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Enhanced DNA Repair of *Serratia marcescens*, *S. typhimurium*, and *Saccharomyces cerevisiae* by Evening Primrose (*Oenothera spp.*) Oil

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ENHANCED DNA REPAIR OF *SERRATIA MARCESCENS*, *SALMONELLA TYPHIMURIUM*, AND *SACCHAROMYCES CEREVISIAE* BY EVENING PRIMROSE (*OENOTHERA* SPP.) OIL

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ENHANCED DNA REPAIR OF *SERRATIA MARCESCENS, SALMONELLA TYPHIMURIUM,* AND *SACCHAROMYCES CEREVISIAE* BY EVENING PRIMROSE (*OENOTHERA* SPP.) OIL

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TABLE OF CONTENTS

INTRODUCTION

Cancer has been a major health concern for well over three decades. Many approaches have been taken in attempt to slow and/or prevent tumor growth. Today, chemotherapy and radiation therapy are two of the most common treatments. Due to the toxicity of these therapies, however, attention has been turned toward natural anticarcinogens. The Chinese medicinal culture still relies on natural products to combat illnesses, and cancer is not an exception. Recent studies have shown that many of the traditional herbs used to treat cancer actually do have anticarcinogenic properties. For example, Shikaron, which is a preparation of eight different Chinese herbs, was found to decrease the incidence of urinary bladder carcinoma in mice treated with a mutagen (15). Another herbal remedy used in China, called PC SPES, showed inhibitory effects toward tumor cell lines, including prostate carcinomas, breast carcinomas, melanoma, and leukemia (12). The development of hepatocellular carcinoma was even prevented in patients with cirrhosis using another Chinese herbal preparation, Shosaiko-to or TJ-9 (20). The method of tumor inhibition appears to be somewhat varied. Some herbal preparations directly affect the G_1 phase of cell growth (12), and others work indirectly by promoting the transformation of T-lymphocytes (25).

The active substances in many herbs have been determined. It has been found that human colon cancer, for example, can be reduced by as much as 50% with the use of β-carotene, which is found in many vegetable oils (5). The β-carotene anticarcinogenic effect may be due to its ability to accept and donate electrons (2). Vitamin A (retinol), vitamin C (ascorbic acid), and vitamin E (α-tocopherol) act as antioxidants and scavenge free radicals. It is also possible that retinol may protect the genome directly through some kind of interaction with DNA (24). Luteolin, a compound found in herbs such as peppermint, thyme, and sage, has been identified as an antimutagen and antioxidant (22).

The natural oils are another class of compounds that have been investigated for their antimutagenic effects. Researchers were able to prevent the growth of chemically-

induced mammary tumors using palm oil as an antimutagen. It was thought that the high amount of α-tocopherol (vitamin E) found in palm oil was responsible for the anticarcinogenic effects (2). An oil that has recently been of interest to researchers is Evening Primrose oil (EPO). EPO has been used to treat everything from atopic eczema and sclerosis to PMS and diabetic neuropathy. It is especially interesting because it contains high levels of linoleic acid, which is a suspected anticarcinogen (1). In one study, the ability of linoleic acid to prevent tumor growth was investigated. It was found that gamma-linoleic acid decreased the incidence of tumors induced by dimethylbenzanthracene (DMBA) in rats (4). In other investigations, it was shown that linoleic acid also has the ability to inhibit the growth of preexisting tumors(9). However, it is not yet known how EPO, or its main constituent, linoleic acid, prevents tumor formation.

The purpose of this research was to investigate the suspected antimutagenicity of EPO. It has already been stated that EPO contains unusually high levels of linoleic acid, which is an essential fatty acid. Linoleic acid is also a precursor of prostaglandin E and its derivatives. Another interesting factor is that the unsaponifiable matter of EPO is very similar to cottonseed oil, containing mostly γ-tocopherols and some α-tocopherols (14). A 1998 study tested the antimutagenicity of various vegetable extracts by exposing a strain of *Escherichia coli his*$^-$ to UV light, adding the mutated cells to a liquid vegetable extract, and then plating the *E.coli* cells onto media deficient in histidine. In this manner, the number of revertant *his*$^+$ could be recorded and used as a measure of the antimutagenicity of the extracts (18). The above method of using bacterial cells as models for tumor cells to test a natural compound for its antimutagenicity, and thereby, its possible action as an anticarcinogen, is similar to the method used in the present investigation. In this experiment, the antimutagenicity of EPO was tested using five different organisms chosen for their diversity: *Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, Serratia marcescens,* and *Saccharomyces cerevisiae.*

E. coli is a gram-negative rod found in the digestive tract of many animals. Many viruses use *E.coli* as a host, including the "T even" coliphages (16). *S. aureus* is a gram-positive, aerobic cocci (16). *S. typhimurium* is another gram-negative rod (13). Strains of *S. typhimurium* are often used in the Ames test to determine if a chemical induces mutations. *S. marcescens* is a pigmented, gram-negative rod. The pigment, known as prodigiosin, gives a reddish color in colonies that are grown below 35°C. Prodigiosin is a secondary metabolite that is made in the late log or stationary phases of growth. The composition of prodigiosin consists mainly of the amino acids proline, histidine, methionine, and alanine (16). *S. cerevisiae*, unlike the bacteria used in this research, is a eukaryotic organism. This strain of yeast, also known as Brewer's yeast, is used primarily for its fermenting abilities, efficiently catabolizing glucose to make CO_2. *S. cerevisiae* is an ascomycete, and therefore has a life cycle similar to *Neurospora* sp. (16).

Mutation, in general, is defined as, "...an inheritable change in the sequence of DNA." Within a population, the organisms that carry this genetic change are known as mutants, and the organisms within that same population that do not carry the particular genetic change are considered wild type individuals. Mutations can occur in a population through two different avenues: random errors in DNA replication or non-repairable damage done to DNA from external forces. The former type of mutation is termed spontaneous mutation, and the rate of spontaneous mutation varies between populations. The latter form of mutation can be caused by a variety of chemical and physical mutagens. Different types of mutations can occur to an organism's genome depending upon the type of mutagen to which the organism is exposed. In general, there are three types of mutation that can occur: substitution, deletion, and insertion of a nucleotide. Substitution involves phenomena such as point mutations and missense mutations, whereas deletions and insertions usually cause a shift in the reading frame (16). Mutations in particular gene sequences can cause the mutated cells to divide at an abnormal rate and form what is commonly known as a tumor (6).

Ultraviolet (UV) light was used as a mutagen in the present study. Ultraviolet radiation is a type of physical mutagen that changes an organism's genome in a very specific manner. It can damage DNA by creating pyrimidine dimers and other products within the double-stranded helix (23). (See Figure 1).

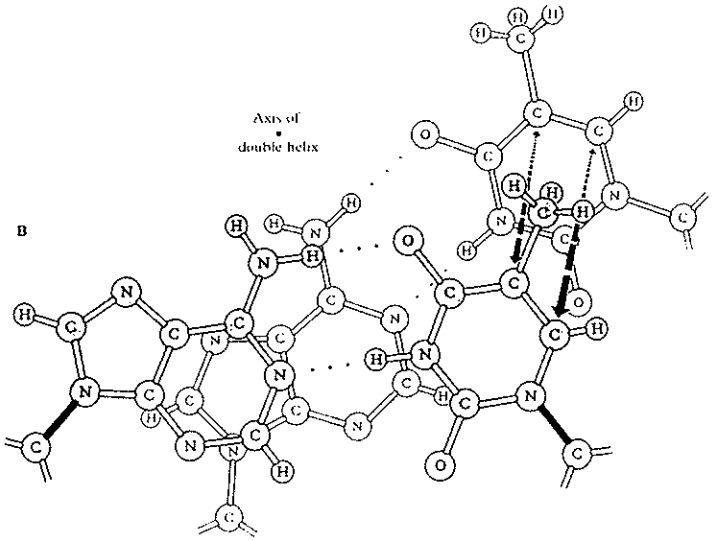


FIG. 1. A molecular level representation of pyrimidine dimers formed within the DNA helix as a result of UV light exposure (23).

These dimers distort the helix, thereby interfering with replication and transcription (16). Generally, the intensity of the UV light application is directly proportional to the number of mutations that occur within an exposed organism's genome.

At the onset of this research, it was suspected that some organisms would have increased resistance to UV light when EPO was available in their environment. This increased resistance would be quantified by a decreased number of lethal mutants when compared to populations that had been exposed to UV light but had no aid from EPO.

However, it was also expected that some organisms would not be aided by the addition of EPO to their environment. This expectation was based solely on the fact that great diversity exists between the organisms tested, and this diversity would manifest itself as varied reactions to treatment with UV light and EPO. However, it was hoped that the diversity of reactions to the mutagen and suspected antimutagen would point the way to discovering the method by which EPO appears to slow, stop, and prevent tumor growth.

MATERIALS AND METHODS

I. Organisms and Media Used

The organisms used in this research can be found in Table 1.

TABLE 1. Organisms used in research, as well as their particular strains and the genetic characteristics and sources of those strains.

Organism Used	Particular Strains	Genetic Characteristics	Source
E. coli B	wild type		Dr. Ellen Jensen
S. aureus	wild type		Dr. Ellen Jensen
S. typhimurium	wild type		Dr. Ellen Jensen
	Ames strain ATCC 29629	inactive *his G* gene, *rfa* gene, and *uvrB* gene	Dr. Ellen Jensen
S. marscecens	wild type		Dr. Ellen Jensen
S. cerevisiae	3-1		Dr. Ellen Jensen
	SX46		Dr. Mike Reagan
	SX46 Δrad2::TRP1	inactive *rad2* gene	Dr. Mike Reagan
	SX46 Δrad1::URA3	inactive *rad1* gene	Dr. Mike Reagan

All bacterial cultures were grown on Difco Tryptic Soy Agar (TSA). The yeast cultures were grown on Yeast Mold Agar (YMA). When necessary, bacteria were grown as a liquid culture by innoculating a 250 mL erlenmeyer flask containing 50 mL Tryptic Soy Broth (TSB) with the desired cells. The flasks were incubated overnight in a 37°C incubator. Yeast cells were obtained by washing actively growing plates with 3.0 mL of TSB. Dilution tubes were prepared by adding 1 g of tryptone to 1 L of distilled deionized water.

Minimal media for Ames tests was prepared according to *Microbiology in Practice: 6th Edition* (3). Variations of TSA plates were also prepared by adding Evening Primrose oil (EPO), peppermint oil (PEP), or corn oil as a control. Ten mL of each oil were emulsified in solutions of 40 mL distilled deionized water and 100 μL Tween 80 with stirring. Thirty mL of the respective emulsified oil solution was then added to 650 mL of autoclaved media that had been cooled to 60°C. The media was stirred for a minute, and plates were poured. Some solutions of minimal media and YMA also had EPO added to them in the same manner. The final concentration of oils in the media was 1.0%

TSA with vitamins A, C, and E was made as reference media, since these vitamins are considered antioxidants. This was accomplished by dissolving one gram of each of the vitamin compounds in 95% ethanol (A and E required 10.0 mL EtOH, C required 6.0 mL EtOH). Three mL of each solution was added to 650 mL autoclaved TSA after it had cooled to 60°C. The entire solution was stirred, and plates were poured. The final concentration of vitamins A and E in the media was 0.0015 g/mL, and was 0.0092 g/mL for vitamin C.

