# HACETTEPE BULLETIN OF
# NATURAL SCIENCES AND ENGINEERING

AN ANNUAL PUBLICATION VOLUME 12 / JUNE 1983

PUBLISHED BY THE
FACULTY OF SCIENCES
HACETTEPE UNIVERSITY
BEYTEPE, ANKARA, TURKEY

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HACETTEPE BULLETIN OF
# NATURAL SCIENCES AND ENGINEERING

CONTENTS

A.BIOLOGICAL SCIENCES

CONTENTS (CONT.)

HACETTEPE BULLETIN OF
NATURAL SCIENCES AND ENGINEERING

# AGEING AND THE LIFE SPAN OF VARIOUS DROSOPHILA MUTANTS

(Çeşitli Drosophila Mutantlarının Ömür Uzunluğu ve Yaşlanması)

Ali Nihat Bozcuk

## INTRODUCTION

Although there is some evidence that the life-span of *Drosophila* is under genetic control, the manner in which this control operates is not clearly understood. A limited amount of work has been done on the relationship between genetics and ageing (reviewed by COMFORT, 1978; LINTS, 1978 and 1980; BOZCUK,1981). Our present knowledge does not allow us, for example, to answer LINTS's (1980) question explicitly: "Does any specific gene, or genes or do all genes exert some type of control on longevity?" In order to understand genetic determination of longevity we have been studying specific or hybridised effects of various autosomal and sexual mutant genes in *Drosophila*. In this contribution some of our results will be evaluated in conjuntion with other related studies of various authors.

## MATERIAL AND PROCEDURE

In this paper, pertinent data of 14 different published works will be reviewed. These are some longevity experiments carried out with *D. melanogaster* in which the life tables were constructed at 25°C,

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but other environmental factors (humidity, food, population density and other treatments) may vary to some extent. In this study, life-table data of various types of (wild type) cultures, autosomal and sexual mutant strains, were examined and the results will be presented.

RESULTS

*Life-Span of w.t. cultures*

In table 1, the mean life-spans of some *D.melanogaster* w.t. stocks as measured by various authors at 25°C are presented. The differences observed may partly be due to differences in some environmental factors or due to strain differenoes. However when the same stock, the oregon w.t. was tested at different times even by the same author, the mean longevity has been found to be quite changeable.

The average life-span for 7 repeats of Oregon w.t. is 61.01; days; and for 9 different kinds of w.t. stocks in 16 experiments it is found to be 54.20 $\bar{+}$ 3.95 days. For 16 repeats of w.t.*D.melanogaster* male populations the mean is 57.39 $\bar{+}$ 3.84 days and for 17 repeats of female populations it is 53.74 $\bar{+}$ 3.52. It seems that there is no apparent difference between the mean duration of life of the sexes of various w.t. cultures of Drosophila tested.

*Life-Span of Autosomal Mutants*

As a further step, 12 different autosomal mutants all affecting morphological characters were studied and their expression in each sex compared (Table 2). In the table, in addition to the chromosomal locus of each gene, percentage decrease in mean life-span of each mutant against the respective w.t. is also calculated and given. Out of 19 populations of mutants studied, 6 of them belonged to the 2$^{nd}$ chromosome *vestigial* (vg) mutant with an average of 32.30 days. The mean duration of adult life of *black body, purple eyes, vestigial wings* (6 repeats) *arc wings, speck wings, vestigial-nipped, ebony* (2 repeats),*spineless, eyeless* (2 repeats), *brown eyes, rolled wings* and *sepia eyes* is 40.52 $\bar{+}$ 3.43 days in males,

and 40.01 ± 2.77 days in females. Although there is a twofold difference between the means of the sexes of the *spineless* mutant in favour of females, there is no pronounced difference between the overall means of males and females of mutants located on 3 different pairs of autosomal chromosomes.

The overall sex-mixed mean longevity for these mutants is 40.25 ± 2.91 days which means that 12 types of autosomal mutant genes located on 3 different chromosome pairs cause in general a 26.40 % decrease in comparison to the mean of the various w.t. cultures (Table 1). No reduction of the mean life-span of *speck* and *black* mutant is observed; on the contrary, their life-span increased compared to that of the wildtype in the paper by GONZALEZ (1923); GONZALEZ's work should be repeated to verify that these mutants indeed cause a lengthened life-span.

*Sexual Mutants*

In Table 3. Various life-span data of single, double and triple combinations of mutants *white eyes*, *miniature wings* and *forked bristles* are presented. Although there is no satisfactory amount of data to draw a conclusion, it can be said at the moment that the decrease caused by 3 different single X-chromosome mutant against the respective w.t. Oregon is 28.62 % on the average while that of a double combinations of *w,m* and *f* mutant genes is 28.52 %. Triple mutant *w m f* has an average cumulative effect of about 45.48 % decrease.

In addition, the effects of these X-chromosome mutants on the life spans of males and females are compared. For the males, the overall average is 53.23 ± 3.21 and for the females it is 54.67 ± 3.29 days. Again, even for the sexual mutants, there is no sex-specific influence.

As it will be seen in these examples and others not mentioned here, that there is no positive correlation between the number of mutant genes and the degree of life span shortening.

Earlier BOZCUK (1978), by combining phenotypes of one sexual (w)

and one autosomal (wg) mutant gene in the same individual did not find a corresponding life-shortening; indeed this genetic manipulation extended life-span. For example, while sex-mixed means of *w* and of *vg* were 54.5 days and 43.32 days respectively, the hybrid obtained in the $F_2$ (with white eyes and vestigial wings-double mutant) lived an average of 55.84 days which is not diminished at all, in fact extended very much against the parental *vg* mutant line, perhaps due to heterosis occurring in the $F_2$. At the same time, the $F_1$ generation of males showing white eye-phenotype lived to a mean of 90.05 days which was lenghtened almost two-fold in comparison with the mean of the mutant parents. It was therefore strongly argued that the result was due to heterosis, and that merely the phenotype (eye-colour or wing shape) is not a determining factor in longevity. Again TROUT and KAPLAN (1970) by using the neurological sex-linked mutants called hyperkinetic and shaker-5 found similar results with females but males were not used in the experiments. The hyperkinetic ($hk^1$) mutant females has a mean life span of 39.9 days, while shaker-5 (sh-5) has 40.5 days. The double-mutant $hk^1$ $sh^5$ females produced a mean of 59.6 days while the w.t. Canton S females had 76.2 days of mean life-span.

*The Measure of Heterosis*

In order to demostrate the degree of heterosis in *D.melanogaster* various studies done with this species are examined, and for the ease of calculations and comparison, PARSONS's (1966) measure of heterosis is used and computed for each lot of life-table data. The summarused results are given in Table 4.

As it will be noticed in the table, some of the data concerns the hybridisation between the wild lines, whereas some others are hybridisation between one w.t. and one mutant and the rest are hybridisations between mutants. Whenever possible the measures are included for the $F_2$ generation. It will be understood from the collected and calculated data that the measure of heterosis is not

dependent in a simple way on whether the parents are w.t. or mutant, or upon the generation at which heterosis is measured. If the autosomal and/or sexual mutant genes are hybridised with each other their detrimental effects in relation to life-span are cured by hybridisation both in the $F_1$ and $F_2$ generation due to heterosis (BOZCUK 1981). However further study is needed in this subject because the mechanism of heterosis may be quite complicated and needs to be understood more clearly.

DISCUSSION

After the review of existing data one is led to the following conclusions:

i. Each w.t. strain of *D.melanogaster* has a characteristic life span, but there is quite a variation among the various repeats of experiments even in the same culture (e.g. Oregon w.t. in Table 1) with the same experimenter.

ii. Contrary to some of the previous reports, there is no clearcut difference of life-span between the sexes of w.t. strains, namely, the females are not the longer-lived sex.

iii. Each mutant gene, whether autosomal or sexual, cause a decrease in life span to a certain degree, and on the average autosomals induce 26.4 % and sexual mutants 22.1% in comparison with respective w.t. oregon (control)flies.

iv. There is no consistent sexual influence of both autosomal as well as sexual mutants on the mean duration of adult life.

v. As the number of sexual mutant genes increased in a individual, this does not result in a proportional decrease in the life span, and there is no direct relationship between the number of mutant genes present and the amount of decrease exerted.

vi. There is no indication of a specific effect of the chromosomes, and the chromosomal locus at which the gene is

located does not have an important bearing on the determination of longevity.

vii. The measure of heterosis (PARSONS, 1966) is not dependent in a simple way on whether the parents are w.t. or mutant or upon the generation at which heterosis is measured (BOZCUK 1981).

Although each mutant gene changes and reduces longevity of flies to a certain degree it is believed that it is not only the single genes themselves but also the whole genome and their interactions and pleitropic effects which may be determinative in this process.This was also found earlier by CLARK and GOULD (1970) where it was stated that the mangnitude of the difference in adult life span between each mutant and its wild type allele is related to genetic background.

Lints et al (1979) considers that "very large phenotypic variability displayed by longevity in wild strains of *D.melanogaster* does not depend on a precise set of specific genes or polygenes with additive action". The fact that the $F_1$ and $F_2$ generations, in the experiments of BOZCUK (1978), express the morphological mutant character but with a lenghtened life-span in comparison with the parents, could be another proof for the above statement of LINTS et al (1979).

Earlier views that "females generally have a longer life span than males" is not varranted by the present data obtained from w.t., autosomal and sexual mutants. In fact, there is also some data showing that life span is affected by the physical activity of the flies (RAGLAND and SOHAL, 1973).

As suggested by SACHER (1978) there is no evidence for the "senescence gene theory". Furthermore, LINTS (1980) adds that there is no evidence of a mutant to prolong life-span, therefore evidence from major genes is not conclusive in favour of specific "longevity genes". A third hypothesis has recently been added to the previous two by SACHER (1978) which is called by him "longevity assurance

genes". While taking the results of HART and SETLOW (1974) as a support he also foresees that acceptance of the hypothesis of longevity-assurance genes implies that by developing appropriate means our evolved and genetically controlled longevity assurance mechanisms could be manipulated.

SUMMARY

Various autosomal and sexual mutant genes of *Drosophila melanogaster* were examined by reviewing the pertinent literature to determine their specific effects on adult longevity. It was concluded that each gene, whether autosomal or sexual may not have a sex-specific influence on the life-span and the comparison of the overall means of different sexes in mutants support this view strongly. It is noticed that not only a morphological character itself but more importantly the complete genetic constitution is determinative in controlling longevity.

If the measure of heterosis is calculated from the data of various authors, it is concluded that it is not dependent in a simple way on whether the parents are w.t. or mutant, or upon the generation at which heterosis is measured. If the autosomal and/or sexual mutant genes are hybridised with each other it will be seen that their detrimental effects in relation to life-span are cured by hybridisation both in the $F_1$ and $F_2$ due to heterosis.

The results are discussed from the point of view of the genetic program of ageing.

ÖZET

Drosophila melanogaster'in çeşitli otozomal ve seksüel mutant genlerinin, ergin ömrü üzerine özel etkilerini saptamak amacıyla ilgili literatür gözden geçirilerek incelendi. Her genin, otozomal ya da eşeysel olsun, ömür uzunluğu üzerine eşeyliğe özgü bir etkiye sahip olmayabileceği sonucu çıkarıldı. Mutantların ayrı sekslerinin genel ortalamasının karşılaştırılması bu görüşü kuvvetle desteklemektedir. Yalnız bir morfolojik karakter kendi başına değil daha önemlisi tüm genetik yapının ömrün denetiminde saptayıcı rol

oynadığı dikkati çekmektedir.

Eğer çeşitli yazarların verilerinden heterozis ölçüsü hesaplanırsa, bunun basit bir şekilde atasoyun normal tip (w.t.) ya da mutant olmasına, ya da heterozis'in ölçüldüğü generasyona bağlı olmadığı sonucuna ulaşılmaktadır. Eğer otozomal ve/veya seksüel mutant genler birbirleri ile melezlenirse bunların ömür uzunluğuna ilişkin zararlı etkilerinin melezleme ile hem $F_1$ ve hem de $F_2$'de heterozis nedeniyle tamir edildiği gözlenmektedir.

Sonuçlar "yaşlanmanın genetik programı" görüş açısından tartışılmaktadır.

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TABLE 1

MEAN LIFE SPANS OF SOME D.MELANOGASTER w.t. CULTURES AS MEASURED BY VARIOUS AUTHORS AT 25°C

| w.t. strain | mean life-spans of males | mean life-spans of females | sex-mixed mean life-span (days) | Authors and Source |
|---|---|---|---|---|
| Old Falmouth | 38.3 | 40.62 | 39.46 | Gonzales (1923) |
| N.and Y.Lines | 44.68 | 33.25 | 38.96 | Parsons (1966) |
| Oregon R. | 67.3 | 56.15 | 61.72 | Clark and Gould (1970) |
| Swedish-C | 37.1 | 29.60 | 33.35 | " " " |
| Canton-S | -- | 76.2 | -- | Trout and Kaplan (1970) |
| Oregon | 40.6 | 44.1 | 42.35 | Woodhams and Hollingsworth (1971) |
| Kaduna | 48.7 | 41.3 | 45.00 | " " " |
| Oregon-R | 38 | 42 | 40 | Biscardi and Webster (1977) |
| Oregon | 78.02 | 74.90 | 76.46 | Bozcuk (1978) |
| Hacettepe | 58.45 | 55.99 | 57.22 | " " |
| Keçiören | 77.79 | 76.39 | 77.09 | " " |
| Hacettepe | 52.37 | 61.47 | 56.92 | Bozcuk et al (1979) |
| Magosa | 62.56 | 53.56 | 58.06 | " " " |
| Oregon | 66.95 | 60.59 | 63.77 | Ünlü and Bozcuk (1979 a) |
| Oregon | 84.98 | 78.67 | 81.82 | Ünlü and Bozcuk (1979 b) |
| Oregon | 59.80 | 62.23 | 61.01 | Ünlü and Bozcuk (1979 c) |
| Hacettepe | 62.72 | 56.64 | 59.68 | Bozcuk (1981) |
| n : | 16 | 17 | 16 | |
| $\bar{X}$ : | 57.39 | 53.74 | 54.20 | (Mean for Oregon-7 repeats: 61.01 days) |
| $S\bar{x}$ : | 3.84 | 3.52 | 3.95 | |

TABLE 2

MEAN LIFE-SPANS OF SOME D.MELANOGASTER AUTOSOMAL MUTANTS AS MEASURED BY VARIOUS AUTHORS AT 25°C

| name of autosomal mutant | chroma-somal locus | mean life span of males (days) | mean life span of females (days) | sex-mixed mean-life span (days) | the mean of the w.t. used as control | % decrease against respective w.t. (sex-mixed) | authors and source |
|---|---|---|---|---|---|---|---|
| black body | 2-48.0 | 41.03 | 40.33 | 40.68 | 39.46 | +3.09 (increase) | Gonzales (1923) |
| purple eyes | 2-53.5 | 27.42 | 21.83 | 24.62 | " | 37.60 | " |
| vestigial wings | 2-69.7 | 14.97 | 20.93 | 17.95 | " | 54.52 | " |
| arc wings | 2-99.2 | 25.20 | 28.40 | 26.80 | " | 32.09 | " |
| speck wings | 2-107.0 | 46.63 | 38.91 | 42.77 | " | +8.38 (increase) | " |
| vg | 2-69.7 | 36.7 | 33.15 | 34.92 | 61.72 | 43.43 | Clark and Gould (1970) |
| vgmp | " | 62.2 | 48.9 | 55.55 | " | 10 | " " " |
| vg | 2-69.7 | 10.0 | 11.5 | 10.7 | 40 | 73.75 | Biscardi and Webster (1977) |
| ebony | 3-77.9 | 35 | 38 | 36.5 | 40 | 8.75 | " " " |
| vg | — | 38.79 | 47.86 | 43.32 | 63.53 | 31.82 | Bozcuk (1978) |
| vg (Hacettepe) | — | 38.35 | 41.01 | 39.68 | 57.49 | 30.98 | Bozcuk et al (1979) |
| vg (Helsinki) | — | 44.53 | 49.97 | 47.25 | " | 17.82 | " " |
| spineless | 3-58.5 | 28.56 | 56.61 | 42.58 | 61.01 | 30.21 | Ünlü and Bozcuk (1979 c) |
| eyeless | 4-2.0 | 53.59 | 46.13 | 49.86 | " | 28.28 | " " |
| brown | 2-104.5 | 63.90 | 48.25 | 55.92 | " | 8.35 | " " |
| rolled | 2-55.1 | 44.28 | 43.65 | 43.96 | 59.68 | 26.35 | Bozcuk (1981) |
| sepia | 3-26.0 | 61.72 | 55.93 | 58.82 | " | 1.36 | " " |
| ebony | 3-77.7 | 45.88 | 46.70 | 46.29 | " | 22.44 | " " |
| eyeless | 4-2.0 | 51.14 | 42.24 | 46.69 | " | 21.77 | " " |
| n : | | 19 | 19 | 19 | (7) | | |
| $\bar{x}$ : | | 40.52 | 40.01 | 40.25 | $\bar{\bar{x}}$: 54.69 | 26.40 % decrease | |
| Sx : | | 3.43 | 2.77 | 2.91 | | | |

TABLE 3: SEXUAL MUTANTS

Mean life spans of some D.melanogaster sexual mutants as measured by various authors at 25°C

| Name of Mutant | Locus | Mean life span of males (days) | Mean life span of females (days) | sex-mixed mean life-span | the mean of the w.t. used as control | % decrease against respective control | author and source |
|---|---|---|---|---|---|---|---|
| w (white eyes) | 1-1.5 | 52.24 | 56.76 | 54.5 | 63.53 | 14.22 | Bozcuk (1978) |
| w | 1-1.5 | 64.05 | 67.63 | 65.84 | 81.82 | 19.63 | Ünlü and Bozcuk (1979 b) |
| m(miniature wings) | 1-36.1 | 52.33 | 60.73 | 56.53 | | 30.91 | " " |
| f(forked bristles) | 1-56.7 | 51.71 | 54.43 | 53.07 | | 35.24 | " " |
| wm | | 54.35 | 60.44 | 57.39 | | 29.86 | " " |
| mf | | 60.42 | 57.73 | 59.07 | | 27.81 | " " |
| wf | | 63.59 | 55.18 | 59.38 | | 27.43 | " " |
| wmf | | 48.10 | 45.70 | 49.90 | | 42.58 | " " |
| wmf | | 32.34 | 33.50 | 32.92 | 63.77 | 48.38 | Ünlü and Bozcuk (1979 a) |
| | n : | 9 | 9 | 9 | (3) | | |
| | $\bar{x}$ : | 53.23 | 54.67 | 54.29 | 69.70 | 22.10 % decrease | |
| | $S_{\bar{x}}$ : | 3.21 | 3.29 | 3.06 | | | |

TABLE 4: MEASURE OF HETEROSİS

| SOURCE | Type of Hybridisation | Parsons's (1966) measure of heterosis for $F_1$ | $F_2$ |
|---|---|---|---|
| Parsons (1966) | N (w.t.) X Y (w.t.) Lines | 0.101 | - |
| Lints and Lints (1968) | Gabarros (w.t.) X Abeele (w.t.) | 0.217 | - |
| Woodhams and Hollingsworth (1971) | Oregon (w.t.) X Kaduna (w.t.) | 0.120 | 0.144 |
| Bozcuk (1978) | vg X w mutants | 0.277* | - |
| Ünlü and Bozcuk (1979 a) | W m f (triple Mutant) X Oregon (w.t.) | 0.236 | 0.132 |
| Bozcuk et al (1979) | Vg (Hacettepe X vg (Helsinki) | 0.149 | - |
| Bozcuk (1981) | rl X se; e X $ey^2$ mutants | 0.149 | 0.173 |

*: misprinted as 0.283 in the original paper.

# THE EFFECTS OF ADENOSINE, NaCl AND KCl ON GROWTH OF HUMAN EPIDERMOID CARCINOMA (Hep-2) CELLS

(Adenosin, KCl ve NaCl'ün İnsan Epidermoid Karsinoma Hücrelerinin Üremesine Etkileri)

Çervin ÇIRAKOĞLU* Meltem ÇEVİK** Gülsel OMURTAY**

## SUMMARY

Effective concentrations of adenosine, NaCl and KCl which inhibit growth of Hep-2 cells were determined. 5mM adenosine inhibit growth of Hep-2 cells.

Hypertonicity effected by elevation of NaCl or KCl concentrations in the growth medium, inhibited growth of Hep-2 cells, accompanied by a complete breakdown of polyribosomes. 150mM NaCl and 200mM KCl were the effective concentrations which inhibited growth of Hep-2 cells. Hep-2 cells started to grow normally upon restoration of isotonicity.

## INTRODUCTION

At present time, a number of inhibitors are available which inhibit growth of cultured mammalian cells (SOBORIO et al ,1974). We wanted to test some of these

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inhibitors on growth of human epidermoid carcinoma (Hep-2) cells.

In this study, we investigated the growth inhibitory effects of adenosine, NaCl and KCl on Hep-2 cells. We found that above compounds which inhibit the growth of cultured mammalian cells, also inhibited the growth of a kind of tumor cell called Hep-2.

## MATERIALS AND METHODS

### I. CELLS AND MEDIA

Human epidermoid carcinoma (Hep-2) cells were obtained from World Health Organization (WHO) Geneva, Switzerland. Hep-2 cells were grown in Eagle's minimal essential medium (MEM) supplemented with 10 % newborn calf serum and intibiotics.

### II. CHEMICALS

Adenosine was obtained from BDH Chemicals Ltd. Poole, England. 50mM stock adenosine solution was prepared and sterilized through millipore filter. NaCl and KCl were obtained from BDH Chemicals Ltd. Poole, England. 1M NaCl and 2M KCl stock solutions were prepared and sterilized through millipore filter.

## RESULTS

### *I. Growth Inhibitory Effect Of Adenosine*

Adenosine was added into the growth medium of Hep-2

cells to give a final concentration of 5mM, immediately after the passage of cells. Hep-2 cells were incubated with adenosine containing medium overnight. After adenosine treatment, cells were examined under microscope. 80 % of the cells detached from the glass surface and floating in the medium. Approximately 20 % of the cells attached to the glass surface but no growth was observed. Morphology of cells changed. Cells rounded and they were smaller than the control cells. When the adenosine treated Hep-2 cells were washed once with MEM gently, cells were lost. This indicates that in the presence of adenosine, cells attached the glass surface very weakly.

*II. Effects of NaCl and KCl on growth of Hep-2 cells*

It was reported that 100mM NaCl inhibited growth of HeLa cells (ROBBINS et. al, 1970). But 100mM NaCl inhibited growth of Hep-2 cells nearly 70 %. We found that 150 mM NaCl was the effective concentration that inhibited growth of Hep-2 cells (Table I). Hep-2 cells shrinked and rounded after treatment with 150mM NaCl. After removing the NaCl from the culture medium, cells recovered in two days and grew, but growth was poor in comparison to the control Hep-2 culture. We tested 100mM, 150mM and 200mM KCl concentrations on growth of Hep-2 cells. We found that 200mM KCl inhibited growth of Hep-2 cells effectively.

Morphological changes induced with KCl were similar to those seen with NaCl. When KCl was removed from the culture medium, Hep-2 cells recovered and grew in 24 hours.

*Table I. Effective concentrations of compounds that inhibit growth of Hep-2 cells*

| *Compound* | *Concentration mM* |
|---|---|
| *Adenosine* | *5* |
| *NaCl* | *150* |
| *KCl* | *200* |

## DISCUSSION

We established the effective concentrations NaCl, KCl and adenosine which inhibited the growth of Hep-2 cells. 5mM adenosine inhibited the growth of Hep-2 cells effectively.

The addition of adenosine to cultured mammalian cells leads to inhibition of pyrimidine synthesis (FOX and KELLEY, 1978), and cause depletion of pyrimidine nucleotide pool (GREEN and CHAN, 1973). Rapidly growing cells like Hep-2 needs more pyrimidine synthesis than slow growing ones.

150mM NaCl and 200mM KCl inhibited the growth of Hep-2 cells by inhibiting protein synthesis. It was reported that hypertonic medium established with high concentrations of NaCl or KCl cause complete breakdown of polyribosomes (WENGLER and WENGLER, 1972). When NaCl or KCl was removed from the medium, upon restoration of isotonicity cells

recovered. This shows that incubation of the cells by hypertonic medium, does not result in irreversible damage to the protein synthesizing machinery.

Although KCL, NaCl and adenosine inhibited the growth of Hep-2 cells effectively invitro. But invivo application of these compounds to inhibit the malignant cell growth is disputable.


