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The Ability of Green Tea to Reduce Dan Damage

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Abstract: *Green tea is recognized for being rich in polyphenols, which are considered as anti-mutagenic as well as anti-carcinogenic agents. These polyphenols are classified as (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG), (-)-epicatechin (EC) and (-)-epicatechin gallate (ECG). (-)-epigallocatechin gallate (EGCG) is identified as one of the most significant catechins in determining the effect of green tea. Green tea has an ability to reduce the blue events, recognized as microsatellite instability. The microsatellite has been stated in some human colon cancer, such as hereditary cancer-prone disorder (HNPCC), which is caused by mutations in mismatch repair proteins. To detect the impact of green tea on DNA, two S.Cerevisiae strains were used as a model system, strains that comprised mutation in mismatch repair msh3 (YN97-147msh3 Δ), and a wild type strain to contrast with the mutated strain which has the ability to correct mismatch repairs.The strains used in this project contain a plasmid known as pKa3-9(AC)41, which is identified to provide a qualitative assay and has a long repetitive tract.After the colonies have grown, the β-galactosidase assay overlay is carried out to allow blue colonies to develop. The tea used in this project was pure green tea and green tea with mint. Both unflavoured and flavoured green tea was able to reduce frame slippage, which is recognized as blue colonies. The reduction of frame slippage depends on the volume of green tea, that is the frame slippage events will decrease as the volume of green tea increases.*

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I. Introduction

Yeast:

Budding-yeast has been used as one of the most effective model system for examining the basic roles of eukaryotic-genes[1].It was the first eukaryote that was transformed by plasmids; the first eukaryote suited for the target gene, and also the first eukaryote wastotally sequenced[2]. Yeast cells possess a nucleus with chromosomes similar to those of human cells. Additionally, Saccharomyces cerevisiae cells split in a just like human cells divide, as well as there are numerous other essential biological properties, which are shared[3]. The requirement of mammalian cells for multiple replicons is usually 50 000 -100 000, therefore yeast (with only 400 replicons) is much simpler[4]. It also is very small (about 1.4 x 10^7 bp/cell) and capableto grow fast, representing a doubling time of about 2 h[3].

In yeast, the life cycle exists as a haploid and a diploid (figure 1.2). There are typically three types of life cycle patterns that are found. These are mainly diplobiontic life cycle, haplobiontic life cycle and haplodiplobiontic life cycle[5]. The diplobiontic life cycle is where the cells are diploid. The direct function of each cell is as ascus. The diploid nucleus of ascus splits meiotically which forms four haploid ascospores[6].

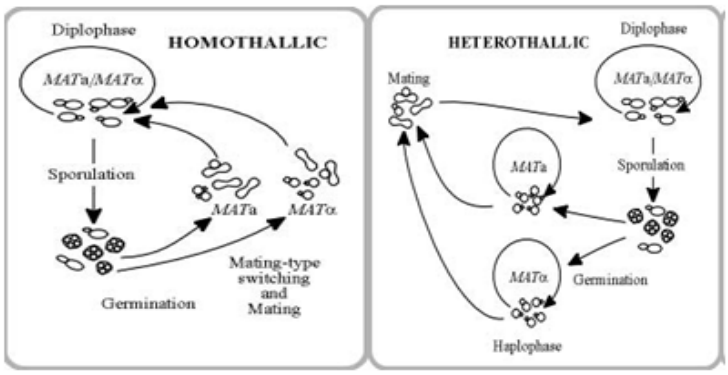


Fig 1.1. Life cycle of yeast **source:**Sherman 2002

Microsatellite Instability

Microsatellites are recurring DNA sequences consisting of short repeated motifs dispersed all over the eukaryotic-genome. There are different types of microsatellite known as mono-, di-, tri-, tetra-, penta- and hexanucleotide repeats. These types are categorised according to their length of the repeated unit[7] .Lengths of microsatellite are substantially polymorphic within human populaces, but seem stable in the life duration of the person.