II. Growth Curves and Standard Curves

Growth curves were generated for *E. coli B*, *S. marcescens*, *S. typhimurium* wild type, and *S. aureus*. A stock culture of each of the bacteria was grown up overnight in TSB. One mL of the stock solution was then added to 100 mL of TSB and placed in a water bath shaker at 37°C and 150 rpm. Optical density (OD) readings were taken in triplicate every 30 minutes using a spectrophotometer (Milton Roy Co., Spectronic 20D) set at a wavelength of 600 nm. When the culture was going through log phase, as indicated by

consecutive doubling of OD readings, the culture was plated on TSA in triplicate at dilutions of 10^{-5} and 10^{-6}. The plates were incubated for 24 hours in a 37°C incubator so that cell density could be associated with an OD reading. Once the culture reached stationary phase, plating was discontinued.

The resulting growth curve (OD vs. time) was plotted for each bacteria, and a standard curve showing the relationship between OD and cell density during log phase was also generated.

III. UV Kill Curves

Since organisms respond differently to UV light, log phase cultures of *E. coli B, S. marcescens, S. typhimurium* wild type, *S. typhimurium his*⁻ Ames strain, and *S. aureus* were exposed to UV light for various amounts of time to determine the sensitivity of these microbes. A January – term class generated the UV kill curve for *S. cerevisiae* 3-1.

Once the culture reached mid-log, 5 mL of the culture was pipetted into an empty petri dish (cover off). A 100 μL sample was diluted and plated in triplicate to determine cell density. The petri dish was then placed on a rocker under UV light. At various time intervals, 0.1 mL samples were again taken, diluted, and plated. All plates were placed in a 37°C incubator for at least 24 hours. After the incubation period, the colonies on each plate were counted, and the plates that represented 10% survivorship (as compared to those plates that were not exposed to UV light) were noted. The colonies growing on these plates were screened for mutation.

IV. Evaluation of Mutation

Mutants of *S. marcescens* and *S. cerevisiae* strains were selected based on a pigment change from red or pink to white.

Mutants of *S. aureus* were selected using a catalase test. Each colony of *S. aureus* growing at 10% survivorship was transferred to another plate using a sterile toothpick. The duplicate plates were then incubated at 37°C until growth was apparent. A drop of hydrogen peroxide was added to each colony on one plate. Bubbling by a treated colony indicated that catalase was present and no mutation had taken place.

E. coli B was tested for mutation in phage receptor genes by streaking out each potentially mutated colony (those at 10% survivorship) onto plates of TSA. Phage T2 and T7 at a 10^{-8} dilution were spotted onto each streak. These plates were incubated for 24 hours at 37°C and checked for plaque formation.

S. typhimurium his^{-} Ames strain was evaluated for mutation by plating on minimal media. Since *S. typhimurium his*$^{-}$ Ames strain is incapable of making histidine, it will not normally grow on minimal media. Therefore, any colonies that grew on the minimal media after exposure to UV light had the ability to make histidine and were the result of a mutation to the *his*$^{+}$ genotype.

V. Toxicity Test

An assay was developed to determine if the suspected antimutagens had any toxic effects on the strains of bacteria tested (*E. coli B, S. marcescens,* wild type *S. typhimurium,* and *S. aureus*). Four mL of TSB was added to 139 cuvettes. Cuvettes 1-30 served as controls, each containing 40 μL of the bacteria in question. Cuvettes 31-139

tested the toxicity of EPO, PEP, and corn oil at various concentrations against the bacterial strains by adding 10, 20, or 40 μL of each oil to 40 μL of a specific bacterial strain. Each trial was done in triplicate. The cuvettes were then placed in a 37°C incubator for at least 24 hours, after which the cuvettes were vortexed and absorbance readings were taken to determine if the oil treatments hindered or enhanced bacterial growth.

VI. Ability to Prevent Mutation Following UV Exposure

Three cultures of *S. marcescens* were started by adding 0.5 mL of stock culture to 500 mL TSB. To one of these cultures, 5 mL EPO was added, to another, 5 mL PEP, and to the last, 5 mL corn oil. All cultures were grown to mid-log populations by incubation in a water bath shaker at 37°C and 150 rpm. Five mL of each of the cultures was exposed to 30 seconds of UV light. As described in the method for the UV kill curve, 100 μL samples of each of the cultures were then taken and plated in triplicate at dilutions of 10^{-4}, 10^{-5} and 10^{-6} . The plates were placed in an incubator at 30°C for 24 hours.

VII. Test For Ability to Enhance DNA Repair Mechanisms Following UV Exposure

A culture of *S. marcescens* was grown to log phase in a water bath shaker set at 37°C. A 100 μL sample of the culture was then plated in triplicate on four different types of media: TSA, TSA + PEP, TSA + corn oil, and TSA + EPO. The plates were exposed to 30 seconds of UV light and placed in an incubator at 30°C for 24 hours.

This experiment was repeated following the same steps, however the culture was plated on TSA, TSA + EPO, TSA + vitamin C, TSA + vitamin A, and TSA + vitamin E.

Also, two different forms of the *S. marcescens* strain were tested for their reaction to UV light: pigmented (incubated at 30°C) and non-pigmented (incubated at 37°C). In all phases of the experiment, viable cell density was determined before the culture was exposed to UV light by plating triplicate dilutions of the bacteria at 10^{-4}, 10^{-5} and 10^{-6}.

Cultures of *S. typhimuruim his*$^-$ (Ames strain) and *S. typhimurium* wild type were grown to log phase in a water bath shaker at 37°C. In the same manner as the *S. marcescens* culture, 100 μL samples of the two *S. typhimurium* cultures were plated in triplicate. The inoculated plates of *S. typhimurium his*$^-$ (Ames strain) were exposed to UV light for 1 second, 5 sec., 10 sec., 15 sec., 30 sec., and 60 sec. The inoculated plates of *S. typhimurium* wild type were exposed to UV light for one minute. Both cultures were plated at dilutions of 10^{-4}, 10^{-5} and 10^{-6}. All plates were incubated at 37°C for 24 hours.

Aliquots of actively growing cultures of *S. cerevisiae* 3-1, SX46, SX46 Δrad2::TRP1, and SX46 Δrad1::URA3 were plated in triplicate on YMA and YMA + EPO. The plates with the 3-1 strain and the SX46 strain were exposed to UV light for two minutes and then incubated for 24 hours in a 30°C incubator. The plates with the SX46 Δrad2::TRP1 strain and SX46 Δrad1::URA3 strain were exposed to UV light for 5 seconds and then incubated for 24 hours at 30°C. All cultures were serially diluted and plated to determine cell concentration.

VIII. Analysis of *S. marcescens* Pigment

An attempt was made to extract the pigment found in *S. marcescens*. Cells were grown overnight at room temperature to produce a very intense red color. Samples were spun down in a centrifuge (International Clinical Centrifuge: Model CL) at 800 rpm until

a pellet collected at the bottom of the centrifuge tubes (approximately ½ hour). The supernatant was poured off. Chloroform, corn oil, and ethanol were added to three different pellets. If extraction appeared successful, i.e., two layers formed and one visually appeared to contain the desired pigment, a UV visible scan using a Beckman DU-64 Spectrophotometer was performed on the extracted pigment. Water was used as a blank. A scan of the *S. marcescens* culture itself was also performed, using an *E.coli B* culture as a blank.

IX. Disk Diffusion Test for Other Mutagens

A search for other mutagens to use in future experimentation was initiated using the disk diffusion method (Lim, 1998). Eleven different mutagens (chloroform, carbon tetrachloride, ortho toluidine blue, urethane, colcicine paste, dimethylsulfoxide (DMSO), ethidium bromide, formamide, toluene, lithium acetate, and cobalt chloride) were chosen. Three different organisms, *S. marcescens*, *S. typhimurium his*⁻ Ames strain, and *S. cerevisiae* 3-1, were used. One hundred µL samples of *S. marcescens* were plated on TSA, *S. typhimurium* was plated on minimal media, and *S. cerevisiae* was plated on YMA. Chemicals were tested three times. The plates of *S. typhimurium* were incubated for 24 hours at 37°C, and the plates of *S. marcescens* and *S. cerevisiae* were incubated for 24 hours at 30°C.

RESULTS

I. Growth Curves and Standard Curves

Growth curves for each of the bacteria tested indicated the following approximate periods of logarithmic growth: *E. coli B*- 120 min. to 210 min.(Figure 2); *S. typhimurium* wild type- 120 min. to 180 min. (Figure 4); *S. marcescens*- 150 min. to 300 min. (Figure 6); *S. aureus*- 180 min. to 270 min. (Figure 8). From this data, it was then possible to determine the approximate time needed to reach mid-log phase for each of the organisms. The approximate mid-log for *E. coli B* is 165 min., and for wild type *S. typhimurium*, *S. marcescens*, and *S. aureus*, 225 min.

Standard curves were prepared by plotting OD against cell density. A line of best fit was drawn on each graph so that any given absorbance could be associated with cell population (Figures 3, 5, 7, and 9).

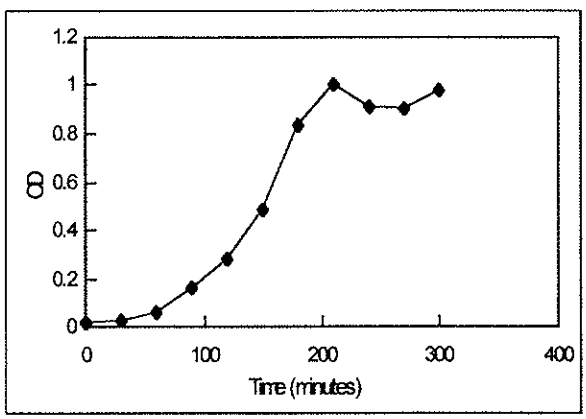


FIG. 2. Growth curve for *E.coli* was established by taking OD (optical density) readings of the growing culture (in TSB at 37°C) every half hour on a Spec20 until log phase had been achieved.

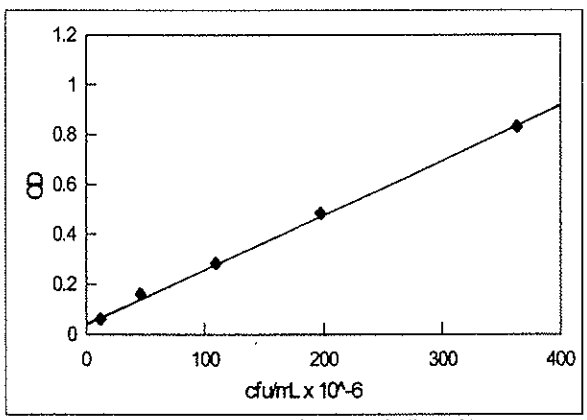


FIG. 3. Standard curve for *E.coli* (in TSB at 37°C) established by comparing the cfu at each half hour interval with the OD (optical density) for the corresponding interval.

