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Studies on the Biosynthesis of L-Serine-Glutamate Transaminase in *Alternaria chlamydospora*

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Abstract: Cell free extracts of the filamentous fungus *Alternaria chlamydospora* grown on L- serine as sole source of nitrogen contained L-serine-glutamate transaminase (EC 2.6.1.52) that catalyzes the transamination between L-serine and α -ketoglutarate to equimolar amounts of pyruvate and glutamate. L- serine-glutamate transaminase was produced during the logarithmic phase of growth. Maximal growth and enzyme formation were obtained after 3 days of growth. The optimum pH for growth and enzyme synthesis was 5.0. L-serine-glutamate transaminase was induced by L-serine, L-alanine,L-threonine and L-glutamic acid. Sucrose was the best carbon source induced synthesis of enzyme. Enzyme activity was repressed by all kind of metal salts. The effect of different vitamins on growth and enzyme production was studied .

Introduction

The occurrence of variety transamination reactions between L-serine and keto acids has been demonstrated in few microorganisms. The three major transamination reaction involvoing L- serine are:

1-L-serine-glutamate transaminase(L-serine- α-ketoglutarate transaminase; EC2.6.1.52)

Which catalyzes the reversible reaction:

L-serine + α-ketoglutarate ↔ 3-hydroxypyruvate+L- glutamate

2-L-serine-glycine transaminase(L-serine-glyoxylate transaminase; EC2.6.1.45)

It catalyzes the following the reversible reaction:

L-serine+ glyoxylate↔ 3-hydroxypyruvate+ glycine

3- L-serine-alanine transaminase(L-serine- pyruvate transaminase; EC2.6.1.51)

Which catalyzes the reversible reaction:

L-serine+pyruvate ↔ 3-hydroxypyruvate+ alanine

The activity of these enzymes was reported to be present in bacteria and fungi. Thus , Large and Qualye (1963)demonstrated that extracts of Pseudomonas AMI catalyzed a transamination between L-serine and α-ketoglutarate or pyruvate. Ragab *et al.*(1991) also reported that cell free extracts of *Cunninghamella elegans* contained three L-serine activities namely L-serine-glutamate, L-serine-glycine and L-serine-alanine transaminase, while those of *Fusarium oxysporum* contained L-serine-glutamate and L-serine-glycine transaminase activities.

Sirirote *et al.*(1986),Yoshida *et al.* (1993), Hagishita *et al.*(1996 a&b)and Pukall *et al.*(2003) show the existence of L-serine –glyoxylate transaminase in a methylotrophic bacterium namly *Protomonas extorquens* NR-1, *Hyphomicrobium methylovorum* (IFO 14180), *Hyphomicrobium methyllovorum* CM2 and *Paracoccus seriniphilus* respectively.

Concering to the induction and repression of L-serine transaminase. Blatt *et al.* (1966)showed the formation of L-serine in extacts of *Escherichia coli* from hydroxy pyruvate by transamination with either L-glutamate, L-alanine or L-aspartate, but L-glutamate was the most active amino group donor.

The present work aims to investigate the ability of extracts of *Alternaria chlamydospora* to catalyze the transamination reactions of L-serine and deals with the biosynthesis of L- serine-glutamate transaminase

in *Alternaria chlamydospora* under different physiologyical conditions.

Material and Methods

Organism

Alternaria chlamydospora was isolated from Al-Qassim region and identified by International Mycological Institute, England, United Kingdom.

Media and culture

The organism was grown on sucrose - Czapek - Dox liquid medium with L-serine replacing $NaNO_3$ on nitrogen equivalent basis. Five ml aliquots of spore suspension of *Alternaria chlamydospora* was used to inoculate 250 ml Erlenmeyer flasks, each containing 50 ml sterile medium. The inoculated flasks were incubated at 28 °C for 3 days, then the mycelia were harvested by filtration, washed thoroughly with distilled water, and finally blotted dry with absorbent paper. The pH of the medium was adjusted to 5.0.