Mismatch repair system

The primary purpose of the Mismatch repair system (MMR) is to remove single-base insertion-deletion loops as well as mismatches, which may arise throughout DNA replication[8]. The 1-5 Insertion-deletion rings result from losses or gains of short replicate units in microsatellite series, also recognized as MSI (microsatellite instability)[3]. E.coli has three proteins involved in mismatch repair, known as MutS, MutL and MutH, whereas there is no MutH homologues have been recognized in Eukaryotes[9]. Eukaryotes, such as Saccharomyces cerevisiae, have six homologues of MutS, known as Msh1- Msh6, and four homologues of MutL, recognized as Mlh1, Mlh2, Mlh3 and PmS1[10].

For mismatch detection, the MSH2 protein creates a heterodimer through either MSH3 or MSH6 depending upon the kind of laceration to be fixed (MSH6 is needed for the rectification of single base mis-pairs, while both MSH6 and MSH3 may add to the rectification of insertion-deletion rings)[8]. In mammalian, a heterodimer of PMS2 and MLH1 directs the interplay amid the mismatch identification complex as well as other proteins essential for Mismatch repair system (MMR).

These extra proteins may consist of at least EXO1 (exonuclease 1), probably helicase(s), PCNA (proliferating cell nuclear antigen), RPA (single-stranded DNA-binding protein), as well as DNA polymerases. On top of PMS2, MLH1 can heterodimerize with 2 extra proteins, PMS1 and MLH3. Recent studies designate that 'PMS2' is needed for the rectification of single-base incongruities, as well asMLH3 and PMS2 both add to the rectification of insertion-deletion rings, while the PMS1's role in Mismatch repair system awaits further exploration . Further homologs of the hominid Mismatch repair system (MMR) proteins are known, which are needed for tasks other than Mismatch repair system[3]. These proteins compriseof MSH4 as well as MSH5, which are essential for meiotic (as well as possibly mitotic) re-permutation although they are not acknowledged to partake in Mismatch repair system (MMR)[8].

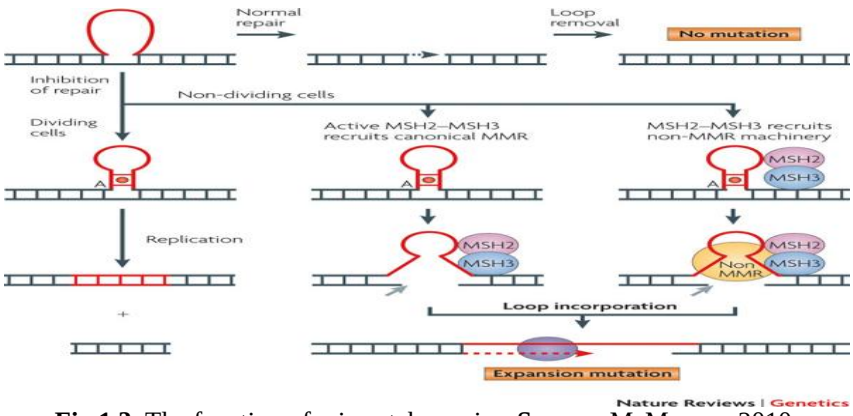


Fig 1.3. The function of mismatch repair **Source**: McMurray, 2010

Green Tea

Green tea is made from plant, known as *Camellia Sinensis,* which prepares with minimum oxidation in the processing. Green tea derivesoriginally from China; nonetheless, it has come to be associated with numerous cultures all over Asia. Additionally, green tea has lately become more pervasive in the West, wherein black tea has been the customarily consumed tea. Green tea has turn out to be the raw-material for extracts that are employed in various health foods, beverages, dietary supplements, as well as cosmetic items[11]. Many types of green tea have been made in the nations where is produced. These types can differ substantively because of the variable growing environments, horticulture, harvesting time, and production processing.