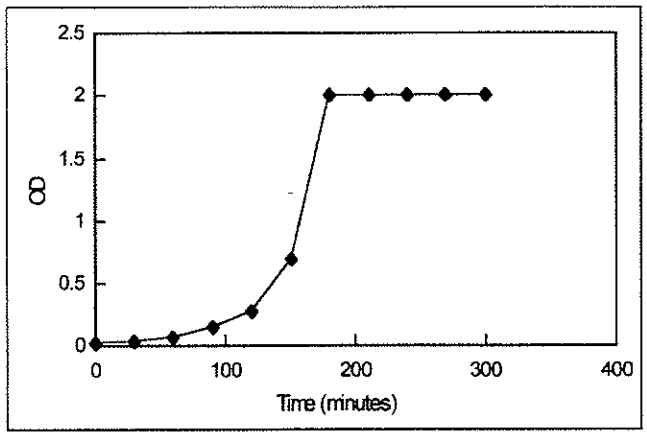


FIG. 4. Growth curve for *S. typhimurium* was established by taking OD (optical density) readings of the growing culture (in TSB at 37°C) every half hour on a Spec20 until log phase had been achieved.

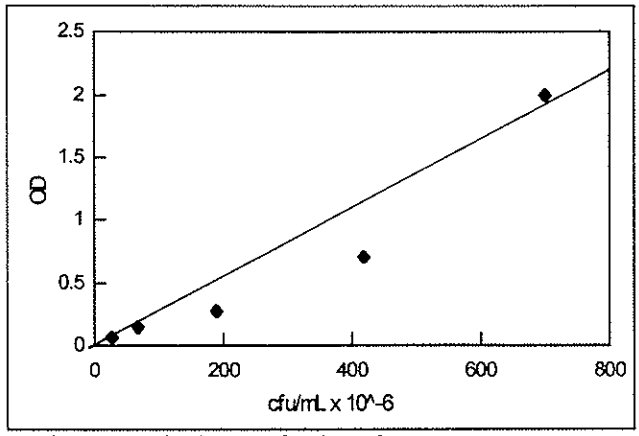


FIG. 5. Standard curve for *S. typhimurium* (in TSB at 37°C) established by comparing the cfu at each half hour interval with the OD (optical density) for the corresponding interval.

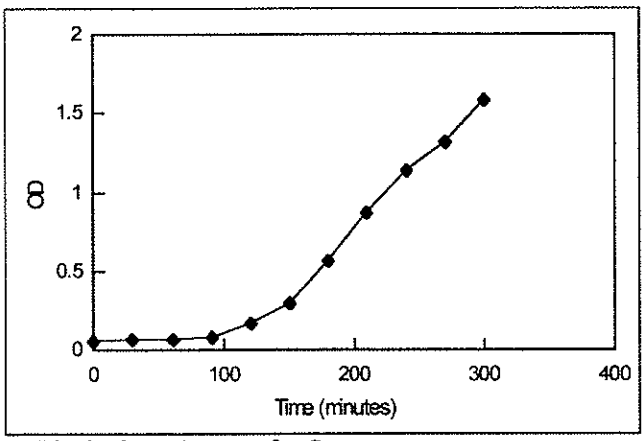


FIG. 6. Growth curve for *S. marcescens* was established by taking OD (optical density) readings of the growing culture (in TSB at 37°C) every half hour on a Spec20 until log phase had been achieved.

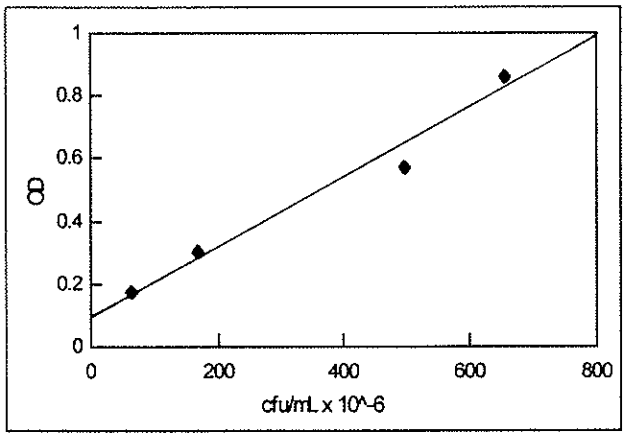


FIG. 7. Standard curve for *S. marcescens* (in TSB at 37°C) established by comparing the cfu at each half hour interval with the OD (optical density) for the corresponding interval.

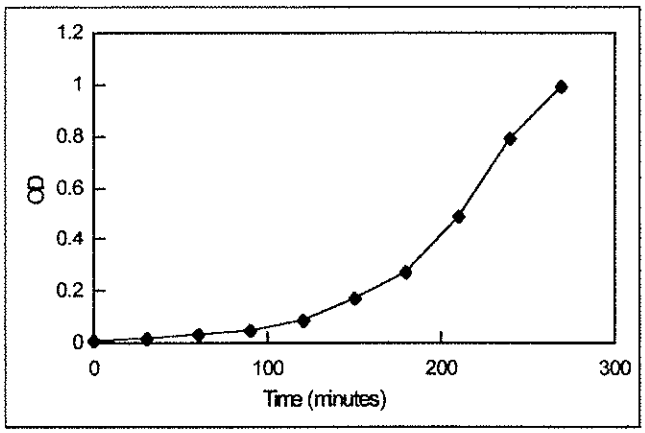


FIG. 8. Growth curve for *S. aureus* was established by taking OD (optical density) readings of the growing culture (in TSB at 37°C) every half hour on a Spec20 until log phase had been achieved.

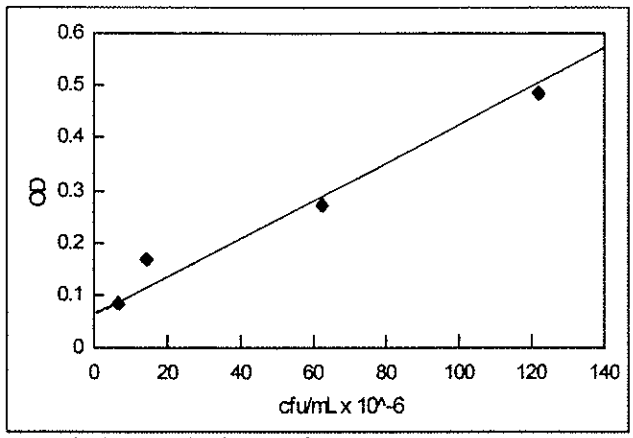


FIG. 9. Standard curve for *S. aureus* (in TSB at 37°C) established by comparing the cfu at each half hour interval with the OD (optical density) for the corresponding interval.

II.UV Kill Curves

As seen in Figures 10–15, all the organisms studied are very sensitive to UV light. *S. typhimurium his*⁻ (Ames strain) appears to be the most sensitive to UV exposure. Its population was decimated after being exposed to UV light for only 60 seconds. From one second of UV exposure to five seconds, average cfu/mL dropped from 1034.67 to 59.67. By 15 seconds of UV exposure, the population was down to an average of seven cfu/mL (See Figure 10).

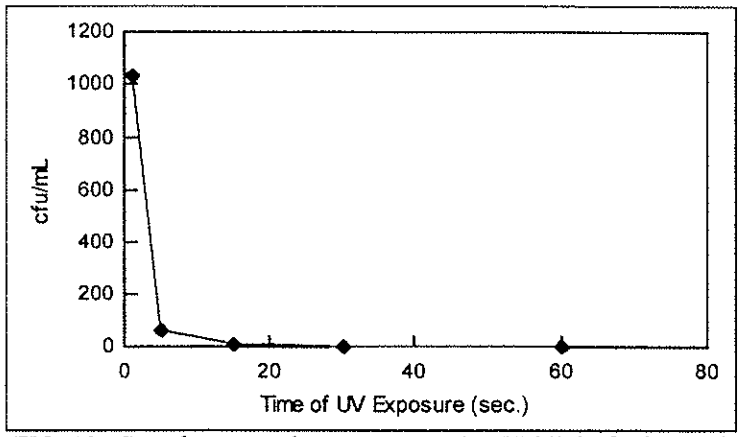


FIG. 10. *S. typhimurium his*⁻ was exposed to UV light for increasing intervals of time to determine its sensitivity to the mutagen.

E. coli B is also extremely sensitive to UV light. At cell density of 10^{-5} cfu/mL, the population of *E. coli B* dropped from an average 938 cfu/mL to 3 cfu/mL after 30 seconds of UV exposure. (See Figure 11).

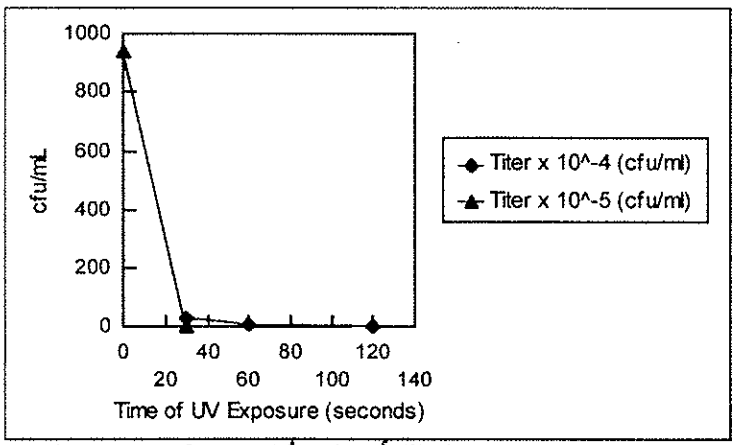


FIG. 11. *E. coli B* at 10^{-4} and 10^{-5} dilutions was exposed to UV light for increasing intervals of time to determine its sensitivity to the mutagen.

As shown in Figure 12, the *S. typhimurium* wild type strain is more resistant to UV exposure than the Ames strain. At cell density of 10^{-4} cfu/mL, an average of 20.56 colonies still grew after 120 seconds of UV exposure.