## ÖZET

Hep-2 hücrelerinin üremesini inhibe eden adenozin, NaCl ve KCl'ün etkin konsantrasyonları tayin edildi. 5mM adenozin Hep-2 hücrelerinin üremesini inhibe etti.

Üreme ortamında NaCl veya KCl'ün konsantrasyonlarının yükselmesi ile oluşan hipertonisite poliribozomların yıkımına neden olarak Hep-2 hücrelerinin üremesini inhibe etti. 150mM NaCl ve 200mM KCl, Hep-2 hücrelerinin üremesini inhibe eden etkin konsantrasyonlardı. İzotonisitenin sağlanması üzerine Hep-2 hücreleri normal olarak üremeğe başladı.


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# THE FISH SPECIES OF THE SAKARYA BASIN AND THEIR ABUNDANCE

## (Sakarya Havzasındaki Balık Türlerinin Dağılımları Üzerine Araştırmalar)*

Füsun ERK`AKAN**


## SUMMARY

The Sakarya basin was divided into three districts separated by flow and adjacent Sarıyar and Gökçekaya dams. At least 7 localities from each district were examined and environmental parameters of them were recorded together with the abundance and distribution of the fish species.


## INTRODUCTION

The purpose of this study is to determine the fish species from the Sakarya basin and their releationship with environmental parameters. The literature on Turkish freshwaters covers no research studies similar to this subject except those of ARAS(1974), SOLAK(1977),KARABATAK (1977) and AKGÜL(1980) and some others who have only considered some environmental parameters such as temperature and vegetation but have not considered the % population. These studies covered only certain species and they were far from being exhaustive.

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## SAMPLING SITES

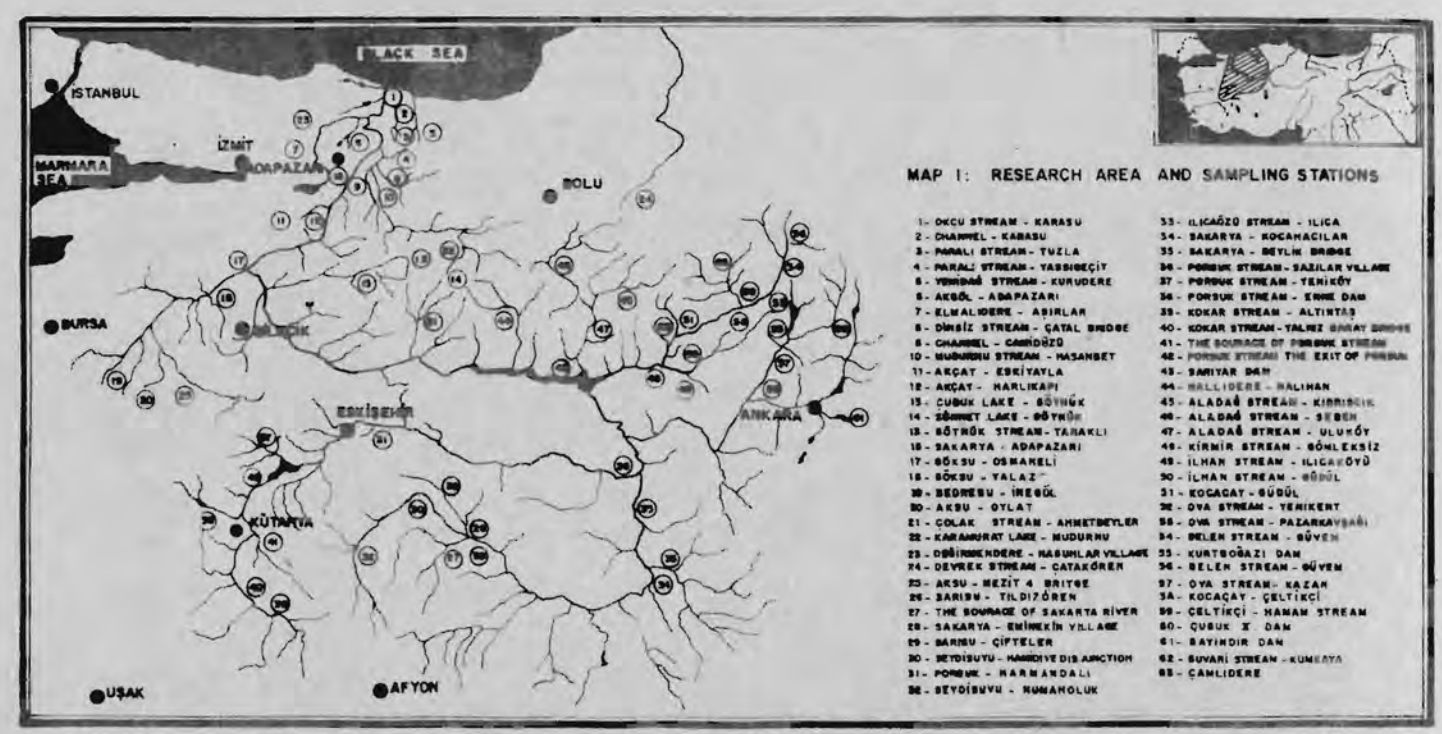
The Sakarya basin was separated into three districts according to the water flow and the Sarıyar and Gökçekaya dams which were accepted as obstacles dividing the natural fauna (Map 1). These three districts were:

1. Lower part of the Sakarya basin
2. Upper Sakarya and Porsuk basins
3. Mürted-Çubuk and Kirmir basins

1. Lower part of the Sakarya basin: This basin extends from the Gökçekaya Dam to the Black Sea into which the Sakarya River empties. From this basin 25 localities having different bottom, current and flow of water were chosen for fishing (Map 1). Of these, 8 localities having characteristics typical of the district were chosen to determine some environmental parameters, which are summarised in Table 1.

2. Upper Sakarya and Porsuk basins: This basin has the source of the Sakarya River and Porsuk stream which is the biggest branch of it. In this basin, 18 localities were chosen for fishing (Map 1). Some environmental parameters from 8 of these localities were recorded and summarised in Table 2.

3. Mürted-Çubuk and Kirmir basıns: The streams of this basin near Ankara and empty into the Sarıyar Dam.20 localities were chosen for fishing and from these,environmental parameters of 7 localities were recorded and summarised in Table 3.




MAP 1: RESEARCH AREA AND SAMPLING STATIONS

1- OKÇU STREAM - KARASU
2- CHANNEL - KARASU
3- PARALI STREAM - TUZLA
4- PARALI STREAM - YASSIBEÇİT
5- YENİDAĞ STREAM - KURUDERE
6- AKGÖL - ADAPAZARI
7- ELMALIDERE - ABIRLAR
8- DİNSİZ STREAM - ÇATAL BRIDGE
9- CHANNEL - CAMİDİZÜ
10- MUDURNU STREAM - HASANBEY
11- AKÇAT - ESKİYAYLA
12- AKÇAT - HARLIKAPI
13- CUBUK LAKE - BÖYNÜK
14- SÜNNET LAKE - BÖYNÜK
15- BÖTNÜK STREAM - TANAKLI
16- SAKARYA - ADAPAZARI
17- BÖKSU - OSMANELİ
18- BÖKSU - YALAZ
19- BEDRESU - İNEGÖL
20- AKSU - OYLAT
21- ÇOLAK STREAM - AHMETDEYLER
22- KARAMURAT LAKE - MUDURNU
23- DÜĞİRMENDERE - HASUMLAR VILLAGE
24- DEVREK STREAM - ÇATAKÖREN
25- AKSU - NEZİT 4 BRITGE
26- BARISU - TILDIZÖREN
27- THE SOURACE OF SAKARYA RİVER
28- SAKARYA - EMİNEKİN VILLAGE
29- BARISU - ÇİFTELER
30- BEYDİSUYU - HAMİDİYE DIS ARRCTION
31- PORSUK - HARMANDALI
32- BEYDİSUYU - KUMANOLUK
33- ILICAĞZÜ STREAM - ILICA
34- SAKARYA - KOCAHACILAR
35- SAKARYA - BEYLİK BRIDGE
36- PORSUK STREAM - SAZILAR VILLAGE
37- PORSUK STREAM - YENİKÖY
38- PORSUK STREAM - ENNE DAM
39- KOKAR STREAM - ALTINTAŞ
40- KOKAR STREAM - YALNIZ GARAY BRIDGE
41- THE SOURACE OF PORSUK STREAM
42- PORSUK STREAM THE EXIT OF PORSUK
43- SARIYAR DAM
44- HALLIDERE - HALIMAN
45- ALADAĞ STREAM - KIRIBIÇIK
46- ALADAĞ STREAM - SEBEN
47- ALADAĞ STREAM - ULUKÖY
48- KİRMİR STREAM - GÖNLEKSİZ
49- İLHAN STREAM - ILICAKÖYÜ
50- İLHAN STREAM - GÖĞÜL
51- KOCAÇAY - GÖĞÜL
52- OVA STREAM - YENİKENT
53- OVA STREAM - PAZARKAVŞAĞI
54- BELEN STREAM - GÜVEN
55- KURTGÖĞAZI DAM
56- BELEN STREAM - GÜVEN
57- OYA STREAM - KAZAN
58- KOCAÇAY - ÇELTİKÇİ
59- ÇELTİKÇİ - HAMAM STREAM
60- ÇUBUK X DAM
61- BAYINDIR DAM
62- SUVARİ STREAM - KUMKAYA
63- ÇAMLIDERE

*TABLE 1. Some environmental parameters in District 1.*

| Localities | Date and time | Water | Temp. water (°C) | Temp. air (°C) | Current | Depth of capture (cm) | Method of capture | Dissolved $O_2$ (ppm) | pH | Conductivity (μMHOS/cm) | Type of Bottom |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Göksu (Yenişehir-Yalaz) | 3.VI.1979 10.30-11.30 | Turbid | 15 | 24 | Rapid | 50 | Electro-shocker | 8.8 | 8.10 | 220 | Sand-gravel |
| | 12.VIII.1979 9.30-10.30 | Clear | 22 | 26 | Medium | 60 | | 7.2 | 7.35 | 435 | |
| Aksu (İnegöl) | 2.VI.1979 14.30-15.00 | Turbid | 15 | 22 | Rapid | 60 | Electro-shocker | 8.4 | 8.25 | 150 | Stony, rubble and sandy |
| | 11.VIII.1979 12.30-13.30 | Clear | 22 | 30 | Rapid | 50 | | 8.2 | 8.20 | 260 | |
| Kocasu (Osmaneli-Bilecik) | 3.VI.1979 16.00-16.30 | Turbid | 16 | 28 | Rapid | 50 | Electro-shocker | 8.4 | 8.10 | 240 | sandy and soft silt |
| | 12.VIII.1979 14.30-16.00 | Turbid | 26 | 32 | Slow | 80 | | 7.8 | 8.25 | 500 | |
| Göynük stream (Taraklı-Göynük) | 15.VI.1979 13.30-14.30 | Clear | 18 | 28 | Medium | 100 | Electro-shocker | 9.4 | 7.70 | 310 | stony and sandy |
| | 26.VIII.1979 15.00-16.00 | Clear | 18 | 32 | Medium | 100 | | 9.2 | 7.75 | 320 | |
| Akçay (Adapazarı) | 15.VI.1979 10.00-11.00 | Turbid | 23 | 26 | Stagnant | 60 | Electro-shocker | 8.4 | 8.05 | 250 | Stony and sandy-gravel |
| | 27.VIII.1979 17.30-19.00 | Turbid | 23 | 27 | Medium | 100 | | 8.2 | 8.25 | 270 | |
| Dinsiz stream (Çatalköprü-Adapazarı) | 16.VI.1979 9.30-11.00 | Turbid | 19 | 25 | Medium | 100 | Electro-shocker | 7.8 | 8.20 | 260 | Rubble, sandy and stony |
| | 27.VIII.1979 11.30-13.30 | Turbid | 23 | 32 | Medium | 100 | | 7.0 | 7.85 | 300 | |
| Kanal stream (Camidüzü-Adapazarı) | 16.VI.1979 12.30-14.00 | Turbid | 23 | 31 | Slow | 100 | Electro-shocker | 9.1 | 7.85 | 500 | Sandy gravel |
| | 27.VIII.1979 17.00-19.00 | Turbid | 26 | 32 | Stagnant | 60 | | 7.4 | 7.80 | 450 | |
| Paralı stream (Tuzla-Karasu) | 17.VI.1979 14.00-17.00 | Turbid | 25 | 32 | Stagnant | 400 | Otter trawl and experimental gill net | 8.0 | 8.20 | 500 | Soft silt |
| | 28.VIII.1979 17.30-1930 | Turbid | 23 | 25 | Stagnant | 400 | | 6.0 | 7.70 | 200 | |

*TABLE 2. Some environmental parameters in District 2.*

| Localities | Date and time | Water | Temp. water (°C) | Temp. air (°C) | Current | Depth of capture (cm) | Method of capture | Dissolved $O_2$ (ppm) | pH | Conductivity (µMHOS/cm) | Type of Bottom |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Seydisuyu (Yıldızören-Çifteler) | 5.V.1979 10.00-11.00 | Clear | 17 | 20 | Slow | 60 | Electro-shocker | 9.0 | 7.80 | 800 | Stony, rubble and sandy |
| | 21.VII.1979 11.30-12.30 | Clear | 25 | 30 | Medium | 60 | | 8.0 | 7.90 | 980 | |
| Sakarya (Eminekin-Çifteler) | 5.V.1979 12.00-13.30 | Clear | 18 | 23 | Medium | 150 | Electro-shocker | 7.5 | 7.50 | 750 | Stony, rubble and sandy |
| | 21.VII.1979 13.30-14.30 | Clear | 25 | 32 | Medium | 150 | | 6.0 | 7.70 | 800 | |
| Kokar stream (Yalnızsaray-Altıntaş) | 19.V.1979 17.00-18.00 | Turbid | 16 | 20 | Rapid | 150 | Otter trawl | 8.2 | 7.65 | 330 | Sandy and soft silt |
| | 4.VIII.1979 15.30-16.00 | Turbid | 24 | 30 | Slow | 60 | | 8.2 | 8.10 | 500 | |
| Seydisuyu (Hamidiye) | 6.V.1979 9.30-10.30 | Clear | 16 | 19 | Slow | 60 | Electro-shocker | 8.0 | 7.65 | 490 | Sandy-gravel |
| | 22.VII.1979 13.00-14.00 | Turbid | 19 | 26 | Slow | 60 | | 7.2 | 7.55 | 710 | |
| Seydisuyu (Numanoluk) | 6.V.1979 13.30-14.30 | Clear | 20 | 23 | Slow | 60 | Electro-shocker | 7.6 | 8.35 | 455 | Muddy and sandy |
| | 22.VII.1979 13.00-14.00 | Turbid | 26 | 32 | Slow | 60 | | 8.0 | 7.50 | 500 | |
| The source of Porsuk stream (Kütahya) | 19.V.1979 18.30-19.30 | Clear | 21 | 17 | Slow | 60 | Electro-shocker | 8.0 | 7.50 | 500 | Rubble |
| | 4.VIII.1979 21.30-22.00 | Clear | 21 | 20 | Slow | 60 | | 7.2 | 7.50 | 500 | |
| The exit of Enne Dam (Kütahya) | 20.V.1979 9.30-11.00 | Turbid | 14 | 17 | Rapid | 50 | Electro-shocker | 8.8 | 8.35 | 410 | Stony and sandy gravel |
| | 5.VIII.1979 10.00-12.00 | Clear | 21 | 26 | Medium | 100 | | 9.6 | 8.25 | 500 | |
| Porsuk stream (Yeniköy) | 20.V.1979 13.30-15.00 | Turbid | 15 | 18 | Rapid | 100 | Electro-shocker | 8.5 | 8.25 | 450 | Sandy-gravel |
| | 5.VIII.1979 14.30-15.00 | Turbid | 23 | 28 | Rapid | 60 | | 8.8 | 8.35 | 700 | |

*TABLE 3. Some environmental parameters in District 3.*

| Localities | Date and time | Water | Temp. water(°C) | Temp. air(°C) | Current | Depth of capture | Method of capture | Dissolved $O_2$(ppm) | pH | Conductivity (μMHOS/cm) | Type of Bottom |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Kocaçay (Güdül) | 22.V.1979 11.00-13.00 | Turbid | 16 | 19 | Rapid | 100 | Electro-shocker | 9.4 | 8.25 | 290 | Stony and rubble |
| | 25.VII.1979 12.00-14.00 | Turbid | 25 | 29 | Rapid | 60 | | 8.0 | 8.40 | 580 | |
| Kirmir stream (Gömlekaiz) | 11.V.1979 11.30-13.00 | Turbid | 23 | 29 | Rapid | 60 | Electro-shocker | 8.0 | 7.60 | 700 | Stony and sandy-gravel |
| | 18.VIII.1979 14.30-15.30 | Turbid | 25 | 33 | Rapid | 100 | | 8.1 | 7.85 | 800 | |
| Süvari stream (Kumkaya) | 22.V.1979 14.00-16.00 | Clear | 18 | 19 | Rapid | 100 | Electro-shocker | 9.2 | 8.20 | 170 | Stony and sandy-gravel |
| | 18.VII.1979 11.30-12.00 | Clear | 19 | 33 | Medium | 40 | | 7.4 | 8.65 | 1000 | |
| Belen stream (Kızılcahamam) | 25.V.1979 13.30-14.30 | Clear | 19 | 21 | Rapid | 65 | Electro-shocker | 8.6 | 8.60 | 100 | Stony and sandy gravel |
| | 11.VII.1979 14.30-15.00 | Clear | 21 | 26 | Medium | 50 | | 8.7 | 8.70 | 330 | |
| Hamam stream (Çeltikçi) | 25.V.1979 12.00-13.00 | Turbid | 17 | 20 | Rapid | 50 | Electro-shocker | 11.0 | 8.40 | 160 | Stony and sandy gravel |
| | 11.VII.1979 16.00-17.00 | Clear | 20 | 26 | Medium | 50 | | 8.0 | 8.35 | 300 | |
| Ova stream (Kurtboğazı) | 25.V.1979 10.30-11.30 | Clear | 17 | 19 | Medium | 70 | Electro-shocker | 8.6 | 8.65 | 200 | Sandy and rubble |
| | 11.VII.1979 11.40-12.30 | Clear | 20 | 24 | Medium | 50 | | 10.4 | 8.70 | 310 | |
| Aladag stream (Çayırhan) | 15.VI.1979 10.30-11.30 | Clear | 19 | 23 | Slow | 150 | Otter trawl and electro-shocker | 9 | 8.15 | 480 | Sandy |
| | 26.VII.1979 9.30-10.30 | Clear | 18 | 30 | Slow | 100 | | 8.4 | 7.75 | 800 | |

## MATERIALS AND METHODS

Specimens were collected using and experimental gill net, an otter trawl and an electroshocker. Fish were fixed in 4 % formalin immediately on capture and placed in plastic bags, carried to the laboratory and stored in 70 % ethanol.

Dissolved oxygen was determined using a portable, 5514 model oxygen meter. Water temperatures were also determined with this apparatus which has a maximum error of 2 % in a range of -5°C to 45°C.

The pH of the water was determined with a portable, 5985-40 model pH meter which can read values as small as 0.05.

The conductivity of water was determined with a portable YSI model 33 S-C-T meter. Salinities between 40-0 ‰ and temperatures between -2 and -45°C can also be determined by using this apparatus.

The bottom and flow and other physical properties of water were observed and recorded.

## THE ABUNDANCE AND DISTRIBUTION OF THE FISHES

The abundance and distribution of the fishes are summarised in Table 4, 5 and 6 for these three districts.

During this study, the effect of the environmental parameters on only 6 species namely *Alburnus orontis*,

*Leuciscus cephalus, Chondrostoma nasus, Capoeta tinca, Capoeta capoeta and Barbus plebejus,* which are considered economically important by the local people, are discussed. The dissolved oxygen level of water increases in the order District 1 < District 2 < District 3. The reason for the highest oxygen level in District 3 is its mountainous nature (Table 3).

TABLE 4. The abundance of the fish species in District 1.

| Localities | Date and time | Fish species and their abundances. |
|---|---|---|
| Göksu Yenişehir-Yalaz) | 3.VI.1979 10.30-11.30 | 50 % L.*cephalus*, 25 % *B.plebejus* 25 % *A. orontis* *Noemacheilus angorae (Total 24 specimens)* |
| | 12.VIII.1979 9.30-10.30 | 41 % *C.nasus*, 15 % *C.capoeta*, 44 % *B. plebejus*, *L.cephalus*, *A.orontis*, *N.angorae* (Total 46 specimens) |
| Aksu (İnegöl) | 2.VI.1979 14.30-15.00 | 46 % *Alburnoides bipunctatus*, 26 % *C.tinca* 28 % *L.cephalus*, *Gobio gobio*, *B. plebejus*, *N. angorae* (Total 71 specimens) |
| | 11.VIII.1979 12.30-13.30 | 44 % *Alburnoides bipunctatus*, 25 % *C. tinca*, 31 % *L.cephalus*, *G. gobio*, *B. plebejus* and *N. angorae* (Total 60 specimens) |
| Kocasu (Osmaneli-Bilecik) | 3.VI.1979 16.00-16.30 | 66 % *L.cephalus 17 % Chondrostoma nasus*, 17 % *C. capoeta* (Total 12 specimens) |
| | 12.VIII.1979 14.30-16.00 | 46 % *C.nasus*, *22 % C.capoeta* 32 % *A.orontis*, *L.cephalus*, *C.tinca*, *B.plebejus*, *Gobius fluvialitis* (Total 50 specimens) |
| Göynük stream (Taraklı-Göynük) | 15.VI.1979 13.00-14.30 | 40 % *A. bipunctatus*, 26 % *L. cephalus*, 34 % *B.plebejus*, *C. tinca*, *N.angorae*, *C.capoeta* (Total 138 specimens) |

TABLE 4-(Continued)

| Localities | Date and time | Fish species and their abundances. |
|---|---|---|
| Göynük stream (Taraklı-Göynük) | 27.VIII.1979 15.00-16.00 | 39 % *A.bipunctatus*, 28 % *L.cephalus*, 33 % *B.plebejus*, *C.tinca*, *N.angorae*, *C.capoeta* (Total 136 specimens) |
| Akçay (Adapazarı) | 15.VI.1979 10.00-11.00 | 63 % *C.tinca*, 14 % *L.cephalus*, 14 % *A.orontis*, *Rhodeus sericeus*, *N.angorae*, *C.nasus*, *G.gobio*, *B.plebejus*, *C.capoeta* (Total 265 specimens) |
| | 27.VIII.1979 17.30-19.00 | 71 % *C.tinca*, 19 % *L.cephalus*, 10 % *A. orontis*, *R.sericeus*, *N. angorae*, *C. nasus*, *G. gobio*, *B. plebejus*, *C.capoeta* (Total 274 specimens) |
| Dinsiz stream (Çatalköprü Adapazarı) | 16.VI.1979 9.30-11.00 | 40 % *R.sericeus*, 34 % *L.cephalus* 26 % *A. orontis*, *Cobitis aurata*, *N. angorae*, *Cyprinus carpio*, *Proterorhinus marmoratus*, *G. fluviatilis*, *Vimba vimba*, *Blicca bjoerkna*, *A. bipunctatus* (Total 250 specimens) |
| | 27.VII.1979 11.30-13.30 | 43 % *R. sericeus*, 23 % *L.cephalus* 34 % *A. orontis*, *C.aurata*, *N. angorae*, *C.carpio*, *P.marmoratus*, *G. fluviatilis*, *V. vimba*, *B. bjoerkna*, *A. bipunctatus* (Total 238 specimens) |
| Kanal stream (Camidüzü Adapazarı) | 16.VI.1979 12.30-14.00 | 55 % *L. cephalus*, 40 % *R.sericeus*, 5 % *C. aurata*, *C.nasus*, *A.bipunctatus* (Total 108 specimens) |
| | 27.VII.1979 17.00-19.00 | 64 % *R. sericeus*, 29 % *L.cephalus*, 7 % *C. aurata*, *A.bipunctatus*, *C. nasus*, *N.angorae*, *C. capoeta* (Total 103 specimens) |