Throughout the past few eras, green-tea has been exposed to many medical and scientific studies to establish the degree of its long-claimed healthiness benefits, with certain evidence proposing that consistent green tea consumers may hold a lower possibility of obtaining heart disease as well as certain kinds of cancer[11]. Though green-tea does not increase the metabolism rate adequate to produce instantaneous weight loss, green tea extracts containing caffeine and polyphenols has been presented to stimulate thermogenesis and accelerate fat oxidation, furthering the metabolism rate 4 per cent without escalating the heart-rate[11].

The average constituent of flavonoids within a mug of green-tea is greater than that within the same amount of other drink and food items, which are traditionally thought of health causative nature, including vegetable juices, fresh fruits or wine. Flavonoids are an assemblage of phytochemicals found within most plant products, which are accountable for health results such as anticarcinogenic and anti-oxidative functions[11]. Nevertheless, the constituent of flavonoids can vary dramatically amid various tea products.

The processing of green tea

is range of methods has been develop to process green tea according to the kind of green tea needed. On account of these ways, high antioxidant quantities are needed to provide full benefits from green tea.There are two simple categories for cultivating setting. One is that bred in the sun, and other is that grown in canopy.The plants should be cultivated in rows before it is being cropped. There are 3 periods per annum to harvest these plants. These harvests occur in May, July and early August. Occasionally, there are a 4th picking. The 1st flush isduring the spring season that provides the finest value leaves, together with good rates to match[11].

During the processing of geen tea, it should be refrigerated (at 32 °F) in low humidity for thirty or sixty kg paper-bags. The aracha (Japan name)is so far to hone throughout this phase, among a finishing firing occurring before blending and choice. In this state, the leaves will be re-fired all through the year like they are required, providing the green-teas a lengthier shelf life as well as better savour[11]. The first harvest must be stored till the next harvestin following year's. Following t re-drying procedure, every crude tea is sieved as well as sorted in accordance to size. Lastly, every lot is blended in accordance to the blend-order by the samplers as well as packed for trade.