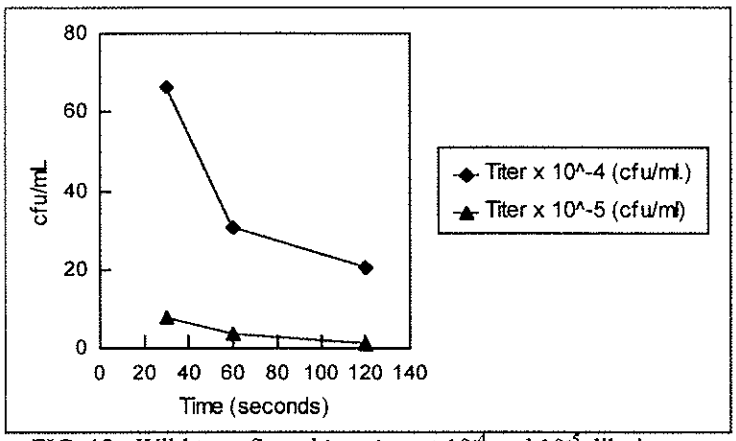


FIG. 12. Wild type *S. typhimurium* at 10^{-4} and 10^{-5} dilutions was exposed to UV light for increasing intervals of time to determine its sensitivity to the mutagen.

The pigmented culture of *S. marcescens* was slightly more resistant than to UV light than the Ames strain *S. typhimurium* and *E. coli B*. After 120 seconds of UV exposure, an average of two cfu/mL still survived at a cell density of 10^{-4} cfu/mL (See Figure 13).

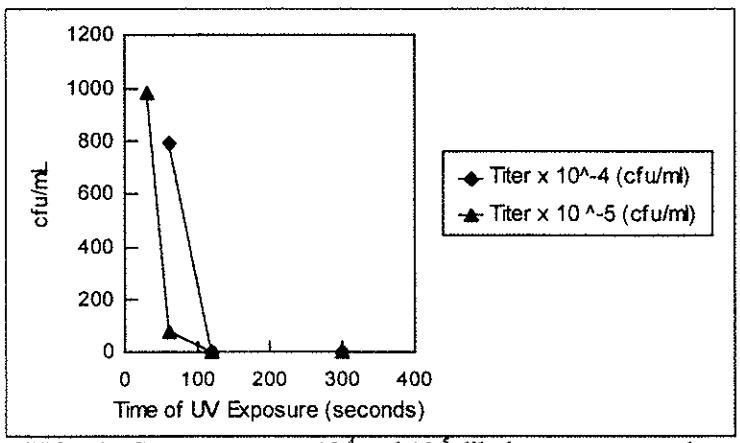


FIG. 13. *S. marcescens* at 10^{-4} and 10^{-5} dilutions was exposed to UV light for increasing intervals of time to determine its sensitivity to the mutagen.

The *S. aureus* culture also appeared to be more resistant to UV light. At cell density of 10^{-4} cfu/mL, an average of 19.5 cfu/mL still survived after 120 seconds of UV exposure. (See Figure 14).

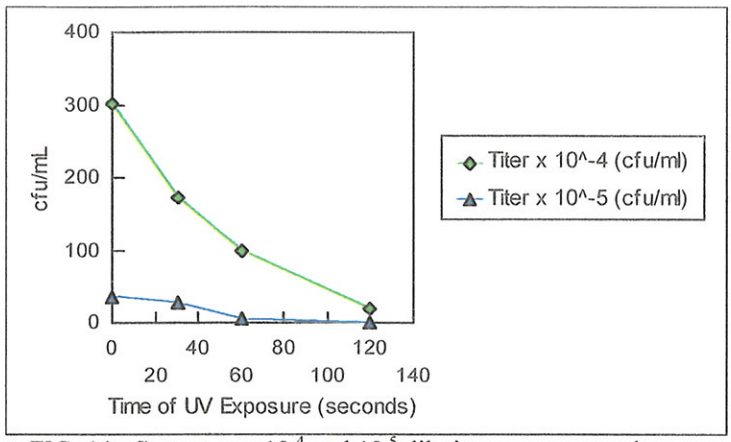


FIG. 14. *S. aureus* at 10^{-4} and 10^{-5} dilutions was exposed to UV light for increasing intervals of time to determine its sensitivity to the mutagen.

S. cerevisiae 3-1 was the most resistant to UV light. After 180 seconds of UV exposure, an average of 22.67 cfu/mL still survived, although approximately 81% of these surviving colonies were mutants (Figure 15).

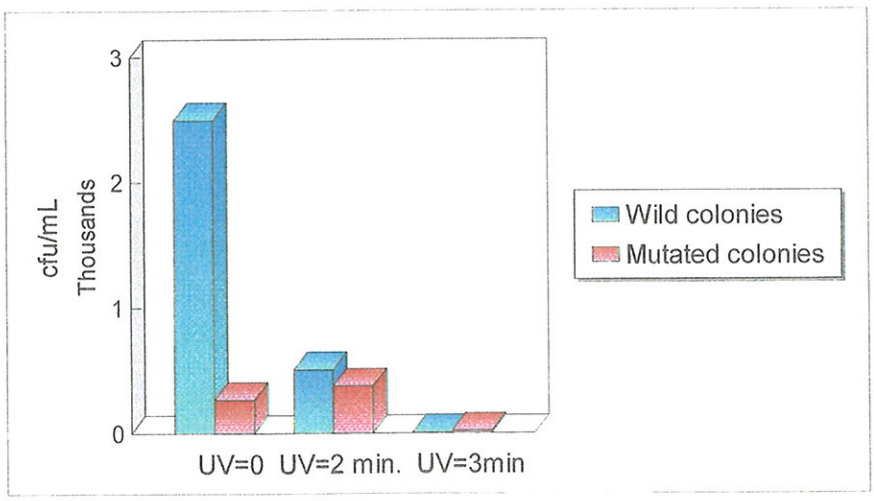


FIG. 15. *S. cerevisiae* 3-1was exposed to UV light for increasing intervals of time to determine its sensitivity to the mutagen as shown in survivability and development of mutants.

From the UV kill curves it was possible to determine the UV exposure time required for appearance of mutants in each organism. A high frequency of mutations usually occurs in the population that represents 10% survival (3). The "mutation time" selected for these organisms was: *S. cerevisiae* 3-1,120 seconds; *S. aureus,* 60 seconds; *S. marcescens* and *E. coli B*, 30 seconds. The mutation time for Ames strain *S. typhimurium* was only one second.

III. Evaluation of Mutation

Relative mutagenic activity (RMA, %) was determined for each of the organisms. RMA is determined using the following equation: [(m/s)/(M/S)] x 100; *m* indicating the number of mutants present after the sample has been exposed to UV light, *s* indicating the number of surviving colonies after UV exposure, *M* indicating the number of spontaneous mutants, and *S* indicating the number of colonies present with no UV exposure (17). The RMA for each organism studied is found in Table 2.

TABLE 2. RMA (Relative Mutagenic Activity) values for *S. typhimurium his*$^-$ and *S. cerevisiae* strains.

	ORGANISMS (cfu/ml.)					
	S. typhimurium		*S. cerevisiae*	*Rad*	*Rad 1*	*Rad 2*
	1 second	15 seconds	3-1	wild type		
No. of mutants present after UV exposure (m)	7.7	49.7	57.3	9	1.3	25.7
No. of surviving colonies after UV exposure (s)	1042.4	56.7	408.3	9	1.3	25.7
No. of spontaneous mutants (M)	22	22	7	65.7	45	119
No. of colonies present prior to UV exposure (S)	1640.7 x 10^6	1640.7 x 10^6	64.3 x 10^6	446.4 x 10^5	427.0 x 10^5	362 x 10^5
Resulting RMA (%)	5.508 x 10^5	653.7 x 10^5	12.89 x 10^5	6.613 x 10^5	9.489 x 10^5	3.042 10^5

S. marcescens, S. aureus, and *E. coli* trials provided no RMA value. Spontaneous mutation was not detected in any of these strains of bacteria. A catalase test to evaluate mutation in *S. aureus* colonies that had been exposed to UV light for 60 seconds yielded positive results (gas formation) in all the colonies. The exposure to UV light did not result in any mutation in the gene responsible for catalase production in *S. aureus*. Of the 27 *E. coli B* colonies (cfu x 10^{-4}) that survived after 30 seconds of UV exposure, none showed growth after being spotted by the T2 phage or the T7 phage. This indicated that there was no mutation in the genes targeted by the phage. Therefore, UV light did not produce any of the viable mutants scanned for in *E coli* or *S. aureus*.

For *S. typhimurium*, RMA was calculated for two different periods of UV exposure – one second and 15 seconds. Ames strain *S. typhimurium* exposed to UV light for 15

seconds resulted in the highest RMA value of all the organisms tested. *S. cerevisiae* 3-1 had the highest RMA value of all the yeast strains tested, and of the *Rad* strains of *S. cerevisiae*, *Rad1* had the highest RMA. Discarding the organisms that had no RMA value, the *Rad2* strain of *S. cerevisiae* had the lowest RMA value..

IV. Toxicity Test

Table 3 shows the mean values, standard deviation, and 95% confidence interval for each of the trials. As shown by the 95% confidence intervals, the toxicity test resulted in no significant inhibition or enhancement of bacterial growth due to the addition of EPO, PEP, or corn oil.

TABLE 3. Resulting absorbance readings for toxicity tests of all natural compounds and organisms used in research.

VOLUME OF OIL ADDED (ml.)	ABSORBANCE			
	E. coli	*S. typhimurium*	*S. marcescens*	*S. aureus*
10 PEP	0.998	1.22	1.45	1.078
20 PEP	1.01	1.22	1.47	1.043
40 PEP	1.028	1.2	1.45	1.12
10 EPO	0.965	1.14	1.22	1.025
20 EPO	0.953	1.103	1.18	1.04
40 EPO	0.973	1.043	1.19	1.042
10 oil	0.985	1.04	1.19	1.048
20 oil	0.987	1.027	1.2	1.035
40 oil	0.98	1.043	1.163	1.025
No treatment	0.98	1.048	1.21	0.97
Mean (of all treatments)	0.986	1.11	1.27	1.04
Std. Deviation	0.0217	0.08016	0.128	0.03834
95% Conf. Interval	0.9703 to 1.001	1.051 to 1.166	1.181 to 1.364	1.015 to 1.07
WITHOUT PEP TREATMENT				
Mean (of all treatments)	0.975	1.06	1.19	1.03
Std. Deviation	0.01212	0.04159	0.01894	0.0263
95% Conf. Interval	0.9635 to 0.9859	1.025 to 1.102	1.176 to 1.211	1.002 to 1.051

Statistical analysis was performed with and without the values from the trials involving PEP because during incubation PEP formed a filmy layer within the culture that may have altered the absorbance readings.

V. Test for Ability to Enhance DNA Repair Mechanisms Following UV Exposure

A. *S. marcescens* Results

The initial results of this phase of the experiment were tainted from the intial use of impure deionized water. Therefore, the results from plating *S. marcescens* on TSA, TSA + PEP, TSA + EPO, and TSA + corn oil are not conclusive. However, this test was repeated using pure deionized water obtained from a different source and plating two separate cultures of *S. marcescens* (pigmented and non-pigmented) on TSA, TSA + EPO, TSA + VitC, TSA + VitE, and TSA + VitA. As shown in Figure 16, the colonies plated on media containing EPO showed considerably more resistance to UV light than any of the other media types. Growth on the EPO plates was so effluent that individual colonies could not even be counted. Also, there were no apparent color mutants growing on the EPO plates.

