml glass tube. The cells were disrupted by sonification for a total time 5 min at output control 4. During sonification the tube containing the sample was maintained cool by soaking in an ice bath. The suspension was centrifuged for 30 min at 4°C and 15,000 x g. Precipitate was again washed with 20 ml 2% $CaCl_2$ and disruption was repeated as above. The suspension was centrifuged for 30 min at 4°C and 15,000 x g. The supernatants were collected and subjected to acetone fractionation. The proportion (%) of cell breakage was determined following the procedure developed by Sutardi and Buckle (1988).

### Acetone fractionation

Acetone fractionation was carried out by slow addition of pre-cooled acetone in an ethano -solid $CO_2$ bath to pre-cooled enzyme preparation to about 60% (v/v) acetone at – 10°C with continuous stirring. The precipitate containing the phytases was dissolved in 0.01 M acetate buffer (pH 4.5), dialysed for 48 h against distilled water, then centrifuged at 4°C for 30 min and 15.000 x g, then freeze-dried (-35°C, 3.75 kPa) to reduce the volume.

### Enzyme assay

.Extracellular bacterial phytases were assayed at 45° ± 1°C for *S. faecium* and *S. dysgalactiae*, while intracellular bacterial phytases were assayed at 45° ± 1 °C and 40° ± 1°C for *S. faecium* and *S. dysgalactiae*, respectively, by measuring the rate of increase of $P_i$ using the ascorbic acid method (Watanabe and Olsen, 1965). The reaction mixture were prepared and assayed with the procedure developed by Sutardi and Buckle (1988) except the buffer solutions were adjusted to pH 4.3 and 4.2 for extracellular phytases of *S. faecium* and *S. dysgalactiae*, respectively, and to pH 4.3 and 4.1 for intracellular phytases of *S. faecium* and *S. dysgalactiae*, respectively. Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

**Effect of pH**

The effect of pH on phytase activity was determined in reaction mixture as described by Sutardi and Buckle (1988). The mixtures were adjusted to pH 3.8 – 4.6 at 0.1 pH intervals for extracellular phytases of *S. faecium* and *S. dysgalactiae*, respectively, and to pH 3.9 – 4.7 and 3.8 – 4.5 at 0.1 intervals for intracellular phytases from *S. faecium* and *S. dysgalactiae*, respectively.

**Effect of temperature**

Phytase assay mixtures were incubated for 30 min over the temperature range 30° – 60°C at 5 degree C intervals.

**Effect of substrate concentration**

Optimum substrate concentration for phytase activities were determined following the procedures developed by Sutardi and Buckle (1988). $K_m$ and $V_{max}$ values were estimated by the method of Lineweaver and Burk (1934).

**Rate of denaturation**

Reaction mixtures before addition of sodium phytate were heated at both 45° and 55°C for the extra- and intracellular phytases of *S. faecium* and for extracellular phytase of *S. dysgalactiae*, and at 40° and 50°C for intracellular phytases of *S. dysgalactiae*, for 0 – 60 min at intervals of 10 min, cooled, sodium phytate added to a final concentration of 1.0 mM and phytase activities were assayed by following the procedure developed by Sutardi and Buckle (1988). The energy of inactivation and temperature coefficient were estimated from the Arrhenius equation.

**Thermal inactivation of phytase**

Enzymes in 0.1 M acetate buffer, pHs as specified were heated in a water bath for 10 min over the temperature 30° – 100°C, cooled, sodium phytate added to a final concentration of 1.0 mM and phytase activities were assayed after 30 min incubation at temperatures as specified.

**Effect of incubation time and temperature**

Phytase activities were assayed during incubation of reaction mixtures at 30°, 40°, 45°, 50° and 60°C for extra- and intracellular phytases of *S. faecium* and extracellular phytase of *S. dysgalactiae*, and at 30°, 35°, 40°, 45°, 50° and 55°C for intracellular phytase of *S. dysgalactiae* for times from 30 to 180 min at 30 min intervals. The energy of activation for phytase was estimated from the Arrhenius equation.

