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L-Serine Dehydratase Formation in *Fusarium moniliforme*

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Abstract: Cell free extracts of *Fusarium moniliforme* grown on L-serine as the sole source of nitrogen contained L-serine dehydratase which catalyzes the cleavage of L-serine into equimolar amounts of pyruvate and ammonia. L-serine dehydratase produced during the logarithmic phase. Maximal growth and enzyme production were obtained after 5 days incubation. Of the tested metal salts, $FeSO_4$ was the best inducer for L-serine dehydratase synthesis by *Fusarium moniliforme*. The optimum pH for growth and L-serine dehydratase formation was 5. L-serine dehydratase was induce by a great variety of nitrogen sources, but L-serine and L-threonine were the best inducer. L-Serine concentration of 2.4 g L^{-1} was the optimal for L-serine dehydratase synthesis. The effect of different carbon sources on growth and enzyme formation was investigated.

Key words: *Fusarium moniliforme,* L-serine dehydratase

INTRODUCTION

L-Serine dehydratase (L-serine hydro-lase, deaminating, EC 4.2.1.13) has been extensively studied in several bacteria including *Aeromonas punctata*[1], anaerobic bacteria[2], *Arthrobacter globiformis*[3], *Bacillus circulans*[4], *Brevibacterium linens*[5], *Campylobacter* spp.[6], Citrobacter[7] *Clostridium acidiurici*[8], *Corynebacterium glycinophilum*[9,10] *Corynebacterium* spp.[11], *Escherichia coli*[12-15], *Eubacterium acidaminophilum*[16], *Helicobacter pylori* heterofermentative lactobacilli[18], homofermentative lactobacilli[19], *lactobacillus murinus*[20], *Lactobacillus fermentum* ATCC14931[21] *Lactobacillus plantarum*[22], *Proteus vulgaris*[23].

Pseudomonas cepacia[24] *Salmonella typhimurium* and *Bacillus cereus*[25], *Sarcina albida*[26], *Staphylococcus epidermidis*[27], *Sterptococcus clavuligerus* *Streptococcus faecalis*[29] and Wild Rhizobia[30]. Its presence was also demonstrated in the yeast *Saccharomyces cerevisia*[31] and the filamentous fungi *Cunninghamella elegans* and *Fusarium oxysporum*[32,33] and *Neurospora crassa*[34].

Nelson[35] reported that L-serine dehydratase was produced to the extent of only 0.04 mg^{-1} dry weight by *Aeromonas punctata* NRRL B-928 when grown on a chemically defined medium. Addition of 2% (w/v) L-serine to this medium increased L-serine dehydratase ten fold or more, indicating a significant inductive effect. D-serine was toxic to the organism, making weights and enzyme titers uncertain, but DL-serine induced specific activity of L-serine dehydratase at least equal to that from the L enantiomorphism.

El-Awamry *et al.*[32] demonstrated that $FeSO_4$ was the best inducers for L-serine dehydratase synthesis by *Cunninghamella elegans* and *Fusarium oxysporum*. The enzyme produced during the logarithmic phase of growth of the two organisms and maximum production was obtained after 3 days incubation. The optimal pH rang for L-serine dehydratase formation in *F. oxysporum* was 4-5, [17]whereas for *C. elegans* enzyme pH 5.0 was the optimal. L-serine dehydratase of both organisms was induced with L-serine, ammonium carbonate, some amino acids and amides, but L-serine was the best inducers. L-Serine concentration of 2.4 g L^{-1} was the optimal for L-serine dehydratase synthesis.

The present investigation deals with the biosynthesis [28]of L-serine dehydratase in *Fusarium moniliforme* under different physiological conditions. Such studied have not been reported before in *Fusarium moniliforme*.

MATERIALS AND METHODS

Organism: *Fusarium moniliforme* was obtained from Cairo Mircen, Faculty of Agriculture, Ain Shams University, Egypt.

Media and culture: The organism was grown on glucose-Czapek-Dox liquid medium with L-serine replacing $NaNO_3$ on nitrogen equivalent basis. In addition, the medium

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supplement with 0.01% $FeSO_4$ for stimulating the synthesis of L-serine dehydratase. The pH of the medium was adjusted to 5.0.

Five ml aliquots of spore suspension of *Fusarium moniliforme* were used to inoculate 250 ml Erlenmeyer flasks, each containing 50 ml sterile medium. The inoculated flasks were incubated at 28 °C for 5 days, then the mycelia were harvested by filtration, washed thoroughly with distilled water and finally blotted dry with absorbent paper.