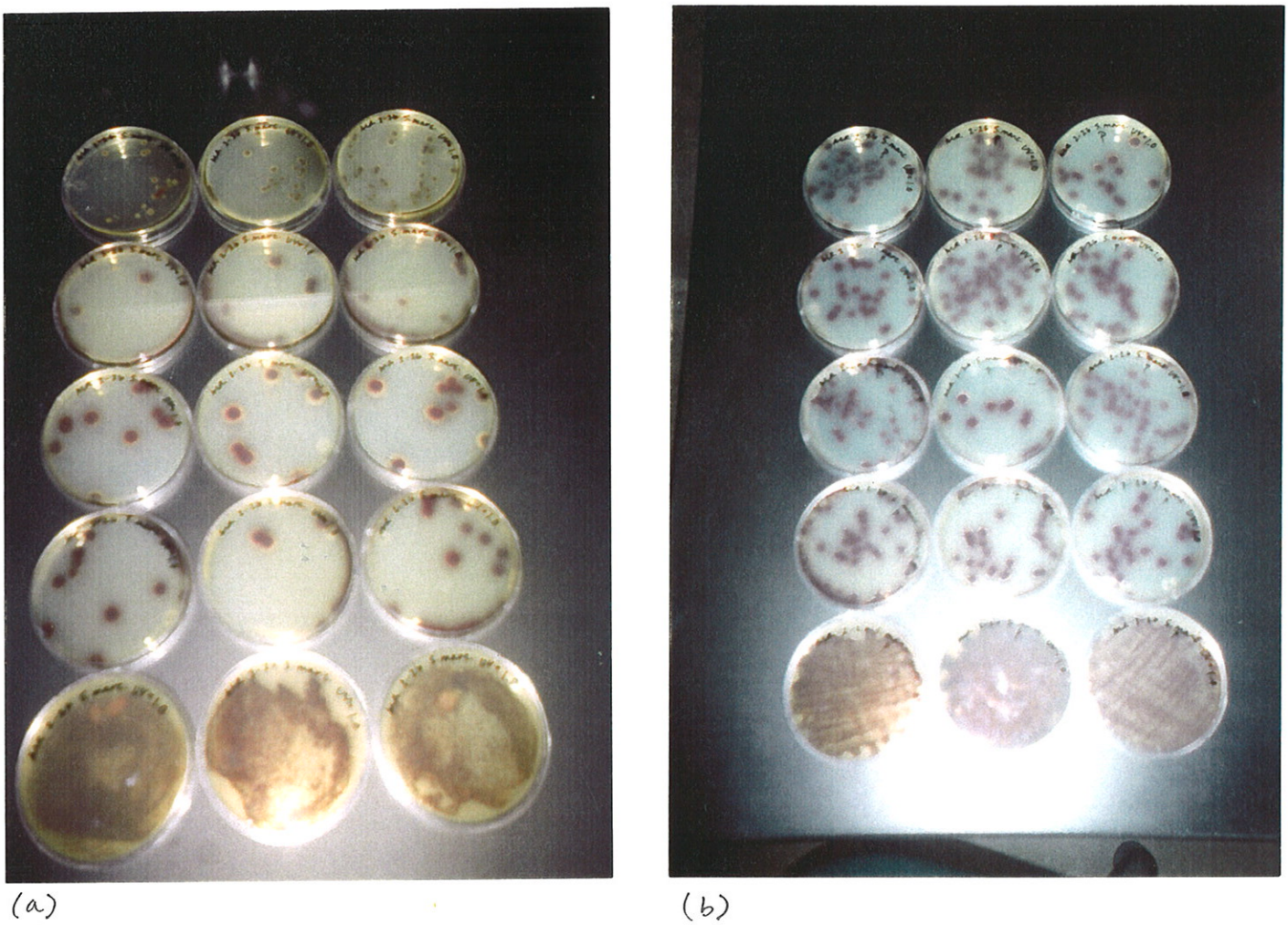


FIG. 16. Non-pigmented *S. marcescens* growth (a) and pigmented *S. marcescens* growth (b) on TSA, Vit. C, Vit. E, Vit. A, and EPO enriched media (top to bottom) after 30 seconds UV exposure.

For the non-pigmented *S. marcescens* colonies, the TSA enhanced with vitamins did not support higher survival rates than TSA alone (Figure 17).

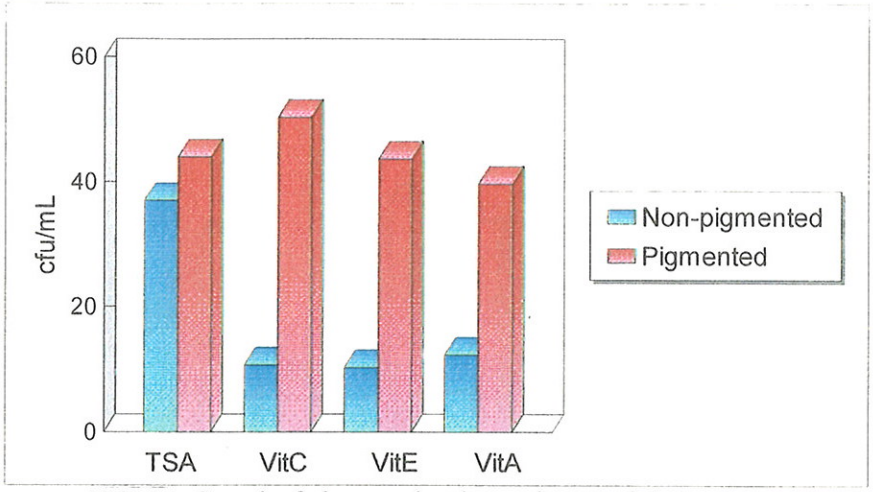


FIG. 17. Growth of pigmented and non-pigmented *S. marcescens* colonies on 4 media types following UV exposure.

TSA enhanced with vitamin C supported the lowest survival of colonies, and TSA alone supported the highest survival of colonies (beyond TSA enhanced with EPO). Analyzing the numbers of colonies growing on TSA and TSA augmented with vitamins with ANOVA resulted in $p = 0.015$, rejecting the null hypothesis of no difference.

For the pigmented *S. marcescens* colonies, TSA with vitamin E added and TSA with vitamin A added did not improve survival when compared to TSA alone (Figure 17). TSA with added vitamin C did yield better colonial survival than TSA alone. However, ANOVA for this data resulted in $p = 0.957$, indicating acceptance of the null hypothesis of no difference.

To determine if there was any difference between the reaction of pigmented and non-pigmented *S. marcescens* colonies when plated on the various media tested, a t-test was run contrasting the two colony types for each media type. Initial titer of both *S. marcescens* colony types (before exposure to UV light) resulted in $p = 0.054$. After one minute of exposure, $p = 0.744$ for TSA; $p = 0.066$ for vitamin C; $p = 0.068$ for vitamin E;

$p = 0.010$ for vitamin A. Therefore, the only significant difference between pigmented and non-pigmented *S. marcescens* growth was observed on TSA + VitA media.

B. *S. typhimurium* Results

No *his*$^+$ revertance was observed on the minimal media + EPO plates that had been inoculated with Ames strain *S. typhimurium*. (See Figure 18).

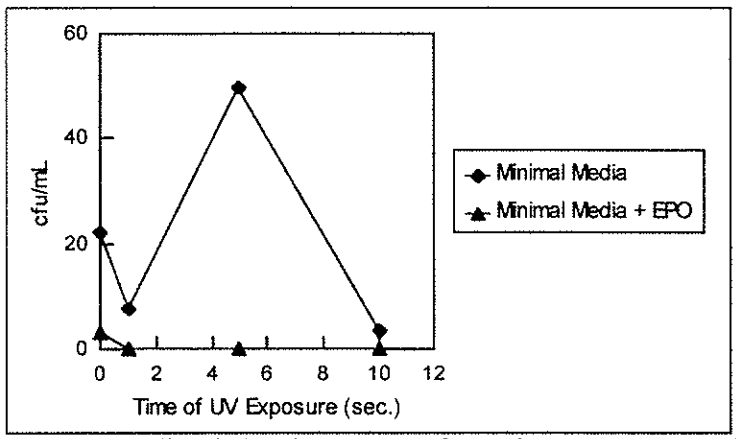


FIG. 18. UV light induced revertance of *S. typhimurium* Ames strain *his*$^-$ colonies to *his*$^+$ colonies with and without EPO in the media.

However, wild type *S. typhimurium* did produce a lawn of growth when plated on EPO and exposed to UV light for one minute.

C. *S. cerevisiae* Results

A lawn of solid growth occurred on the plates of YMA + EPO that had been inoculated with *S. cerevisiae* 3-1 and exposed to UV light for 120 seconds (See Figure 19).

FIG. 19. *S. cerevisiae* 3-1 growth on YMA (top row) and EPO enriched media (bottom row) after 120 seconds UV exposure.

When plated on YMA + EPO, all the *Rad* strains not only survived exposure to UV light with only a few color mutants, but also grew wild type colonies in a consistent lawn (See Figure 20).

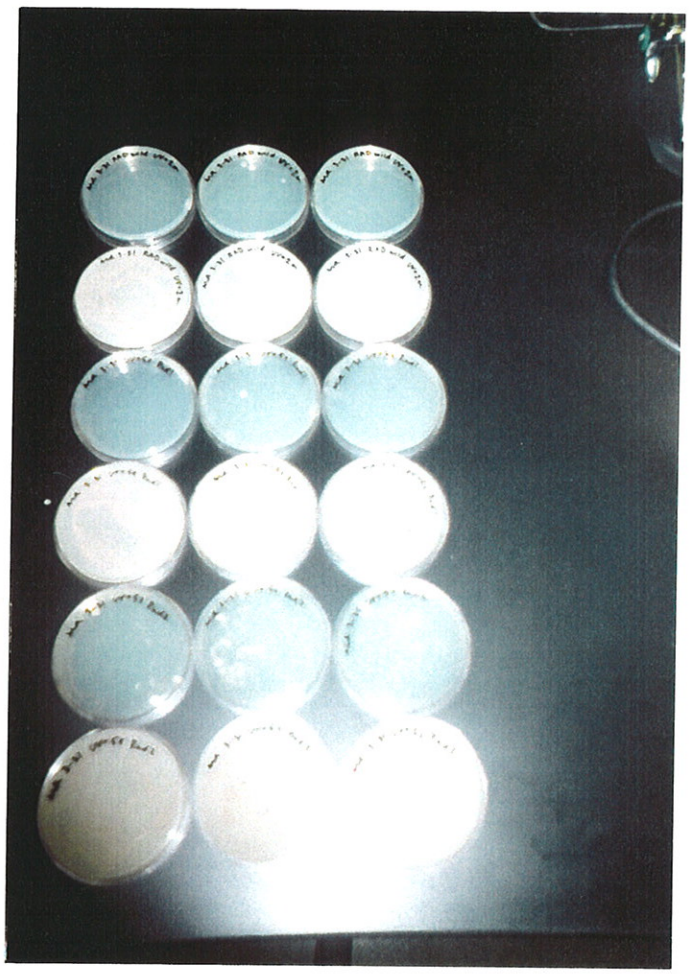


FIG. 20. (Alternating rows YMA and EPO enriched media) *S. cerevisiae Rad* wild type growth after 2 min. UV exposure (top 2 rows); *Rad1* (middle 2 rows) and *Rad 2* (bottom 2 rows) growth after 5 sec. UV exposure.