## RESULTS AND DISCUSSION

**Production of phytases**

*S. faecium* and *S. dysgalactiae* grown in MRS broth produced both extra- and intracellular phytases (Table 1). After acetone fractionation, *S. faecium* produced extra- and intracellular phytases with a higher activity than those of *S. dysgalactiae*. The extracellular phytases of *S. faecium* and *S. dysgalactiae* had a higher total activity than did the intracellular phytases.

**Isolation and purification of phytase**

Both extra- and intracellular phytases were isolated from growth media and bacterial cells, respectively, and partially purified. Yield and specific activities of the enzymes at various stages of purification are presented in Table 1. At each stage of purification, total activities of the extracellular phytases from *S. faecium* and *S. dysgalactiae* were higher than those of the intracellular phytases. Specific activities of the intracellular phytases were higher than those of the extracellular phytases except for the intracellular phytases of *S. faecium* after acetone fractionation, dialysis and freeze drying. As reported by Wang *et al.* (1980), some strains of *Aspergillus oryzae* grown in rice media produced extracellular phytases with a higher activity than the intracellular phytases.

The extra- and intracellular phytases from *S. faecium* were purified 4.2 and 2.0 fold with recoveries of 2.8 and 52.9%, respectively, while the extra- and intracellular phytases from *S. dysgalatiae* were purified 8.3

Table 1. Yield and specific activities during purification of extra-and intracellular phytases from *S. faecium* and *S. dysgalactiae*.

| Purification Stage | Species of bacteria | Extracellular phytase | | | | | | Intracellular phytase | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total Volume (ml) | Total Protein (mg) | Total Activity (unit)* | Specific Activity (unit/mg | Recovery (%) | Purification (fold) | Total Volume (ml) | Total Protein (mg) | Total Activity (unit)* | Specific Activity (unit/mg) | Recovery (%) | Purification (fold) |
| *Cullture Filtrate* | *S. faecium* | 2935 | 8307 | 2.01 | 0.0003 | 100 | - | 105 | 172 | 0.10 | 0.0006 | 100 | - |
| | *S. dysgalactiae* | 2927 | 8147 | 0.98 | 0.0001 | 100 | - | 108 | 93 | 0.07 | 0.0008 | 100 | - |
| *Freeze drying and dialysis* | *S. faecium* | 673 | 1842 | 0.70 | 0.0004 | 35 | 1.3 | 100 | 102 | 0.06 | 0.0006 | 60 | 1.1 |
| | *S. dysgalactiae* | 660 | 1603 | 0.26 | 0.0002 | 27 | 2.0 | 103 | 52 | 0.06 | 0.0012 | 86 | 1.4 |
| *Aceton Fractionation, Dialysis and Freeze drying* | *S. faecium* | 77 | 56 | 0.06 | 0.0011 | 3.0 | 3.7 | 40 | 46 | 0.05 | 0.0011 | 50 | 1.8 |
| | *S. dysgalactiae* | 83 | 48 | 0.05 | 0.0010 | 5.0 | 10.0 | 43 | 13 | 0.03 | 0.0023 | 30 | 3.9 |

and 10.8 fold with recoveries of 4.9 and 39.4%, respectively. The recovery of the extracellular phytases was significantly lower than that of the intracellular phytases, possibly due to the higher loss of extracellular enzyme associated with high levels of impurities present in growth media during enzyme preparation. On the other hand, the intracellular phytases are more easily separated by centrifugation from cell debris with negligible loss of enzyme, and presumable the intracellular supernatants contain less impurities than those the growth media.

As shown in Table 1, the specific activity of the both extra- and intracellular phytases from *S. faecium* and *S. dysgalactiae* increased significantly during purification. In the current study, acetone purified extra- and intracellular bacterial phytases gave satisfactory result for all assays.