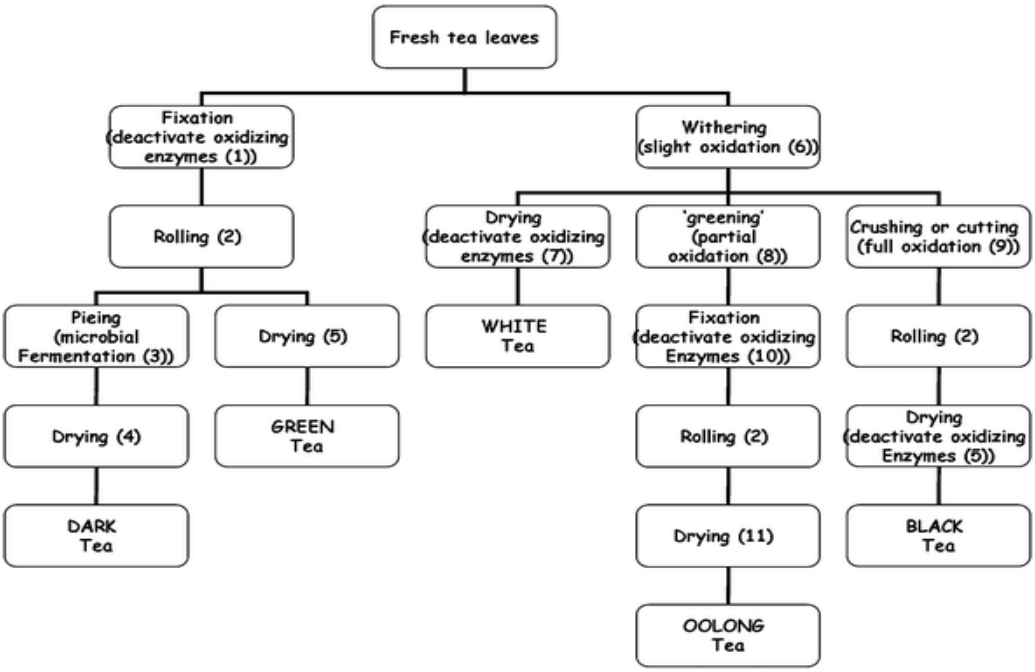


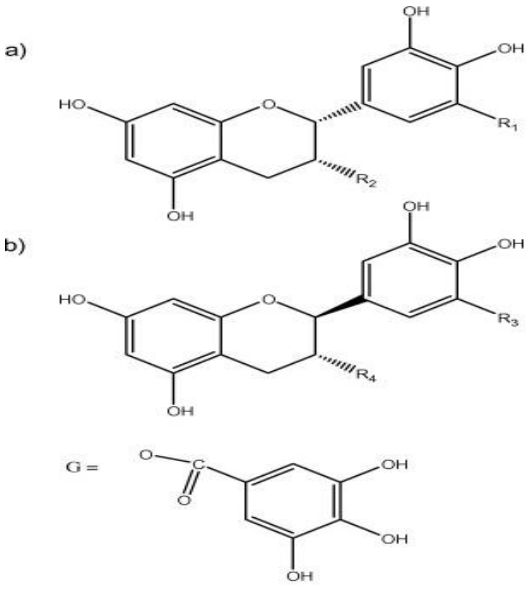
Figure 1.4 a synopsis of the simple processing approaches for diverse green teas.
Source: Dong et al. 2011

Chemical composition of green tea

They are four types of catechain, classified as (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG), (-)-epicatechin (EC) and (-)-epicatechin gallate (ECG), and their matching epimers are (-)-gallocatechin (GC), (-)-gallocatechin gallate (GCG), (+)-catechin (C) and (-)-catechin gallate (CG), respectively[12]. A report has been revealed that EGCG and EGC were the most chatechins, containing more than 50% of the amount of catechins[13]. In addition, the two chemical component catechains (EGCG and EGC) have the same gallate structure as well as effectiveradical scavenging capabilities. Therefore, EGCG and EGC are used as a health marker of green tea quality[14]. There are common backbones in the structure of green tea catechains with specific differences in the substituents at the C-3 and C-5^0 positions (figure)[12]. Green tea contains a complex chemical composition. Table 1.1 shows the percentage of the mean composition in green tea leaves[15].

Table1.1. The percentage of the mean composition in green tea leaves

Constituent	Percentage
Polyphenols	37.0
Carbohydrates	25.0
Caffeine	3.5
Protein	15.0
Aminoacids	4.0
Lignin	6.5
Organic acids	1.5
Lipids	2.0
Ash	5.0
Chlorophyll	0.5



Component	Abbrev.	R_1	R_2	R_3	R_4
-Epigallocatechin gallate	(-)-EGCG	OH	G	-	-
(-)-Epicatechin gallate	(-)-ECG	H	G	-	-
(-)-Epigallocatechin	(-)-EGC	OH	OH	-	-
(-)-Epicatechin	(-)-EC	H	OH	-	-
)-Gallocatechin gallate	(-)-GCG	-	-	OH	G
(-)-Gallocatechin	(-)-GC	-	-	OH	OH
(-)-Catechin gallate	(-)-CG	-	-	H	G
(+)-Catechin	(+)-C	-	-	H	OH

Fig 1.5. The structures of mean catechins components: (a) epicatechins and (b) non-epicatechins
Source: Sharma & Zhou, 2010

Health benefits associated with green tea

Green tea is recognized for holding rich polyphenols, which are deemed as anti-mutagenic as well asanti-carcinogenic agents[11]. Numerous studies have established that EGCG steers to cell-cycle arrest as well as apoptosis of particular cancer cells. Green-tea polyphenols are explained to trigger NER (nucleotide excision repair) conduit to eradicate UVB facilitated DNA damage. Furthermore, green teas' polyphenols are shown to stimulate microsatellite DNA-stability as well as lower sister-chromatid exchange level. Cells that have high rate of MSI (microsatellite DNA instability) and SCE (sister-chromatid exchange) are linked with carcinogenesis. A research has been employed to check the upshot of hydroxyurea upon DNA slippage. It has been found that Hydroxyurea agent is considered to inhibit frame slippage events, which causes the instability of microsatellite[1].