In comparing Figures 21 and 22, it can be deduced that UV light exposure dramatically decreased the number of wild colonies growing on unenriched YMA. Most of the colonies remaining on the YMA after UV exposure were mutants. On the other hand, media enriched with EPO produced a lawn of wild type colonies dotted with a few white mutant colonies.

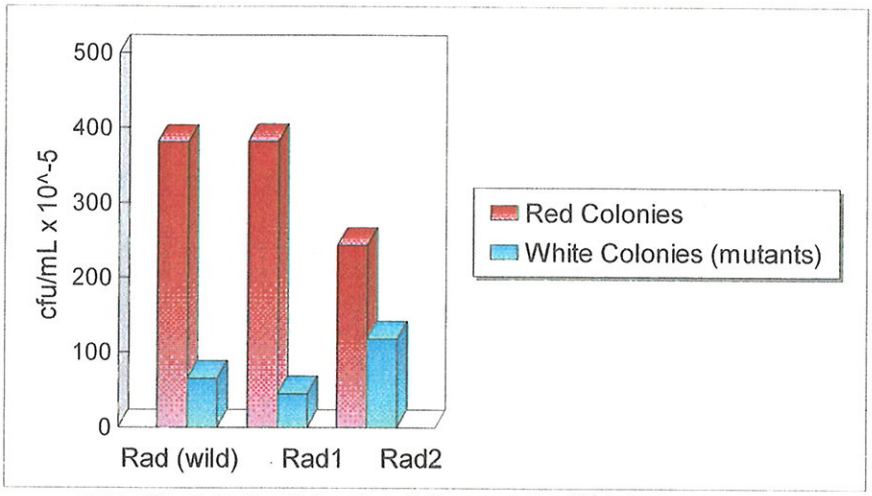


FIG. 21. Initial cell density of *S. cerevisiae Rad* strains.

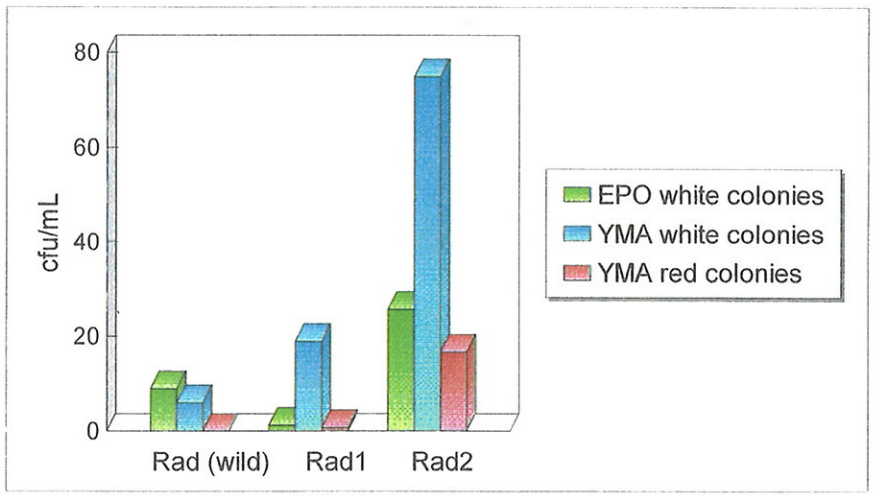


FIG. 22. Effect of UV light on *S. cerevisiae Rad* strains' mutant formation and survival of wild type colonies (*Rad* wild, UV=2 min., *Rad1* & *Rad2*, UV=5 sec.).

Suprisingly, the number of mutants growing on YMA + EPO media after UV exposure appeared to be smaller than the number of spontaneous mutants in all of the *Rad* strains. In order to validate this observation that EPO possibly decreased the spontaneous mutation rate for *S. cerevisiae* strains, *S. cerevisiae* 3-1 was plated on both YMA and YMA + EPO (in triplicate) and incubated at 30°C for 24 hours. The results of this experiment can be seen in Figure 23. A paired t-test was run to compare the spontaneous mutation rate of *S. cerevisiae* 3-1 with and without EPO in the media, resulting in a p value of 0.423. This value accepts the null hypothesis that no difference exists between the spontaneous mutation rate of the yeast with and without EPO in the media.

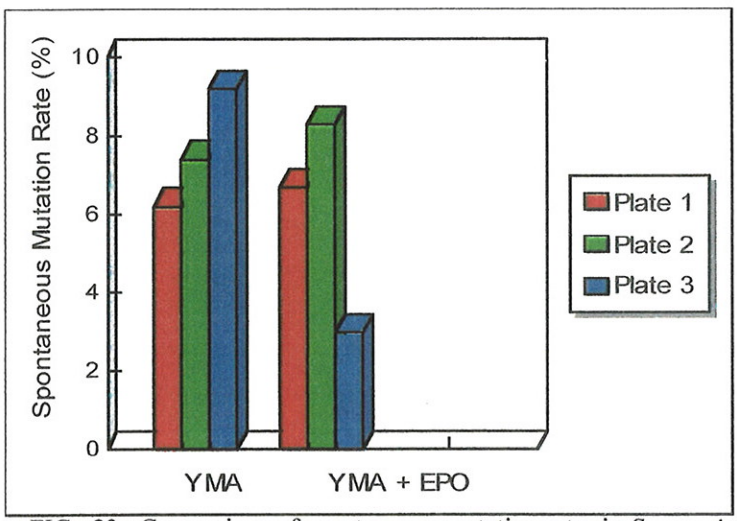
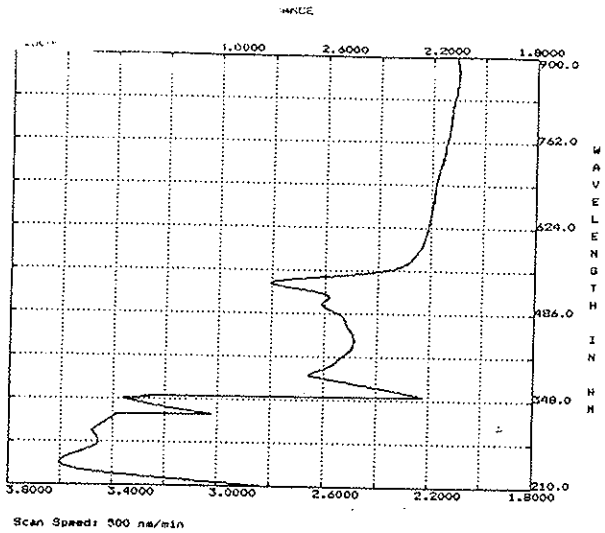


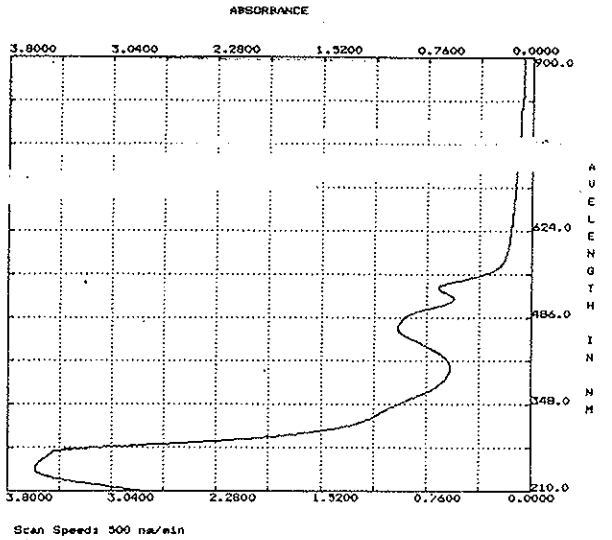
FIG. 23. Comparison of spontaneous mutation rates in *S. cerevisiae* 3-1 with and without EPO in the media. P=0.423.

VI. Analysis of *S. marcescens* Pigment

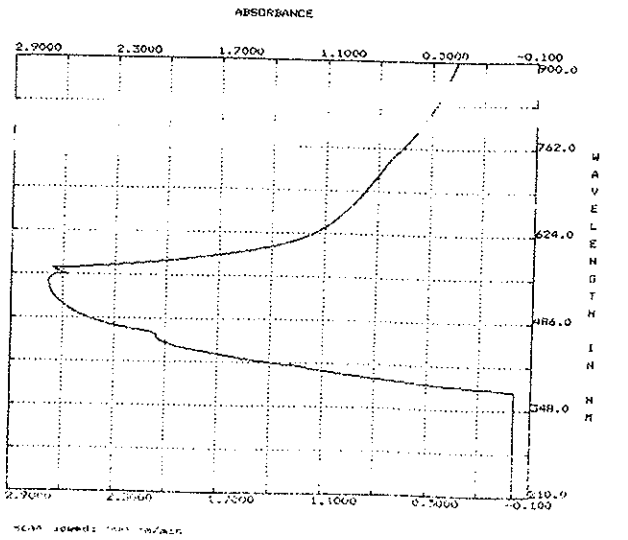
Results of the spectrophotometric analysis of the *S. marcescens* pigment can be seen in Figure 24.



UV Scan of *S. marcescens* Pigment Extracted with Vegetable Oil



UV Scan of *S. marcescens* Pigment Extracted with Ethanol



UV Scan of *S. marcescens* Culture

FIG. 24. UV visible scan of various extractions of the *S. marcescens* pigment, prodigiosin.

Chloroform did not successfully extract the pigment. The pigment extracted with corn oil shows a substantial peak at approximately 235 nm and minor peaks at 469 nm and 532 nm. The pigment extracted by ethanol resulted in a range of high absorbance from approximately 348 nm to 245 nm and from 480 nm to 380 nm. The culture of *S. marcescens* blanked with *E. coli B* resulted in one major peak around 550 nm.

VII. Disk Diffusion Test for Other Mutagens

As seen in Table 4, chloroform killed both *S. marcescens* and *S. cerevisiae*. Cobalt chloride (at low concentrations) induced mutation in *S. cerevisiae* and *S. marcescens* to white colonies. Ortho-toluidine blue also induced mutation in *S. marcescens*, from red to white colonies. The histidine control yielded positive results for *S. typhimurium his*$^-$ Ames strain plated on minimal media.