**Effect of pH**

The extra- and intracellular phytases of *S. faecium* and *S. dysgalactiae* acted over a narrow pH range. Figure 1 shows optimum pH at 4.3 and 4.2 for extracellular phytases of *S. faecium* and *S. dysgalactiae*, respectively; while the intracellular phytases had pH optima at 4.3 and 4.1, respectively. These pH optima characterize the enzyme as acid phosphohydrolases as reported for bacterial phytase (Irving and Cosgrove, 1971). Both extra- and intracellular phytase activities decrease sharply before and after the pH optima. More than 75% loss of activity was recorded when the pH fell below 3.9 or rose above 4.5. The optimum between 4.1 – 4.3 is within the range (pH 2.2 – 5.6) reported for microbial phytases (Wang *et al.*, 1980; Nayini and Markakis, 1984).

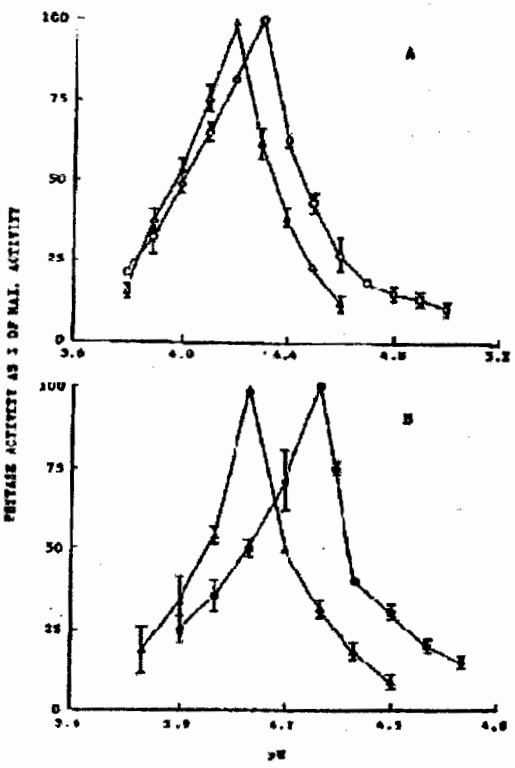


Figure 1. pH-activity profile of extra- (A) and intracellular (B) phytases from *S. faecium* (o,·) and *S. dysgalactiae* (D,s). Vertical bars indicate range of duplicate determinations on each of 2 enzyme preparation.

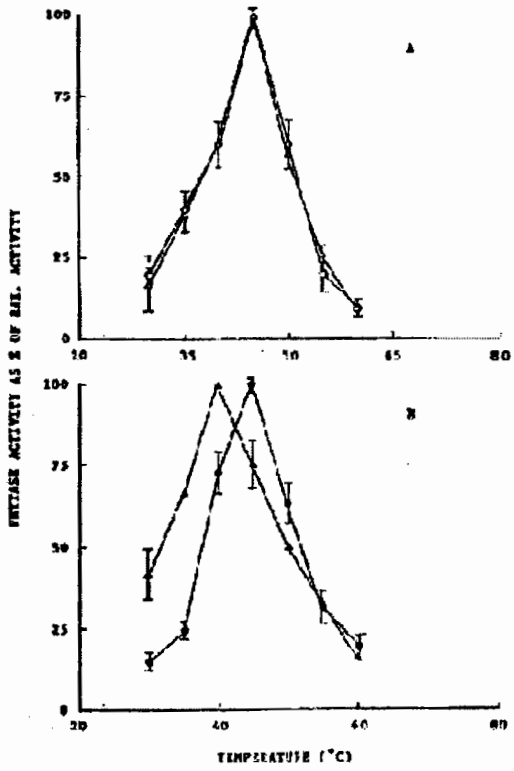


Figure 2. Temperature-activity profile of extra- (A) and intracellular (B) phytases from *S. faecium* (o,•) and *S. dysgalactiae* (Δ,s). Vertical bars indicate range of duplicate determinations on each of 2 enzyme preparation.

**Effect of temperatures**

The temperature-activity profile of the extra- and intracellular phytases from *S. faecium* and *S. dysgalactiae* (Figure 2) shows that there was phytase activity over the range 30° to 60°C, with optima at 45°, 45°, 45° and 40°C, respectively. These temperature optima were relatively low compared with the optimum temperatures of other microbial phytases that ranged between 45° – 70°C (Sutardi, 1988) and the bacterial phytases in the present study showed optimum temperatures in the range 45° – 70°C, except for intracellular phytase of *S. dysgalactiae*.