Some research has proposed that the regular ingestion of green tea decreases the risk of cardiovascular disease. It has been established that green tea is able to influence this cardiovascular role through the mechanisms of low-density lipoprotein oxidation[16]. The oxidation of this low-density lipoprotein has been found to be associated with some chronic diseasessuch as atherosclerosis and heart disease. This oxidation can be prevented by consumption of green tea containing EGCG antioxidant components[16].

The drinking of green tea on a regular basis is known to prevent obesity-associated diseases such as diabetes and arthrosclerosis. It has been found that EGCG has an ability to decrease or maintain the body weight in obese Zucker rates . It has been found that EGCG in in vivo experiment can prevent lipid oxidation and also change glucose levels . A study in humans established that the ingestion of green tea significantly improved glucose tolerance,however, there was no effect on glucose levels. In addition, once fructose was fed to normal rats, green tea was established to inhibit the growth of insulin and hyperglycemia[17].

Mint

There are factually countless types of green teas available. One of these types is mint green tea. Green tea with mint is a prevalent blend, which delivers a fresh flavor as well as a health benefits.Mint is a well-known herb and is used for many years ago as one of the most medical herbs in the world. It was used in several historical ancient such as Roman, and Egyptian ancients as cooking flavor andmedicine. Mint leaf is identified to increase a mood and make relaxing. The smell and flavorof the mint not only cause good mood, it also aidsto improve brain function[18].

The growth of mint genus is slightly different, especially in pattern and form of growth, but it participatesseveral common features. Mint herbs form vertical stems and horizontal rhizome.it grows very quickly in a moisture soil,and has a prevalent underground and overground stolon[18]. Mint can be classified in three categories, known as peppermint, spearmint and apple mint.

Chemical composition of mint

Mints are well known for its rich content of minerals such as magnesium, potassium, phosphorus, calcium and iron. It also holds good quantities of Vitamin A, citric acid, K, B_9, B_1, B_2 and B_3. Certain2nd metabolites of Lamiaceae containmany terpenoids and phenolic compounds . Mint leaves contain a high amount of polyphenols (catechins and flavonoids). The highest significant amounts of phenolic acids are Caffeic and rosmarinic acids. However, there is a good content of catechins such as (-)-epigallocatechin (EGC).Mint contains an essential oil. This essential oil is known for its chemicallycomplicated in peppermint. There is approximately 0.5-5% of oil in peppermint, while there is about 0.5% of oil in spearmint[18].

Health benefits associated with mint

Mint herb has been used as herb treatment for several problems such as stomach illnesses, respiratory aliments, mouth hygiene, and skin diseases . The properties of mints give them a powerful effectiveness against stomach-ache, as it was found that peppermint's oil has an ability to treat irritable bowel syndrome and spasms . Mint has been used as soothing treatments for skin problems such as acne and nurns. The flavour and aroma of mint has also been used for cold remedy and respiratory tract infections .

Mint can be used in antibacterial capacity, and it has also been found that mints are able to increase food shelf life. Studies have established thatneomenthol and carvacrol compounds, which are found in peppermint oil, have effective antimicrobial actions.Mints furthermorehave antifungal properties. The essential oil in peppermint hasan ability to prevent fungi growth such as Fusarium sulphureum at concentrations 0.5 and 1.0% actions.The research also established that There are about 70mg of polyphenols found in 100 ml of mint leaf. These polyphenols are known to be affective at preventing cancer growth[18]. Vitamin C, which is found in mint leaves, is recognised as having antioxidant actions due to its electron donating which inhibits other compounds from being oxidised[19]. Ascorbic acid is able to eliminate the free radicals resulting from oxidative stress.

