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EFFECT OF GRAPE EXTRACT ON GROWTH OF YEAST AND BACTERIA

Praveen Kumar Gupta[1], Ullhas Kaarthi. M[2], Saranya G[3], Vidyashree.S[4], Aayush S[5]
Department of Biotechnology, R. V College of Engineering,
Bangalore-59, India.
E- mail: praveenkgupta@rvce.edu.in

Abstract
Grape is known to have a naturally existing compound known as resveratrol, which is found in the skin of red grapes and also in Japanese knotwood plant. The phytochemical resveratrol (3, 5, 4'-transhydroxystilbene) is one of the most widely studied representatives of the plant-produced polyphenols. As a component of grape skin (up to 0.1%), it is extracted, along with the coloured anthocyanins during red wine fermentation. The health multiple beneficial effects of red wine includes anticarcinogenic effect and effects on low-density lipoproteins, is believed to result from its high concentration of polyphenols, including resveratrol. Resveratrol has been claimed to be an activator of Sirtuin1 a gene responsible for aging. Hence an effort has been made to study the details of it in the model organisms such as S. cerevisiae and bacteria.

Keywords- *Grape extract, resveratrol, E. coli, S. cerevisiae, Red wine.*

I. INTRODUCTION

The grape is a non-climacteric fruit, botanically a true berry, that grows on the perennial and deciduous woody vines of the genus *Vitis*. The skin, stem, seeds and juice of the grape are used in making wine. However, they have also been frequently used in producing nutritional supplements.[1]

Resveratrol (3, 5, 4'-transhydroxystilbene) is a polyphenolic phytoalexin. Nearly 50 to 100 µg of resveratrol is present per gram of fresh grape skin. This is particularly true for muscadine grapes, whose skin and seeds have about one hundred times the concentration as the pulp. Moreover, the amount of resveratrol found in grape skin also varies with the grape cultivar, its geographic origin and exposure of fungal infection, while the amount of fermentation time a wine spends in contact with grape skins is an important determinant of its resveratrol content.[2,3] Resveratrol is known to prolong both human and animal life spans. [4] Thus resveratrol is recommended as an important ingredient in the diet plan.

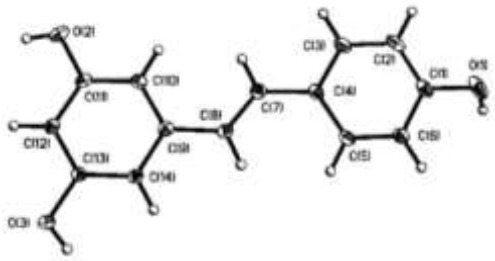


Fig.1 Molecular structure of trans-resveratrol obtained from crystal X-ray diffraction. [5]

Resveratrol minimizes the risk of developing Alzheimer's disease or Parkinson's disease to a great extent. Since it can also be a great energy booster by significantly increasing the level of patience and stamina, it is an important component for athletes. Additionally, studies showed that resveratrol could check harmful types of cancer like that of the skin, breast, colon, esophageal, gastric and leukemia. Although adoption of wine consumption is not recommended by some health authorities, a significant volume of research indicates that moderate consumption, such as one glass of red wine a day for women and two for men, may confer health benefits. Emerging evidence is that wine polyphenols like resveratrol provide physiological benefit whereas alcohol itself may have protective effects on the cardiovascular system [3].

Epidemiologic studies have been able to associate the consumption of foods or dietary supplements and various health outcomes. Though animal experiments can demonstrate what can happen in the species tested, only human clinical trials can determine whether supplementation is useful for humans or not. Resveratrol has not been tested in clinical trials, and most clinical trials of other antioxidants have failed to demonstrate the benefits of anti-aging suggested by preliminary studies. Some substances—most notably beta-carotene have even produced adverse effects. [6-9]

E.coli a model organism: *E. coli* is frequently used as a model organism in microbiology studies. Cultivated strains (e.g. *E. coli* K12) are well-adapted to the laboratory environment and unlike wild type strains, have lost their ability to thrive in the intestine. Hence this strain of *E.coli* was chosen for the present study. [10-11]

Sacchromyces cerevisiae as model organism: It is believed that it was originally isolated from the skins of grapes (one can see the yeast as a component of the thin white film on the skins of some dark-colored fruits such as plums; it exists among the waxes of the cuticle). Many proteins important in human biology were first discovered by studying their homologs in yeast; these proteins include cell cycle proteins, signaling proteins, and protein-processing enzymes. The petite mutation in *S. cerevisiae* is of particular interest. Hence this strain of *Sacchromyces cerevisiae* was chosen for the present study[12].