More than 75% loss of activities were observed when extracellular phytases of *S. faecium* and *S. dysgalactiae* were incubated at 30° or above 55°C. The same loss of activity was recorded when the intracellular phytase of *S. faecium* was incubated at temperature below 35° or at 60°C, or at 60°C for the intracellular phytase of *S. dysgalactiae*. More than 50% loss of activity was observed for the intracellular phytase of *S. dysgalactiae* at 30°C. The relatively low optimum temperature for these bacterial phytases are advantageous in hydrolyzing phytic acid during soaking of soybeans since soaking is carried out at ambient temperature, providing that the phytases either are able to gain access to the phytate in the beans by diffusing into the beans, or act on phytate extracted into the soaking solution.

### Effect of substrate concentration

The rate of enzyme activity increased up to a substrate level of 2.0 mM, although activity increased less rapidly above 1.0 mM (Figure 3). A typical Michaelis-Menten curve was obtained. The $K_m$ and $V_{max}$ values (Figure 4) of 1.2 x $10^{-4}$ M and 0.005 µmole $P_i$ liberated/min/ml enzyme, and 2.2 x $10^{-4}$M and 0.007 µmole $P_i$ liberated/min/ml enzyme, respectively were determined for the extra- and intracellular phytases from *S. faecium*, respectively, while the extra- and intracellular phytases of *S. dysgalactiae* had $K_m$ and $V_{max}$ values of 1.0 x $10^{-4}$ and 0.5 x $10^{-4}$ M, and 0.003 and 00.3 µmole $P_i$ liberated/min/ml enzyme, respectively. Among the estimated $K_m$ values, the value of 0.5 x $10^{-4}$ M for the intracellular phytase of *S. dysgalactiae* was the lowest, indicating strong affinity of the enzyme for the substrate. The $K_m$ values for microbial phytases has been reported to range between 0.013 mM and 0.47 mM (Irving and Cosgrove, 1974; Wang *et al.*, 1980).

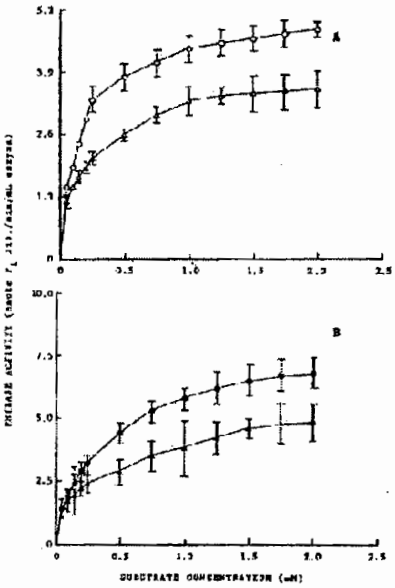


Figure 3. Effect of substrate concentration of extra- (A) and intracellular (B) phytases from *S. faecium* (o,•) and *S. dysgalactiae* (Δ,▪). Vertical bars indicate range of duplicate determinations on each of 2 enzyme preparation.

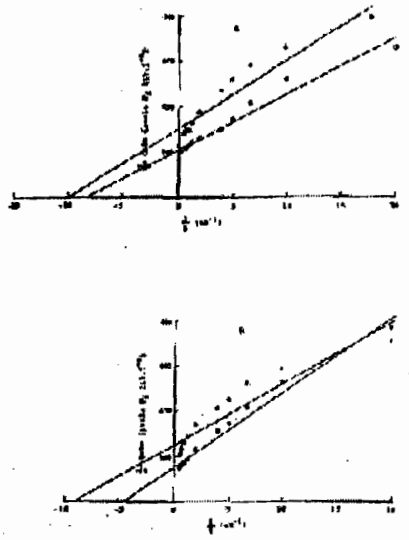


Figure 4. Lineweaver-Burk plot acyivity of extra- (A) and intracellular (B) phytases from *S. faecium* (o,•) and *S. dysgalactiae* (Δ,▪). Data obtained from Figure 3 are plotted as reciprocal of the initial rate (1/v, min. mole$^{-1}$) versus reciprocal of the substrate concentration (1/S, mM$^{-1}$).