Preparation of cell-free extract: The harvested mycelia were ground with cold sand in a cold mortar and extracted with 0.05 M Tris-HCl buffer at pH 8.5. The obtained slurry was then centrifuged at 12 g for 10 min and the supernatant was used as the crude enzyme preparation.

Chemical methods: Pyruvate was estimated by the method of Friedmann and Haugen[36]. The method is based on the interaction of pyruvate with the 2, 4-dinitrophenylhydrazine reagent. The procedure can be summarized as follows: To 1 ml of the reaction mixture, 1 ml of 0.1 2, 4-dinitrophenylhydrazine in 2 N HCl was added. After 5 min, 5 ml of 2.5 N NaOH were added and the color intensity was measured spectrophotometrically at 520 nm. Protein was determined according to the method of Sutherland *et al.* [37].

Assay of L-serine dehydratase: L-serine dehydratase activity was routinely assayed by determining pyruvate formation from L-serine. One unit of enzyme activity is defined as the amount of protein which catalyzes the formation of one μmole pyruvate in 90 min at 30 °C.

All data were statistically analyzed using Person coefficient[38].

RESULTS AND DISCUSSION

L-Serine dehydratase activity at different stages of growth of *F. moniliforme*: The growth was measured by the dry weight of the mycelium and L-serine dehydratase activity in extracts of the experimental fungus were determined at different periods of incubation. Figure 1 shows that the highest specific enzyme activity was obtained at the 5th day of growth after which the enzyme activity decreased. Maximal growth was also obtained after 5th day incubation.

Effect of different pH values on L-serine dehydratase synthesis and growth of *F. moniliforme*: To study the effect of the original pH value of L-serine-containing medium on the intensity of growth and the activity of

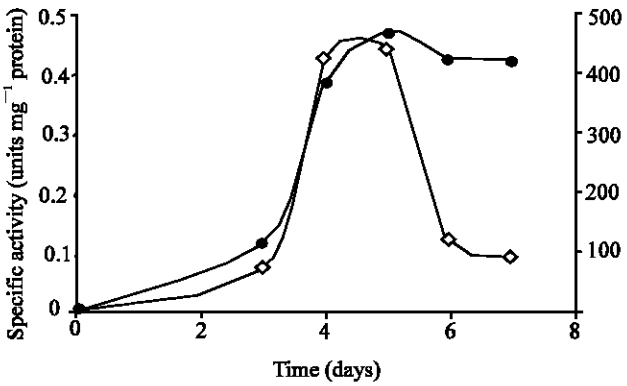

Fig. 1: L-Serine activity at different stages of growth of *F. moniliforme* (●) specific activity. (◇) Dry weight

Table 1: Effect of different pH values on L-serine dehydratase synthesis and growth of *F. moniliforme*

Initial pH	Final pH	Specific activity (units mg^{-1} protein)	Mycelial dry weight (mg 100 ml^{-1} cul. med.)
2	2.0	0.000	0.00
3	3.0	0.250	280.80
4	3.0	0.340	384.00
5	4.0	0.390	507.20
6	5.0	0.300	180.80
8	5.5	0.176	133.60
10	6.0	0.080	179.00

The final pH of the culture medium was measured after 5 days incubation

Table 2: Growth and L-serine dehydratase synthesis of *F. moniliforme* on various nitrogen source

Nitrogen source	Specific activity (units mg^{-1} protein)	Mycelial dry weight (mg 100 ml^{-1} culture medium)
Sodium nitrate	0.020	131.3
L-Serine	0.400	384.0
L-Glutamic	0.113	159.0
L-Glycine	0.140	183.2
L-Alanine	0.056	226.8
L-Threonine	0.400	200.2
Ammonium chloride	0.080	181.0

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

L-serine dehydratase in *F. moniliforme*, 7 pH values were chosen they rang from 2.0 to 10. It was found that no growth occurred at pH 2.0. It is clear that *F. moniliforme* could grow and synthesis L-serine dehydratase within a wide pH range (3-10). It is clear that the growth rate was more or less equal at these pH values. Maximum growth and enzyme formation were obtained at pH 5 (Table 1).

In addition, the pH values of the culture media were measured at the end of incubation. No significant change in the pH values 3, 4, 5 and 6 of these media was detected, while there was significant change in the pH values 8 and 10 of these media. The acid production by the fungus causes decrease in the pH values of the medium. These results are in close agreement to those reported for *C. elegans* and *F. oxysporum*[32].

