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# HACETTEPE BULLETIN OF NATURAL SCIENCES AND ENGINEERING

*A BULLETIN PUBLISHED BY*
FACULTY OF SCIENCES OF HACETTEPE UNIVERSITY

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# NATURAL SCIENCES AND ENGINEERING

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# NATURAL SCIENCES AND ENGINEERING

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# CONTROL OF THE CELLULASE SYNHESİS İN CELLULOMONAS FLAVIGENA

(SELÜLAZ SENTEZİNİN KONTROLÜ)

N.Kolankaya[X]

## SUMMARY

In this study, effect of some external factors which control the cellulase enzyme synthesis in Cellulomonas flavigena ATCC 482 was investigated. The synthesis of enzyme was found to be repressed by readily metabolized carbon sources added into C.flavigena cultures growing on cellulose. In addition to the carbon sources, the concentrations of dissolved oxygen and yeast-extract in culture media were also effective on cellulase formation. 2,4-DNP at final concentration of $5x10^{-5}$M in culture medium increased differantial rate of the enzyme synthesis. The existence of an inverse relationship between growth rate and enzyme production of C.flavigena was a comman observation of the study.

## INTRODUCTION

Synthesis of cellulases by fungi and bacteria is considered to be induced by cellulose substrates. However, since cellulose is high-molecular weight compound its penatration into cell is problematical. According to the concept of JACOB and MONOD(1961) inducer is an exogenous substance that penatrates into cell and derepresses the synthesis of enzyme protein. The data obtained in studies dealing with cellulase formation suggests that there was inverse relationship between metabolic rate and cellulase production of bacteria and fungi(YAMANE et.al.1970;HULME and STRANKS, 1971).

In present work, control of the cellulase synthesis in C.flavigena cultures was studied.

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## MATERIALS AND METHODS

### I. Organism and Culture Conditions.

Cellulomonas flavigena ATCC 482 was provided from American Type Culture Collection, U.S.A. Stock cultures were maintained in slants made of stock-culture. Agar(Difco). Working basal medium was consisting of 0.1%$K_2HPO_4$,0.05%KCI,0.05$MgSO_4$ and 0.05% yeast-extract(Difco). Into the basal medium, water-soluble derivative of cellulose, CMC(Carboxymethylcellulose) and other carbon sources were added after they were sterilizedseparately.Erlenmeyer flaks containing 50 or 100 ml of sterile culture medium were inoculated with bacterial suspension prepeared by suspending the bacterial cells in slant tubes with 10 ml of sterile saline solution, at ratio of 0.1 ml suspension per 100 ml of medium. Culturations were carried out with Psycrotherm Incubator Shaker(New Brunswick Co.) at 30°C with different period of growth time. Dissolved oxygen concentrations in culture flasks containing 50 ml of medium were measured with the aid of Oxygen electrode(YSI model) for each shaking speeds(100-300 r.p.m). And then, rate of Oxygen transfer into medium was found as suggested by ARNOLD and STEELE(1958).

### II.Measurement of Growth and Cellulase Activity

The growth of C.flavigena was measured by reading optical density of culture fluids at 600 nm by means of spectronic 20 model of Bosch and Lomb spectrophotometer.

Culture supernatants were used as enzyme source in enzyme assays. The method described by NISIZAWA et. al.(1971) was used for measuring the cellulase activity as CMCase. In experiments, a unit CMCase activity was described as the amount of enzyme producing 1µg reducing sugar in a minute at the experimental conditions. The amounts of reducing sugars in enzyme and incubation solutions were measured by DNS(Dinitrosalicilic acid) procedure(MILLER,1959), and calculated as glucose on standard curve prepeared with glucose. Since the enzyme synthesis is occured during exponantio-

nal growth phase of C.flavigena, the differantal rates of enzyme synthesis were found using the equation offered by WELKER and CAMPBELL(1963) for amylase producing organism, as follows,