TABLE 4-(Continued)

| Localities | Date and time | Fish species and their abundances. |
|---|---|---|
| Paralı stream Tuzla (Karasu) | 17.VI.1979 14.00-17.00 | 46 % *A.brama*, 31 % *B.bjoerkna*, 23 % *V. vimba*, *Tinca tinca*, *Perca fluviatilis*, *Esox lucius*, *G. fluviatilis*, *G.gymnotrachelus*, *C. aurata*, *Chalcalburnus chalcoides*, *Alburnus alburnus*, *A. orontis*, *R. sericeus*, *L.cephalus*, *L.borysthenicus*, *C.nasus*, *C. capoeta* (Total 646 specimens) |
| | 28.VII.1979 17.30-19.30 | 50 % *A. brama*, 33 % *B.bjoerkna*, 37 % same species with 17.VI. 1979 (Total 619 specimens) |

TABLE 5. The abundance of the fish species in District 2.

| Localities | Date and time | Fish species and their abundances |
|---|---|---|
| Aydisuyu (Yıldızören Çifteler) | 5.V.1979 10.00-11.00 | 27 % *L.cephalus*, 16 % *C.capoeta*, 57 % *A.bibunctatus*, *B.plebejus*, *C. tinca*, *A. orontis*, *G. gobio*, *C. nasus*, *Aphanius chantrei*, *V. vimba*, *C. taenia*, *N. angorae* (Total 94 specimens). |
| | 21.VII.1979 11.30-12.30 | 34 % *L. cephalus*, 14 % *C. capoeta* 52 % *A. orontis*, *C.nasus*, *A. chantrei*, *C. tinca*, *A.bipunctatus*, *B.plebejus*, *G.gobio*, *V. vimba*, *N. angorae* (Total 108 specimens). |
| Sakarya (Emineköy - Çifteler | 5.V.1979 12.00-13.30 | 57 % *A. orontis*, 12 % *L.cephalus*, 31 % *B. plebejus*, *C.capoeta*, *A. bipunctatus*, *C. tinca*, *Silurus glanis*, *C.carpio*, *C. vimba*, *A. chantrei* (Total 122 specimens). |
| | 21.VII.1979 13.03-14.30 | 41 % *A. orontis*, 18 % *L.cephalus* 41 % *B. plebejus*, *C.capoeta*, *S. glanis*, *A.bipunctatus*, *C.carpio*, *V. vimba* (Total 73 specimens). |
| Kokar stream | 19.V.1979 17.00-18.00 | 82 % *A.orontis*, 9 % *A.chantrei*, 9 % *A. bipunctatus*, *N.angorae*, *B. plebejus* (Total 194 specimens). |

TABLE 5-(Continued) .

| Localities | Date and time | Fish species and their abundances. |
|---|---|---|
| Yalnızsaray-Altıntaş) | 14.VIII.1979<br>15.30-16.30 | 85 % *A.orontis*, 10 %.*A.chantrei*, 5 % *A. bipunctatus*, *C. Capoeta*, *N. angorae* (Total 224 specimens). |
| Seydisuyu (Hamidiye) | 6.V.1979<br>9.30-10.30 | 31 % *L.cephalus*, 20 % *C.capoeta*, 49 % *C.tinca*, *A.orontis*, *C. nasus*, *G.gobio*, *A.bipunctatus*, *B.plebejus*, *N.angorae* (Total 193 specimens). |
| | 22.VII.1979<br>13.00-14.00 | 33 % *L.cephalus*, 19 % *C.tinca*, 51 % *C.capoeta*, *G.gobio*, *A. orontis*, *C.nasus*, *N.angorae* (Total 155 specimens) |
| Seydisuyu (Numanoluk) | 6.V.1979<br>13.30-14.30 | 32 % *L.cephalus*, 25 % *C.capoeta*, 33 % *C.tinca*, *C.nasus*, *G.gobio*, *B.plebejus*, *A.orontis*, *A.bipunctatus* (Total 95 specimens) |
| | 22.VII.1979<br>13.00-14.00 | 41 % *C.capoeta*, 20 % *L.cephalus*, 39 % *C.tinca*, *A.orontis*, *G.gobio* *C. taenia* (Total 97 specimens) |
| The source of Porsuk stream | 19.V.1979<br>18.30-19.30 | 50 % *B.plebejus*, 30 % *C.tinca*, 20 % *A.bipunctatus*, *C.capoeta*, *N.angorae* (Total 24 specimens) |
| | 4.VIII.1979<br>21.30-22.00 | 65 % *B.plebejus*, 14 % *A.bipunctatus*, 20 % *N.angorae*, *L. cephalus*, *C.tinca* (Total 37 specimens) |
| The exit of Enne Dam Kütahya) | 20.V.1979<br>9.30-11.00 | 33 % *B.plebejus*, 33 % *L.cephalus*, 34 % *A.bipunctatus*, *C. capoeta*, *C.tinca*, *G.gobio*, *A orontis* (Total 43 specimens) |
| | 5.VII.1979<br>10.00-12.00 | 47 % *L.cephalus*, 20 % *A.orontis*, 33 % *A.bipunctatus*, *B.plebejus*, *C.capoeta*, *C.tinca*, *G.gobio* (Total 60 specimens) |

TABLE 5-(Continued)

| Localities | Date and time | Fish species and their abundances. |
|---|---|---|
| Porsuk stream (Yeniköy) | 20.V.1979 13.30-15.30 | 33 % *A. orontis*, 17 % *C.tinca*, 50 % *C. capoeta*, *G. gobio*, *N. angorae*, *C.taenia* (Total 18 specimens) |
| | 5.VIII.1979 14.30-15.30 | 43 % *C.tinca*, 22 % *L.cephalus*, 35 % *A.orontis*, *C.capoeta*, *N. angorae*, *G.gobio*, *L.cephalus*, *B. plebejus*, *C. taenia* (Total 74 specimens) |

TABLE 6. The abundance of the fish species in District 3.

| Localities | Date and time | Fish species and their abundances. |
|---|---|---|
| Kocaçay (Güdül) | 22.V.1979 11.00-13.00 | 31 % *C. tinca*, 25 % *A.bipunctatus*, 34 % *B.plebejus*, *C. capoeta*, *V.vimba*, *L.cephalus* (Total 32 specimens) |
| | 25.VII.1979 12.00-14.00 | 33 % *L.cephalus*, 26 % *C.tinca*, 41 % *C. capoeta*, *B.plebejus*, *A. bipunctatus*, *N. angorae*, *A. orontis*, *V. vimba* (Total 126 specimens) |
| Kirmir stream (Cömlek-siz) | 11.V.1979 11.30-13.30 | 36 % *L.cephalus*, 20 % *C. capoeta* 44 % *A.orontis*, *V. vimba*, *A. bipunctatus*, *B.plebejus*, *C. tinca*, *N. angorae*, *C.taenia* (Total 83 specimens) |
| | 18.VIII.1979 14.30-15.30 | 36 % *L. cephalus*, 27 % *C.capoeta*, 37 % *C. tinca*, *A.orontis*, *A. bipunctatus*, *B. plebejus*, *C. taenia*, *N. angorae* (Total 56 specimens) |
| Süvari stream (Kumkaya) | 22.V.1979 14.00-16.00 | 50 % *A. bipunctatus*, 38 % *B.plebejus*, 12 % *L.cephalus*, (Total 16 specimens) |
| | 18.VII.1979 11.30-12.00 | 29 % *C. tinca*, 21 % *A.orontis*, 50 % *C. capoeta*, *L.cephalus*, *V. vimba*, *B.plebejus*, *C.nasus*, *A. bipunctatus*, *N. angorae*, *C. taenia* (Total 191 specimens) |

TABLE 6-(Continued)

| Localities | Date and time | Fish species and their abundances. |
|---|---|---|
| Belen stream (Kızılca-hamam) | 25.V.1979 13.30-14.30 | 38 % *C.tinca*, 31 %*L.cephalus*, 36 % *N.angorae*, *C.taenia*, *A. orontis*, *A. bipunctatus* (Total 47 specimens) |
| | 11.VII.1979 14.30-15.00 | 33 % *C. tinca*, 31 % *L.cephalus*, 30 % *N.angorae*, *C.taenia*, *A. orontis*, *A. bipunctatus* (Total 187 specimens) |
| Hamam stream (Çeltik-çi) | 25.V.1979 12.00-13.00 | 57 % *A. bipunctatus*, 27 % *N. angorae*, 16 % *L. cephalus*, *A. orontis*, *C.taenia* (Total 71 specimens) |
| | 11.VII.1979 16.00-17.00 | 74 % *L.cephalus*, 11 % *C. tinca*, 15 % *N.angorae*, *C. taenia*, *Cobitis simplicispinna* (Total 71 specimens) |
| Ova stream (Kurtbo-ğazı) | 25.V.1979 10.30-11.30 | 41 % *A.orontis*, 25 % *C. tinca*, 33 % *L.cephalus*, *C.taenia*, *N. angorae* (Total 126 specimens) |
| | 11.VII.1979 11.40-12.30 | 53 % *A. orontis*, 15 % *L.cephalus*, 32 % *C. tinca*, *N.angorae*, *C.taenia*, *C.simplicispinna* (Total 295 specimens) |
| Aladağ stream (Cayır-han) | 15.VI.1979 10.30-11.30 | 43 % *A. orontis*, 24 % *L.cephalus*, 33 % *C.capoeta*, *C.tinca*, *V.vimba*, *C.nasus*, *B.plebejus*, *A.bipunctatus*, (Total 124 specimens) |
| | 26.VIII.1979 9.30-10.30 | 38 % *A. orontis*, 26 % *L.cephalus*, 36 % *C.capoeta*, *C.tinca*, *V.vimba*, *B.plebejus*, *C.nasus*, *N.angorae*, *A. bipunctatus* (Total 117 specimens) |

The population densities of *Alburnus orontis, Leuciscus cephalus, Chondrostoma nasus, Capoeta tinca, Capoeta capoeta, Barbus plebejus* (Fam. Cyprinidae) and other species from different families are shown in Table 7.

Table 7. The population densities of the fish species.

| Species | District 1 | District 2 | District 3 |
|---|---|---|---|
| *Alburnus orontis* | 10 % | 24 % | 23 % |
| *Leuciscus cephalus* | 12 % | 26 % | 22 % |
| *Chondrostoma nasus* | 10 % | 10 % | 5 % |
| *Capoeta tinca* | 9 % | 11 % | 23 % |
| *Capoeta capoeta* | 7 % | 9 % | 7 % |
| *Barbus plebejus* | 5 % | 10 % | 6 % |
| *Others* | 47 %* | 10 % | 13 % |

*Alburnus orontis* has the highest population density in District 2 which indicates that this species prefers a stream which has a sandy-gravel bottom, high dissolved oxygen level, low pH values and generally high conductivity.

*Leuciscus cephalus* also has a highest population density in District 2 which indicates that it prefers the same kind of stream as *Alburnus orontis*.

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** In this district, the variety of the species was high but their population densities were low.*

*Chondrostoma nasus* has the same and the highest population density in District 1 and 2 so, we may conclude that this species prefers a muddy-sand bottom and medium or slow flow of water. Oxygen level, pH and conductivity determined during this study has no effect on this species.

*Capoeta tinca* has the highest population density in District 3. This species prefers water which has a sandy-gravel bottom, high dissolved oxygen level, pH values between 7.5-8.70. The conductivity determined during this study has no effect on *Capoeta tinca*.

*Capoeta capoeta* has the highest population density in District 2 which means that this species prefers medium or slow flow, muddy or sandy gravel bottom and water which has generally high oxygen level. pH values and conductivity determined during this study have no effect on this species.

*Barbus plebejus* also has the highest population density in District 2 indicating that it prefers sandy-gravel or rubble bottom and generally rapid flow of water with a high oxygen level, between 7.50-8.35 pH values and conductivity of 400-500 $\mu$MHOS/cm.

## DISCUSSION

In general, most of the species show low population densities in District 1. In fact, except *C. nasus* which shows its highest population density in this district, all the other five species (*A. orontis, L.*

*cephalus, C. tinca, C. capoeta* and *B.plebejus)* show low population densities as can be observed in Table 7.

I relate this observation to the environmental factors. District 1 is a fertile area so it is largely used for agricultural purposes. As a necessity, in order to project the vegetation, agricultural pesticides are used in large amounts. These pesticides contaminate the water and effect the fist production. Moreover, this area is also industrially well developed so industrial by-products are also outlet to the river causing pollution and as a concequence effect similarly the vitality of all the fishes in varying degrees.

I also observe that in District 3, most of the species show medium population densities. District 3 in neither highly developed industrially nor a largely agricultural region but it is an area where the number of population centers is large. Especially at the sampling stations near the villages, pH values of water were determined to be relatively high and foamy water was observed. Foam as a result of the contamination of water by the detergents used for cleaning purposes.

In District 2, most of the species show highest population densities. Environmental factors are more suitable for fishes than in District 1 and 3. Because the source of the Sakarya River exists in this district

and it is not a highly developed industrially not a largely agricultural region environmental pollution is not marked.

Because of these reasons, I conclude that District 2 is a more favourable region for the fishes.

## ÖZET

Sakarya havzası, Sarıyar ve Gökçekaya barajları doğal birer engel olarak kabul edilerek üç bölgeye ayrılmıştır. Her bölgeden en az 7 istasyon seçilerek bu istasyonlardaki bazı ekolojik özellikler, balık türlerinin dağılımları ve populasyon yoğunlukları saptanmıştır.

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# THE FISHES OF THE THRACE REGION

(Trakya Bölgesi Balıkları)

Füsun Erk'akan*

SUMMARY

In this study, 23 species which belong to 10 families have been identified from the Thrace Region. Of these, *Lepomis gibbosus* is new for Turkey. Also, some *Phoxinellus* specimens were compared with the *Phoxinellus* species described by Karaman in his revision (1972). But, as our specimens were detected to be different than the species described in the above mentioned revision, those specimens are actually undetermined.

## INTRODUCTION

Up till now, the important part of the freshwater fish fauna of Turkey were determined by the earlier authors (Banarescu, 1968; Battalgil, 1940; 1941, 1942, 1944; Hanko, 1924; Karaman, 1969, 1971; Kuru, 1971, 1972, 1975; Ladiges, 1960, 1964). There are also some records from Thrace (Berg, 1949; Karaman, 1971; Kuru, 1980). To have more fully document about the fish species of this region, fishes were caught from the different water systems during the excursion in October 1982. The fish species were determined and listed in this article.

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## MATERIALS AND METHODS

Specimens were collected using seines (experimental gill net, Trammel net) and a dip net. Fishes were fixed in 4% formalin immediately on capture, carried to the laboratory and stored in 70% ethanol for further systematical examinations. Measurments were taken with a milimetric ruler.

## RESULTS

As will be seen from the fallowing list, 23 species belonging to 10 families were found from the different water systems of the Thrace region.

I.Fam. ANGUILLIDAE

Anguilla anguilla (LINNAEUS, 1758)

Gala lake, İpsala-Edirne 16.X.1982, 3 specimens.

II.Fam. ESOCIDAE

Esox lucius LINNAEUS, 1758

Channel, İpsala-Edirne, 16.X.1982, 4 specimens.

III.Fam. CYPRINIDAE

Rutilus rutilus (LINNAEUS, 1758)

Channel, İpsala-Edirne, 16.X.1982, 5 specimens.

Leuciscus (Squalius) cephalus (LINNAEUS, 1758)

Hayapaşa stream, Lalapaşa-Edirne 16.X.1982, 2 specimens.

Dokuzdere Lake, Keşan-Edirne 15.X.1982, 3 specimens.

Phoxinellus sp.

Channel, İpsala-Edirne 16.X.1982, 7 specimens.

Scardinius erythrophtalmus (LINNAEUS, 1758)

Channel, İpsala-Edirne 16.X.1982, 4 specimens.

Tinca tinca (LINNAEUS, 1758)

Channel, İpsala-Edirne 16.X.1982, 1 specimen.

Chalcalburnus chalcoides (GULDENSTADT, 1772)

Karahisar stream, Keşan-Edirne, 15.X.1982, 5 specimens.

Alburnoides bipunctatus (BLOCH, 1758)

İncirli stream, Enez-Edirne 15.X.1982, 6 specimens.

Vimba vimba tenella (NORDMANN, 1840)

Channel, İpsala-Edirne, 16.X.1982, 3 specimens.

Rhodeus sericeus amarus (BLOCH, 1782)

Karahisar stream, Keşan-Edirne, 15.X.1982, 10 specimens.

Channel, İpsala-Edirne, 16.X.1982, 15 specimens.

Carassius carassius (LINNAEUS, 1758)

Channel, İpsala-Edirne, 16.X.1982, 3 specimens.

Cyprinus carpio (LINNAEUS, 1758)

Channel, İpsala-Edirne, 16.X.1982, 5 specimens.

IV.Fam. COBITIDAE

Cobitis taenia LINNAEUS, 1758

Karahisar stream, Keşan-Edirne, 15.X.1982, 4 specimens.

Hayapaşa stream, Lalapaşa-Edirne, 16.X.1982,3 specimens.

Dokuzdere Lake, Keşan-Edirne, 15.X.1982, 4 specimens.

V.Fam. SYNGNATHIDAE

Syngnathus abaster RISSO, 1826

İncirli stream, Enez-Edirne, 15.X.1982, 3 specimens.

VI.Fam. POECILIDAE

Gambusia affinis (BAIRD and GIRARD, 1835)

Karahisar stream, Keşan-Edirne, 15.X.1982, 28 specimens.

Channel, İpsala-Edirne, 16.X.1982, 19 specimens.

VII.Fam. PERCIDAE

Perca fluviatilis LINNAEUS, 1758

Karahisar stream, Keşan-Edirne, 15.X.1982, 5 specimens.

Channel, İpsala-Edirne, 16.X.1982, 4 specimens.

VIII.Fam. CENTRARCHIDAE

Lepomis gibbosus (LINNAEUS, 1758)

Channel, İpsala-Edirne, 16.X.1982, 5 specimens.

IX.Fam. GOBIIDAE

Proterorhinus marmoratus (PALLAS, 1811)

Karahisar stream, Keşan-Edirne, 15.X.1982, 6 specimens.

Channel, İpsala-Edirne, 16.X.1982, 4 specimens.

Gobius (Zosterisessor) ophiocephalus PALLAS, 1811

Bücürmene stream, Enez-Edirne, 15.X.1982, 1 specimen.

Pomatoschistus (Bubyr) caucasicus (KAWRAJSKY, 1899)

İncirli stream, Enez-Edirne, 15.X.1982, 4 specimens.

X.Fam. PLEURONECTIDAE

Platichthys flesus (LINNAEUS, 1758)

Gala Lake, İpsala-Edirne, 16.X.1982, 2 specimens.

Of these, except *Lepomis gibbosus* and *Phoxinellus* sp., the other species have the same systematical characteristics described before by the earlier authors.

*Lepomis gibbosus* (LINNAEUS, 1758) New record for Turkey.

According to the characteristics of the 7 specimens from İpsala-Edirne:

Body oval-shaped and laterally compressed. Head enters 2.6-3.3 times in standart length, body depth 2.0-2.5. Dorsal fin has 10 spines and 11-12 soft rays, anal fin 3 spines and 9-10 soft rays. Scales on body ctenoid. There are 33-36 scales in lateral line and 10-11 gill rakers. Back olive to brown with vertical bands of same colour crossing sides, a red spot on dark tip of gill cover, dark spots on the dorsal, caudal and anal fins.

Distribution: This species is known from North America, England, France, Holland, Belgium, Germany, the basin of the Danube River, Greece and the Thrace Region.

The specimens may be result of introductions to Greece of Bulgaria as Edirne is near these countries.

7 specimens from İpsala-Edirne.

Leg. S. Subakar and G. Yurdatapan.

*Phoxinellus* sp.

Diagnose:

Body small, laterally compressed. Maunth terminal, lips thin, upper lip developed. Lateral line complete. Head length equal to Dorsal fin height. Eye diameter equal to snout length. Dorsal half of the body greyish-brown, ventral side whitish or yellowish. A blue band above lateral line.

Description:

D. III/7; A. III/6(7); L.lat: 33-36. Small fish. Mostly

under 100 mm. The greatest height of the body is less than, sometimes equal to head length. Head length 3.7-4.4 times in the body length (without caudal fin). Mount terminal. Eyes medium size, their diameter 4.3-5.0 times in the head length. The number of gill rakers short, on the first arch ranges from 10-12. Pharyngeal teeth uniseriate, 4-5, 5-5 or 4-4. Dorsal fin starts in front of the middle of the body, its height equal to sometimes shorter than the head length with 7 soft rays. Ventral fins start just below the anterior end of the dorsal. Body covered with medium sized scales. Lateral line complete. Peritoneum light and marked with large, black stellate pigment cells. Described from 7 specimens, İpsala-Edirne.

Leg: S. Subakar and G. Yurdatapan.

## DISCUSSION

According to D. E. Mc Allister and B.W. Coad (1974), the dorsal fin of *Lepomis gibbosus* with 9 or 10 spines and 11-13 soft rays. Anal fin 3 spines and 10-12 soft rays. There are 35-44 scales in Lateral line and 11-13 gill rakers. Our specimens has 3 spines and 9-10 soft rays in anal fin. There are 33-36 scales in lateral line and 10-11 gill rakers. Other important systematical characteristics are the same as indicated.

Differences of our *Phoxinellus* specimens from the other *Phoxinellus* species which were indicated in the "Revision of certain dwarf genera of Cyprinidae (*Phoxinellus, Leucaspius, Acanthobrama*, etc.) from southern Europe, Asia minor, the Middle

East and North Africa" by Karaman (1972) are as following:

From *Ph. zeregi*: Differ with having higher dorsal fin starting in front of the middle of the body, complete lateral line with 33-36 scales, 10-12 gill rakers on the first arch, ventral fin starting just below the anterior end of the dorsal fin.

From *Ph. chaignoni*: Differ with having longer snout, variations for pharyngeal teeth (5-5;5-4;4-4), higher dorsal fin starting in front of the middle of the body, lateral line not forming a wide arc along the flank (strait lateral line), shorter pectoral fins.

From *Ph. sojuchbulagi*: Differ with having higher number of gill rakers on the first arch (10-12), lower number of soft rays in dorsal fin (7 rays), complete lateral line and laterally compressed body.

From *Ph. crassus*:Differ with having lower number of scales in lateral line, dorsal fin begining behind the ventrals, medium sized scales.

From *Ph. handlirschi*: Differ with having lower number of scales in lateral line, higher dorsal fin begining in front of the middle of the body, variations for pharyngeal teeth (5-5; 5-4; 4-4).

From *Ph. eğridiri*: Differ with having complete lateral line with lower number of scales, dark spots on the dorsal portion and greater size from our specimens.

From *Ph. pleurobipunctatus*: Differ with having lower number of soft rays in dorsal and anal fins, lower number of scales in

lateral line, higher dorsal fin that has same height with the head length and not straight posterior margin, medium sized scales, not having two black lines bounding the lateral line.

From *Ph. stimphalicus*: Differ with having complete lateral line with lower number of scales, higher dorsal fin starting in front of the middle of the body, ventral fins starting just belowe the anterior end of the dorsal, light peritoneum with large, black stellate pigment cells and large eyes.

From *Ph. adspersus*: Differ with having complete lateral line with lower number of scales, higher dorsal fin and it's height is generally equal to head length, medium sized and well developed scales, light peritoneum with large, black stellate pigment cells.

ÖZET

Bu çalışmada, Trakya bölgesinden 10 familyaya ait 23 tür saptanmıştır. Bunlardan *Lepomis gibbosus* Türkiye için yeni kayıttır. *Phoxinellus* örnekleri ise Karaman tarafından 1972'de yapılan revizyondaki tüm *Phoxinellus* tür'leriyle karşılaştırılmış ve tür'ü kesinlikle saptanamamıştır.

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# RE-DISCUSSION OF SYSTEMATICAL STATU OF *VARICORHINUS ANTALYENSIS* BATTALGIL, 1944.

(*Varicorhinus antalyensis* BATTALGİL, 1944'in Sistematik Durumunun Yeniden İncelenmesi)

Füsun Erk'akan*-Mustafa Kuru*

SUMMARY

*Varicorhinus antalyensis* BATTALGİL, 1944 was accepted as *Hemigrammocapoeta* by KARAMAN(1969) and later *Hemigrammocapoeta kemali* by the same author (1971) because of the scale number in lateral line. In the same study it was indicated that because of the deficient description of BATTALGİL, it is impossible to determine the definite systematical statu of these specimens and also it is thought that it could be placed in *Tylognathoides*.