TABLE 4. Results of disk diffusion test for antimutagenicity, testing a variety of mutagens and three different organisms.

	S. marcescens	*S. cerevisiae* 3-1	*S. typhimurium* Wild type
Histidine	***	***	***
Chloroform	*	*	*
Carbon tetrachloride	***	***	*
Ortho-toluidine blue	**	***	*
Urethane	***	***	*
Colcicine	***	***	*
Dimethylsulfoxide	***	***	*
Ethidium bromide	***	***	*
Formamide	***	***	*
Toluene	***	***	*
Lithium acetate	***	***	*
Cobalt chloride	**	**	*

* No growth
** Mutation
*** Growth

DISCUSSION

I. Support for the Antimutagenicity of EPO

The current research supports the proposal that Evening Primrose oil is an effective antimutagen against UV light induced mutation in *S. marcescens, S. typhimurium,* and *S. cerevisiae*. EPO increased the survival rate of these organisms after UV exposure, indicating that EPO possibly lowers the frequency of lethal mutations. EPO also virtually eliminated the color mutations in *S. marcescens* and *S. cerevisiae* colonies that were able to survive. However, RMA percentage was not a practical measure of the antimutagenicity in the present research because EPO appears to decrease the incidence of deleterious mutations more profoundly than those mutations that can be identified in surviving colonies.

Many researchers have proposed mechanisms for the action of natural antimutagens. Monoterpenes, which are found in the essential oil of many plants, including EPO, have shown some antimutagenic properties. Monoterpenes inhibit the isoprenylation of small G proteins. Preventing isoprenylation could affect gene expression via altered signal transduction, as well as reduce the physiologic functioning of the protein. It is also believed that the monoterpenes have the ability to change the rate at which free radicals are detoxified by inhibiting the synthesis of ubiquinone (Co-Q) (11). As stated earlier, many natural antimutagens, such as luteolin, can also act as antioxidants to reduce mutation within an organism's genome. However, it is not believed that EPO employs any of these methods in mutation prevention in *S. marcescens, S. typhimurium,* and *S. cerevisiae* colonies. A few observations support this hypothesis.

First of all, vitamins A, C, and E are reported to be natural antimutagens acting via their antioxidative properties. In fact, the message that these vitamin are "cancer fighters" has become so mainstream that some women who are at high risk for a second case of breast cancer purposely take supplemental vitamin pills; 8.0% use vitamin C, 4.6% use vitamin E, and 1.4% use vitamin A (19). In the present research, however, vitamins A, C, or E did not significantly protect *S. marcescens* against UV light induced damage (Figure 17). The number of cells surviving on the vitamin-enriched TSA media was lower than the number of surviving cells on TSA media alone. However, TSA enriched with EPO reduced the number of lethal mutations.

In previous studies, it was shown that vitamin E had to have direct interaction with a chemical mutagen to prevent mutation (24). It is possible that the vitamins tested in the present study can only be effective as antimutagens when they are in direct contact with a chemical mutagen. They may have no effect on mutations derived by a physical mutagen, such as UV light. Therefore, the data seems to indicate that EPO decreases the mutation rate in a different manner than vitamins A, C, and E.

A second observation elucidating the pathway for EPO antimutagenicity is that EPO actually resulted in an increase in the growth of many of the organisms after exposure to UV light. For *S. marcescens*, *S. typhimurium* (wild type and Ames strain), and *S. cerevisiae* (3-1 and *Rad* strains), adding EPO to the media protected the colonies from UV light, increasing the number of wild type colonies and decreasing the number of mutated colonies (as compared to populations plated on media that was not enhanced with EPO). Also, EPO prevented any white mutants from forming in the *S. marcescens* populations after they were exposed to UV light.

It could be hypothesized that EPO's protective effect for many organisms is due solely to its action as a "sunscreen," i.e. actively absorbing UV rays. As a matter of fact, EPO does absorb light within the UV range (see Figure 25).

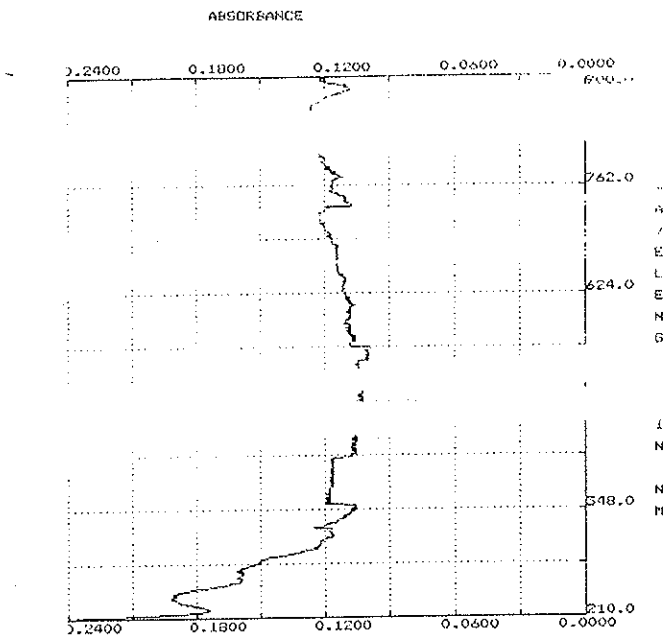


FIG. 25. A UV visible scan of 1.0% EPO. A peak is visible within the accepted range for UV light absorbance.

However, it is possible that simply absorbing UV light does not account for the lawn of population growth on EPO media that occurred with each of the organisms investigated in the current research. Therefore, more investigation into the repair systems of *S. marcescens, S. typhimurium,* and *S. cerevisiae* is warranted.

It was initially believed that if EPO does somehow affect the repair of *S. cerevisiae* or *S. typhimurium* DNA, its primary target would probably be the *Rad* gene system of *S. cerevisiae* and the *uvr* gene system of *S. typhimurium.*

The *Rad* system is a collection of over 30 loci is responsible for the repair of any mutations due to UV light exposure. *Rad1* and *Rad2* are genes within the system that are a part of the *RAD3* group of *Rad* genes. A mutation in any of the genes in the *RAD3*

group results in severe sensitivity to UV light. When a piece of DNA is damaged by UV light, the some of the proteins coded for by the *Rad* genes perform incision of the damaged piece of DNA. Once the intial cuts have been made, other *Rad* proteins perform the excision of the damaged polynucleotide. A new strand of DNA is formed by the action of polymerase, and ligase fills any nicks. *Rad1* (in conjunction with *Rad10*) and *Rad2*, specifically, code for the synthesis of endonucleases (8). A representation of the hypothesized nucleotide excision directed by *Rad2* and *Rad1/Rad10* can be seen in Figure 26.

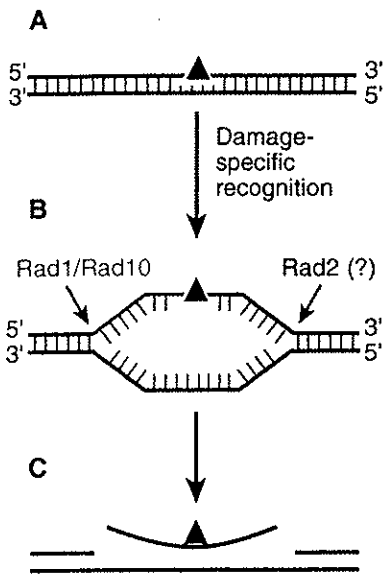


FIG. 26. Model for the incision of damaged DNA by Rad1/Rad10 and Rad2 proteins of *S. cerevisiae.* After the sight of base damage has been identified (A), the Rad endonucleases recognize opposite ends of the damaged DNA strand (B), and exonucleotides excise the resulting polynucleotide (C) (8).

Bacteria have also evolved elegant systems for repairing DNA damaged by UV light. This repair system is most commonly based on the *uvr* system of genes: *uvrA, uvrB*, and *uvrC*. A *uvrD* gene also exists, but very little information about its importance is

currently available. The *uvrABC* system recognizes any change in the covalent structure of the DNA helix (7). A map of the *uvr* gene system in the *E.coli* genome can be seen in Figure 27.

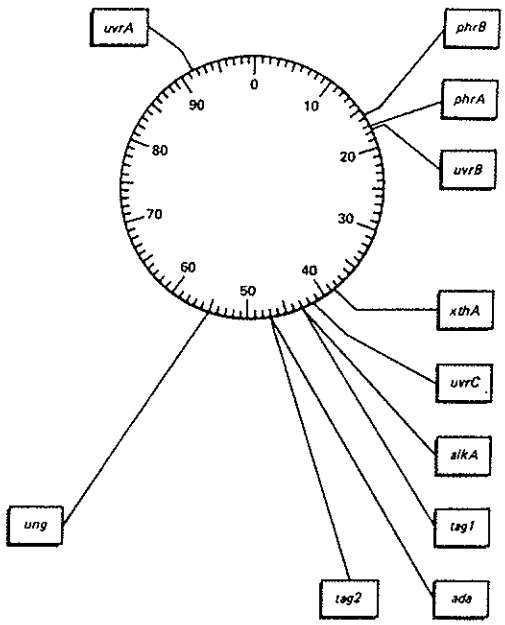


FIG. 27. A genetic map of the *E. coli* genome, indicating the location of the uvrA, uvrB, and uvrC genes (7).

Using a multi-step process, a bacterial cell is able to direct the excision of a damaged piece of DNA from its genome. The gene products of the *uvrABC* system have endonucleic properties and, when aggregated, make an incision of the DNA helix at the phosphodiester bond (10). It appears as though *uvrC* encodes for the actual incising

enzyme, while *uvrA* and *uvrB* encode polypeptide subunits of the enzyme (23). An exonucleotide then excises an oligonucleotide (usually around 12 nucleotides long) containing the dimers. Next, the intact strand is used as template, and the free hydroxyl end of the broken strand is used as a primer to "rebuild" the mutated strand in correct form. Finally, ligase covalently links the free ends of the repaired patch of DNA (10). A model for the mechanism of the *uvrABC* repair system can be seen in Figure 28.