Materials and Methods:

- ***Saccharomyces cerevisiae*** **(MTCC 170) and** ***Escherichia coli*** **(MTCC 1687) cultures** MTCC plates were procured.
- **Local variety of** Red grapes was purchased from the market.
- Red wine made from local variety of grapes was purchased.

Chemicals:
The following were ordered from HiMedia (Mumbai, India):

- LB medium
- YPD medium
- Ethanol 80% v/v

Experimental methods:

Media preparation:

LB broth and YPD broth were prepared as in Table 1 and Table 2 respectively.

Sl. No.	Constituent	g/1000ml	g/350ml
1.	LB broth powder	25	8.75
2.	Distilled water	1000ml	350ml

Table 1: *E.coli*media:LB broth (pH 6.8) was prepared as given below.

Sl. No.	Constituent	g/1000ml	g/350ml
1.	Yeast Extract	10	3.5
2.	Peptone	20	7.0
3.	Dextrose	20	7.0
4.	Distilled water	1000ml	350ml

Table 2*: S.cerevisae*media: YPD medium (pH 7.2) was prepared as below.

Culturing of *E.coli:*

- The inoculated media were incubated overnight at 31^{o} C in a shaking incubator to reach OD 0.3 - 0.6.
- One loopful of *E.coli* culture was streaked on LB agar media and incubated at 37^{o} C for 24 hours in a bacteriological incubator.
- The OD of the flasks was checked every 2 hours and the growth curve was plotted

Culturing of *S.cerevisae:*

- The inoculated media were incubated overnight at 31°C in a shaking incubator to reach OD 0.3 - 0.6.
- One loopful of *S.cerevisae* culture was streaked on YPD agar media and incubated at 37°C for 24 hours in a bacteriological incubator.
- OD was measured every 2 hours and Growth curve was plotted.

Preparation of grape extract:

- Red grapes (*Vitis vinefera*) were purchased from a vendor shop and washed thoroughly under tap water and distilled water.
- The grapes were peeled, deseeded and crushed using 80% v/v ethanol in a mortar and pestle.
- The crushed homogenate was centrifuged at 6000rpm for 8 minutes.
- The supernatant was distilled at 65^{o}C for 30 mins to eliminate ethanol from the extract.
- The distilled extract was filter sterilized using Whatmann filter paper.
- 10ml of the filtrate was diluted to 25% with distilled water to get 40ml solution.
- The diluted solution was dispensed equally into 4 clean test tubes.
- 20ml of the filtrate was diluted to 50% to get 40ml of solution.
- The diluted solution was dispensed equally into 4 clean test tubes [13-15].

Study of the effect of wine and grape extracts on test organisms:

The culture medium for *E. coli* and *S. cerevisae* were prepared as mentioned below.

E. coli:

- 25ml of LB medium was dispensed into 2 conical flasks, cotton plugged and autoclaved at 15lbs pressure for 15 mins.
- 25ml of wine was added to each of the flasks in the LAF.
- 0.2ml of *E.coli* culture was inoculated in 1 flask and the other was maintained as control.
- OD was taken at 600nm at different times to plot a curve.
- Comparative graph was plotted.

S. cerevisae:

- 25ml of YPD medium was dispensed into 2 conical flasks, cotton plugged and autoclaved at 15lbs pressure for 15 mins.
- 25ml of wine was added to each of the flasks in the LAF.
- 0.2ml of *S.cerevisae* culture was inoculated in 1 flask and the other was maintained as control.
- OD was taken at 600nm at different times to plot a curve.
- Comparative graph was plotted

Grape extract:

E.coli:

- 50ml of LB medium was dispensed into 6 conical flasks; cotton plugged and autoclaved at 15lbs pressure for 15 mins.
- 10ml of grape extract was added to 4 of the flasks in the LAF.
- 2 flasks were used for control.
- 0.1ml of *E.coli* culture was inoculated in 3 flasks and the other 3 were maintained as control.
- OD was taken at 600nm at different times to plot a curve.
- Comparative graph was plotted