Preparation of cell-free extract

The harvested mycelia were ground with cold sand in a cold mortar and extracted with cold distilled water. The obtained slurry was then centrifuged at 12.000 g for 10 min and the supernatant was used as the crude enzyme preparation.

Chemical analysis methods

The amounts of L-serine or glutamate were determined by quantitative paper chromatography, using Whatman No.1 filter paper and water-saturated phenol as a solvent system (Kay *et al.*,1956). The chromatogram was continuously sprayed with the solvent for 16 hr, then left to dry at room temperature for 24 hr. Drying was completed in an oven at 65 °C. The dried chromatogram was then sprayed with 0.2 % ninhydrin in acetone and heated at 65 °C for 20 min. The colored spots of the two amino acids were located (in comparsion with standard samples), cut from the chromatogam and each was placed in tube. To each tube 5 ml of 71 % ethanol was added and the tubes were shaken for 5 min. The resulting colored solution was measured spectrophotometrically at 575 nm. Ablank was prepeard by cutting from the chromatogram a white of paper of the same size at the colored spots, then treating this piece as described before. The concentration of glutamate were determined in reaction mixture using standared curve for it, which was prepeared at the same method. Protein was determined according to the method of Lowry *et al.* (1951).

Identification of products

L-Serine and glutamate were identifed by paper chromatography using Whatman No.1 filter paper and two solvent systems. Solvent I consisted of n-butanol-aceton-acetic acid-water (70: 70: 20: 40) (Erasser and Smith,1976) and solvent II was water-saturated phenol (Kay *et al.* ,1956). The R_f values of identified spots were identical to those of the standard samples. The keto acid, α-ketoglutarate was identified by paper chromatography of its 2,4-dinitrophenylhydrazine derivative (Friedemann and Haugen,1943), using Whatman No.1 filter paper and two solvent systems. Solvent I consisted of n-butanol-ethanol-water (40:10: 20) (Germano and Anderson, 1968) and solvent II consisted of n-butanol-ethanol-0.5 N NH_4OH (70:10 : 20) (Seakins *et al.*, 1976). The identification spot had the same R_f values as standard sample.

Assay of transaminase activity

L- Serine- glutamate transaminase activity was assayed by following the formation of glutamate, when α-ketoglutarate and L- serine were incubated with crud cell-free extracts. Unless otherwise specified, the standard reaction of the transaminase contained in a total volume of one ml : amino donor (L-serine 4 μmoles) ; amino acceptor (α-ketoglutarate 5μmoles); Tris-HCl buffer at pH 8, 80 μmoles and the appropriate amount of enzyme. All the reactions were initiated by the addition of substrates and stopped by boiling for 2 min in water bath. One unit of enzyme activity is defined as the amount of protein which catalyzes the formation of one μmole glutamate in 60 min at 40 °C and pH 8. There were 3 replicates per expermint. All data were statisticaly analysis using Person coefficient (Marija, J. and Norusis / spss Inc., 1990).

Results and Discussion

Identification of amino acids formed from L-serine and keto acids through transamination by extracts of *A. chlamydospora*

In this experiment *A. chlamydospora* was grown for 3 days on sucrose-Czapek - Dox medium with L-serine as sole source of nitrogen. Table (1) show that *A. chlamydospora* extracts catalyzed transamination between L-serine and α-ketoglutarate only. This result indicates that these extracts contained L-serine –glutamate transaminase. No transamination between L-serine and glyoxylate, L-

serine and pyruvate or L-serine and oxalacetate could be detected. Ragab *et al.*(1990)reported that extracts of *Cunninghamella elegans* contained L-serine-glutamate, L-serine-glycine and L-serine-alanine transaminase activities, while those of *Fusarium oxysporum* contained L-serine-glutamate and L-serine-glycine transaminase.