### Rate of denaturation

Figure 5 shows the rate of denaturation of extra- and intracellular phyatses from *S. faecium* and *S. dysgalactiae*. Prolonged heating up to 60 min at various temperature (Sutardi, 1988) caused denaturation of enzyme and thus the activities remaining after heating decreased significantly.

The activation-time relationships for extra- and intracellular phytase activities remaining after heating at various temperatures deviated significantly from linearity. Inactivation energies of 13,700 and 23,800 cal/mole were determined for extracellular phytases of *S. faecium* and *S. dysgalactiae* with $Q_{10}$ values of 2 and 3, respectively (Figure 5), while the intracellular phytases showed inactivation energies of 24,200 and 12,000 cal/mole with $Q_{10}$ values of 3 and 2, respectively. The relatively low $Q_{10}$ values indicate that heating of the enzyme at 10 deg. C above the optimum temperature caused a marginal decreased in activity compared to that at the optimum temperature. Although no data have

been published on the energy of inactivation and temperature coefficient for bacterial phytases, Sutardi and Buckle (1988) reported that an energy of inactivation were 28.300 and 33.200 cal/mole for the extra- and intracellular phytase of *R. oligosporus*, and the temperature coefficient of inactivation ($Q_{10}$) over the interval 55° – 65°C were about 4 and 5 for extra- and intracellular phytase of *R. oligosporus*, respectively.

**Thermal inactivation of phytase**

Heating enzyme preparations for 10 min at temperatures above 30°C significantly depressed extra- and intracellular phytase activities of *S. faecium* and *S. dysgalactiae*, and at 80°C the activities were reduced by about 75%, except for the extracellular phytase of *S. dysgalactiae* where more than 90% loss of activity was observed; at 90°C the activity was practically destroyed (Figure 6).

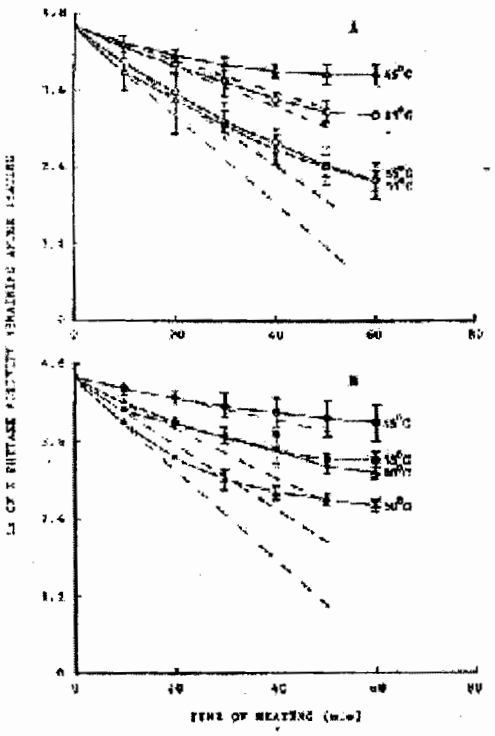


Figure 5. Rate of denaturation of extra- (A) and intracellular (B) phytases from *S. faecium* (o,•) and *S. dysgalactiae* (Δ,▲). Vertical bars indicate range of duplicate determinations on each of 2 enzyme preparation.

**Effect of incubation time and temperature**

Table 2 and 3 show phytate hydrolysis by extra- and intracellular phytases of *S. faecium* and *S. dysgalactiae* as a function of time at different temperature. Liberation of $P_i$ from phytic acid increased with increase in temperature up to 45°C for 180 min incubation for both extra- and intracellular phytases of *S. dysgalactiae*, respetively. Activity then decreased significantly on further rise of temperature. At 60°C there was about 25% of the activity of that at 45°C after 180 min incubation for the intracellular phytase of *S. faecium* but, 20% loss of activity was observed for the extracellular phytase of *S. faecium*.