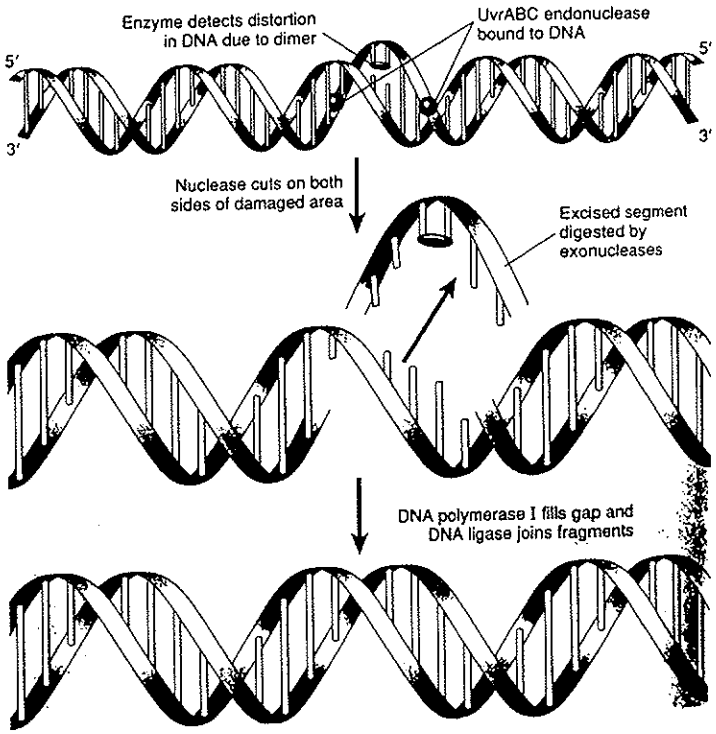


FIG. 28. A model of the nucleotide incision performed by the uvrA, B, C protein complex during repair processes (16).

Mutants of the *uvrABC* system have been developed in order to better understand the roles of the individual genes in the incision process. For example, it was found that incision is possible without *uvrC*, but the actual strand breaks occur more slowly without *uvrC* than when the entire *uvr* system is intact (7).

Using *uvrABC* mutants, much information has also been gathered on the individual gene, *uvrB*. A map of the gene can be seen in Figure 29.

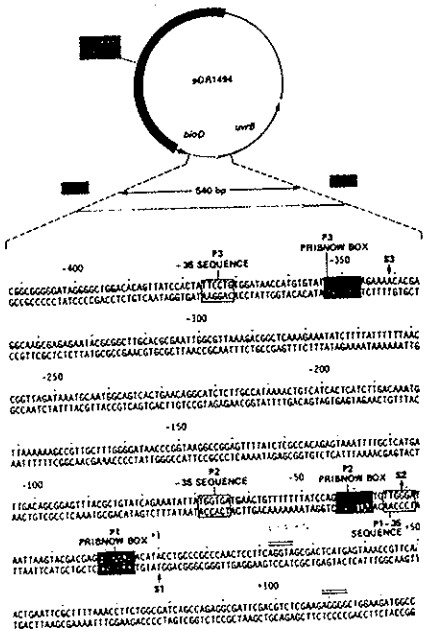


FIG. 29. A map of the uvrB gene and the location of its three promoter regions: P1, P2, and P3 (7).

When the *uvrA* gene product is present, the *uvrB* protein binds very tightly to DNA. About one or two dimers per molecule can be bound when both the A protein and the B protein are present. However, without the *uvrA* gene product, the *uvrB* protein is

virtually useless in DNA repair. Addition of the *uvrB* protein to the *uvrA* product also results in a smaller amount of *uvrA* protein required in creating the binding complex. Therefore, more DNA can be bound by the same amount of *uvrA* protein when the *uvrB* gene product is available (7).

Mutant strains of both *S. cerevisiae* and *S. typhimurium* did not produce the expected results, however. When the mutant strains of the *Rad* gene system were plated on EPO and exposed to UV light, their reaction was no different than the wild type *Rad* strain that was plated on EPO. Based on these results, it does not appear that the antimutagenicity of EPO stems from enhancing the action of either the *Rad1* or *Rad2* gene in *S. cerevisiae.* Also, when *S. typhimurium his*$^{-}$ was plated on minimal media + EPO after UV exposure, no revertant *his*$^{+}$ grew, indicating that EPO worked as an antimutagen without the presence of the *uvrB* gene. It is possible, therefore, that EPO confers antimutagenicity in *S. cerevisiae, S. typhimurium,* and *S. marcescens* by two methods not involved with specific UV repair processes: intially acting as a sunscreen by absorbing UV light (which would account for increased colonial survival), and augmenting the proofreading system or mismatch repair system.

Spontaneous mutations within an organism's DNA come about because of random errors in replication. Many errors can occur in the replicative process, but the majority of these errors are corrected by biological "proofreaders" or the mismatch repair system. The proofreaders are actually polymerases that can act as endonucleases. In many prokaryotes and eukaryotes, the polymerases can "read" a strand of DNA in the 3' to 5' direction and edit out mispaired nucleotides at the 3' terminal. Proofreading efficiency has been shown to increase with the addition of deoxyribonucleotides, tri- and

monophosphates, and accesory proteins. The deoxyribonucleotides and tri- and monophosphates are precursors in DNA synthesis. Accesory proteins are usually single-stranded DNA-binding proteins. The addition of an array of 2^+ charged ions decreases the efficiency of the proofreading system (8).

Mismatch repair also occurs after replication of the DNA. It is a similar process in both prokaryotes and eukaryotes. Short patch and long patch mismatch repair both exist, but short patch is very specific to the type of error that has occurred. The major genes involved in the long patch mismatch repair system of *S. cerevisiae* are: *mutH*$^+$, *mutL*$^+$, and *mutS*$^+$. The protein produced by *mutL*$^+$ and *mutS*$^+$ binds to a site of mismatch (a base pair that has been incorrectly replicated). Then an endonuclease, produced by *mutH*$^+$, excises the error in a manner similar to nucleotide excision repair. *S. marcescens* also has a gene that encodes for the same type of endonuclease, however its gene is called *MLH1*. The proteins of the mismatch repair system are able to recognize an erroneous strand of DNA by its state of methylation of GATC sites. If a strand displays low methylation at these particular sites, it is recognized by the mismatch repair system and the errors within the strand are excised (8).

Surprisingly, it appears that the mismatch repair system and the UV repair system are somehow connected. Once the endonuclease of the mismatch repair system has successfully cut out a piece of DNA, polymerase reforms a new strand and ligase fills the nicks. However, when an organism is exposed to UV light, new nicks are made in the DNA strands in order to excise the nucleotides that have been damaged by the UV exposure. The repair of UV damage is directed by a completely different repair system than the mismatch repair system, as eluded to in the previous discussion of the *Rad*

repair system in *S. cerevisiae* and the *uvr* system in *S. typhimurium*. The nicks introduced in DNA by the UV repair system make the DNA itself more susceptible, once again, to mismatch repair (8). This phenomenon may explain why it appeared that EPO did not decrease mutation rates in *S. cerevisiae* populations when they were not exposed to UV light. Only once an organism was radiated was the EPO able to enhance mismatch repair and decrease the mutation rate profoundly from the spontaneous mutation rate.

Based on the results of the present research, it is proposed that EPO may enhance DNA repair in many ways. First of all, EPO is able to absorb UV light and act as a sunscreen for exposed colonies. Secondly, it appears that EPO augments the proofreading capabilities and/or mismatch repair of *S. cerevisiae, S. typhimurium,* and *S. marcescens*. It is possible that EPO somehow binds up free 2^{+} ions within an organism and thereby increases the efficiency of the proofreading system. Linoleic acid has a typical fatty acid structure, and therefore has a negatively-charged carboxyl tail that could bind positive ions. On the other hand, EPO may act to increase the transcription of accessory proteins, which would also improve the proofreading system. EPO may somehow increase the recognition of sites of low methylation by the mismatch repair system, or increase the efficiency of binding to damaged DNA by *mutL*$^{+}$ and *mutS*$^{+}$ proteins.

All of these possibilities are completely hypothetical, therefore more research is warranted to discriminate more clearly the antimutagenic pathways of EPO.

II. Analysis of *S. marcescens* Pigment

The function of the pigment found in *S. marcescens*, prodigiosin, is unknown. It has been suggested, on the basis that prodigiosin is so rich in amino acids, that the pigment is possibly responsible for the removal of excess metabolites (16). However, it may also be possible that the pigment offers an organism protection from UV light by absorbing radiation within that wavelength range. Strains of *S. marcescens* responsible for infections in humans do not produce prodigiosin. Only strains found in the environment produce the deep red pigment. *S. marcescens* would have no need for UV protection in a human body, but UV protection would probably be very important to a strain of bacteria growing in the soil. Data from this research somewhat supports the hypothesis that prodigiosin offers some UV protection. Pigmented strains of *S. marcescens* had a higher survival rate after being exposed to UV light when compared to non-pigmented strains of *S. marcescens* (Figure 17). However, this difference was not significant.

In the spectrophotometric scan performed (Figure 24), peaks occurring at wavelengths ranging from 210 to 400 nm accounted for ultraviolet light absorption. Both the product extracted by ethanol and the product extracted by corn oil displayed strong peaks in this range, suggesting that the products absorb UV light. However, the scan of the culture of *S. marcescens* (blanked by an *E. coli* culture) did not display a major peak within this range. Therefore, it was not possible to prove that the trials using ethanol and corn oil successfully extracted the desired pigment.

Results from the current research tentatively suggest that prodigiosin may have some role in UV light protection for *S. marcescens*, but the data is not strongly supported. More research needs to be performed before this hypothesis is validated.

III. Future Research

The Disk Diffusion Test performed suggests low concentrations of cobalt chloride have the potential to mutate both *S. marcescens* and *S. cerevisiae* to white colonies. Also, ortho-toluidine blue can cause *S. marcescens* color mutants. Future research could be performed to investigate if EPO has the same effect on mutation frequency of the two organisms when chemical mutagens are used in place of UV light. This would further elucidate the pathway by which EPO allegedly protects *S. marcescens* and *S. cerevisiae* from UV light.

In outside research, it has been shown that when bacteria are grown in the presence of an antibiotic, such as penicillin, drug-resistant cells develop very quickly (21). Because of the ability of bacteria to mutate and develop resistance to drugs, many strains of bacteria now have plasmids that contain antibiotic resistance genes. For example, strains of both *E. coli* and *S. aureus* with plasmid-encoded resistance exist (13). However, it has also been determined that when bacteria is grown in the presence of penicillin and an antimutagen, such as putrescine, the frequency of mutation decreases (21). Since it appears that EPO has antimutagenic properties, it may be possible to use it to decrease mutations for antibiotic resistance. For instance, when a patient gets a prescription for penicillin to treat a bacterial infection, he or she may also be required to take EPO tablets to reduce incidents of resistance to the antibiotic. EPO is also safe for human consumption, unlike many other antimutagens. Based on the above factors, the role of EPO in reducing antibiotic resistance in pathogenic bacteria should also be explored more thoroughly.

CONCLUSION

Evening Primrose oil has shown potential in this research as a very powerful, natural antimutagen. However, the scenarios studied were very specific, examining a particular mutagen and only a few of the millions of organisms that exist. Therefore, future research expanding upon the classes of mutagens and organisms tested should be performed. The present research tentatively suggests that the pigment found in *S. marcescens*, prodigiosin, plays a role in UV protection for the organism. The data compiled was by no means complete, however, and more experimentation should be performed to isolate the pure pigment and test its ability to absorb UV light. Evening Primrose oil has the potential to be an exciting, new anticarcinogen. With further study and research, it is my belief that it will pave the way for the use of other natural products within the medicinal and scientific communities.

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