In this study, the specimens caught from Aksu and Köprü streams near Antalya were compared with *Tylognathoides*, *Hemigrammocapoeta kemali* and *Varicorhinus antalyensis* according to important systematical characters. Because of the similarities, it was concluded that these specimens belong to *Varicorhinus antalyensis* BATTALGİL, 1944. So,it was proved that *Varicorhinus antalyensis*, described as a new species by BATTALGİL, 1944 does exist but, according to the Revision of genus *Capoeta* (syn. *Varicorhinus*) by KARAMAN (1969) and as a systematical rule, *Varicorhinus*

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*antalyensis* BATTALGİL, 1944 must be accepted as *Capoeta antalyensis* (BATTALGİL, 1944).

## INTRODUCTION

Until now the systematical status of the freshwater fishes of Turkey has been completely determined with the exception of some minor problems. BATTALGİL (1944) described some fish specimens from the streams near Antalya, as a new species, *Varicorhinus antalyensis*. This species was distinguished from *Varicorhinus tinca* with having a lower scale number in lateral line and other *Varicorhinus* species having two pairs of barbels. According to the revision of genus *Capoeta* by KARAMAN(1969), this species was accepted as *Hemigrammocapoeta antalyensis*.Later in the revision of genus *Barbus* (KARAMAN(1971), it has been advanced that because of the resemblance of the scale number of lateral line, this species could be accepted as *Hemigrammocapoeta kemali*. But these specimens have two pairs of barbels contrary to *Hemigrammocapoeta kemali*. KARAMAN also claimed that BATTALGİL`s specimens might be introduced in *Tylognathoides*. But as a consequence of the above ideas, it was decided by KARAMAN that it was difficult to determine exactly the systematical statu of this species because of the deficient description of BATTALGİL.

BALIK has studied the Taxonomic Revision of Southern Anatolia (1979) and notified that he couldn`t catch any

*Capoeta (syn. Varicorhinus)* specimens from the streams flowing into the Mediterranean sea.

In spite of these, KURU(1980) indicated the figure and the distribution area of *Varicorhinus antalyensis* according to BATTALGİL's description in the Catalogue of Freshwater Fishes of Turkey.

On account of this, specimens were collected from Aksu and Köprü streams near Antalya to determine the definite systematical statu of *Varicorhinus antalyensis*.

## MATERIALS AND METHODS

Specimens were collected using experimental gill nets from the Aksu and Köprü streams. Fishes were fixed in 4 % formalin immediately on capture and placed in plastic bags, carried to the laboratory and stored in 70 % ethanol. Some morphometric characters of the specimens were measured by Kanon LKSM model compas as mm. (Table 1). These characters are given below:

Standart length (SL)*: Distance from the tip of snout to the end of the scales of caudal peduncle.

Head length (HL)*: Distance from the tip of the snout to the end of operculum.

Head depth (HD)*: Measured at occiput, i.e.,above where the first vertebra attached to cranium.

Body depth(BD)*: Maximum depth of body exclusive of fins.

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*Notations in tables

Predorsal length (PrDL)*: Distance from the tip of snout to the base of first dorsal fin ray along the mid-line of body longitidunally.

Postdorsal length (PDL)*: Distance from the base of the first dorsal fin ray to the end of scales of caudal peduncle along the mid-line of body longitidunally.

Caudal peduncle length (CPL)*: Distance from anal aperture to the end of scales along mid-line of body longitidunally.

Caudal peduncle depth (CPD)*: Minimum depth of this region

Eye width (EW)*:Longitidunal diameter of eye.

Snout length (SnL)*: Distance from the tip of the snout to the anterior margin of eye.

Interorbital distance (ID)*: Bony distance between orbits.

Dorsal fin height (DH)*: Length of its longest ray.

Anal fin heigth (AH)*: Legnth of its longest ray.

Pectoral fin length (PL)*: Distance from anterior edge to tip.

Ventral fin length (VL)*: Distance from anterior edge to tip.

Mouth width (MW)*: Distance between corners of mouth.

Ventral fin-Vent distance (V-A)*: Distance between ventral fin base and anal aperture.

Second pair of barbels length (SBL)* : Distance from base to the tip of second pair of barbels.

On the other hand, some other important systematical characters of the specimens as; dorsal (D)* , anal (A)* , pectoral (P)* , ventral (V)* and caudal (C)* fin rays, scales in lateral series (L.lat)* , the number of scales between lateral line and anterior of the ventral and dorsal fins (L.trans)* , gill rakers on the outher side of the first arch (GR) and the structure of the pharyngeal teeth (PhT)* were determined (Table 2).

Values for body depth, head length, head depth, predorsal length, postdorsal length, caudal peduncle length, caudal peduncle depth, ventral fin-vent distance, pecto-ventral distance and snout length were entered as a proportion of standart length. Head depth, eye width, interorbital distance, dorsal fin height, anal fin height, pectoral fin length and ventral fin length were entered as a proportion of head length. Second pair of barbels length was entered as a proportion of eye width and caudal peduncle depth was entered as a proportion of caudal peduncle length (Table 3).

Ranges, means and standard deviations of these values were computed and shown in table 4.

Table 1. Some morphometric characters of Antalya specimens (as mm.)

| Characters | Number of fish specimens | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| SL | 121 | 123 | 146 | 156 | 110 | 151.5 | 112 | 97 |
| HL | 29 | 29.5 | 34 | 37 | 26 | 36.5 | 28 | 25 |
| HD | 21.5 | 21 | 25 | 25 | 17 | 23.5 | 18 | 16.5 |
| BD | 33 | 30 | 34 | 39 | 26 | 36 | 26.5 | 22 |
| PrDL | 62 | 64 | 72 | 78 | 54 | 76 | 55 | 46 |
| PDL | 66 | 68 | 76 | 81 | 60 | 79 | 57 | 51.5 |
| CPD | 14 | 14 | 16 | 18 | 12 | 18 | 12.5 | 10.5 |
| CPL | 22 | 21 | 28 | 29 | 21 | 31.5 | 22 | 20 |
| EW | 6 | 6 | 6.5 | 7 | 6 | 7 | 6 | 5.5 |
| SnL | 10.5 | 10 | 12 | 14 | 10 | 13 | 10 | 9 |
| ID | 12 | 11.5 | 13 | 14 | 9 | 13 | 10 | 8.5 |
| DH | 21 | 23 | 30 | 27.5 | 20.5 | 29 | 22 | 19 |
| AH | 19 | 21 | 25 | 25 | 18 | 26 | 19 | 17 |
| PL | 21 | 23 | 26 | 26 | 18 | 27 | 20 | 18 |
| VL | 18 | 20 | 22 | 23 | 16 | 23 | 18 | 15 |
| MW | 10 | 10 | 10 | 12 | 8 | 12 | 8 | 7.5 |
| P-V | 39.5 | 38 | 47 | 50 | 35 | 47 | 37 | 30 |
| V-A | 25 | 26 | 32 | 32.5 | 24 | 33 | 25 | 21 |
| SBL | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |

Table 2. Some important systematical characters of Antalya specimens.

| Characters | Number of fish specimens | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| D.fin rays | 11 | 12 | 12 | 12 | 11 | 12 | 11 | 11 |
| A.fin rays | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| D-fin rays | 18 | 18 | 16 | 16 | 16 | 16 | 16 | 16 |
| V.fin rays | 8 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| C.fin rays | 19 | 17 | 17 | 19 | 17 | 19 | 18 | 18 |
| L.lat. | 52 | 50 | 54 | 53 | 52 | 53 | 54 | 50 |
| L.trans. | $\frac{10}{6}$ | $\frac{10}{6}$ | $\frac{10.5}{6}$ | $\frac{11}{6}$ | $\frac{10}{5}$ | $\frac{11}{6}$ | $\frac{10}{6}$ | $\frac{9.5}{5}$ |
| PhT | 2.3.5<br>5.3.2 | 2.3.5<br>5.3.2 | 2.3.5<br>5.3.2 | 2.3.5<br>5.3.2 | 2.3.5<br>5.3.2 | 2.3.5<br>5.3.2 | 2.3.5<br>5.3.2 | 2.3.5<br>5.3.2 |
| GR | 12 | 15 | 15 | 13 | 14 | 15 | 15 | 15 |

Table 3. Some proportional characters of Antalya specimes.

| Characters | Number of fish specimes | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| SL/BD | 3.7 | 4.1 | 4.3 | 4.0 | 4.2 | 4.2 | 4.2 | 4.4 |
| SL/HL | 4.2 | 4.2 | 4.3 | 4.2 | 4.2 | 4.2 | 4.0 | 3.9 |
| SL/HD | 5.6 | 5.9 | 5.3 | 6.2 | 6.5 | 5.9 | 6.2 | 5.9 |
| SL/PrDL | 2.0 | 1.9 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.1 |
| SL/PDL | 1.8 | 1.8 | 1.9 | 1.9 | 1.8 | 1.9 | 2.0 | 1.9 |
| SL/CPL | 5.5 | 5.9 | 5.2 | 5.4 | 5.2 | 4.8 | 5.1 | 4.9 |
| SL/CPD | 8.6 | 8.8 | 9.1 | 8.7 | 9.2 | 8.4 | 9.0 | 9.2 |
| SL/V-A | 4.8 | 4.7 | 4.6 | 4.8 | 4.6 | 4.6 | 4.5 | 4.6 |
| SL/P-V | 3.1 | 3.2 | 3.1 | 3.1 | 3.1 | 3.2 | 3.0 | 3.2 |
| SL/SnL | 2.8 | 3.0 | 2.8 | 2.6 | 2.6 | 2.8 | 2.8 | 2.8 |
| HL/HD | 1.3 | 1.4 | 1.4 | 1.5 | 1.5 | 1.6 | 1.6 | 1.5 |
| HL/EW | 4.8 | 4.9 | 5.2 | 5.3 | 4.3 | 5.2 | 4.7 | 4.5 |
| HL/ID | 2.4 | 2.6 | 2.6 | 2.6 | 2.9 | 2.8 | 2.8 | 2.9 |
| HL/DH | 1.4 | 1.3 | 1.4 | 1.5 | 1.4 | 1.4 | 1.3 | 1.3 |
| HL/AH | 1.5 | 1.4 | 1.4 | 1.5 | 1.4 | 1.4 | 1.5 | 1.5 |
| HL/PL | 1.4 | 1.3 | 1.3 | 1.4 | 1.4 | 1.4 | 1.4 | 1.4 |
| HL/VL | 1.6 | 1.5 | 1.5 | 1.6 | 1.6 | 1.6 | 1.6 | 1.7 |
| EW/SBL | 1.5 | 1.5 | 1.6 | 1.8 | 1.5 | 1.8 | 1.5 | 1.4 |
| CPL/CPD | 1.6 | 1.5 | 1.8 | 1.6 | 1.8 | 1.8 | 1.8 | 1.9 |

Table 4. Ranges, means and standard deviations of some characters of Antalya specimens (measurements in mm.).

| Characters | Range | Mean | Standart deviation |
|---|---|---|---|
| SL | 97-156 | 127 | ± 21.60 |
| HL | 25-37 | 30.6 | ± 4.63 |
| HD | 16.5-25 | 20.9 | ± 3.46 |
| BD | 22-39 | 30.8 | ± 5.72 |
| PrDL | 46-78 | 63.4 | ± 11.40 |
| PDL | 51.5-81 | 67.3 | ± 10.77 |
| CPD | 10.5-18 | 14.4 | ± 2.76 |
| CPL | 20-31.5 | 24.3 | ± 4.45 |
| EW | 5.5-7 | 6.3 | ± 0.53 |
| SnL | 9-14 | 11.1 | ± 1.74 |
| ID | 8.5-14 | 11.4 | ± 2.01 |
| DH | 19-30 | 24 | ± 4.22 |
| AH | 17-26 | 21.3 | ± 3.58 |
| PL | 18-27 | 22.4 | ± 3.66 |
| VL | 15-23 | 19.4 | ± 3.11 |
| MW | 7.5-12 | 9.7 | ± 1.75 |
| P-V | 30-50 | 40.4 | ± 6.91 |
| V-A | 21-33 | 27.3 | ± 4.54 |
| SBL | 4 | 4 | ± 0 |

Table 4. Continued.

| Characters | Range | Mean | Standart deviation |
|---|---|---|---|
| D.fin rays | 11-12 | 11.5 | ± 0.53 |
| A.fin rays | 8 | 8 | ± 0 |
| P.fin rays | 16-18 | 16.5 | ± 0.93 |
| V.fin rays | 8-9 | 8.9 | ± 0.35 |
| C.fin rays | 17-19 | 18 | ± 0.93 |
| L.lat. | 50-54 | 52.3 | ± 1.58 |
| L.trans | 9.5-11/5-6 | 10.25/5.8 | ± 0.53/0.46 |
| GR | 12-15 | 14.3 | ± 1.16 |

## RESULTS

In this study 8 fish specimens from Aksu and Köprü streams near Antalya were examined and their characters as following:

Body elongated and fusiform, scales moderate. D.III/8-9; A.III/5; P.I/15-17; V.I/7-8; C.17-19. L.lat. 50 $\frac{9.5-11}{5-6}$ 54.

Body depth 3.7-4.4, head length 3.9-4.3, head depth 5.8-6.5, predorsal length 1.9-2.1, postdorsal length 1.8-2.0, caudal peduncle length 4.8-5.9, caudal peduncle depth 8.4-9.2, ventral-vent distance 4.5-4.8, pecto-ventral distance 3.0-3.2 and snout length 2.6-3.0 times in standart length. Head depth 1.3-1.6, eye width 4.3-5.3, dorsal fin

height 1.3-1.5,anal fin height 1.4-1.5, ventral fin length 1.5-1.7, pectoral fin length 1.3-1.4 and interorbital distance 2.4-2.9 times in head length. Caudal peduncle height 1.5-1.9 times in caudal peduncle length and second pair of barbels length 1.4-1.8 times in eye width.

Mouth ventral, large longitidunal, upper lip not fimriate, lower lip developed only in the corners of the mouth. Barbels two pairs, gill rakers 12-15. Last unbranched ray of dorsal fin thickened, slightly emerginate and denticulated up to 2/3 of its length. Origin of dorsal fin some what in advance of the ventral origin. Keel extend in front of Dorsal. Pectoral fins long and rounded. Sides grey to blackish, abdomen silver-grey.

Previous records from Turkey: Antalya; BATTALGİL, 1944.

Localities of our specimens: Aksu stream, Aksu-Antalya; 16.V.1982, 2 specimens (number 1 and 2 in tables); Köprü stream, Serik-Antalya, 16.V.1982, 6 specimens (number 3,4, 5,6,7 and 8 in tables). Leg. F.ERKAKAN and M.KURU,

## DISCUSSION

Our specimens resemble the specimens which were caught from Antalya and determined as *Varicorhinus antalyensis* by BATTALGİL (1944) because of the similarities existing in the total number of D.,A.,P.and V.rays, scale number of lateral line, some proportional characteristics such as SL/BD, HL/EW, HL/MW, EW/SBL, DH/HL, number of pharyngeal teeth and barbels, structures of mouth and

pharyngeal teeth (Table 5). On the other hand the dorsal fins of our specimens are slightly truncated, predorsal distance is equal to postdorsal, the base of ventral fin just below the first ray of the Dorsal, Caudal origin remotely behind the anal fin end. Because of these characters our specimens also resemble BATTALGİL`s specimens. "t" test can not be used to determine the significance level between these values because of BATTALGİL`s deficient description.

Table 5. Comparison of some characters of Antalya specimes with *V. antalyensis* BATTALGİL, 1944.

| Characters | BATTALGİL`s specimens (*V. antalyensis*) | Antalya specimens |
|---|---|---|
| D.fin rays | III/9 | III/8-9 |
| A.fin rays | III/5-6 | III/5 |
| P.fin rays | I/16 | I/15-17 |
| V.fin rays | I-II/8 | I/7-8 |
| L.lat | 55-56 | 50-54 |
| L.trans | 12.5-13.5/7.5 | 9.5-11/5-6 |
| SL/BD | 4.1-4.3 | 3.7/4.4 |
| SL/HL | 4.6-4.7 | 3.9-4.3 |
| HL/EW | 3.8-5.0 | 4.3-5.3 |
| ID/EW | 1.3-1.5 | 1.5-2.0 |
| HL/MW | 3.0-4.5 | 2.9-3.5 |
| EW/SBL | 1.3 | 1.4-1.8 |
| PhT | 2.3(4).5-5.3.2 | 2.3.5-5.3.2 |
| DL/HD | 1.3-1.7 | 1.1-1.2 |

In the revision of genus *Capoeta* by KARAMAN (1969), *Capoeta* distinguished from *Varicorhinus* because of having moderate scales, slightly thickened and denticulated third spine of dorsal fin, narrow Lacrimalia covers only small part of the snout area, long and norrow Suborbitalia, Maxilla having posterior extension not reaching the Jugularis, long Mandibula especially long Articulare. Our specimens also have these characteristics, so we distinquished our specimens from genus *Varicorhinus* and accepted them as *Capoeta* (Figure 1).

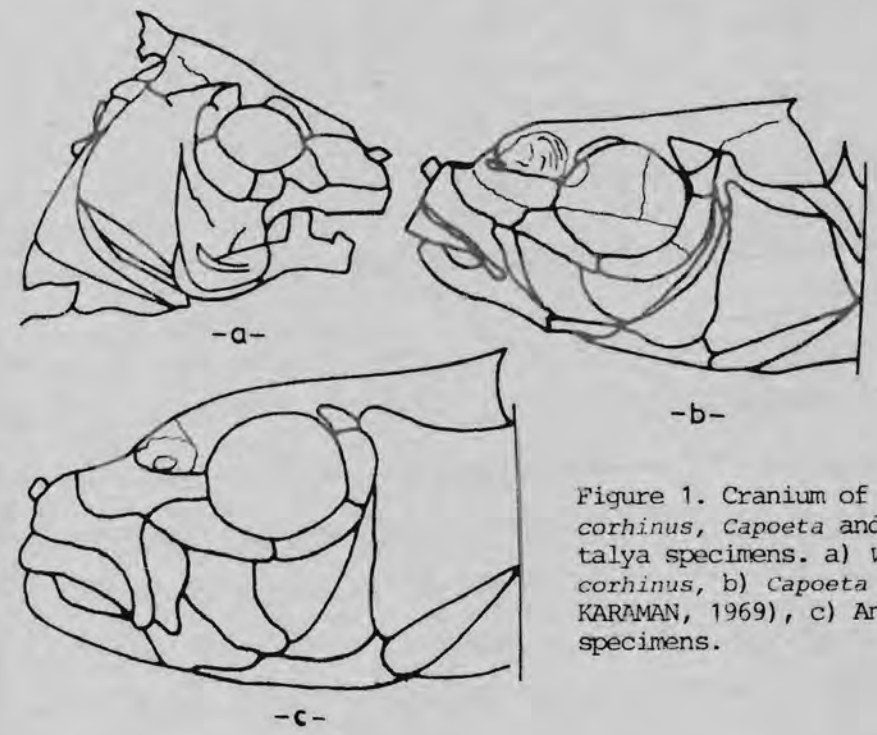


Figure 1. Cranium of *Varicorhinus*, *Capoeta* and Antalya specimens. a) *Varicorhinus*, b) *Capoeta* (from KARAMAN, 1969), c) Antalya specimens.

In the revision of genus *Barbus*, KARAMAN (1969) defended that *Varicorhinus antalyensis* specimens could belong to genus *Hemigrammocapoeta* or *Tylognathoides*. But mouth structure and other important systematical characters of these specimens never resemble these two genera (Figure 2).

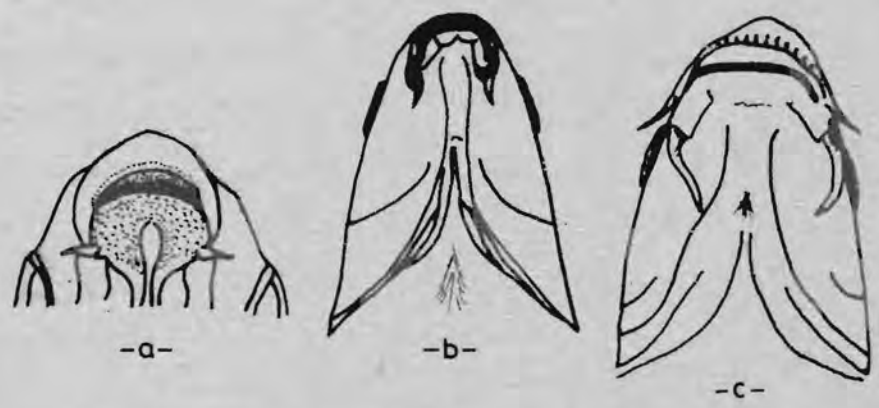


Figure 2. Mouth structures of *Hemigrammocapoeta*, *Tylognathoides* and Antalya specimens. a) *Hemigrammocapoeta*, b) *Tylognathoides* (from KARAMAN, 1971), c) Antalya specimens.

On the other hand, one of the most important systematical characters of *Capoeta* is the number of barbels. Barbel number is used to distinguish *Capoeta* species from each other. Our specimens differ from most of the *capoeta* species, because they have two pairs of barbels. In *Capoeta*, only *C.tinca* and C.*c.heratensis* have two pairs of barbels. From these *C.tinca* is found only in the streams of the Black sea and C.*c.heratensis* in Turkmenistan and North Afghanistan. Because of some characters, shown in table 6, and the zoogeogra-

phical distribution, our specimens can neither be accepted as *C.tinca* nor as *C.c.heratensis*.

According to the revision of genus *Capoeta* by KARAMAN (1969) all of the *Varicorhinus* species from Turkey were introduced into *Capoeta*, so our specimens must be accepted as *Capoeta antalyensis* (BATTALGİL, 1944).

Table 6. Comparison of some characters of Antalya specimens with *C.tinca* and *C.c.heratensis*.

| Characters | *C.tinca* (according to KARAMAN,1969 | *C.c.heratensis* (according to KARAMAN,1969) | Antalya specimens |
|---|---|---|---|
| D.fin rays | III/7-8 | III-IV/8 | III/8-9 |
| A.fin rays | III/5 | III-IV/5 | III/5 |
| L.lat. | 64-80 | 50-60 | 50-54 |
| GR | 10-15 | 20-25 | 12-15 |
| SL/VH | 3.8-5.0 | (*Capoeta capoeta*) 3.5-5.0 | 3.7-4.4 |
| SL/HL | | 3.9-5.3 | 3.9-4.3 |
| HL/SBL | 3.6-9.9 | 4.4-12.8 | 5.3-9.3 |
| HL/EW | | 3.9-7.2 | 4.3-5.3 |
| HL/MW | | 2.2-3.9 | 2.9-3.5 |
| HL/DH | | 3.9-6.2 | 1.3-1.5 |
| P-V/CPL | 1.2-1.8 | 1.1-1.9 | 1.7-1.9 |
| CPL/min.BD | 2.0 | 2.0 | 0.7-1.3 |

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ÖZET

BATTALGİL tarafından 1944 yılında yeni bir tür olarak tanımı yapılan *Varicorhinus antalyensis* örnekleri, KARAMAN (1969) tarafından önce *Hemigrammocapoeta* cinsine dahil edilmiş ve daha sonra (1971) yan çizgisindeki pul sayısı açısından *Hemigrammocapoeta kemali* olacağı ileri sürülmüştür. Aynı çalışmada bu örneklerin *Tylagnathoides* cinsine de dahil edilebileceği belirtilmiş, fakat BATTALGİL (1944)`in tam olmayan tanamına göre sistematik yerinin kesinlikle saptanamıyacağı bildirilmiştir. Antalya yöresindeki Köprü ve Aksu çaylarından yakalanan örneklerimiz, sistematik özellikler açısından incelenmiş, *Hemigrammocapoeta kemali, Tylognathoides* ve *V. antalyensis* örnekleri ile karşılaştırılmıştır. Örneklerimizin *Hemigrammocapoeta* ve *Tylognathoides* cinslerinden önemli farklılıklar gösterdiği, buna karşın BATTALGİL (1944)`in tanımına uygunluk gösterdikleri saptanmıştır. Yalnız Türkiye`deki *Varicorhinus* örnekleri, daha sonra KARAMAN (1969) tarafından *Capoeta* cinsine dahil edildiğinden ve örneklerimizin de bu özelliklere uygunluk göstermesinden dolayı, *V.antalyensis* BATTALGİL, 1944' ün sistematik bir kural olarak *Capoeta antalyensis* (BATTALGİL, 1944) seklinde yazılması gerekir.