$\Delta^{II} = \Delta E / \Delta O.D$

## RESULTS

### I.Effect of Carbon Sources on Cellulase Synthesis

When the growth and cellulase synthesis in C.flavigena were considered, it was observed that the organism produced the enzyme throughout logarithmic phase of growth in presence of 1.0% CMC as carbon source(Fig 1). Reducing sugar in the culture was also increased during the logarithmic growth phase. The CMCase formation in terms of differantial rates has been effected by CMC concentrations. Synthesis of the enzyme was increased up to the 0.5% CMC and then slightly decreased (Fig.2).While the addition of glucose and cellobiose were activating the growth in cultures grown on CMC, these two sugars were both repressing the cellulase formation (Fig 3 and 4).Same phenomenon was observed with the other carbon sources which were added into the culture media at 24. hr of culturation(Table 1). The most activily repressing carbon sources were of that on which C.flavigena was shown to grow with higher growth rates.

### II.Effect of Physiological Conditions on Cellulase

Growth of C.flavigena was found to be proportional to the concentration of dissolved oxygen in culture medium(Fig.5).But, the formation of cellulase was negatively correlated wit the oxygen concentration. Amount of yeast-extract in culture medium has also affected growth and cellulase formation in cultures.There was an inrease in the growth of organism due to the increase in amount of yeast extract concentration up to 0.20% in the medium. In contrast to increase in growth, enzyme formation in culture was decreased (Fig. 6).

2,4 DNP has influenced the enzyme synthesis and growth of the organism inversly(Table 2).Presence of 2,4 DNP in culture medium at $5 \times 10^{-5}$ M concentration caused decrease in growth but the increase in CMCase synthesis of C.flavigena.

TABLE I.Effect of various carbon sources on synthesis of cellulase in C.flavigena cultures.

| Carbon Source[X] | CMCase ($\Delta U/\Delta O.D_{600nm}$) | Growth Rate [XX] ($\Delta O.D_{600nm}/hr \times 10^3$) |
|---|---|---|
| CMC(Control) | 21.32 | 8 |
| Lactose | 8.53 | 18 |
| Na-acetate | 7.25 | 20 |
| Mannose | 7.20 | 21 |
| Glycerol | 7.20 | 25 |
| Fructose | 4.50 | 50 |
| Galactose | 2.65 | 60 |

X: Carbon sources were added into media at 24.hr of culturation to give final concentration of 002%. Before the addition of carbon sources, o.5% CMC was sole source of carbon in media.

XX: Growth rates for each carbon sources were found by growing C.flavigena in medium containing relavant carbon source at 0.1% concentration.

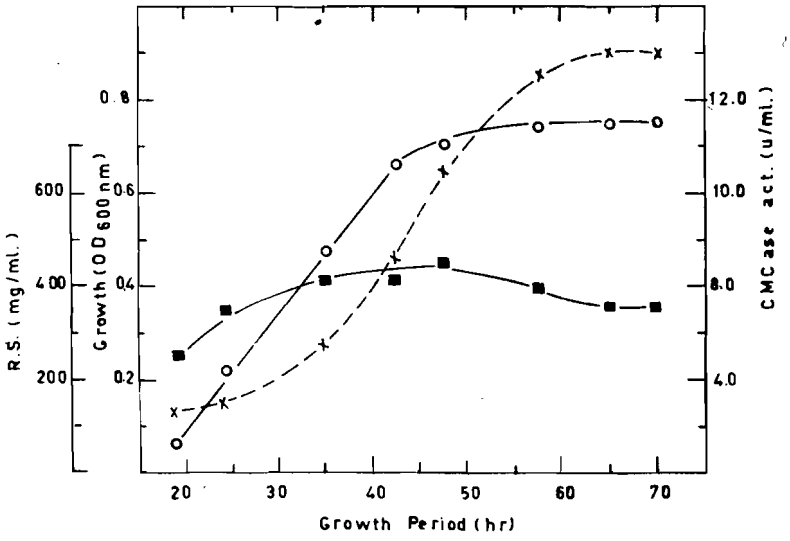


Fig.1.Growth and Cellulase Formation in C.flavigena. Organism was grown in basal-medium containing 1.0 %CMC.
X:CMCase activity (U/ml),o:Growth ($O.D_{600nm}$),: Reducing sugar (µg/ml).