S. cerevisae:

- 50ml of YPD medium was dispensed into 6 conical flasks, cotton plugged and autoclaved at 15lbs pressure for 15 mins.
- 10ml of grape extract was added to 4 of the flasks in the LAF.
- 2 flasks were used for control.
- 0.1ml of *S. cerevisae* culture was inoculated in 3 flasks and the other 3 were maintained as control.
- OD was taken at 600nm at different times to plot a curve.
- Comparative graph was plotted

Results:

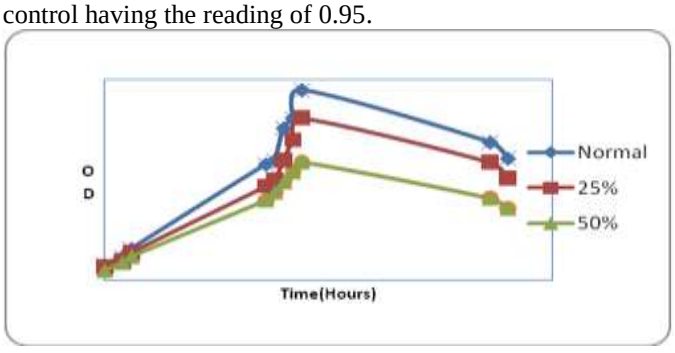
The effect of wine on *E. coli* in LB Medium was studied (Table 1 and Graph 1) by taking the OD values at different time intervals. The table shows that OD value was highest with 0.15 at 22minutes with the control having the reading of 1.79

The effect of wine on ***Saccharomyces cerevisiae*** in YPD medium was studied (Table 2 and Graph 2) by taking the OD values at different time intervals. The table shows that OD value was highest with 0.92 at 30 minutes with the control having the reading of 0.86.

OD= Optical Density at 600nm

Graph 1: The effect of wine on *Saccharomyces cerevisiae* in YPD Medium.

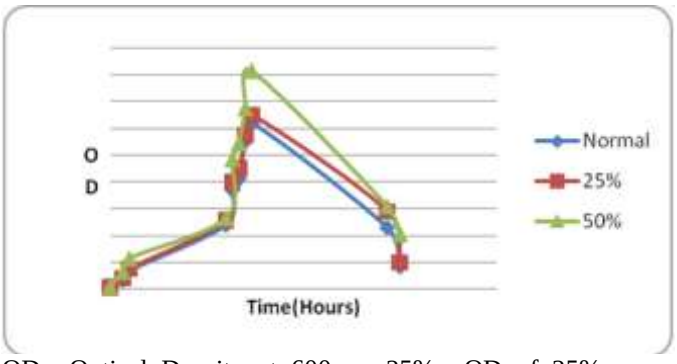
The effect of grape extract on ***E. coli*** in L.B medium was studied (Table 3 and Graph 3) by taking the OD values at different time intervals. The table shows that OD value was highest with 0.81 at 25% and 0.59 at 50% at 22 hours.The control having the reading of 0.95.



OD= Optical Density at 600nm

Graph 2: The effect of Grape extract on *E.coli* in L.B Medium.

The effect of grape extract on ***Saccharomyces cerevisiae*** in YPD medium was studied (Table 4 and Graph 4) by taking the OD values at different time intervals. The table shows that OD value was highest with 1.15 at 25% and 1.63 at 50% at 20 and 22 hours respectively. The control having the reading of 1.24.



OD= Optical Density at 600nm; 25%= OD of 25% grape extract; 50% = OD of 50% grape extract; Control= 10ml grape extract without inoculation.

Graph 3: The effect of Grape extract on *Saccharomyces Cerevisiae* in YPD Medium.

Discussion:

In the present investigation**,** wine showed maximum enhancement of growth followed by grape extract in yeast and minimum in bacteria respectively. We noticed that among the two products, wine seemed to be promising. However the study on yeast (*S. cerevisiae*) showed overexpression of the Sirutin (Sir2) gene that had resulted in a lifespan extension of about 30% [16-17].

Resveratrol found in skins and seeds of grapes vary widely. For instance, the skin and seeds of muscadine grapes have about one hundred times the concentration as the pulp. Among all the research been conducted, one would notice the ambiguity in the results which could be attributed to the geographic variation that affects the grape cultivar and its constituents. Grapes of all colors offer comparable benefits. This is because of the various nutrients in the soil, the climate and water availability. Obviously the difference in the effect of wine and grape extract was observed in bacteria and yeast [18].

When noticing the benefits of this wonder compound and the fears associated with commercial products, we were inquisitive to know how best a developing country like India could use this knowledge for a healthy living.

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