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# ISOLATION OF A MUTATOR STRAIN OF ESCHERICHIA COLI K12*

(Bir Escherichia coli K12 mutator suşunun izolasyonu)

Ali KALAYCIOĞLU**


## ABSTRACT

This work concerns mutator mutant of *Escherichia coli* K12 which have been obtained by examining colonies on eosin-methylene blue lactose agar of an F-merodiploid strain, H2 *lac*$^-$/F'*lac*$^-$, with different mutations in the *lac* Z genes, for those which showed an increased number of *lac*$^-$ papillae. Mutator gene in H2 strain have been mapped on the E.coli chromosome. The marker was co-transduced by phage P1 with the *thy* A gene with frequencies close to 60 %. The mutator mutation was called *mut* H21 and the strain H2110.


## INTRODUCTION

Strains of *E.coli* are known in which the spontaneous mutation frequency is from one to three orders of magnitude higher than in the wild type. These are called mutator strains, and the increase in mutation rate occurs in the absence of mutagens such as ionizing radiation,ultraviolet

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light and base analogs. Many of these genes have been mapped [COX et al.(1972), COX(1973), HOESS and HERMAN (1975), SIEGEL(1973), SIEGEL and IVERS(1975) BACHMAN and LOW(1980)] on the chromosome and their products, or lack of products, cause a variety of mutations ranging from single base-pair changes through small additions and deletions to quite large deletions and insertions.

Mutators have been shown to increase the mutation rate in most, if not all, genes on the chromosome.

How new mutations arise is poorly understood, but there are growing experimental data to suggest that many spontaneous mutations arise as errors in all three metabolic processes affecting DNA replication, recombination and repair.

H2110 gave the highest yield of azauracil resistant mutant and relatively low yield of *lac*$^+$ cells compared to hyper-rec strains, which will be published in another paper. HOESS and his collaborators (HOESS and HERMAN 1975, HOESS and FAN 1975) published data concerning mutants of the *mutR* gene, which are probably alleles of *mutH*, first identified by HILL(1970), which is located close to *thyA*. We believe the mutation in H2110 to be in this gene.

# MATERIALS AND METHODS

## Bacterial strains list

Table 1 list the bacterial strains used in this work. The list shows the relevant genotype of each strain. In cases where the spesific cistron or allele is unknown, no letter or number is given. Stock cultures of bacterial strains were maintained in small screw capped bottles on Dorsett egg medium.

## Media

The various types of solid and liquid bacteriological media used in this work are listed here. Contents are given per litre of medium prepared in sterile distilled water.

Nutrient broth : 25 g Oxoid broth No.2

Nutrient agar : 25 g Oxoid broth No.2 and 12.5 g Davis New Zealand agar No.3

Minimal agar : 20 g of Davis New Zealand agar No.3,20 mg of L-amino acid, 5 g of carbon source, 1 mg of appropriate vitamins and 100 ml of minimal salts, 0.005 or 0.05 g of thymine if necessary. Eosin Methylene Blue (EMB agar): 8 g Difco bacto casamino acids, 1 g Difco bacto yeast extract, 5 g NaCl, 2 g $K_2HPO_4$,0.4 g Eosin yellow, 0.065 g Methylene blue, 10 g sugar, 15 g agar.

The eosin, methylene blue, nutrient base and sugar solutions were prepared and sterilised seperately and were mixed together just before pouring the plates.

TABLE I

Bacterial strains

| Strain No | Mating type | Characteristic |
|---|---|---|
| X7184 | $F^-$ | *thi*$^-$, *sup*$^-$, *lac*Z$^-$ 10b,M15 *ara*$^-$, $\lambda^R$ $T_6^S$ $AzU^S$ $Str^R$ |
| AB1157 | $F^-$ | *thr*$^-$, *leu*$^-$, *his*$^-$, *proA*$^-$ *argE*$^-$, *thi*$^-$, *lac*$^-$, *ara*$^-$ *xyl*$^-$, $\lambda^-$, $T_6^R$ $Str^R$ |
| AB2070 | $F^-$ | *metE*$^-$, *ilv*$^-$, *trp*$^-$, *his*$^-$ *pro*$^-$, *leu*$^-$, *ara*$^-$, *thi*$^-$ $Str^R$ |
| KL398 | $F^-$ | *metE*$^-$, *leu*$^-$, *proC*$^-$, *hisF*$^-$, *thyA*$^-$, *thi*$^-$, *lac*$^-$Z36, *ara*$^-$ *mtl*$^-$, *xyl*$^-$, $Str^R$, $Spc^R$ |
| KL398 mut$^-$ | $F^-$ | as KL398 except *thy*$^+$, *mut* H21 |
| KL400 | $F^-$ | as KL398 except *met*$^+$ |
| H2 | F-prime | as X7184 except *pro*$^-$,F' *lac*$^-$Z$_{(am)}$u131 |
| H2110 | F-prime | as H2 except *mut* H21 |
| KL16 | Hfr | $\lambda^+$ |
| F143 | F-prime | *lysA* *tyrA* (←\|—\|) |

Chemicals

N-methyl-N-nitro-N' nitrosoguanidine (MNNG)(Aldrich) was dissolved in warm distilled water at varying concentrations. Streptomycin (Glaxo) and spectinomycin (donated by Upjohn Ltd.) were dissolved in distilled water at 10 mg/ml, 6 azauracil (Sigma) at 3.5 mg/ml and trimethoprim lactate (Burroughs Wellcome) 1 mg/ml. Nalidixic acid (Calbiochem) was dissolved in 100 ml distilled water 2.5 mg/ml by adding 2 ml of 2M NaOH.

Conjugation

Log phase nutrient broth cultures of a donor at $2 \times 10^8$ cells/ml were mixed with a recipient at $2\text{-}4 \times 10^8$ cells/ml in the ratio of 1 donor cell to 10-20 recipient cells. Mating mixture were incubated on an angled rotator or in a waterbath at $37^\circ$ C. The incubation period usually depended upon the type of donor and selective markers.1 ml of the mating mixture was withdrawn at appropriate times and agitated vigorously with a Gallenkamp flask shaker to interrupt mating.

Appropriate dilutions were plated on selective plates, and incubated 2-4 days at either $30^\circ$ C or $37^\circ$ C. Recombinants were picked and repurified on the same selective media testing for the inheritance of unselected markers.

Generalized P1 transduction

Suspensions of phage P1 were obtained by the confluent-lysis plate technique (MILLER 1972). Bacteria to be transduced were grown in 10 ml nutrient broth until the titre was between 2-4 x $10^8$/ml at 37°C, centrifuged and resuspended in 1 ml of P1 adsorption fluid (MILLER 1972) 0.1 ml of a stock P1 phage suspension was mixed with 0.9 ml bacterial cells and the mixture incubated for 25-30 minutes at 37°C in water bath. The multiplicity of infection aimed at was usually about one. The mixture was then diluted and plated out on selective minimal agar plates. After 2-3 days incubation recombinants were counted, repurified and checked for co-transducible markers on minimal agar plates.

As a control, phage and bacterial cells were plated on the same type of selective plates before mixing.

Mutagenesis

1 ml aliquots containing 20 µg of nitrosoguanidine were added to 5 ml of log phase bacterial cultures in minimal medium and the culture was incubated for 20 minutes with aeration at 37°C. The culture was then centrifuged or filtered and the cells washed and resuspended in buffer. The mutagenised cells were plated immediately onto EMB lactose agar and incubated at 37°C for four days at dilutions that gave about 300 colonies per plate. After the

incubation those colonies which showed many papillae were picked and purified for further study.

Investigation of potential mutants with the mutator phenotype

After the MNNG mutagenesis of *E.coli* K12 strain H2 with the genotype $lacZ_{10b,M15}/F'$ *lac* $Zu_{131}$, some survivors produced many papillae on EMB lactose agar.

Those potential mutants were restreaked, to see if the phenotype was stable, on EMB-lactose agar. Those that passed this test, were restreaked onto master plates and incubated to form master plates for replica plating. This allowed a classification into mutator mutants.

Penicillin treatment for the isolation of auxotrophic amino acid marker

An overnight culture was subcultured into nutrient broth by diluting 10 to-20 fold. The cultures was aerated for 2-3 hours at 37°C and then filtered and washed. 0.1-0.ml of washed cell suspension was transferred into a bubler tube containing 10 ml of M9 glucose medium (the cell titres were usually about $5 \times 10^7$/ml). This tube was aerated 20 minutes at 37°C before adding 400 unit penicillin per ml. Cells were incubated with aeration 5-6 hours then 0.1 ml of several dilutions were spread on nutrient agar plates and incubated overnight. Plates were then replica plated onto nutrient agar and minimal agar plates supplemented

with the original requirements of the strain. After overnight incubation those colonies which appeared on the nutrient agar but not on the minimal agar were purified and their requirements were identified.

### Selection of thymine requiring strains with trimethoprim

A fresh overnight bacterial culture was diluted into M9 glucose medium containing 50 µg/ml thymine and 10 µg/ml trimethoprim (STACEY AND SIMPSON, 1965). After an overnight incubation a few drops of the culture were subcultured into 5 ml of the identical medium. They were allowed to grow overnight to saturation. Suitable dilutions were spread on nutrient agar plates supplemented with 50µg/ml thymine and incubated overnight. Single colonies were picked and streaked onto two glucose minimal plates one supplemented with 50 µg/ml thymine, and the other not. Thymine requiring clones did not grow on plates without thymine supplementation.

### Mutation frequencies

Several tests were used to determine the mutator character of recombinants and parent strains. Recombinants were usually grown with aeration in 2 ml cultures in small tubes. Then suitable dilutions were spread on minimal glucose and selective plates. Mutation frequencies were

calculated after discarding any plate in each series which had a very high number of resistant clones and revertants so as to avoid "jackpots".

Reversion frequencies

0.1 ml of each bacterial culture (approximately $10^9$/ml) was plated on selective medium containing all the requirements for growth with the exception of one requirement. Samples were also plated for viable cell counts. The number of revertants was calculated after 2 days of incubation at the required temperature.

## RESULTS

Isolation of H2110

1 ml nitrosoguanidine (20 µg/ml) was added to a nutrient broth culture of strains H2 at 37°C as it reached a cell density of 1 x $10^8$ cells/ml and the incubation continued for a further 20 mins. The culture was diluted, plated on EMB-lactose agar (LEDERBERG at all.,1952) at a density that yielded 200-250 colonies per plate, and plates were incubated for 3 days at 37°C. Those clones that showed more than the normal number of papillae were picked and restreaked on the same medium. These streaks that still showed unusually many papillae were picked into broth, incubated overnight and the cultures were plated on minimal agar containing 100 mg/ml azauracil, minimal lactose agar

and minimal glucose agar to measure the frequencies of azauracil resistant mutants and lactose positive cells. H2110 gave the highest yield of azauracil resistant mutants and, by comparasion, a relatively low yield of *lac*$^+$ cells. It seemed likely that this was a mutator strain rather than one with a hyper-recombination phenotype.

Mutation frequencies

Table II shows that the frequency of mutants in cultures of H2110 was highest for loss mutations but the yield of missense mutations (reversions) was also high.

TABLE 2

Effect of H2110 and H2 on mutation frequencies

| | H2 | H2110 |
|---|---|---|
| (1) $Ara^-$ → $Ara^-$ | $3.3\times10^{-7}$ | $2.4\times10^{-7}$ |
| (1) $Amp^R$ (10μg/ml) | $4.2\times10^{-7}$ | $7.6\times10^{-6}$ |
| (1) $Tm^R$ (10μg/ml) | $3\times10^{-7}$ | $7\times10^{-7}$ |
| (2) $Tm^R$ (10μg/ml) | $4\times10^{-6}$ | $3.2\times10^{-4}$ |
| $Nal^R$ (50μg/ml) | $2.3\times10^{-8}$ | $1.5\times10^{-5}$ |

(1) *Symbols: Ara:Arabinose, Amp:Ampicillin, Tm:Trimethoprim, Nal:Nalidixic acid.*

(2) *Minimal agar plates also supplemented with thymine (50μg/ml).*

Penicillin selection with a culture of H2110 yielded a variety of auxotrophic mutants while the same procedure applied to a culture of H2 gave only rare *his*$^-$ (histidine) mutants (Table III).

TABLE 3

5 hours penicillin (400 units/ml) treatment of H2 and H2110(1)

| Time (hours) | Viable count | Type and number of mutants obtained |
|---|---|---|
| | H2 | |
| 0 | $7 \times 10^7$ | |
| 2 | $8 \times 10^6$ | |
| 5 | $8 \times 10^4$ | 3 $His^-$ |
| | H2110 | |
| 0 | $6.3 \times 10^7$ | |
| 2 | $1.4 \times 10^7$ | |
| 5 | $3.5 \times 10^5$ | 1 $Thr^-$, 1 $Cys^-$, 1 $Ilv^-$, 8 $His^-$, 4 $Trp^-$, 4 $Met^-$ |

(1) Twenty five nutrient agar plates, each containing clones, were replicated onto minimal agar and nutrient agar plates.

Mapping

In crosses between H2110 and AB1157 $F^-$ recombinants selected for $Thr^+$, $leu^+$, $Arg^+$, $His^+$, were not mutators (Table IV). None of the selected recombinants from cross between H2110 x AB2070 inherited mutator gene (Table V).

TABLE 4

Mapping data for H2110, linkage analysis of selected and unselected markers.

From the cross between H2110 x AB1157/nal (50 μg/ml).

| Selected | Colonies | % linkage | | | | |
|---|---|---|---|---|---|---|
| Markers | Scored | leu | arg | thr | his | mut$^-$ |
| Thr | 200 | 55 | 2 | - | 0 | 0 |
| Leu | 200 | - | 2 | 60 | 2 | 0 |
| Arg | 200 | 68 | - | 78 | 3 | 0 |
| His | 50 | 0 | 4 | 0 | - | 0 |

TABLE 5

Mapping data for H2110; linkage analysis of selected and unselected markers from the cross between H2110xAB2070/nal

| Selected | Colonies | % linkage | | | | |
|---|---|---|---|---|---|---|
| Markers | Scored | ilv | trp | his | met | mut$^-$ |
| Met | 100 | 98 | 0 | 0 | - | 0 |
| Ilv | 100 | - | 0 | 0 | 60 | 0 |
| His | 50 | 0 | 0 | - | 0 | 0 |

All these recombinants were scored for the mutation frequencies of other auxotrophic markers and $Tm^R$, $Val^R$, and $AzU^R$ (Table 4 and 5 also show the linkage percentage for unselected markers). Further conjugation experiments suggested that the mutant gene was linked to *thyA*. At this

point we learned of the work of HOESS and HERMAN(1975) and tried, therefore, to transduce *thy*$^-$ strains using phage P1 grown on H2110 and obtained 60 % contransduction of high mutability (Table 6).

TABLE 6

Transduction of *mut*H21 into different recipients

| P1(H2110) | Colonies Scored (Thy$^+$) | % linkage Mut$^-$ phenotype | Mutation frequency (50µg/ml Nal) |
|---|---|---|---|
| X KL398 | 100 | 64 | $3.19 \times 10^{-5}$ |
| X KL400 | 100 | 62 | $2 \times 10^{-5}$ |

The tight linkage of the mutator activity and *thy*A was confirmed by crosses between H2110 as donor and KL400 and KL398 as recipients (Table 7 a,b) and between KL16 as donor and H2110 *thyA*$^-$, as recipient. In this last cross 83 % of *thy*$^+$ recombinants were *mut*$^+$ (Table 8).

TABLE 7

a) Cross between H2110 and KL400/spc (150 µg/ml)

| Selected Markers | Colonies Scored | % linkage his | thy | uraP$^-$ | leu | mut$^-$ |
|---|---|---|---|---|---|---|
| (1) Leu | 100 | 2 | 60 | 22 | - | 42(2) |
| Thy | 100 | 0 | - | 30 | 25 | 75 |
| His | 100 | - | 40 | 25 | 10 | 30(2) |

(b) Cross between H2110 and KL398/spc (150 μ q/ml)

| Selected Markers | Colonies Scored | % linkage leu | his | thy | uraP$^-$ | met | mut$^-$ |
|---|---|---|---|---|---|---|---|
| Met | 100 | 32 | 0 | 88 | 8 | - | 4(2) |
| Thy | 100 | 20 | 0 | - | 2 | 12 | 76 |
| His | 100 | 16 | - | 88 | 8 | 4 | 4(2) |

(1) These results suggest that there was considerable mating on the plate despite the level of spectinomycin used to counter select the donor.

(2) All the mut$^-$ recombinants were also thy$^+$

TABLE 8

Cross between KL16 and H2110 *thy*A$^-$/str

| Selected Marker | Colonies Scored | mut$^-$ | mut$^+$ |
|---|---|---|---|
| *thy*A$^+$ | 100 | 17 | 83 |

As excepted diploids of F143 which carries the wild type alleles of *mut*H$^+$ and *thy*A$^+$;H2110 *thy*A$^-$ *mut*H21/F143 had the same mutation frequencies as the original strain, H2.Table 9 shows the mutation frequencies of some markers in KL398 *mut*$^-$ and the isogenic parent *mut*$^+$ strain.

TABLE 9

Frequencies of mutants in *mut*$^+$ and *mut*$^-$ cultures of KL398 *mut*$^-$ H21 and KL398 *mut*$^+$

| Mutant phenotype | KL 398 mut$^+$ | KL398 mut$^-$ H21 |
|---|---|---|
| *met*$^-$ → Met$^+$ | $2.6 \times 10^{-8}$ | $1.2 \times 10^{-7}$ |
| *pro*$^-$ → Pro$^+$ | $3.4 \times 10^{-7}$ | $2.4 \times 10^{-7}$ |
| *leu*$^-$ → Leu$^+$ | $2.6 \times 10^{-8}$ | $3.8 \times 10^{-6}$ |
| *lac*$^-$ → Lac$^+$ | $2.6 \times 10^{-8}$ | $3.7 \times 10^{-6}$ |
| *nal*$^S$ → Nal$^R$ | $2.6 \times 10^{-8}$ | $4.2 \times 10^{-5}$ |

## DISCUSSION

The mutator strain of E.coli K12,H2110, which have been obtained by an examination of colonies on eosin-methylene blue (EMB) lactose agar of an F-merodiploid strain, H2 *lac*$^-$/F'*lac*$^-$ with different mutations in the *lac*Z genes, for those which showed an increased number of *lac*$^+$papillae. H2110 has been mapped and studied in some detail (will be published in another paper). The mutator phenotype was found to be associated with a mutation in the gene *mut*H (HILL 1970) and we have designated it *mut*H21. The marker was co-transduced by phage P1 with *thy*A gene with frequencies close to 60 %. The phenotype, it produced especially when the allele had been transferred to another genetic background resembled closely that described by HOESS and FAN(1975); as in their mutants, the mutator activity was great for missense mutations.

## ÖZET

Bu projede *Escherichia coli* K12 suşundan izole edilen bir mutator mutantının kromozom üzerinde haritalanması ile ilgili calışmalara yer verilmiştir. Lac Z geninde farklı mutasyonlar içeren bir F-kısmi diploid suşunun, H2 *lac*$^-$ / F'*lac*$^-$ , EMB-laktoz agarda çok fazla sayıda *lac*$^+$ papillalar içeren kolonilerinden biri mutator mutantı olarak izole edilmiştir.



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# RESEARCHES ON THE PLECOPTERA (Insecta) SPECIES OF TURKEY

(Türkiye Plecoptera (Insecta) Türleri Üzerine Araştırmalar)

Nilgün Kazancı*

## SUMMARY

In this paper, 28 species of Plecoptera which belong to 4 families and 10 genus from Middle, Northern, Eastern and Southern Anatolia are given and, *Amphinemura standfussi* RIS, 1902, *Leuctra fusca* (LINNE), 1758, *Isoperla lesbica* ZWICK, 1978, *Isoperla chius* ZWICK, 1978, *Perla kiritschenkoi* ZHILTZOVA, 1961, *Plesioperla sakartvella* (ZHILTZOVA), 1956 are new records for Turkey.

## INTRODUCTION

The plecoptera is a very ancient order of insects and known since Permian period. Our knowledge of Plecoptera fauna in Anatolia is relatively deficient. Sixty-two species of Plecoptera have previously been recorded from Anatolia. These sixty-two species have been listed below according to AUBERT (1964), ZWICK (1971, 1973, 1975, 1978 I and II), THEISCHINGER (1975, 1976), ILLIES (1978), KAZANCI (1982, (1983).

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I. Fam. Taeniopterygidae

Brachyptera

*Brachyptera demirsoyi* KAZANCI

*Brachyptera sislii* KAZANCI

*Brachyptera transcaucasica* ZHILTZ.

*Brachyptera zwicki* BRAASCH-JOOST

II. Fam. Nemouridae

Amphinemura

*Amphinemura mirabilis* MART.

*Amphinemura standfussi* RIS.

*Amphinemura trialetica* ZHILTZ.

Nemoura

*Nemoura brevipennis* MART.

*Nemoura cinerea* RETZ.

*Nemoura dromokeryx* THEISCH.

*Nemoura flexuosa* AUBERT

*Nemoura martynovia* CLSSN.

*Nemoura subtilis* KLP.

*Nemoura taurica* ZHILTZ.

*Nemoura wittmeri* ZWICK

Protonemura

*Protonemura bacurianica* ZHILTZ.

*Protonemura besucheti* ZWICK

*Protonemura bifida* MART.

*Protonemura bithynica* AUBERT

*Protonemura capitata* MART.

*Protonemura eumontana* ZHILTZ.

*Protonemura karabagi* KAZANCI

*Protonemura microstyla* MART.

*Protonemura ressli* ZWICK

*Protonemura teberdensis* ZHILTZ.

*Protonemura vernalis* ZHILTZ.

III. Fam. Leuctridae

Leuctra

*Leuctra aspoeckorum* THEISCH.

*Leuctra collaris* MART.

*Leuctra furcatella* MART.

*Leuctra fusca* L.

*Leuctra hippopus* KMP.

*Leuctra kurui* KAZANCI

*Leuctra martynovi* ZHILTZ.

*Leuctra minuta* ZHILTZ.

*Leuctra sanainica* ZHILTZ.

*Leuctra schistocerca* ZWICK

*Leuctra svanetica* ZHILTZ.

*Leuctra zangezurica* ZHILTZ.

*Leuctra zhiltzovae* THEISCH.

IV. Fam. Capniidae

Capnia

*Capnia arensi* ZHILTZ.

*Capnia sevanica* ZHILTZ.

*Capnia tuberculata* ZHILTZ.

V. Fam. Perlodidae

Bulgaroperla

*Bulgaroperla mirabilis* RAUSER

Isoperla

*Isoperla armeniaca* ZHILTZ.

*Isoperla bithynica* KEMPNY

*Isoperla chius* ZWICK

*Isoperla grammatica* PODA

*Isoperla rhododendri* ZHILTZ

*Isoperla tripartita* ILLIES

Perlodes

*Perlodes microcephala* PICTET

Eoperla

*Eoperla ochracea* KOLBE

Perla

*Perla illiesi* BRAASCH ve JOOST

*Perla kritschenkoi* ZHILTZ.

*Perla marginata* PANZ.

*Perla pallida* GUERIN

Phasganophora

*Phasganophora werneri* KEMPNY

VI, Fam. Chloroperlidae

Chloroperla

*Chloroperla zhiltzovae* ZWICK

Plesioperla

*Plesioperla sakartvella* ZHILTZ.

Pontoperla

*Pontoperla teberdinica* BAL.

Siphonoperla

*Siphonoperla burmeisteri* PICTET

Xanthoperla

*Xanthoperla yerkoyi* KAZANCI

## MATERIAL AND METHODS

All the material have been obtained from Middle, Eastern, Northern and Southern Anatolia between 1978 and 1982. The specimens have been collected with an insect net from surroundings area of various running waters. Ethyl alchol (%80 percent) used for the preservation of speciments and warm KOH (%4 per cent) for clearing genital segments at the end of abdomen. The binocular (Nikon-SN Model) used for examinations of species. The new records for Turkey have been given according to ILLIES (1978) except *Isoperla chius* ZWICK, 1978 and *Isoperla lesbica* ZWICK, 1978 (ZWICK, 1978). All the material collected was deposited in the Biology Department of Hacettepe University.

## RESULTS

The data concerning the species are given below.

I. Fam. Nemouridae

Amphinemura

*Amphinemura mirabilis mirabilis* (MARTYNOV), 1928

Erzurum: Boğaz, 15.6.1981, 2♂, 2♀.

*Amphinemura standfussi* RIS, 1902

New record for Turkey! Antalya: Elmalı: Çalpınar Village, 14.5.1982, 1♀

Bolu: Göynük: Örencik Village, 12.6.1982, 12♂ 10♀

*Amphinemura trialetica* ZHILTZOVA, 1957

Bolu: Yedigöller, 13.6.1982, 2♂

Nemoura

*Nemoura flexuosa* AUBERT, 1949.