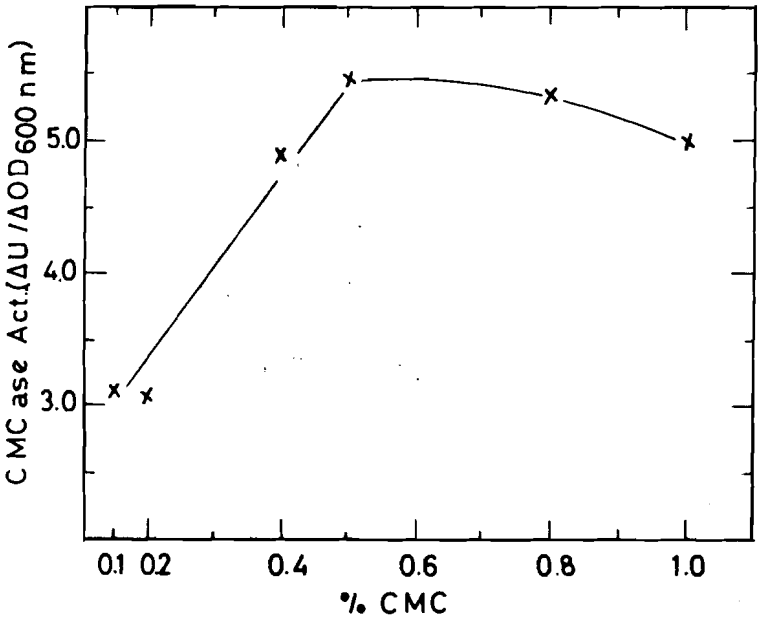


Fig.2.Differantial rates of CMCase synthesis in the presence of various concentration of CMC in medium.Cells were grown for 24 hrs at 30°C, 150r.p.m.

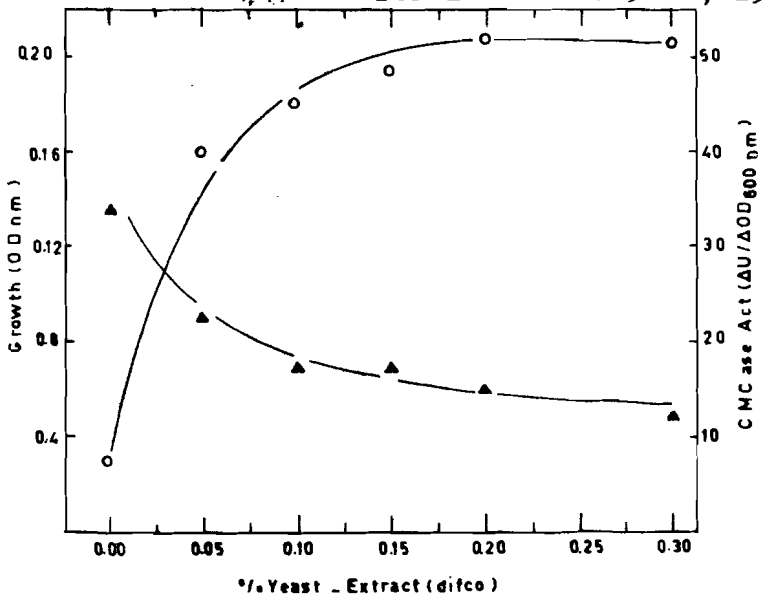


Fig.6.Effect of yeast-extract on the growth and rate of CMCase synthesis of C.flavigena. o:Growth of organism, ▲:Rate of CMCase synthesis. Culturations were carried out in presence of 0.5%CMC for 24 hr at 30°C.