Table 1. Identification of amino acids formed from L-serine and keto acids through transamination by extracts of *A. chlamydospora*

Sample	r
Authentic L- serine	0.33
Authentic L-glutamic acid	0.28
Authentic glycine	0.42
Authenic aspartic acid	0.13
Authentic L-alanine	0.57
Reaction mixture contaning L-serine +α -ketoglutarate	0 .28
Reaction mixture contaning L-serine +glyoxylate	0.31
Reaction mixture contaning L-serine +oxalacetate	
Reaction mixture contaning L-serine+ pyruvate	0.32

Solvent system : Water – saturated phenol.

L-Serine –glutamate transaminase activity at different stages of growth of *Alternaria chlamydospora*

The growth was measured by the dry weight of the mycelium and L-serine –glutamate transaminase activity in extracts of the experimental fungus was determined at different periods of incubation. Fig. 1 shows that the highest specific enzyme activity was obtained at the 3rd day of growth then the enzyme level decreased. Maximal growth was also obtained after 3 days incubation.

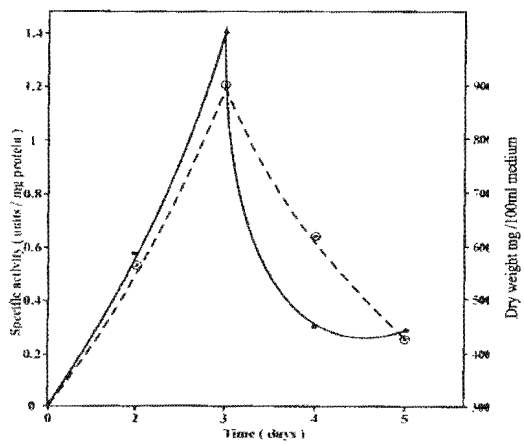

Fig. 1. Effect of incubation time on growth and Induction of L- serine -L-glutamate transaminase by *A.. chlamydospora*...
O--------- O Dry weight • _____ • Specific activity

Effect of different pH values on growth and L-serine –glutamate transaminase synthesis of *Alternaria chlamydospora*

For study the effect of the original pH value of L-serine cotaining medium on the amount intensity of growth and the level of L-serine – glutamate transaminase in *A. chlamydospora* 6 pH values of Tris-HCl buffer were chosen for this investigation (from 3.5 to 8) since it was known that the pH of the growth medium affects the utilization of amino acids by microorganism. The data obtained are presented in table 2. It was found that no growth or synthesis L-serine – glutamate transaminase occurred at pH 3.5 and 4. It is clear that *A. chlamydospora* could grow and synthesis serine – glutamate transaminase within a wide L- pH range (5-8). Maximum growth and enzyme formation was obtained at pH 5.

Table 2. Influence of different pH values on L-serine-L-glutamate transaminase synthesis and growth of *A. chlamydospora*

pH values	Specific activity units\ mg protein	Mycelial dry weight mg\100 ml medium
3.5	0.00	0.00
4.0	0.00	0.00
5.0	1.519	628
5.5	0.457	548
7.0	0.283	606
8.0	0.1012	820

Growth and formation of L-serine–glutamate transaminase of *A. chlamydospora* on various nitrogen sources

The aim of this experiment was to study the effect of various nitrogen sources on the growth rate and the level of L-serine-glutamate transaminase in *A. chlamydospora*. Also this experiment aim to demonstrated which of these compounds work as inducer or repressor for enzyme synthesis. Table 3 demonstrates that *A. chlamydospora* can grow with a variety of nitrogen nutrients. L-Threonine, L-alanine and L-glutamic, supported growth which was more or less equal to that on L-serine. However, growth on sodium nitrate and urea was suppressed as compared with L- serine. Results shown in Table 3 indicate that the synthesis of *A. chlamydospora* L-serine -glutamate transaminase was induced by L-serine, L-threonine, L-alanine and L-glutamic, while another nitrogen sources used in this experiment compeletly repressed the enzyme synthesis. L-serine and L-threonine were the most potent inducers for L-serine-glutamate transaminase.

Table 3. Effect of different nitrogen sources on synthesis of L-serine–glutamate transaminase and growth of *A.. chlamydospora*.