Bolu: Abant, 29.5.1981, 1♂, Bolu: Yedigöller, 13.6.1982, 2♂, Çankırı: Ilgaz Mountains: Derbent and Soğuksu, 19.6.1982, 6♂.

*Nemoura subtilis* KLAPALEK, 1895

Bolu: Yedigöller, 6.6.1981, 1♂:Antalya:Avlan: Göltarla Village, 14.5.1982, 1♂, Bolu:Gölcük Lake, 12.6.1982, 1♂.

*Nemoura taurica* ZHILTZOVA, 1967

Ankara: Çamkoru, 6.5.1981, 2♂, Ankara: Çamkoru, 26.6.1981, 1♂, Antalya-Elmalı Road:Karamanbeli, 14.5.1982, 3♂, 1♀, Antalya: Avlan:Göltarla Village, 14.5.1982, 4♂, 2♀.

Protonemura

*Protonemura bacurianica* ZHILTZOVA, 1957

Erzurum, 27.7.1981, 1♀, Gümüşhane: Bayburt: Yoncalı Village, 3.8.1981, 1♀.

*Protonemura bifida* MARTYNOV, 1928

Gümüşhane: Bayburt: Yoncalı Village, 28.7.1981,1♀, Gümüşhane: Bayburt: Yoncalı Village: Kırklar Mountain, 2.8.1981, 2♀, Çankırı: Ilgaz Mountains: Soğuksu 13.10.1982 2♂.

*Protonemura bithynica* AUBERT, 1964

Bolu-Ankara Road, 26.5.1981, 5♂, Bolu: Abant, 29.5.1981, 2♂, Ankara: Kızılcahamam: Soğuksu, 26.6.1981, 2♂, Bolu: Yedigöller, 6.6.1981, 5♂, Erzurum Gümüşhane Road: Kop Pass, 27.8.1981,8♂, Bolu: Göynük: Örencik Village, 12.6.1982, 1♂, Bolu: Sünnet Lake, 12.6.1982, 5♂, Bolu: Yedigöller, 13.6.1982, 8♂, Çankırı: Ilgaz Mountains: Soğuksu, 19.6.1982, 1♂.

*Protonemura karabagi* KAZANCI, 1982

Bolu: Yedigöller, 29.10.1982, 2♂.

II. Fam. Leuctridae

Leuctra

*Leuctra collaris* MARTNOV, 1928

Erzurum: Yeşildere, 3.5.1975, 6♂, 5♀,(Leg. DEMİRSOY) Bolu: Yedigöller, 29.10.1982, 4♂, 4♀, Kastamonu-Tosya Road:Evciler Village,20.6.1982,2♂

*Lecutra furcatella* MARTNOV, 1928

Gümüşhane: Bayburt: Yoncalı Village, 3.8.1981, 15♂, 10♀, Bolu: Göynük: Örencik Village,12.6.1982, 3♂, 5♀, Çankırı: Ilgaz Mountains, Soğuksu, 19.6. 1982, 5♂, 5♀, Çankırı: Ilgaz Mountains: Derbent, 19.6.1982, 1♂.

*Leuctra fusca* (LINNE), 1758

New record for Turkey! Bolu: Yedigöller,21.10.1980, 2♂, 4♀, Bolu: Abant, 22.10.1980, 1♂, 2♀, Bolu-Ankara Road: Çamtur, 28.10.1982, 1♂, 2♀, Bolu: Yedigöller, 29.19.1982, 1♂, Bolu: Abant,30.10.1982 7♂, 14♀: Kastamonu: Araç: Gemi Village, 30.10.1982, 2♂, 3♀.

*Leuctra hippopus* KEMPNY, 1899

Bolu: Abant, 29.5.1981,1o; Kastamonu-Tosya Road: Evciler Village, 20.6.1982. 1♂,3♀.

*Leuctra kurui* KAZANCI, 1983

Bolu: Yedigöller, 29.10.1982, 5♂, 4♀,Kastamonu: Araç: Gemi Village, 30.10.1982, 1♂, Çankırı:Ilgaz

Mountains, Soğuksu, 31.10.1982, 5♂, 6♀, Çankırı: Ilgaz Mountains: Kadınçayırı, 31.10.1982, 1♀.

***Leuctra martynovi*** ZHILTZIVA, 1960.

Çankırı: Ilgaz: Derbent, 19.6.1982, 1♂, 1♀.

***Leuctra minuta*** ZHILTZOVA, 1960

Çankırı: Ilgaz Mountains: Soğuksu, 19.6.1982, 3♂.

III. Fam. Perlidae

Isoperla

***Isoperla armeniaca*** ZHILTZOVA, 1961

Çankırı: Ilgaz: İnköy, 19.6.1982, 2♂, Tunceli: Munzur Valley, 27.7.1982, 9♂.

***Isoperla lesbica ZWICK, 1978***

New record for Turkey, Aydın 10 km. south of Çine, 30.4.1975, 1♀ (leg. Besuchet and Löbl)(Coll.Museu Geneva)

***Isoperla chius*** ZWICK, 1978

New for Turkey! Bolu-Ankara Road: Akyarma Pass, 20.6.1980, 40♂.

***Isoperla rhododendri*** ZHILTZOVA, 1956

Bolu: Göynük: Çubuk Lake, 12.6.1982, 20♂, 10♀, Çankırı: Ilgaz Mountains: Derbent, 19.6.1982, 4♂, 6♀, Kastamonu Tosya Road, 20.6.1982, 6♂, 1♀, Erzincan: Çağlayan, 26.7.1982, 6♂, 3♀, Erzincan-Tunceli Pass: Pülümür Stream, 27.7.1982, 1♂, 1♀.

Perla

*Perla illiesi* BRAASCH and JOOST, 1973

Bolu: Sünnet Lake, 12.6.1982, 2♂.

*Perla kiritschenkoi* ZHILTZOVA, 1961

New record for Turkey! Artvin: Yusufeli, 23.6.1974, 5♂, 7♀, (Leg. DEMİRSOY); Artvin: Şavşat: Yavuzköy, 23.7.1981, 1♂.

*Perla marginata* (PANZER), 1799

Giresun-Dereli Road: Tekke Village, 22.7.1981.2♂, Ankara: Çeltikçi-Güdül Road, 7.7.1982, 1♂.

Phasganophora

*Phasganophora werneri* (KEMPNY), 1908

Kütahya: Domaniç, 5.7.1980, 6♂, 5♀.

IV. Fam. Chloroperlidae

Chloroperla

*Chloroperla zhiltzovae* ZWICK, 1967

Bolu: Göynük: Çubuk Lake, 10.6.1979, 1♂, Kıbrıscık, 3x.6.1979, 4♂, Kastamonu: Diphan, 19.6.1982, 1♂, 3♀, Çankırı: Ilgaz Mountains: Derbent, 19.6.1982, 1♂.

*Plesioperla sakartvella* (ZHILTZOVA), 1956

New record for Turkey! Gümüşhane: Bayburt: Yoncalı Village, 3.8.1981, 2♂.

Pontoperla

*Pontoperla teberdinica* (BALINSKY), 1950

Bolu: Yedigöller, 6.6.1981, 9♂, 6♀, Erzurum, 15.6.1981, 3♂, 3♀, Erzurum, 27.7.1981, 1♂, Gümüşhane:

Bayburt:Yoncalı Village, 3.8.1981, 10♂, 7♀, Hakkâri: Berçalan, 17.8.1981, 1♂, 3♀, Hakkâri: Çukurca, 25.8.1981 2♂, Bolu: Göynük: Örencik Village, 12.6.1982, 4♂, 4♀, Çankırı: Ilgaz Mountains, 19.6.1982, 23♂, 18♀, Kastamonu-Tosya Road, 20.6.1982, 6♂, 4♀.

## DISCUSSUION

*Amphinemura standfussi* RIS, 1902, *Leuctra fusca* (LINNE), 1758, *Isoperla lesbica* ZWICK, 1978, *Isoperla chius* ZWICK, 1978, *Perla kiritsichenkoi* ZHILTZIVA, 1961, *Plesioperla sakartvella* (ZHILTZOVA), 1956 are new record for Turkey. Also, they are known from adjoining countrie to Turkey (ILLIES, 1978).

The autmn species of Plecoptera are little known in Anatolia. In this study, the autmn species *Leuctra fusca* (LINNE), 1758 is a new record and *Protonemura karabagi* KAZANCI, 1982, *Leuctra kurui* KAZANCI, 1983 are recorded second time since they have been described by Kazancı (1982, 1983).

The specimens of *Amphineumura standfussi* RIS,1902 examined in this study are brachypterous.

*Plesioperla sakartvella* (ZHILTZOVA),1956 was know endemic to Caucasica, but the new record of this specie revealed that its geographical distribution extends i west as far as to Gümüşhane (Bayburt).

## ÖZET

Bu çalışmada Orta Anadolu, Kuzey Anadolu, Doğu Anadolu ve Güney Anadolu'dan 4 familyaya ve 10 cinse ait 28 Plecoptera türü verilmiştir. Bu türlerden *Amphinemura standfussi* RIS, 1902, *Leuctra fusca* (LINNE),1758 *Isoperla lesbica* ZWICK, 1978, *Isoperla chius* ZWICK, 1978, *Perla kiritschenkoi* ZHILTZOVA, 1961, *Plesioperla sakartvella* (ZHILTZOVA), 1956 Türkiye için yeni kayıtlardır.



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# THE EFFECTS OF VARIOUS PHYSIOLOGICAL CONDITIONS ON FAT SYNTHESISING YEAST CANDIDA ALBICANS CBS 562

(Bazı Fizyolojik Koşulların Yağ Üreten *C.albicans CBS 562* Üzerindeki Etkileri)

Nevin Keskin* Ali Matur*

SUMMARY

In this study the effect of pH on biological growth, the utilization of glucose-nitrogen and, eventually, the production of lipids were examined, and it was observed that the pH.5,5 was more appropriate than pH 6,8 for all above. The effect of temperature on lipid production and metabolism of *C.albicans 562* was tested. It was also observed that the aeration rate of 150 rpm is the most suitable level, which was determined by adjusting, the agitation rate.

The production of total lipid was observed to be dependent on the C:N ratio, therefore the glucose and nitrogen used in media were also analysed.

ÖZET

Bu çalışmada pH'nın üremeye, şeker-azot kullanımına ve total

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lipid sentezine olan etkisi incelendi ve pH 5,5'un pH 6,8'e kıyasla daha uygun olduğu sonucuna varıldı. Sıcaklığın, metabolizmaya ve lipid sentezine etkisine bakıldı. Aynı zamanda en ekonomik havalandırmayı (çalkalama hızının ayarlanması ile) saptamak için çalışıldı ve 150 rpm'lik çalkalama hızının optimum olduğu bulundu.

Total lipid verimi C:N oranına bağımlı olduğu için ortamdaki şeker ve azot kullanımı incelendi.

## INTRODUCTION

As in all microbiological processes, the most economic way of bio-oil production depends upon there being an abudence of a cheap and easily awaliable substrate. It is known that the lipid production and the composition of it are greatly influenced by cultural conditions (Ratledge and Hall, 1977; Hall and Ratledge, 1977).

In general, a change in substrate from one carbohydrate to another does not lead to major changes in the fatty acid synthesised, although it can influence the amount of oil produced (Withworth and Ratledge, 1974). It is expectable that the changes in aeration, temperature and pH have effects on the quantity and composition of microbial fats. All work done with changes in these variables upon oleaginous microorganisms has been performed in batch cultures (Kessel, 1968; Rattray et al, 1975).

An adequate supply of oxygen and removal of carbondioxide is essential to maintain growth and metabolic activity of fat synthesising microorganisms. Although it is well established that aeration is required to produce lipid there is no evidence to suggest that

excess aeration results in a higher lipid levels (Babij, et al, 1969; Brown and Rose, 1969).

The lipid content of microbial cells can be significantly effected by the environmental temperature (Thorpe and Ratledge, 1973).

The control of pH is necessary to optimise the growth rate andmicrobial fat production (Withworth and Ratledge, 1974).

In this study the effects of some physiological conditions on fat synthesising yeast *Candida albicans CBS 562* were studied.

## MATERIALS AND METHODS

*C.albicans CBS 562* was used in this study. The modified fermentation media was of originally Murray and Walker (1956). It contained in a liter of distilled water: $(NH_4)_2SO_4$ 1,5 g, $KH_2PO_4$ 0,36 g, $CaCl_2.6H_2O$ 0,1 g, $MgSO_4.7H_2O$ 0,5 g, $FeSO_4.7H_2O$ 0,05g, $ZnSO_4.7H_2O$ 0,02 g, NaCl 0,1 g, Glucose 10 g. Instead of biotin which is rather expensive commerrial producet the dried ethyl alcohol yeast was aimed to use (Keskin, 1981). Dried ethyl alcohol was obtained as a waste material of ethyl alcohol producing factory in Eskişehir.

Batch cultivation was carried out in 250 ml erlenmayer flaks containing 100 ml media in a rotary shaker for 3 days. This period was determined by previous studies (Keskin,1981).

Sugar was determined by phenol-sulfuric acid method according to Keleti and Lederer (1974). Nitrogen was determined by Nesslera-tion method (Rand et al, 1975). Total lipid was determined according to Wilson and Hanner (1955).

## RESULTS

*C.albicans CBS 562* was grown on glucose medium at 30°C and 150 rpm. We tried two different initial pH 5,5 and 6,8. The results are summarized in Table I.

TABLE I. The effects of pH on *C.albicans CBS 562* cultures.

| pH | Growth O.D.490 nm | Sugar utilization (%) | Nitrogen utilization (%) | Total lipid µg/ml |
|---|---|---|---|---|
| 6,8 | 0.490 | 97 | 46 | 238 |
| 5,5 | 0.950 | 97 | 77 | 248 |

It is seen that pH 5,5 was superior to comparision to pH 6,8 for total lipid production.

Three different fermentation temperatures were tested, 23, 30 and 37°C, to find the optimum one. For this, the cells were grown at pH 5,5, 150 rpm. In this test, maximum yield of total lipid was obtained at 30°C (Fig.1). In order to find out the optimum agitation rate, the cultures were prepared in the same way and incubated at 30°C, pH 5,5. Various levels of agitation rates, 100, 150 and 250 rpm, were examined, and agitation rate of 150 rpm was observed to be the most appropriate (Fig.2).

## DISCUSSION

Although the growth of *Candida* yeasts in media pH ranging 3.0 to 7.0 were quite applicable (Dyatlavtskaya et al, 1969) Hall and Ratledge (1977) reported that total lipid content has been

obtained with *Candida 107* grown at pH 5,5. We also found that pH 5,5 was superior to pH 6,8 for microbial fat production (Table I).

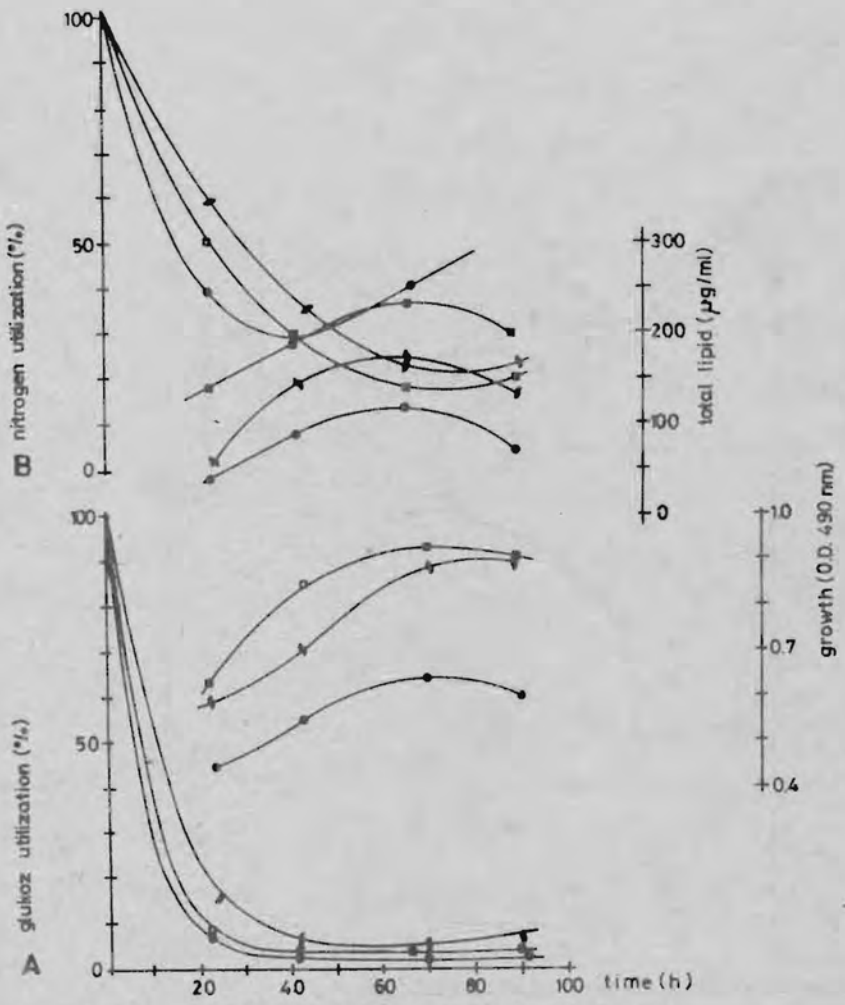
The influence of temperature on growth and metabolism of yeasts has been examined by Stokes (1971). The lipid contents of thermotolerant yeast *C.tropicalis* was shown to be decreased with increasing environmental temperature (Thorpe and Ratledge, 1973). Growth of *C.lipolytica* and *S.cerevisiae* at temperature higher than optimal values was also shown to be resulted in decreased lipid production levels (Kates and Baxter, 1962; Hunter and Rose, 1972).

Decrease in the growth temperature for *C.lipolytica* has been determined to results in unimportant decrease in lipid levels, but an increase in the unsaturated fatty acid ratio (Kates and Paradis, 1973). The results presented in this paper confirm those above. The influence of temperatures, 19, 24, 27, 30, 37$^{o}$C, on the growth and total lipid of *Candida 107* have been examined by Hall and Ratledge (1977). They found that in one-stage system, lipid accumulation was highest at 30$^{o}$C. We also found that 30$^{o}$C was more suitable temperature for lipid production.

Aeration has a pronounced effect on the growth, general metabolism and lipid composition of yeast (Babij et al, 1969). Growth of *C.lipolytica* on hexadecene at low oxygen tension resulted in decrease of lipid levels (Kluq and Markovetz, 1969). It would, therefore, appear that low oxygen inhibits both the elongation and oxygen-dependet desaturase systems. The fatty acid patterns of

total lipid of *Candida utilis* grown under low oxygen tension have been found to be in agreement with our results (Babij et al,1969). The results presented in this paper for the low aeration agrees closely with the findings of them.

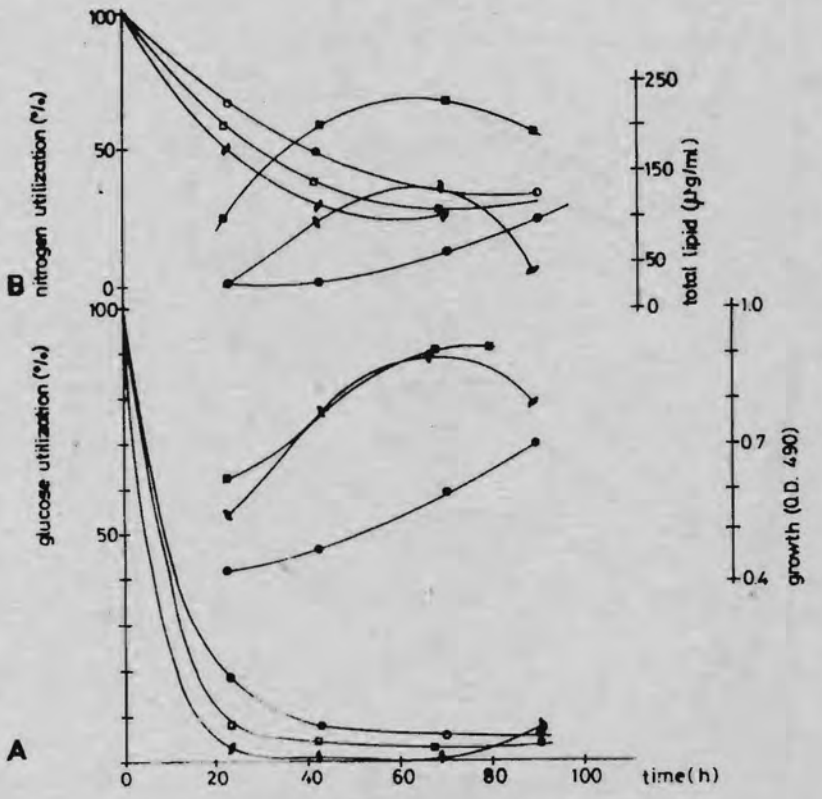
Although formation of saturated fatty acids were predominated, the total lipid levels were lower at higher aeration rates (Klug and Markovetz, 1969). In case of aeration ir required to produce lipids, there is no evidence that excess aeration results in a higher lipid production (Babij et al, 1969; Brown and Rose, 1969). We found that low total lipid levels at agitation rate of 250 rpm, and 150 rpm was more effective agitation rate for total lipid production.




Figures 1: The effect of different temperatures on C. albicans CBS 562

A ∘ The glucose utilization ● The growth at 23°C ▪ " " " 30C ▪ " " " 37C

B □ The Nitrogen utilization ■ Total lipid at 23°C ■ " " " 30C ● " " " 37C




Figures 2: The effect of aeration on C.albicans CBS 562

A ● The growth ○ The glucose utilization at 100 rpm
■ " " □ " " " " 150 rpm
◆ " " ◇ " " " " 250 rpm

B ● Total lipid ○ The Nitrogen utilization at 100 rpm
■ " " □ " " " " 150 rpm
◆ " " ◇ " " " " 250 rpm

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# MEMBRANE-BOUND AND FREE POLYRIBOSOMES IN SOYBEAN

(Soya fasulyesinde membrana-bağlı
ve
serbest poliribozomlar)

Avni Kuru

SUMMARY: The capacity of both free and membrane bound polyribosomes from developing soybean seeds and young soybean hypocotyl to program protein synthesis in a wheat germ cell-free translational system has been examined. In hypocotyl tissue and in developing seeds the two types of ribosomes are in different functional state; it means that the free polyribosomes synthesize more protein than membrane-bound polyribosomes in hypocotyl tissue while the opposite is true for developing seeds of soybean.

## INTRODUCTION

Lately many research workers have focused their effort on isolation and *in vitro* translation of polyribosomes (SUN *et al.*,1975; LARKINS and DALBY, 1975; BURR and BURR, 1976; JONES *et al.*,1977; BEACHY *et al*;1978;

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KRAFT-CREECH and LOCHMANN, 1978; TORRIGIANI *et al.*,1978; EVANS *et al.*, 1979; MORI *et al.*,1979; KURU and CHERRY, 1980; KURU, 1980, 1981a, 1981b) and messenger RNAs(TOBIN and KLEIN, 1975; LARKINS *et al.*,1976b; HIGGINS and SPENCER, 1977; HALL *et al.*,1978; BEACHY *et al.*, 1978; EVANS *et al.*, 1979; KURU, 1980, 1981b; PARK *et al.*,1980; BEACHY *et al.*, 1980a-c; MATTHEWS *et al.*,1981) in plant systems.

In this work polyribosome population and distribution of free and membrane-bound polyribosomes were comparatively investigated in young hypocotyl tissue and in developing seed of soybean. Besides, *in vitro* translation capacity of the free and membrane-bound polyribosomes and messenger RNAs partially purified from polyribosomal preparation was also investigated.