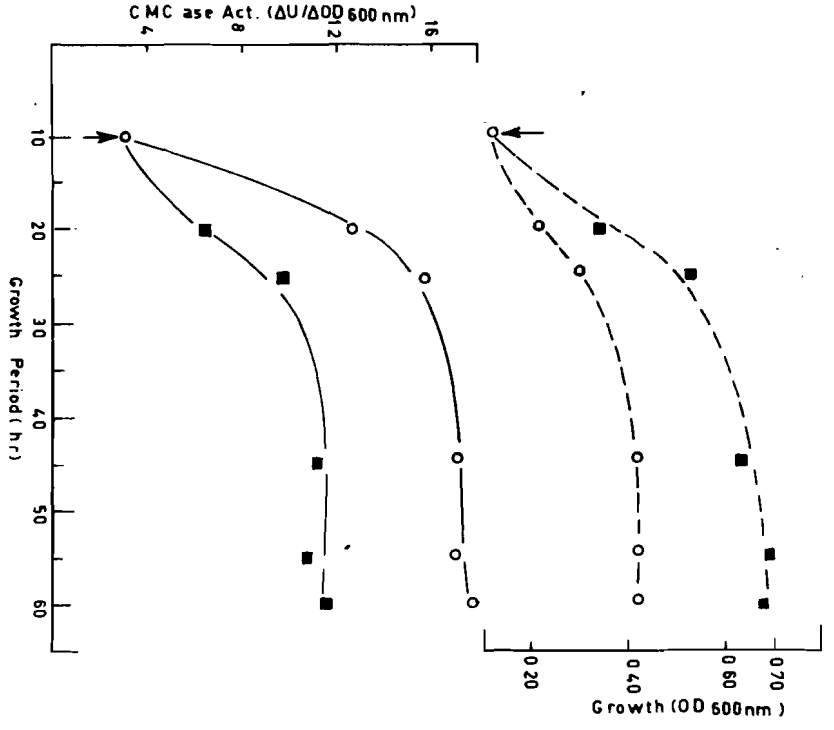


Fig.3. Effect of glucose on the growth and rate of CMC-ase synthesis of C.flavigena. 0.02% glucose was added into culture growing on 0.5% CMC. at 10.hr of culturation.
--- o: growth of control, ■; Growth of control plus glucose,
—— o: Rate of CMCase synthesis of control, ■——: Rate of CMCase synthesis of control plus glucose.