Table 3: Influence of different carbon sources on L-serine dehydratase synthesis and growth of *F. moniliforme*

Carbon source	Specific activity (units mg^{-1} protein)	Mycelial dry weight ($mg100ml^{-1}$ culturemedium)
Glucose	0.440	278
Lactose	0.344	98
Galactose	0.276	124
Maltose	0.930	100
Sucrose	0.456	88
Starch	0.610	274

Table 4: Effect of some metal salts on L-serine dehydratase synthesis and growth of F. *moniliforme*

Addition	Specific activity (units mg^{-1} protein)	Mycelial dry weight (mg 100 ml^{-1} culture medium)
None	0.184	278.0
$CaCl_2$	0.274	130.0
$CoCl_2$	0.470	130.0
$CuSO_4$	0.410	114.8
$FeSO_4$	0.431	507.2
$MnCl_2$	0.488	134.6

Each metal salts was added at concentration of 10 mg L^{-1}

Table 5: Growth and synthesis of L-serine dehydratase as a function of L-serine concentration in the culture medium of *F. moniliforme*

L-serine in medium g L^{-1}	Specific activity (units mg^{-1} protein)	Mycelial dry weight (mg 100 ml^{-1} culture medium)
0.6	0.07	154
1.2	0.20	202
2.4	0.30	380
4.8	0.20	321

Growth and L-serine dehydratase synthesis of *F. moniliforme* on various nitrogen sources: Table 2 shows that *F. moniliforme* can grow with a great variety of nitrogen nutrients. L-Glutamic, L-glycine and ammonium chloride supported growth which was more or less equal to that of cultures grown on sodium nitrate . However, growth on L–serine was superior as nitrogen sources for sodium nitrate, while L-threonine and L-alanine supported moderate growth.

Results shown in Table 2 indicated that the synthesis of *F. moniliforme* L–serine dehydratase was induced by L-serine, L-glutamic, L-glycine, L-alanine, L-threonine and ammonium chloride as compared with that of nitrate-grown cultures. L-serine and L-threonine were the most potent inducers. Comparable result were obtained by Nelson[35] who found that L-serine dehydratase was produced to the extent of only 0-04 mg^{-1} dry weight by *Aeromonas punctata* NRRL B-928 when grown on a chemically defined medium. Addition of 2%(w/v) L-serine to this medium increased L-serine dehydratase ten-fold or more, DL-serine induced a specific activity of L-serine dehydratase at least equal to that from the L enantiomorphism. El-Awamry *et al.*[32] demonstrated that L–serine dehydratase of *C. elegans* and *F. oxysporum* was induced with L–serine, ammonium carbonate, some amino acids and amides, but L–serine was the best induced.

Influence of different carbon sources on L-serine dehydratase synthesis and growth of *F. moniliforme*: Table 3 demonstrated that the growth of *F. moniliforme* was not significantly affected when glucose was replaced by starch, but significantly affected when glucose was replaced by galactose, maltose, sucrose and lactose.

Concerning the synthesis of L-serine dehydratase by *F. moniliforme,* it is clear from the results that maltose was the best inducer, it caused about 111%increase in specific enzyme activity. While, starch and sucrose caused 38 and 3% increased in specific enzyme activity. However, Lactose and Galactose caused 32 and 37.3% repression in enzyme formation.

Effect of some metal salts on L-serine dehydratase synthesis and growth of *F. moniliforme*: Table 4 shows that $FeSO_4$ stimulated fungal growth and L-serine dehydratase formation by about 82 and 134%, respectively. $CaCl_2$, $CoCl_2$, $CuSO_4$ and $MnCl_2$ resulted a decrease in mycelial dry weight by about 53.2, 53.2 , 58.7 and 51.6%, respectively, while L-serine dehydratase formation was stimulated by $CaCl_2$, $CoCl_2$, $CuSO_4$ and $MnCl_2$ about 48, 155, 122 and 165%, respectively. This result agreed with that reported by El-Awamry *et al.*[32] who demonstrated that L-serine dehydratase of *C. elegans* and *F. oxysporum* was induced by metal salts and $FeSO_4$ was the best inducer.

Growth and synthesis of L-serine dehydratase as a function of L-serine concentration in the culture medium of *F. moniliforme*: L-Serine was added to the basal medium as the sole source of nitrogen in concentrations ranging from 0.6 to 4.8 g L^{-1}. In Table 5 the optimum concentration of L-serine for growth was 2.4 g L^{-1}. The mount of nitrogen in this concentration is equivalent to its amount in 2 g sodium nitrate. The specific activity of L-serine dehydratase was increased with increasing L-serine concentration in the medium. Optimum concentration of L-serine for enzyme synthesis was 2.4 g L^{-1}. This result agreed with that reported for L-serine dehydratase of *C. elegans* and *F. oxysporum*[32].

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