## MATERIALS AND METHODS

### 1. Extraction of polyribosomes

Polyribosomes were isolated from developing seeds (250 mg each) of soybean *(Glycine max)* grown in a greenhouse and from the hypocotyl of three days old dark grown soybean seedling by modifying the methods of JACKSON and LARKINS (1976) and LARKINS *et al.*, (1976a) according to BEACHY *et al.*, (1978). Frozen plant materials were powdered in a cold mortar under liquid nitrogen and then 5 volumes of grinding buffer (0.2 M Tris HCl,pH 8; 0.2 M sucrose; 400 mM KCl; 50 mM $MgCl_2$; 5 mM dithioerythritol) were added, and allowed to thaw. The homogenate was cleared by centrifugation (100xg for 5 min) and the supernatant was centrifuged at 10.000xg for 15 min. The supernatant fraction was saved in ice-box in a cold room as a source of free polyribosomes. The pellet was re-extracted with the same buffer including 1% (V/V) Triton

*X-100*, and 10.000xg supernatant saved as a source of membrane-bound polyribosomes. The supernatans containing the free and membrane-bound polyribosomes were layered on 4 ml of 1.75 M sucrose pad (1.75 M sucrose; 40 mM Tris HCl, pH 8; 20 mM KCl; and 1 mM $MgCl_2$) and polyribosomes were pelleted by centrifugation at 50.000 rpm for 3 hours in the Beckman 50-Ti rotor. After centrifugation, the supernatant was aspirated and the pellet was rinsed briefly with steril, cold deionized $H_2O$, and then resuspended in a 100 ul resuspension buffer ( 40 mM Tris-HCl, pH 8; 20 mM KCl; and 1 mM $MgCl_2$). For sucrose gradient analysis about 5 $A_{260}$ units of polyribosomes were layered on a 12 to 36% linear sucrose gradients prepared in 40 mM Tris-HCl, pH 8; 20 mM KCl; and 10 mM $MgCl_2$, and centrifuged in the Beckman SW-25-1 rotor at 20.000 rpm for 3 hours. Gradients were monitored at 254 nm with an ISCO density gradient analyzer.

2. Purification of messenger RNA

Messenger RNA ( Poly(A)-containing RNA) was purified from polyribosomal material by the method of KRYSTOSEK *et al.*,(1975) as modified by BEACHY *et al.*,(1978). Polyribosome pellets were suspended in a buffer containing 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, and 0.5 % sodium dodecyl sulfate (SDS). After the solution was made 0.5 M with respect to NaCl and heated in a 60°C water bath for 2 min, it was cooled to room temperature and applied to a column containing 1 g of oligo (dT)-cellulose equilibrated in 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.5 M NaCl and 0.5% SDS. After the $A_{260}$ of the efluent returned to the pre-sample application level the column was eluted with buffer without NaCl and 260 nm absorbing material was collected, made 0.2 M with sodium acetate (pH 5.5) and 2 volumes of ethanol were added. The precipitate which formed after standing at least 12 hours at-30°C was collected by centrifugation at 12.000 rpm for 20 min. The

RNA was suspended in glass distilled $H_2O$ and reprecipitated twice with ethanol to remove residual SDS. The final RNA precipitate was dried *in vacuo* and suspended in glass-distilled $H_2O$ and stored frozen.

### 3. *In vitro* translation of polyribosomes and mRNA

*In vitro* translation of polyribosomes and mRNA was performed essentially as described by BEACHY *et al.*, (1978). Reactions were done in 50µl volumes containing 12 mM KCl, 0.4 mM spermidine, 20 mM HEPES (pH 7.5), 0.1 mM $MgCl_2$, 10 mM EDTA, 2 mM dithioerythritol, 80 µM each of GTP and CTP, 1.36 mM ATP, 8.6 mM creatine phosphate, 1 µg creatine phosphokinase, 40 µM each of the protein amino acids (except leucine), 0.25 µCi L-($^{14}$C)-leucine (310 µCi/µM), and varying amounts of polyribosomes or purified mRNAs. Magnesium acetate and potassium acetate concentrations were used 3 mM and 100 mM for polyribosomes and 2.2 mM and 120 mM for mRNAs respectively. Reactions were carried out at 25°C for 45 min. Hot trichloroacetic acid-insoluble radioactivity in 50 µl samples was determined using Whatman GF/A glass fiber filters in a Packard-Tri-Carb Liquid Scintillation Spectrometer.

## RESULTS AND DISCUSSION

Distribution of the free and membrane-bound polyribosomes in hypocotyl and developing seeds are shown in Table I.

Table I. Polyribosome population and distribution of total free and membrane-bound polyribosomes in hypocotyl tissue and developing seed.

| | Polyribosomes (OD/gr.Fr.Wt.) | |
|---|---|---|
| | Free | Membrane-bound |
| Hypocotyl | 4.9 | 3.5 |
| Developing seed | 8.5 | 11.3 |

From the results presented in Table I, it can be concluded that polyribosomal yield is higher in developing seeds than in hypocotyl tissue, and free polyribosomes in hypocotyl tissue and membrane-bound polyribosomes in developing seeds are dominant.

Both the total free and membrane-bound polyribosomes isolated from developing seeds or the hypocotyl tissue gave essentially the same polyribosomal profile with high polyribosomes and low level monoribosomes (Figure 1 and Figure 2).

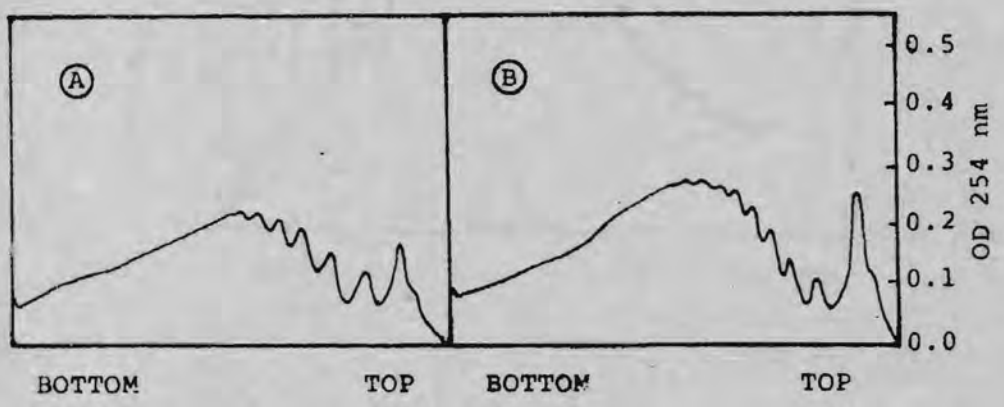


Figure 1. Sucrose gradient sedimentation of total free and membrane-bound polyribosomes from developing seeds (A=Free, B=Membrane-bound polyribosomes). Approximately 5 $A_{260}$ units of total polyribosomes were sedimented on 12 to 36% linear sucrose gradient and monitored at 254 nm.

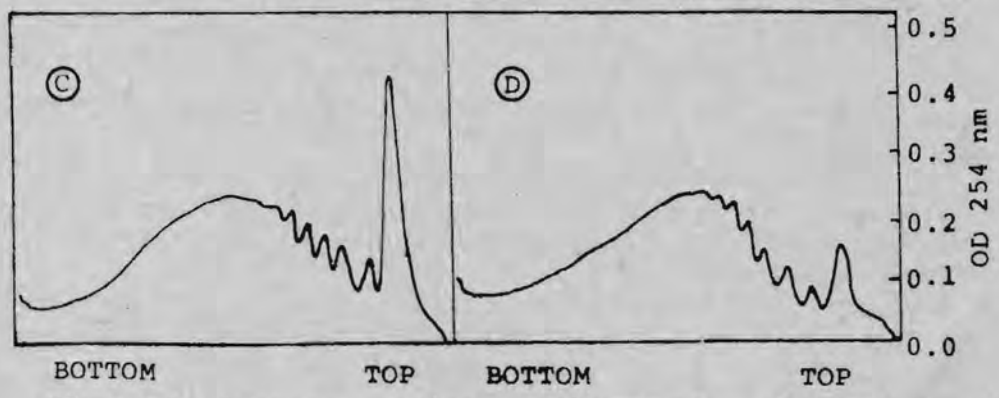


Figure 2. Sucrose gradient sedimentation of total free(C) and membrane-bound(D) polyribosomes from hypocotyl tissue.

Purity of the polyribosomal extract was tested with ribonuclease. After treatment of polyribosomal extract with ribonuclease $T_1$ (20 µg/ml) for 20 min at 20°C before gradient centrifugation, all polyribosomal peaks disappeared and a big monoribosomal peak appeared(Figure 3).

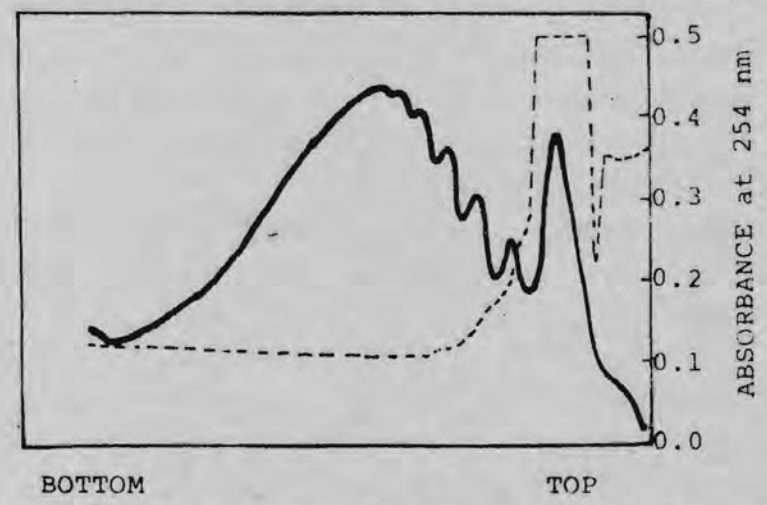


Figure 3. Effect of ribonuclease treatment on polyribosomal profile. Polyribosomes were untreated (—) or treated (---) with 20 µg/ml of ribonuclease $T_1$.

*In vitro* translation capacity of the polyribosomes extracted from young hypocotyl tissue and developing seeds were also tested in a wheat germ cell-free system. As it is shown in Table II, in hypocotyl tissue proteins are synthesized dominantly on free ribosomes. But this is not true for developing seeds of soybean. In cotyledonary tissue proteins are synthesized dominantly on membrane-bound ribosomes.

Table II. *In vitro* translation capacity of the polyribosomes extracted from young hypocotyl tissue and developing seeds. 15 µl polyribosomes were used for each 50 µl assay tube.

| | $^{14}$C-leucine incorporation (CPM) | |
|---|---|---|
| | Free Polyribosomes | Membrane-bound Polyribosomes |
| Hypocotyl | 30860 | 22475 |
| Developing seed | 35155 | 50751 |
| Unincubated control | 338 | 389 |

This result supports the idea that in some plant tissue membrane-bound polyribosomes are active in protein synthesis, BURR and BURR (1976), LARKINS *et al.*, (1976a), KRAFT-CREECH and LOCHMANN (1978). LARKINS and DAVIES (1975) have also showed that approximately 45% of the ribosomal material was membrane-bound (released by detergent) and the remaining 55% consisting primarily of free polyribosomes. According to LUTHE and PETERSON (1977) membrane-bound polyribosomes synthesize about twice the amount of protein as do free polyribosomes in developing oat seeds. Similar results were also observed in 2,4-dichloroacetic acid (a synthetic plant growth regulator) treated soybean hypocotyl tissue, KURU (1981a) and in developing seeds of soybean KURU (1981b).

On the other hand, as far as we are aware, nothing has been published on the level of free and membrane-bound polyribosome distribution in the young hypocotyl tissue of soybean. In this work it is shown that in the young hypocotyl tissue and in the developing seeds of soybean the two types of ribosomes are also in different functional state.

Polyribosomal RNA (Messenger RNA =Poly(A)$^+$ RNA) was also isolated from purified polyribosomes and its template activity tested in an *in vitro* protein synthesizing system. Figure 4 shows the elution profile of seed polyribosomal RNA absorbed to Oligo (dT)-cellulose.

When Poly (A)$^+$ RNA, purified from polyribosomal material, was translated in an *in vitro* system derived from wheat germ, a low level of incorporation was obtained, perhaps due to the other ribosomal RNA contamination or some other reasons which are to be investigated.

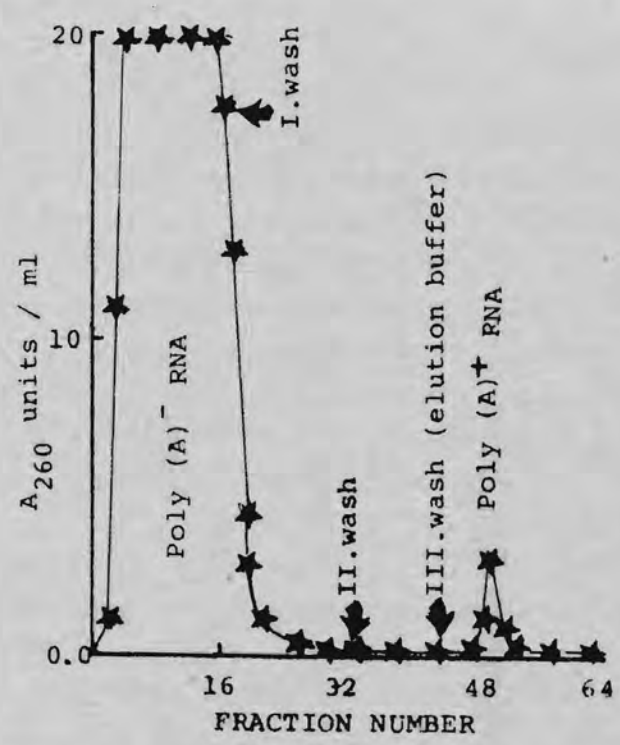


Figure 4. Elution profile of seed polyribosomal RNA absorbed to Oligo (dT)-cellulose.

Table III. $^{14}$C- leucine incorporation directed by Poly (A)$^{+}$ RNA.

| mRNA (µg/50 µl) | $^{14}$C-leucine incorporated (CPM) |
|---|---|
| 1.875 | 7535 |
| 0.900 | 8222 |
| 0.075 | 6280 |
| 0.045 | 4218 |
| 0.000 | 194 |

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*ÖZET*

Gelişmekte olan soya fasulyesi tohumlarından ve genç soya fasulyesi hipokotillerinden ekstre edilen serbest ve membrana-bağlı poliribozomların protein sentezleme kapasiteleri buğday özüne dayalı bir *in vitro* protein sentezleyen sistemde araştırıldı. Her iki tip poliribozomların, hipokotil dokusunda ve gelişmekte olan tohumlarda, farklı fonksiyonel düzeyde oldukları görüldü; diğer bir deyimle, hipokotil dokusunda serbest poliribozomların membrana-bağlı poliribozomlardan daha fazla protein sentezlemelerine karşın, gelişmekte olan tohumlarda bunun tersi geçerliydi.

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# A COPRO-PARASITOLOGICAL INVESTIGATION IN BEYTEPE AND GÜLVEREN PRIMARY SCHOOLS

(Beytepe ve Gülveren İlkokullarında Parazitolojik Bir Araştırma)

Nurdan Özer*

SUMMARY

In this parasitological investigation we have examined 102 stool specimens obtained from Primary School children in Beytepe village and Gülveren district of Ankara. The results showed that 58.8 % of the whole population were infected. A total of 7 species were observed. They were Ascaris lumbricoides (16 %), Hymenolepis nana (4.9 %), Trichuris trichiura (0.9 %), Giardia lamblia (16.6 %), Entamoeba coli (17.6 %), Enterobius vermicularis (1.9 %) and Taenia saginata (0.5 %). In this study we arrived in a conclution that parasitic infection remains to be one of the major health problems in both Beytepe and Gülveren district.

## INTRODUCTION

The parasites have many important pathological effects on human and animals which lead to the production of unhealthy and unproductive new generations, economical loses and even deaths (MERDİVENCE, 1978., MİMİOĞLU, 1977., ÇETİN, 1979., ÜNAT, 1956, 1979., YAŞAROL, 1978). Therefore the parasitological investigations are essential for puplic health in every area of Turkey which is

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convenient for increasing and spreading of many parasites because of the poor cultural and socia-economic situation of the people and insufficient control of the hygienic elemination of human stool and the other relevant environmental factors. There are a lot of studies carried out on this subject in several areas of the world (ACARER, 1963, 1965., BAYKAN, 1969., COSGROVE, 1960., DALMAK,1958., DEANER, 1956., EYLES, 1954., İSFENDİYAROĞLU, 1968., JOSEPH, 1955., KELLEY, 1955., KUNTZ, 1953, 1958, 1960., MERDİVENCİ, 1960., SELLİOĞLU, 1980). In this paper, the results of the parasitological investigation of 102 stool specimens obtained from two rural areas of Ankara were discussed.

## MATERIALS AND METHODS

This study was carried out in the Parasitology laboratory of Beytepe in 1981. A total of 43 and 59 stool specimens were obtained respectively from the students of Gülveren and Beytepe Primary Schools. As in our previous surveys, fecal samples were fixed by the "MIF" method since the materials preserved in this manner are well stained and fixed immediately and can be examined at a later date with very expectation of obtaining reliable idendification of protozoa and helminths. We used two methods in the copro-parasitological investigation. In the direct smear method, the samples having the size of a garden pea were selected from three different part of each stool and mixed with a dorp of Lugol's iodine solution on the slide. The second method was concentration method called formalin-ether techique. In this method, the samples of stool specimens were centrifugated with formalin, ether was used to melt the fats and then the sediment was prepared with lugol. These two methods were essayed together for each sample (MERDİVENCİ, 1979).

Lugol's iodine solution was prepared as follows:

Iodine ..... 1 gr

KI ..... 2 gr

Distilled water..50 cc

## RESULTS AND DISCUSSION

We identified 3 species of helminth eggs and 2 species of protozoon cysts in 59 stool specimens obtained from Beytepe Primary School students (57.6%) and 4 species of helminth eggs and 2 species of protozoon cysts in 43 stool specimens from Gülveren Primary School students (60.4%). The species that we found and their percentages are as follows.

Table I. Incidence of parasitic infection in Beytepe Primary School.

| Parasites | Number of infected children | % |
|---|---|---|
| Ascaris lumbricoides | 14 | 23,7 |
| Hymenolepis nana | 4 | 6,7 |
| Trichuris trichura | 1 | 1,6 |
| Giardia lamblia | 3 | 5 |
| Entamoeba coli | 12 | 20 |
| Toxal examined | 34 | 57,6 |

Table II. Incidence of parasitic infection in Gülveren Primary School.

| Parasites | Number of infected children | % |
|---|---|---|
| Enterobius vermicularis | 2 | 4,6 |
| Ascaris lumbricoides | 2 | 4,6 |
| Hymenolepis nana | 1 | 2,3 |
| Taenia saginata | 1 | 2,3 |
| Giardia lamblia | 14 | 32,5 |
| Entamoeba coli | 6 | 13,9 |
| Toxal examined | 26 | 60,4 |

Beytepe village, located 27 km. west of Ankara, has a population of about 500. The present conditions in the village are convenient for increasing and spreading of many parasites. Although strong sunlight, drough and dryness of the soil prevailed in this

region are probably important factors in the destruction of many parasite eggs and cysts, intense tree shadows and current fountain waters in the village prevent them from the harmfull effects of these factors. Water leakage from outdoor toilets, the close association of the population and the other insanitary conditions are also the factors which create epidemies. Gülveren district with 7000-8000 population has the similar conditions. All these conditions facilitate human-soil-stool circuit and enable the parasites to survive.

Ascariasis caused by Ascaris lumbricoides was the most prevalent and wide-spread parasite infection in both districts. The high prevalance of this parasite and the other helminths such as Trichuris trichiura which is more resistance to climatic factors, is undoubtedly due to the resistance of their ability to develop under a wide range of environment factors. The prevalance of Ascaris lumbricoides in Beytepe (23,7 %) is higher than in Gülveren (2 %) because of the poor sanitary conditions found in Beytepe.

The eggs of Hymenolepis nana and the cysts of Giardia lamblia and Entamoeba coli are infective when they leave the human body (MERDİVENCİ, 1978., UNAT, 1979). So, the high incidence of these parasites are due to poor sanitary conditions, habits and closeness of groups and families rather than climatic factors.This parasitic infection rates found in this study, might be higher if more stool specimens were examined from each individual.

The diagnosis of Enterobius vermicularis requires special techniques and the diagnosis of Taenia saginata is incidental, therefore the findings with these two parasites may to reflect the exact results.

More surveys on the parasitic infections in many parts of Turkey will help cure of infected populations. Additionaly, by coordinated efforts, the great social and health problem of parasite infection can be minimized.

These findings are similar to the surveys of intestinal

parasites of other districts which are similar social and sanitary conditions and climatic factors (ACARER, 1963,1965., BAYKAN, 1969., DALMAK, 1958., SELLİOĞLU., ÖZCAN, 1980).

ÖZET

Bu parazitolojik araştırma, Beytepe ve Gülveren ilkokullarındaki toplam 102 öğrencide yapılmış ve sonuçta %58,6'inin enfekte olduğu saptanmıştır. Yaklaşık olarak, çocukların yarısının 7 ayrı parazit türüyle enfekte olduğu görülmüştür. Bunlar, sıklık sırasıyla, Ascaris lumbricoides (%16), Hymenolepis nana (%4,9), Trichuris trichiura (%0,9), Giardia lamblia (%16,6), Entamoeba coli (%17,6), Enterobius vermicularis (%1,9) ve Taenia saginata (%0,5)'dır.
Bu çalışmanın sonucu olarak, parazit enfeksiyonlarının Beytepe ve Gülveren'de en önemli sağlık problemlerinden biri olarak varlığını sürdürmekte olduğu gözlenmiştir.

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# EFFECTS OF AGITATION AND AERATION ON THE MORPHOLOGY OF THE CULTURE IN PENICILLIN FERMENTATIONS

(Penisilin fermantasyonlarında karıştırma ve havalandırmanın kültür morfolojisine etkisi)

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## SUMMARY

The relationship between morphology and the different environmental conditions in a fermenter has not yet received the attention it deserves in biotechnological investigations. However, tailoring the environmental conditions to obtain the desirable morphology holds a great industrial potential in fungal processes.

This study consists of semi-quantitative data on morphological changes due to operating conditions observed in a high producing strain of Penicillium chrysogenum, in penicillin fermentations.

## INTRODUCTION

The relationship between morphology and the environmental conditions is very important with respect to product formation and the economics of an industrial fungal process. Effects of agitation on microbial morphology and biosynthesis in molds have been reported by many investigators. Increasing the agitation damages the organism and increases the operating costs of a fermenter while providing favorable conditions by enhancing mass, heat and momentum transfer within the vessel. Understanding this relationship and its mechanism may enable us to modify the conditions to suit our requirements, that is inducing the most suitable morphology to provide good mass and heat transfer within the vessel while ob-

taining high product yields at low operating costs.

## GENERAL INFORMATION

Agitation has two main functions in a fermenter. The first is to mix the culture and so maintain relatively homogeneous conditions throughout the culture. For a given fluid, the nature of the mixing process and the mixing time depends on the overall reactor geometry, the impeller type (MOO-YOUNG et al., 1972; NOVAK and RIEGLER, 1975; VAN'T RIET et al., 1976) and the impeller speed. Suspending the cells or the mycelial cultures uniformly throughout the working volume of the fermenter is relatively easy, due to the differences in the densities. Reducing the temperature gradient in the broth and enhancing heat transfer rates between the broth and the cooling surfaces is a more demanding duty, though both can be achieved at low power inputs (BRYANT, 1977).

The second and the most demanding function of agitation is to assist mass transfer between the different phases present in the culture. One of the problems in designing a fermenter is the choice of the correct form of mechanical agitation system. Further complication exists because of the performance characteristics of impellers and the variation of these characteristics with the rheological properties of the broth (BRYANT, 1977).

Generally, the viscosity of most mycelial suspensions is dependent on a number of parameters:

-shape, size and mass of particles,
-flexibility and deformability of particles,
-concentration of particles,
-surface forces on particles,
-rate of shear.

and in fermenters, these parameters depend on:

-age of the broth,
-surface structure of the particles,
-the presence or the production of extracellular substances (MYERS, 1962).

In mycelial cultures, the branched network forms a three dimentional structure which imparts rigidity to the suspension. The long hyphae get entangled with each other at high shear rates, in much the same way as linear polymer molecules. This leads to a very viscous suspension (several thousand cp.) generally with non-Newtonian rheology. Cultures will exhibit low viscosities in the regions of high

shear rate(near the impeller) and very high viscosities in the regions of low shear rate (near the wall).

Hyphal flexibility; that varies with changes in the cell wall composition, changes in branching pattern and variation of turgor pressure; affects the rheological properties of the culture broth. Cell wall composition is influenced by the culture conditions. Increased hyphal branching decreases flexibility and causes an increase in viscosity. Turgor pressure is easily influenced by changes in the osmotic pressure of the culture fluid or the intracellular fluid. Higher osmotic pressures give a lower turgor pressure, making the hyphae more flexible.

So, the operating conditions of a fermenter affect the growth and the morphology of the culture that in turn affects product formation and all of these factors influence the viscosity of the broth.