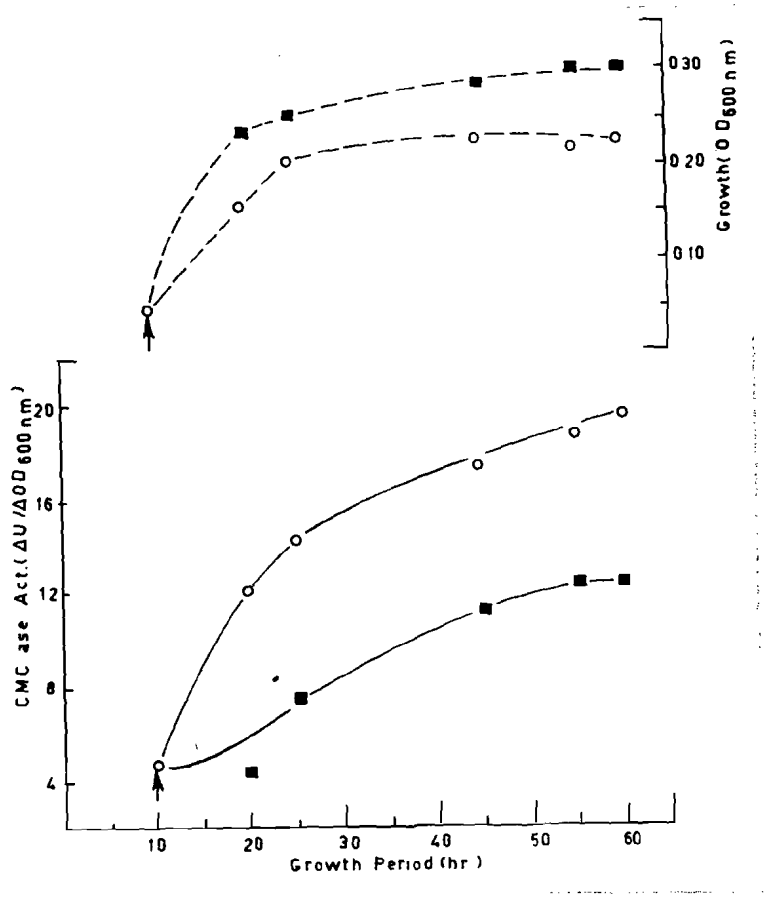


Fig.4. Effect of cellobiose on the growth and rate of CMCase synthesis of C.flavigena. 0.02% cellobiose was added into culture growing on 0.5%CMC, at 10.hr of culturation.
---o:Growth of control,---■Growth of control plus cellobiose, —o:Rate of CMCase synthesis of control,
—■:Rate of CMCase synthesis of control plus cellobiose

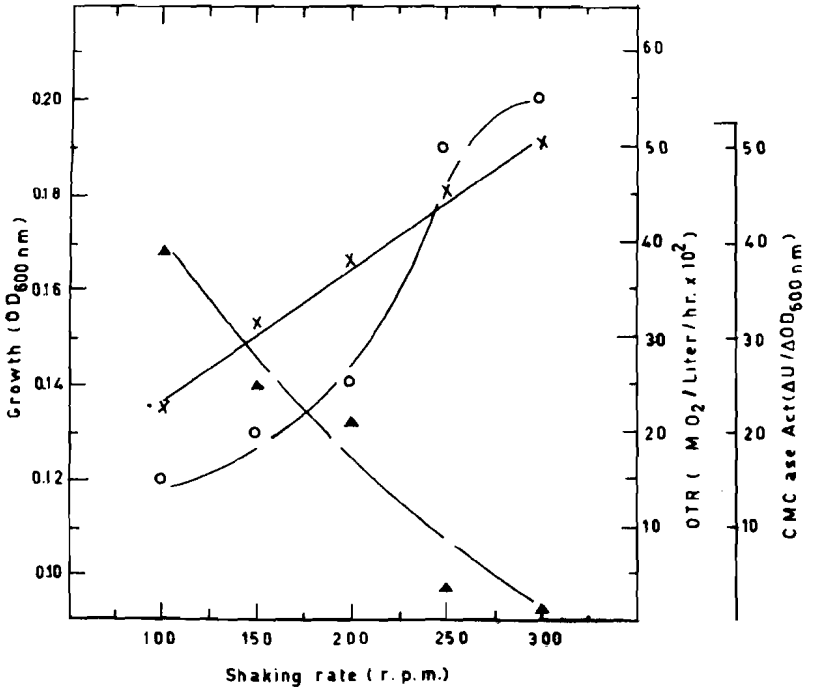


Fig.5. Effect of dissolved $O_2$ concentrations on the growth and CMCase synthesis of C.flavigena. X: $O_2$ transfer rate into medium, o: Growth of organism, ▲: Rate of CMCase synthesis. Culturation was carried out in presence of 0.5% CMC for 24 hr at 30°C.

Table 2. Effect of 2,4 DNP on the growth and cellulase synthesis of C.flavigena[X].

| 2,4DNP (Mol.) | Growth ($O.D._{600nm}$) | CMCase ( U/ $O.D._{60nm}$) |
|---|---|---|
| 0.00 (Control) | 0.16 | 21.20 |
| $5x10^{-5}$ | 0.12 | 31.60 |

X: Culturations were carried out in presence of 0.5% CMC for 48 hr at 30°C.