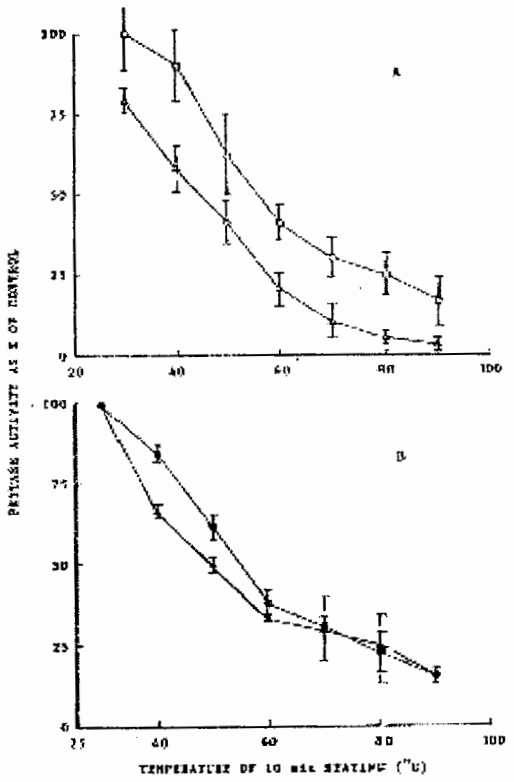


Figure 6. Thermal inactivation of extra- (A) and intracellular (B) phytases from *S. faecium* (o,•) and *S. dysgalactiae* (Δ,▲). Vertical bars indicate range of duplicate determinations on each of 2 enzyme preparation.

Table 2. Effects of incubation time and temperature on the liberation of $P_i$ from phytate by extra- and intracellula bacterial phytase from *S. faecium*.

| Tempe rature | Extracellular phytase activity at incubation time (min)* | | | | | | Intracellular phytase activity at incubation time (min)* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (°C) | 30 | 60 | 90 | 120 | 150 | 180 | 30 | 60 | 90 | 120 | 150 | 180 |
| 30 | 43 ± 1.8 | 47 ± 2.4 | 50 ± 0.0 | 52 ± 0.4 | 54 ± 1.7 | 56 ± 0.0 | 26 ± 0.0 | 33 ± 0.4 | 40 ± 0.2 | 40 ± 0.0 | 40 ± 0.1 | 40±0.6 |
| 40 | 50 ± 0.2 | 54 ± 2.7 | 56 ± 2.3 | 58 ± 0.6 | 59 ± 0.3 | 60 ± 0.4 | 43 ± 0.1 | 50 ± 0.1 | 53 ± 0.3 | 57 ± 0.2 | 60 ± 0.1 | 60 ± 0.4 |
| 45 | 56 ± 1.0 | 60 ± 0.4 | 63 ± 0.2 | 63 ± 0.4 | 63 ± 0.3 | 63 ± 0.3 | 84 ± 1.4 | 95 ± 0.8 | 100 ± 0.0 | 100 ± 0.4 | 100 ± 0.3 | 100 ± 0.0 |
| 50 | 40 ± 0.3 | 45 ± 0.4 | 48 ± 1.2 | 50 ± 1.1 | 52 ± 0.7 | 53 ± 0.3 | 21 ± 0.5 | 25 ± 0.2 | 26 ± 0.0 | 27 ± 0.7 | 28 ± 0.2 | 28 ± 0.4 |
| 60 | 36 ± 0.0 | 39 ± 0.3 | 42 ± 0.8 | 45 ± 2.6 | 48 ± 1.7 | 50 ± 1.3 | 0 | 0 | 0 | 18 ± 0.1 | 25 ± 0.3 | 26 ± 0.5 |