Nitrogen source	Specific activity units\ mg protein	Mycelial dry weight mg\100 ml medium
L-Serine	1.64	824.2
L-Threonine	1.535	683.2
L-Alanine	0.485	726.73
L-Glutamic	0.392	842.33
$NaNO_3$	00	510
Urea	00	303

Each nitrogen source was added in amounts equivalent, on nitrogen basis ,to the amount of nitrogen in sodium nitrat in Czapek-Dox medium.

Table 4. Effect of different carbon sources on synthesis of L-serine– L–glutamate transaminase and growth of *A. chlamydospora*.

Carbon Sources	Specific activity units\ mg protein	Mycelial dry weight mg\100 ml medium
Sucrose	1.6015	547.4
Glucose	0.551	381.8
Fructose	0.607	517.4
Citric acid	0.0	0.0
Starch	0.907	1053

Each carbon source was added at concentration of 3%

Influence of different carbon sources on L-serine–glutamate transaminase production and growth of *A. chlamydospora*.

Table 4 demonstrates that the rate of growth of *A. chlamydospora* on fructose was more or less equal to that on sucrose. Glucose was inferior to sucrose. Where as starch was superior for growth. Citric acid repressed growth of *A.. chlamydospora*, when sucose was replaced by fructose, glucose, starch or citric acid on the basal medium.

Concerning the synthesis of L-serine dehydratase by *A. chlamydospora*, it is clear from the results that sucrose was the best inducers for enzyme synthesis, while citric acid repressed L-serine dehydratase synthesis

Effect of some metals on induction of L-serine–glutamate transaminase and growth of *A. chlamydospora*

Metal salts was added to the culture medium in concentration 100 µg/100ml medium. Table 6 shows that the growth of *A.. chlamydospora* varied greatly with kind of metal. $CuCl_2$ increased growth. However, $NiCl_2$, $CoCl_2$, $FeSO_4$ and $ZnSO_4$ resulted in a decreased in mycelial dry weight. Table 6 demonstrated that all metal salts used in this experiment decreased specific activity of L-serine dehydratase.

Table 5. Effect of some metals on induction of L-serine–glutamate transaminase and growth of *A. chlamydospora*.

Metal	Specific activity units\ mg protein	Mycelial dry weight mg\100ml medium
None	1.415	824.2
$CuCl_2$ 100µg	0.549	898.8
$NiCl_2$ 100µg	0.678	500.8
$CoCl_2$ 100µg	0.430	613
$ZnSO_4$ 100µg	0.527	556.7
$FeSO_4$ 100µg	0.770	591

Table 6. Effect of vitamins on L-serine–glutamate transaminase formation and growth of *A. chlamydospora*

Vitamin	Specific activity units\ mg protein	Mycelial dry weight mg\100ml medium
None	1.68	824
Riboflavin 100 µg	0.490	590.5
Folic acid 100 µg	0.3104	655.2
Barbiturate 100µg	0.600	947.6
Yeast (B_{12}) 0.1gm	0.727	679.4

Effect of vitamin on L-serine–glutamate transaminase formation and growth of *A. chlamydospora*.

The aim of this experiment was to study the effect of various vitamin in synthesis of L-serine-glutamate transaminase and growth of *A. chlamydospora*. Riboflavin, folic acid and Barbiturate(vitamin A) were added to the culture medium in concentration 100 µg/l, while Yeast (B_{12}) was added to the culture medium in concentration 0.1g/l. Results cited in table (6) indicate that synthesis of L-serine-glutamate transaminase and growth of *A. chlamydospora* affected with the type of vitamin. Riboflavin, folic acid and Barbiturate with concentration 100 µg/l resulted in a decreased in specific activity of enzyme by about 70.84%, 81.52 and 64.28% respectively, while vitamin(B_{12}) decreased enzyme synthesis by about 56.27 % Table (6) also Riboflavin, folic acid and Yeast decreased mycelial dry weight by about 28.33 %, 20.48 and17.54% respectively Barbiturate increased fungal growth synthesis by about 14.97%.

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