## DISCUSSION

On the basis of our present data, limitation in metabolism of the C.flavigena is resulted in induction of cellulase synthesis. It is apparent that the cellulase synthesis in C.flavigena cells is more effectively repressed by the easily assimilated carbon sources (Fig.3,4 and Table 1). Induction of some depolymerases in culture of bacteria and fungi was shown to be possible when the growth of organisms was slowed down by limiting the consumption of carbon sources(HSU andVAUGHN, 1969; LABONAK and PAVLOVSKAYA,1975; HULME and STRANKS 1971). Comparatively recently, initiation of the cellulase synthesis by lactose in Trichoderma lignorum was regarded as a result of slow consumption of sugar by the organism (LABONAK and PAVLOVSKAYA 1978). Induction of cellulase formation of C.flavigena by cellulase is probably dependent on slow degradation of cellulose by the enzyme already present in small amounts in the cell. Therefore, cellulase may be semi-consitutive in C.flavigena as in Pseudomonas fluorescens (YAMANE et.al.1978)

In experiments, the cellulase formation of C.flavigena was enhanced due to dissolved $O_2$ and yeast extract concentrations which are not optimal for growth (Fig.5,6). Restriction in ATP bynthesis and on growth of C.flavigena was also resulted in increase in cellulase formation(Table 2). As proposed by HULME and STRANKS 1970), for celluloytic microorganisms, limitation in metabolism of C.flavigena may be the main mechanism for its cellulase synthesis.

Manuscript Received in April 1980

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## ÖZET

Bu çalışmada Cellulomonas flavigena ATCC482 suşu üzerinde selülaz sentezini kontrol eden dış etkenler araştırılmaya çalışıldı. Selüloz varlığından üretilen C.flavigena kültürlerinin üreme ortamına eklenecek kolay metabolize edilen karbon kaynaklarının selülaz sentezini baskıladıkları saptandı. Eklenen karbon kaynakları dışında, selülaz sentezi üzerinde ortamdaki oksijen ve maya-ekstresi konsantrasyonları da etkin olmakta idi. Ayrıca, 2-4-DNP'nin kültür ortamlarında $5x10^{-4}$ konsantrasyonunda bulunması durumunda enzim sentezinin differasiyel hızını arttırdığı gözlendi. Genel olarak, C.flavigena kültürlerinde organizmanın üreme hızı ile enzim üretimi arasında ters bir ilişkinin var olduğu saptandı.

# L-Serine Dehydratase Synthesis By Pseudomonas aeruginosa

## Pseudomonas aeruginosa'da L-Serin Dehidratas Sentezi

Atilla Atalay*

SUMMARY

L-serine dehydratase (L-serine hydro-lyase, EC 4.2.1.13)production was studied in Pseudomonas aeruginosa OMB-1468.Maximum L-serine dehydratase activity was obtained when 0.5 % $NH_4Cl$ was supplied as nitrogen source in the basal medium containing 1 % Beef extract,0.3 % $K_2HPO_4$ and 0.01 % $MgSO_4.7\ H_2O$. Various carbon sources inhibited or did not effect the enzyme formation.

## INTRODUCTION

REGAN and co-workers (1969) showed that chronic granulocytic leukemia as well as normal human marrow cells apparently require serine for growth. This finding suggests that L-serine dehydratase (LSD)might

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be usefull in leukemia therapy.

For this purpose NELSON et al (1973) screened 117 bacterial strainer and found that Chromobacterium viscosum has the highest L-serine dehydratase activity. NOLANTAYA et al (1975) also screened a series of bacteria and fungi for L-serine dehydratase activity and found that Pseudomonas aeruginosa was the best source for this enzyme.

In the present study the **nutritional factors affecting the enzyme formation were studied.**

## MATERIAL AND METHODS

### I.ORGANISM

Pseudomonas aeruginosa CMI-1468 was obtained from Quartermaster Research and Development Center U.S.Army Natick,Mass.The culture was maintained on a medium containing(grams per liter):Pepton,10;Beef extract,6; $K_2HPO_4$,3.3;and agar,2.