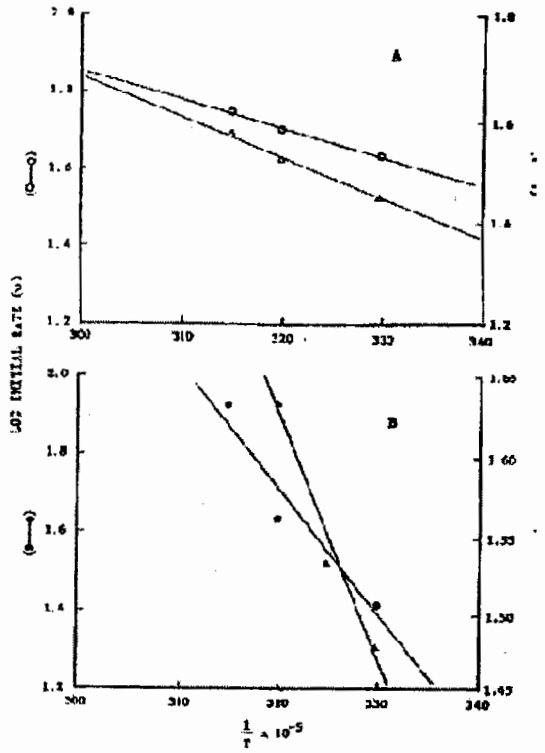
* Activity defined as nmole $P_i$ liberated per ml acetone purified enzyme.
\# Mean ± s.d. of duplicate determinations on each of two enzyme preparartions.

Table 3. Effects of incubation time and temperature on the liberation of $P_i$ from phytate by extra- and intracellul bacterial phytase from *S. dysgalactiae*.

| Tempe rature | Extracellular phytase activity at incubation time (min)* | | | | | | Intracellular phytase activity at incubation time (min)* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (°C) | 30 | 60 | 90 | 120 | 150 | 180 | 30 | 60 | 90 | 120 | 150 | 180 |
| 30 | 28 ± 2.4 | 36 ± 0.0 | 39 ± 0.6 | 41 ± 0.7 | 42 ± 0.7 | 43 ± 0.0 | 30 ± 0.2 | 36 ± 0.0 | 40 ± 0.5 | 40 ± 0.4 | 40 ± 0.0 | 40±0.5# |
| 40 | - | - | - | - | - | - | 34 ± 0.8 | 40 ± 0.3 | 42 ± 1.1 | 44 ± 0.9 | 45 ± 0.6 | 46 ± 0.3 |
| 45 | 33 ± 2.1 | 40 ± 0.4 | 43 ± 1.7 | 44 ± 0.3 | 45 ± 0.5 | 45 ± 0.6 | 43 ± 0.0 | 50 ± 0.1 | 54 ± 0.4 | 57 ± 0.4 | 60 ± 0.1 | 60 ± 0.0 |
| 50 | 28 ± 0.0 | 32 ± 1.3 | 33 ± 0.0 | 34 ± 0.4 | 35 ± 0.2 | 35 ± 1.2 | 26 ± 1.4 | 31 ± 0.4 | 33 ± 0.7 | 34 ± 1.5 | 35 ± 0.3 | 35 ± 0.2 |
| 60 | 25 ± 1.9 | 29 ± 0.0 | 30 ± 0.0 | 31 ± 0.0 | 31 ± 1.9 | 31 ± 1.2 | - | - | - | - | - | - |

* Activity defined as nmole $P_i$ liberated per ml acetone purified enzyme.
\# Mean ± s.d. of duplicate determinations on each of two enzyme preparartions.

Extra- and intracellular phytases of *S. dysgalactiae* showed 50 and 60% loss of activity at 60° and 50°C, respectively, for 180 min incubation. The highest activities were observed at 45°C for the extra- and intracellular phytases of *S. faecium*, and at 45° and 40°C for the extra- and intracellular phytases of *S. dysgalactiae*, respectively, which are consistent with results shown in Figure 2. From the data in Table 2 and 3 (30 min incubation), the activation energy for hydrolysis of phytic acid by extra- and intracellular phytases of *S. faecium* and *S. dysgalactiae* were calculated by Arrhenius equation to be 3,400, 3,600; 14,600 and 7, cal/mole, respectively (Figure 7), as compared to 6, and 9,500 cal/mole for extra- and intracellular phyta from *R. oligosporus* (Sutardi and Buckle, 1988).



ure 7. Arrhenius plot for estimation of activation energy from hydrolysis of phytic acid by extra- (A) and intracellular (B) phytases from *S. faecium* (o,·) and *S. dysgalactiae* (D,s). Initial rate (v) estimated from data of Tables 2 and 3, T = absolute temperature.