High viscosity of the mold culture prevents the conditions of fully developed turbulent flow from being established. With such cultures, the flow conditions within the fermenter lie in the transition region where the relationship between operating variables and power consumption are complex. The shear and flow fields have a marked effect on the observed viscosity.

In aerated mycelial cultures, most of the gas flow is in the vicinity of the impeller, resulting in:

-greater gas channeling and lower residence time
-increased bubble size
-deterioration of bulk mixing
-poor distribution of oxygen throughout the vessel.

Poor oxygen transfer results in low dissolved oxygen tension in the vessel which in turn increases the viscosity due to changes in the mycellium morphology. Increasing the degree of mixing improves culture homogeneity and affects the final productivity in a microbial process. This is achieved by preventing aggregation of solid particles and cells, assisting gas to liquid and liquid to cell mass transfer by a reduction in viscosity. There may also be some desirable morphological characteristics that can only be obtained above certain shear rates (METZ and KOSSEN, 1977; KOSSEN and METZ, 1976). However, the more viscous a culture is, the less it is affected by shear in the impeller region. As the viscosity in-

creases, the power dissipated to the broth decreases when the impeller is flooded due to increased bubble size and poor distribution of bubbles throughout the broth.

The effect of shear on microorganisms is difficult to describe quantitatively due to the variability of tissue fragility. In most cases, shear affects morphology and growth. The difficulty arises in separating the shear effects from other effects of agitation (such as oxygen transfer) that are completely interrelated. This work summarizes the morphological changes observed under various conditions during our penicillin fermentations.

## MATERIALS AND METHODS

The culture was a mutant strain of Penicillium chrysogenum, developed by Pan Labs Inc. It is identified as P1 and mainly produces penicillin V. The fermentations were carried out in a baffled 7-liter Chemap fermenter with two sets of four-bladed turbine impellers as described previously (VARDAR and LILLY, 1982) and the conditions were controlled at 26°C, pH 6.7 at different stirrer speeds. Special care was taken during these fermentations to keep the dissolved oxygen tension and all the other conditions at optimum levels and to isolate the effects of various variables. Continuous exit gas analysis was carried out for oxygen and carbon dioxide.

The cultures were examined under a light microscope as unstained and stained slides. The unstained slides were prepared by mixing several drops of culture broth with several drops oflactophenol solution containing: 100 ml lactic acid, 100 g phenol, 100 ml glycerol and 100 ml water. Three different stains were used for the stained slides, trypan blue, cotton blue and a 1:1 mixture of methyl blue and neutral red. These were applied according to the methods described by GURR and MACCONAILL (1965) and HARRIGAN and MCCANCE (1966).

## RESULTS

Microscopic examination of cultures grown under different conditions of agitation and aeration were carried out. In a fermentation under our standard conditions (26°C, pH 6.7, 750 rpm, impeller tip speed 3.14 m/s), the organism existed in three distinct morphological forms simultaneously (Figures 1, 2 and 3):

-free mycelia,
-entangled hyphae forming a mycelial clump,
-pellets.

As the fermentations progressed, the percentage of vacuolation and irregularities in the hyphae increased, the effect being more significant at very low dissolved oxygen tensions. These were in the form of thicker or swollen (A, B) hyphal sections (Figure 1). They also appeared to be more heavily stained and less transparent. Death of individual sections was observed as leakage of protoplasm (C) (Figure 1). When the mixture of methyl blue and neutral red was used for staining, two distinct sections could be clearly seen in especially larger pellets. This stain is known for staining the living and dead organic material in different colors. A thin uniform layer on the outside of the pellet sphere was observed to be living while the inside was dead due to increased limitations in mass transfer.

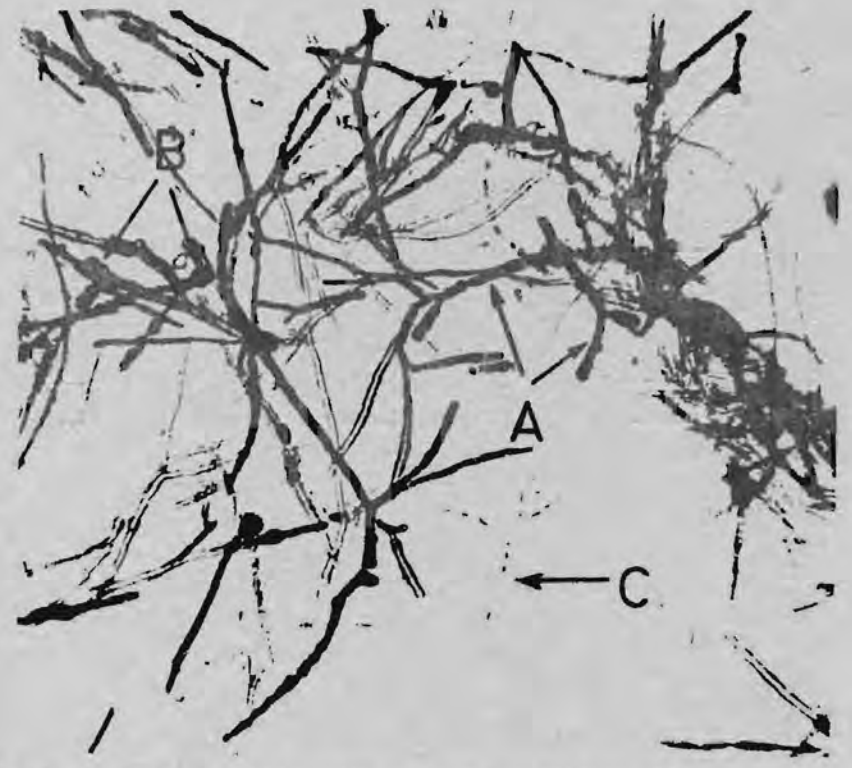


Figure 1: Free mycelia under normal conditions.350X.
Stained with cotton blue.

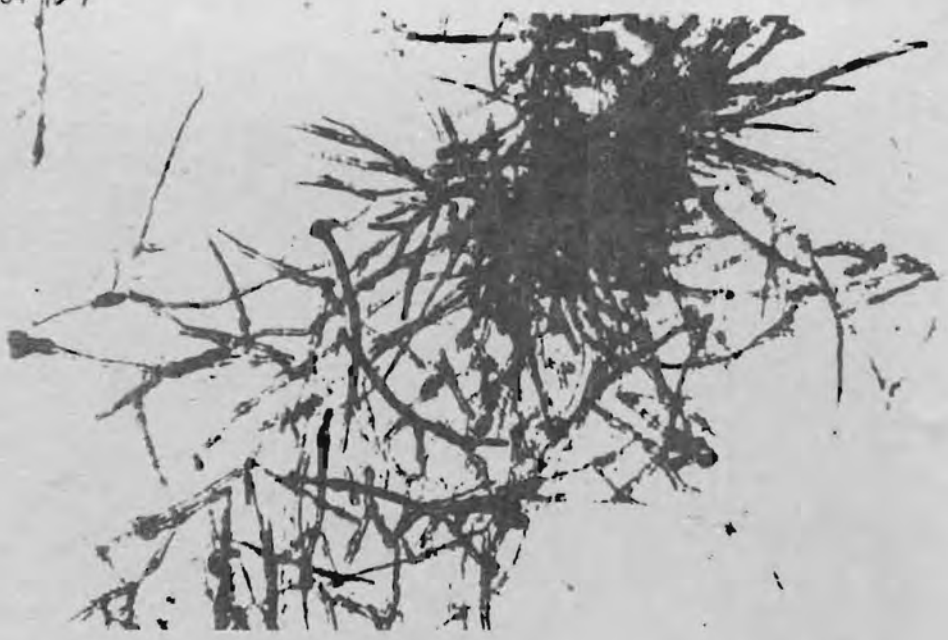

Figure 2: Entlangled hyphae forming a mycelial clump.Stained with cotton blue.Magnification 175X.

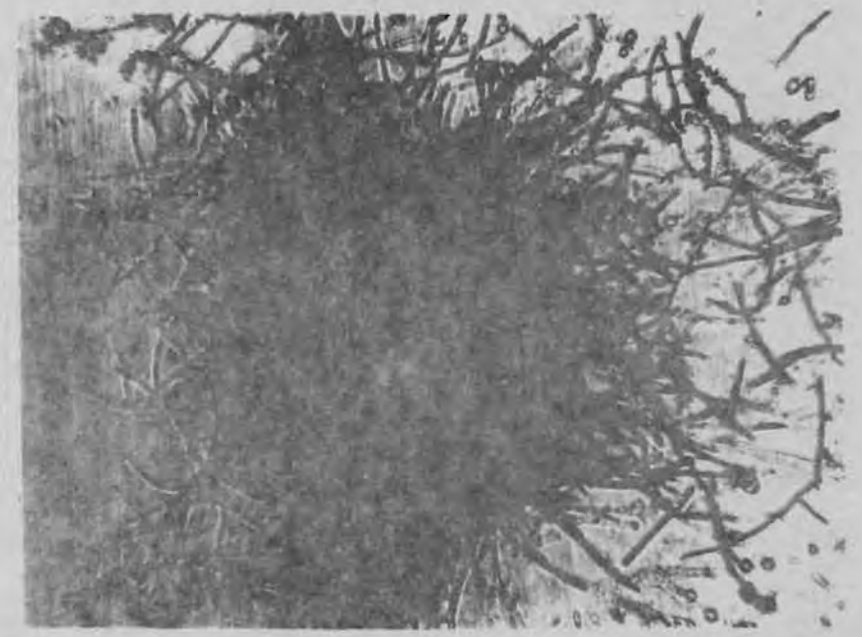

Figure 3: Pellets under normal conditions.Stained with cotton blue.Magnification 175X.

In a standard fermentation, the free mycelia were about 150 - 300 µm long and 3 - 5 µm thick. The pellets ranged between 200 - 500 µm in diameter. In cultures grown under conditions of severe agitation, at high stirrer speeds (1000 - 1100 rpm and impeller tip speeds of 4.19 - 4.61 m/s), free mycelia were shorter and less branched (Figure 4), pellets were smoother with few hyphae extending from the pellet (Figure 5). Furthermore, a significant decrease in the specific penicillin production rate and an increase in the oxygen uptake rate of the culture accompanied this morphological change (VARDAR and LILLY, 1982).

Figure 4: Free mycelia under high shear conditions. Stained with trypan blue, 350 X.

A morphological change in the culture was also observed when small inoculum sizes were used to start the fermentation. A higher percentage of the culture consisted of pellets of approximately 500 µm in diameter with a 2.5 % inoculum. 10 % inoculum gave a higher percentage of free mycelia accompanied by the highest specific penicillin production rate (VARDAR and LILLY, 1982).

Figure 5: Pellets under high shear conditions. Stained with trypan blue, magnification 350X.

The apparent difference in the average thickness of the hyphae in Figures 1 - 3 and Figures 4 and 5 was due to the characteristics of the two different stains used. This difference was not observed in unstained slides where photography was extremely difficult.

## DISCUSSION

There is a fine balance between the establishment of favorable conditions for growth and product formation, such as adequate mass, heat and momentum transfer and the initiation of detrimental shear conditions. Morphological changes in molds due to environmental conditions have been investigated by a number of authors on qualitative (DION and KAUSHAL, 1959; DION et al., 1954; CAMPOSANO et al., 1959) and on quantitative basis (VAN SUIJDAM and METZ, 1981; METZ et al., 1981). In most cases, the observed agitation effects have been traced to different artefacts such as improved mass transfer rates for oxygen, substrates and waste products or increase in temperature. Few attempts have been made to isolate the effects of these interrelated factors.

Intense agitation has been reported to induce the formation of smaller and more compact pellets (METZ and KOSSEN, 1977; CLARK and LENTZ, 1963). DION et al. (1954) showed that impeller tip speeds above 2.5 m/s affected the morphology of Penicillium

chrysogenum and the rate of penicillin synthesis. STEEL (1959) and KÖNIG et al. (1981) observed similar morphological changes as well as significant decreases in antibiotic titres in Streptomyces and Penicillium cultures.

The results in this study agree with the observations of these investigators. However, the elucidation of the relationship between morphology and environmental conditions and the nature and mechanism of these high shear effects in molds require more detailed quantitative studies.

ÖZET

Bir fermantörde farklı ortamsal koşullar ile organizmanın morfolojisi arasındaki ilişki, biyoteknolojik araştırmalarda henüz hakettiği ilgiyi görememiştir. Halbuki arzu edilen morfolojiyi elde edebilmek için ortamsal koşulları değiştirmek, endüstriyel fungi proseslerinde özel ve büyük bir potansiyel ortaya koymaktadır.

Bu çalışmada, yüksek üretici bir Penicillium chrysogenum suşu ile yapılan penisilin fermantasyonlarında çalışma koşullarının doğurduğu morfolojik değişiklikler hakkında yarı kantitatif sonuçlar sunulmuştur.

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# THE EFFECT OF CARBON SOURCES ON THE β-GLUCOSIDASE SYNTHESIS IN CELLULOMONAS FLAVIGENA CULTURES

(Karbon Kaynaklarının *Cellulomonas flavigena* kültürlerinde β-glucosidase sentezine etkisi)

N. KOLANKAYA** and L. DAĞAŞAN

SUMMARY

Since the β-glucosidase enzyme general takes part in the hydrolysis of cellulose into glucose, the synthesis of this enzyme was examined in presence of variour carbon sources in *Cellulomonas flavigena* cultures. There was an inverse relationship between the rate of comsumption of sugars such as glucose; lactose; cellobiose, etc. and inductional rate of the enzyme synthesis. In addition to this, the synthesis of the β-glucosidase has been found to be induced by carboxymethycellulose (CMC), up to a concentration of 1% CMC the medium. The cellobiose, the degration product of cellulose hydrolysis by cellulase also induces β glucosidase synthesis in limited concentrations.

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## ÖZET

β-glukosidaz enzimi genel olarak selülozun glukoza yıkımında yer aldığından, kültür ortamlarında yer alan çeşitli şekerlerin varlığında *Cellumonas flavigena* kültürlerinde β-glukosidaz enziminin sentezi araştırıldı. Enzim sentezi ile şekerlerin kullanımları arasında ters orantılı bir ilişkinin varlığı saptandı. Ayrıca, β-glukosidaz sentezinin karboksimetilselulaz (CMC) tarafından %1 konsantrasyona kadar indükte edildiği bulundu. Düşük konsantrasyonlarda sellobioz'un da β-glukosidaz sentezini indükte ettiği saptandı.


## INTRODUCTION

Analytical studies with the cellulase complex of *Trichoderma viride* have shown that at least three enzymatic components were actively responsible in conversion of cellulose into glucose (SELBY and MAITLAND 1967, WOOD 1968, BERGHEM and PETTERSON 1974). Last step in the enzymatic breakdown of the cellulose to produce glucose is achieved by β-glucosidase, a component of the cellulase complex. Due to the results of various studies carried out with cellulolytic microorganisms, it is possible to say that the sugars such as glucose and cellobiose have repressing effect on the synthesis of β-gluconase

(NISIZAWA;et al. 1971; FUSEE and LEATHERWOOD 1972· FUSEE and LEATHERWOOD 1972: MANDELS and REESE 1960: NISIZAWA et. al. 1971)$_a$

In this paper the carbon sources affecting the β-glucosidase synthesis in *C. flavigena* cultures are examined.

## MATERIALS AND METHODS

The microorganism. *Cellulomonas flaviaena* ATCC 482 used in this study was obtained from American Type Culture Collection, U.S.A. Culture conditions were the same as reported previously (KOLANKAYA, 1980). The cells grown in culture media were harvested by centrifugation and washed twice in 0.1M $K_2HPO_4$ buffer, pH 7.0. The cell suspension prepeared in 5 ml of phosphate buffer was subjected to sonification and then centrifuged to remove cell debris. Supernatant solution so obtained was used as enzyme source to measure the β-glucosidase activity.

Two different subtrates, cellobiose and paranitrophenly-β-D-glucoside (Sigma Co.) were used in determination of β-glucosidase activity. For measuring cellobiase activity 0.25 ml of 0.05 % cellobiose solution prepared in phosphate buffer was mixed with 0.25 ml of enzyme and incubated at 37°C for 20 minutes. The amount of glucose produced in incubation mixture was determined with glucose

oxidase procedure as described by WASHKO and RICE (1961). To determine the arly-β-D-glucosidase activity, the method suggested by HAN and SRINIVASAN (1969) was used. For this purpose, 1 ml of $5x10^{-3}$M of paranitrophenly-β-D-glucoside solution was mixed with 0.5 ml of enzyme and allowed to incubate for 30 minutes at 37°C. After the reaction was stopped by adding 1 ml of 1N $Na_2CO_3$, the intensity of yellow colour formed due to formation of paranitrophenol as result of enzyme activity was determined spectrophotometricaly by means of Coleman spectrophometer at 420 nm wave length.

Since similiar type of activity patterns were obtained for both cellobiase and aryl-β-D-glucosidase (Fig.1); the measurement of the aryl-β-D-glucosidase activity was preferred in most of our experiments. An unit aryl-β-D-glucosidase activity was expressed as amount of enzyme releasing 1μM of paranitrophenol from the substrate in a minute at 37° C. Protein determinations were made by using Lowry's method (LOWRY. et.al.,1951).

## RESULTS AND DISCUSSION

On basis of data obtained by growing *C. flavigena* cells in medium containing 0.1 % Carboxymethylcellulose (CMC), it seems that β-glucosidase synthesis occurs during the exponential growth phase of culture (Fig.2), as in the cellulase synthesis which was shown previously

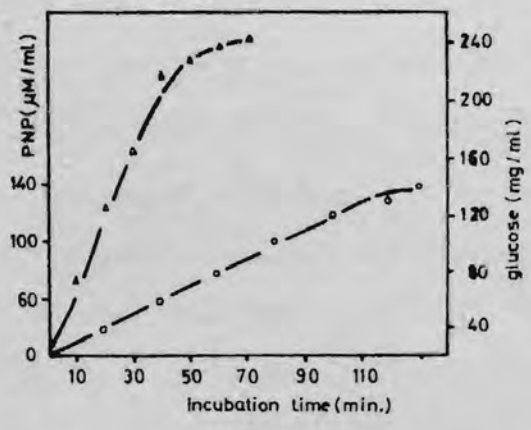


*Figure 1*: Aryl-β-D-glucosidase and Cellobiase activities of β-glucosidase enzyme obtained from *C. flavigena*
▲--:Cellobiase activity, 0--:arly-B-D-glucosidase activity.

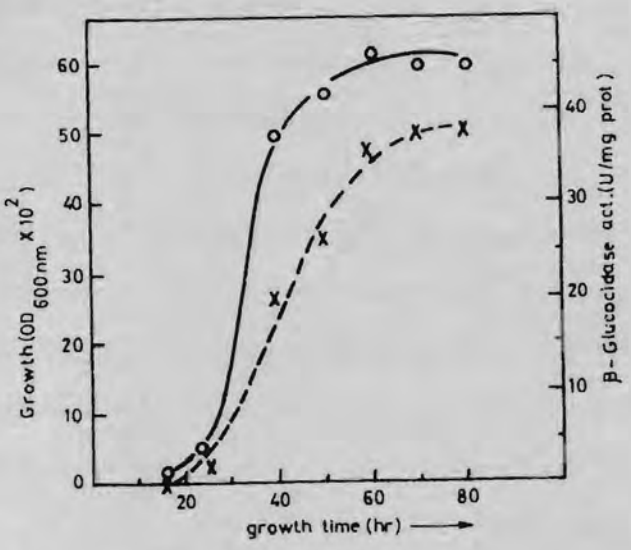


*Figure 2*: Growth and B-Glucosidase activity of *C. flavigena* Organisms was grown in medium containing 0.1%CMC
----0.Growth, -----x:Enzyme activity.

(KOLANKAYA, 1980). Because of this proportionality between the cell growth and enzyme synthesis, the differential synthesis rate of β-glucosidase ( Δ E:ΔU/ΔOD) was calculated as suggested by WELKER and CAMPBELL (1963).

1 % CMC in growth medium was found to be as maximum substrate concentration for the induction of β-glucosidase synthesis (Fig.3). The slight decrease in the rate of β-glucosidase synthesis, for the CMC concentrations more than 1 % in culture media, is probably due to the repressing effect of reducing sugars produced in media as result of cellulase activity of the organism.

According the results given Table I, it can easily be said that there is an inverse relationship between consumptional rate of carbon sources in media and rate of enzyme synthesis. For this reason, the inductive role of CMC for β-glucosidase synthesis can be associated with the slow consumption of this substrate by the organism, due to its high polymeric structure. Already the inductive ability of some sugars for different depolymerase activities was attributed to their slow metabolization by microorganisms (HSU and VAUGH, 1969; LABONAK and PAVLOSKAYA, 1975, 1978; KOLANKAYA, 1979).

Since cellobiose has previously shown chromotographicaly to be as main product of cellulose hydrolysis with the cellulase obtained from *C. flavigena* (KOLANKAYA,1980),

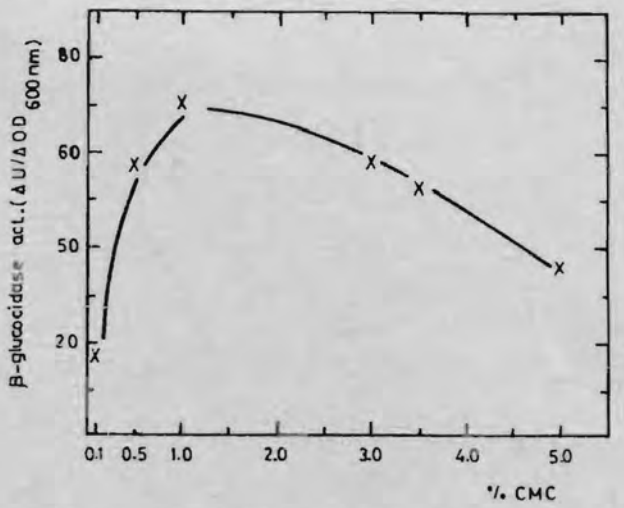


*Figure 3*: Effect of different CMC concentrations of β-glucosidase synthesis of *C. flavigena*.

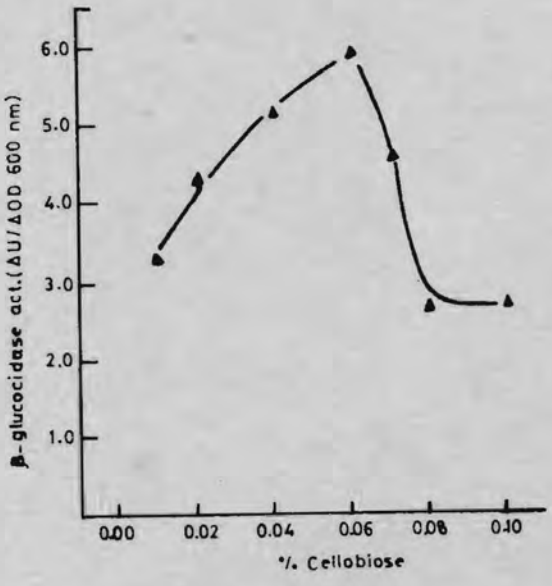


*Figure 4*: Effect of Different Cellobiose concentrations on the β-glucosidase synthesis of *C. flavigena*.

TABLE I: THE SYNTHESIS OF β-GLUCOSIDASE BY C.FLAVIGENA GROWN ON DIFFERENT CARBON SOURCES

| Carbon Source (0.2 %) | Diff.Rate of β-glucosidase Synt. ($\Delta U/\Delta O.D$) | Rate of Growth (mg. Dr.Wt/ml/hr) $x10^3$ |
|---|---|---|
| Glicerol | 6.50 | 0.5 |
| Lactose | 16.25 | 2.0 |
| Cellobiose | 2.98 | 13.0 |
| Glucose | 1.88 | 14.0 |
| Galactose | 0.78 | 14.0 |

Growth rates and β-glucosidase activities of cultures were measured at $36^{th.}$ hour of the growth period.

the possibility of the inducing effect of cellobiose for β-glucosidase synthesis in *C-flavigena* cultures was examined. Consequently, the cellobiose concentrations between the concentrations of 0.01 % and 0.06 % was found to be responsible for induction of β-glucosidase synthesis. Fig.4. Whereas cellobiose concentrations more than 0.06 % in medium have repressed the enzyme synthesis. Consequently, it can be concluded that the β-glucosidase synthesis in *C.flavigena* cultures is under the control of metabolization rate of the carbon sources present in culture media.

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