### II.Bacterial growth and preparation

For L-serine dehydratase production by P.aeruginosa the media indicated in the respective tables were used. Corn steep liquor was adjusted to pH 7 with sodium hydroxide and boiled for 10 min.The precipitate was removed by filtration and the clear filtrate was used for the preperation of media.The media were distributed in 50 ml amounts to 250 ml shaking flasks,sterilized at 121°C at pressure for 20 min,and inoculated with a loop-full of bacteria.Culturation was carried out for 20 hours at 37°C on a rotary shaker(New Brunswick Sci.Co) at 175 rev/min.Cells were harvested by centrifugation (6000xg for 10 min) and resuspended in 5 ml of water.

III.Enzyme assay

The keto acid produced by the enzyme was determined by the direct method of FRIEDEMANN and HAUGEN (1943). A 0.1 ml of cell suspension was mixed with 0.9 ml of (A) buffer (0.1 M borate,pH 8.6, 0.01 M $MgCl_2$ and 0.1 M L-serine) and incubated in a 10 X )( mm tube with reciprocal shaking in a water bath at 37°C.Duplicate sample tubes were incubated with a control tube in (A) buffer without L-serine.After 10 min the reaction was stopped by adding 1 ml of 1 N HCl and 0.3 ml of 0.1 % 2,4-dinitrophenylhydrazine in 2 N HCl.The mixture was further incubated for 10 min at 37°C,and then 2 ml of 2 N NaOH was added. The absorbance of the solution was determined at 417 nm in a Coleman spectrophotometer. The amount of pyruvate liberated was calculated by reference to a standard curve prepared with crystalline sodium pyruvate.

A unit of L-serine dehydratase was defined as the amount of enzyme catalyzing the formation of 1 μM of pyruvate per min under the conditions described above.

The remainder of the cell suspention was dried (overnight at 100°C) and the dry weight determined.

## RESULTS AND DISCUSSION

Different nitrogen sources were added to the basal medium containing 1 % Beef extract,0.3 % $K_2HPO_4$ and 0.01 % $MgSO_4.7\ H_2O$ to examine the effect on the enzyme formation.Among the nitrogen sources tested only $NH_4Cl$ increased the enzyme activity (Table 1).

The other nitrogen sources increased the growth rate of culture but inhibited the enzyme formation.The effect of $NH_4Cl$ on the yield of LSD was further studied.

synthesis from 0.084 to 0.221 IU/mg dry weight(Table 2).

Table 1.Effect of nitrogen sources on the formation of L-serine dehydratase[x]

| Nitrogen source | L-serin dehydratase IU/mg dry wt of cells | Dry wt of cells mg/ml |
|---|---|---|
| Control | 0.085 | 0.98 |
| Yeast Extract | 0.011 | 1.82 |
| Tryptone | 0.006 | 1.90 |
| Casein Hydrolysate | 0.008 | 2.07 |
| Peptone | 0.015 | 1.51 |
| Corn Steep Liquor | 0.005 | 1.23 |
| $NH_4Cl$ | 0.160 | 0.56 |

[x]The medium contained 1 % beef extract, 0.3 % $K_2HPO_4$, and 0.01 % $MgSO_4 \cdot 7\ H_2O$

Table 2.Effect of ammonium chloride concentration on the formation of L-serine dehydratase[x]

| $NH_4Cl$ (%) | L-serine dehydratase IU/mg dry wt of cells | Dry wt of cells mg/ml |
|---|---|---|
| 0 | 0.084 | 0.98 |
| 0.1 | 0.083 | 0.98 |
| 0.5 | 0.221 | 0.77 |
| 1.0 | 0.068 | 0.68 |
| 1.5 | 0.055 | 0.60 |
| 2.0 | 0.074 | 0.54 |

[x]The basal medium was the same as in Table 1.

Addition of 0.5 % $NH_4Cl$ increased the enzyme synthesis from 0.084 to 0.221 IU/mg dry weight (Table 2). In their previous studies PARDEE and PRESTIDGE (1955) found the highest activity of L-serine deaminase when they used Casein hydrolysate and yeast extract as a nitrogen source in E.coli. In addition, ISENBERG and NEWMAN (1974) showed the inhibition of enzyme formation in E.coli K 12 with the addition of ammonium sulphate to the minimal medium containing proline, leucine and

glycine.These finding suggest that the Pseudomonas enzyme may be different from that of E.coli.