## CONCLUSSION

S. *faecium* and *S. dysgalactiae* grown in MRS broth, ated from soaked soybeans and partially purified acetone fractionation produced both extra- and acellular phytases. The extra- and intracellular tases from *S. faecium* were purified 4.2 and 2.0 fold ı recoveries of 2.8 and 52.9%, respectively, while extra- and intracellular phytases from *S. dysgalatiae* e purified 8.3 and 10.8 fold with recoveries of 4.9 39.4%, respectively.

Both extra- and intracellular phytases of *S. faecium* *S. dysgalactiae* had optimum pH between 4.1 – 4.3, le optimum temperatures at 40° – 45°C.

$K_m$ of extra- and intracellular phytases from *S. faecium* and *S. dysgalactiae* were between 1.2 - 2.2 x $10^{-4}$ M and 1.0 – 0.5 x $10^{-4}$ M, respectively; while $V_{max}$ were between 0.005 – 0.007 µmole $P_i$ liberated/min/ml and 0.003 µmole $P_i$ liberated/min/ml.

The activation energy for hydrolysis of phytic acid by extra- and intracellular phytases of *S. faecium* and *S. dysgalactiae* were calculated by the Arrhenius equation to be 3,400; 3,600; 14,600; and 7,100 cal/mole, respectively.

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## REFERENCES

Greaves, M.P., Anderson, G. and Webly, D.M. 1967. The hydrolysis of inositol phosphates by *Aerobacter aerogenes*. Biochem. Biophys. Acta. 132: 412 – 418.

Irving, G.C.J. and Cosgrove, D.J. 1971. Inositol phosphatases of microbiological origin. Some properties of a partially purified bacterial (*Pseudomonas sp.*) phytase. Aust. J. Biol. Sci. 24: 547-557.

Irving, G.C.J. and Cosgrove, D.J. 1974. Inositol phosphate phosphatases of microbial origin. Some properties of the partially purified phosphatases of *Aspergillus ficum* NRRL 3135. Aust. J. Biol. Sci. 27: 361 – 368.

IUB. 1979. Enzyme nomenclature. Recommendations (1978) of the nomenclature committee of the International Union of Biochemistry. Academic Press, New York. pp. 243, 247.

Jaffe, G. 1981. Phytic acid in soybeans. J. Am. Oil Chem. Soc. 58: 493 – 495.

Lineweaver, H. and Burk, D. 1934. The determination of enzyme dissociation constant. J. Am. Oil Chem. Soc. 56: 658 – 666.

Lolas, G.M. and Markakis, P. 1977. The phytase of navy beans (*Phaseolus vulgaris*). J. Food Sci. 42:1094 – 1106.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randel, J.R. 1951. Protein estimation with folin phenol reagent. J. Biol. Chem. 193: 265 – 275.

Nayini, N. and Markakis, P. 1984. The phytase of yeast. Lebensm Wiss-u-Technol. 17: 24 – 26.

Sutardi. 1988. Phytase activity during tempe production. PhD. Dissertation, University of New South Wales. Australia.

Sutardi and Buckle, K.A. 1986. The characteristics of soybean phytase. J. Food Biochem. 10: 197 – 216.

Sutardi and Buckle, K.A. 1988. Characterization of extra- and intracellular phytases from *Rhizopus oligosporus* used in tempeh production.

Wang, H.L., Swain, E.W. and Hesseltine, C.W. 1980. Phytase of moulds used in oriental food fermentation. J. Food Sci. 45: 1262 – 1266.

Watanabe, E.S. and Olsen, S.R. 1965. Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soil. Soil Sci. Soc. Am. Proc. 29: 677 – 678.