When amino acids were added to basal medium together with 0.5 % $NH_4Cl$,growth rates of cultures were increased but the enzyme fonmation either inhibited or unaffected (Table 3).

However,previous studies of PARDEE and PRESTIDGE (1955) and ISENBERG and NEWMAN (1974) have reported LSD from E.coli induced by glycine and leucine.The effect of carbohydrates and some carboxylic acids as a carbon source on the production of LSD was tested in a basal medium containing 1 % Pepton,0.3 % $K_2HPO_4$ and 0.01 % $MgSO_4.7\ H_2O$.It was found that carbon sources with the exception of galactose depressed the enzyme formation (Table 4).

Galactose increased the enzyme activity about 30 per cent.As with a number of enzymes,formation of LSD is also inhibited by the addition of sugars,particulary glucose.The published results of BOYD and LICHSTEIN (1951),BOYD and LICHSTEIN (1955), EPPS and GALE (1942) showing that LSD activity is depressed in various cultures grown in a medium containing glucose.Although, carboxylic acids increased the growth rate of the culture,they inhibited the enzyme formation (Table 5).

As a result of this study we found that the best medium for the production of LSD by P.aeruginosa was 1 % Beef extract, 0.3 % $K_2HPO_4$, 0.01 % $MgSO_4.7\ H_2O$ and 0.5 % $NH_4Cl$.

Table 3. Effect of amino acids on the formation of L-serine dehydratase[x]

| Amino acid % 0.5 | L-SDH IU/mg dry wt of cells | Dry wt of cells mg/ml |
|---|---|---|
| None | 0.168 | 0.648 |
| L-serine | 0.060 | 1.200 |
| L-glutamic acid | 0.043 | 1.220 |
| L-threonine | 0.082 | 1.140 |
| L-alanine | 0.102 | 1.100 |
| L-tryptophane | 0.041 | 1.250 |
| L-asparagine | 0.164 | 1.120 |
| L-leucine | 0.107 | 1.300 |
| Glycine | 1.160 | 1.320 |

[x]The basal medium contained 1 % Beef extract, 0.3 % $K_2HPO_4$, 0.01 $MgSO_4.7H_2O$, and 0.5 % $NH_4Cl$.

Table 4. Effect of carbon compounds on the formation of L-serine dehydratase[x]

| Carbon compound | L-SDH IU/mg dry wt of cells | Dry wt of cells mg/ml |
|---|---|---|
| Control(no C added) | 0.033 | 0.723 |
| Glucose | 0.011 | 1.188 |
| Fructose | 0.014 | 1.228 |
| Maltose | 0.035 | 0.762 |
| Galactose | 0.043 | 0.644 |
| Sucrose | 0.036 | 0.696 |
| Lactose | 0.034 | 0.716 |
| Dextrine | 0.036 | 0.776 |

x The medium contained 1% peptone, 0.3% $K_2HPO_4$, and 1% carboxylic acid.

Table 5. Effect of carboxylic acids on the formation of L-serine dehydratase[x]

| Carboxylic acid | L-SDH IU/mg dry wt of cells | Dry wt of cells mg/ml |
|---|---|---|
| Control(no added) | 0.046 | 0.54 |
| Lactate | 0.007 | 1.12 |
| α-Ketoglutarate | 0.017 | 0.94 |
| Fumarate | 0.009 | 0.91 |
| Malate | 0.006 | 1.12 |
| Pyruvate | 0.008 | 1.06 |
| Citrate | 0.010 | 0.72 |

[x]The medium contained 1% peptone, 0.3% $K_2HPO_4$, and 1% carboxylic acid.

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Iwish to thank L.M.Drown for helpful discussions and for assistance in preparing the manuscript.

ÖZET

P.aeruginosa'da L-serin dehidrataz aktivitesi çalışıldı.Amonyum klorürün enzim aktivitesini arttırdığı,değişik karbon kaynaklarının ise inhibe ettiği gözlendi.

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