II. Materials and Methods

Chemical

Majority of the chemicals were sourced from Formedium in Norwich and Houstanston within the United Kingdom. The other chemicals were sourced from different suppliers

Media

Yeast extract Peptone Dextrose (YEPD) medium

The yeast strains that were used during the research were produced through Yeast Extract Peptone Dextrose (YEPD) media.

To produce 1 liter of the media;

i. 20 grams of Yeast was extracted
ii. 10 grams of Peptone was used
iii. 20 grams of Glucose were used

The elements were dissolved in distilled water which then produced a final volume of 1 liter.

20 grams of agar were then added on each liter.

Finally, it was autoclaved at 121°C for a period of 15 minutes.

Synthetic complete Dextrose (SCD) Medium

To produce 1 liter of media;

i. 1.7 grams of Yeast Nitrogen base was required
ii. 5 grams of (NH4)2SO4 was obtained from Fisher Scientific, Leicester, in the United Kingdom
iii. 20 grams of Glucose was used
iv. 25 Milliliter of amino acid was used

The Amino acid mix contained of

Arginine 0.2 g
Histidine 0.1 g
Isoleucine 0.6 g
Leucine 0.6 g
Lysine 0.4 g
Methionine 0.1 g
Phenylalanine 0.6 g
Threonine 0.6 g
Adenine (hemiSO4 0.1 g
Tryptophan 0.4 g
Uracil 0.1 g

In order to make a final volume of 1 liter of the media, the chemicals were distilled in water. 20 grams of agar were added on each liter.Finally, it was autoclaved at 121°C for a period of 15 minutes. For the purpose of this particular study, Uracil was not used in the Amino acid mix which meant that the Synthetic Complete Dextrose was without Uracil. The selective media was used to ensure that the transformed yeast strains grew.

Test components

Tea used

Two different type of tea that was flavouredand unflavoured green tea. Table 2.1. Shows that the two different types of green tea were used in this project.

Table2.1Teaused.

Nameof Tea	**Ingredients**
Greenteawithmint	Green tea contains 10% Peppermint leaves and 7% Natural mint flavour.
Pure greentea	Only greentea.

The green tea were Twinning brand of green tea and were purchased from the local Tesco Express store that is located in Fountain Pridge, Edinburgh, Scotland, UK. As per the information contained in the packaging, the Twinning Green Tea was manufactured and packaged by R. Twinning and Company Ltd that is based in Hampshire, England, United Kingdom. Each tea pack weighed 50 grams and contained 25 tea bags with each tea bag weighing 2grams.

Making the tea

Two tea bags with a total weight of 4grams were added into 200 ml of boiling distilled water and then continuously stirred for three minutes. Two different concentrations were used in this study, with regard to the green tea that contained mint, 50ml and 100ml of mint tea were added to 450ml and 400ml of SC-U media correspondingly resulting in a total volume of 500 ml, and for a low concentration, 25ml and 50 ml were added to 450ml and 400ml of SC-U media respectively to make 500 ml. similarly with the pure green tea, 50 ml and 100 ml of the pure green tea were added to 450 ml and 400 ml of SC-U media after it had autoclaved in order to make a total volume of 500 ml of high concentration tea, as well as 25 ml and 50 mlbeing added to 450 ml and 400 ml to make 500 ml of low concentration tea. The media were then emptied into the plates and left to cool and rest at room temperature.

Strains applied

In table 2.2, there are two S.Cerevisiae strains, which were applied in this particular study. The Two S.Cerevisiae strains were the wild type YN94-1 and a strain that contained mutation in the DNA mismatch repair gene YN97-147. The strains were cultured on YEPD plates and then incubated at 30°C for 2 days before being transformed.

Table2.2Types ofYeastStrains Used

Strain	Genotype	Source/Reference
YN94-1	MATa,*ade2-1,his3-11,leu2-3, 112,trp1-1,ura3-1,can1-100*	Laboratory Collection
YN97-147	MATa, YN94-1*Δmsh3::LEU2*	Laboratory Collection

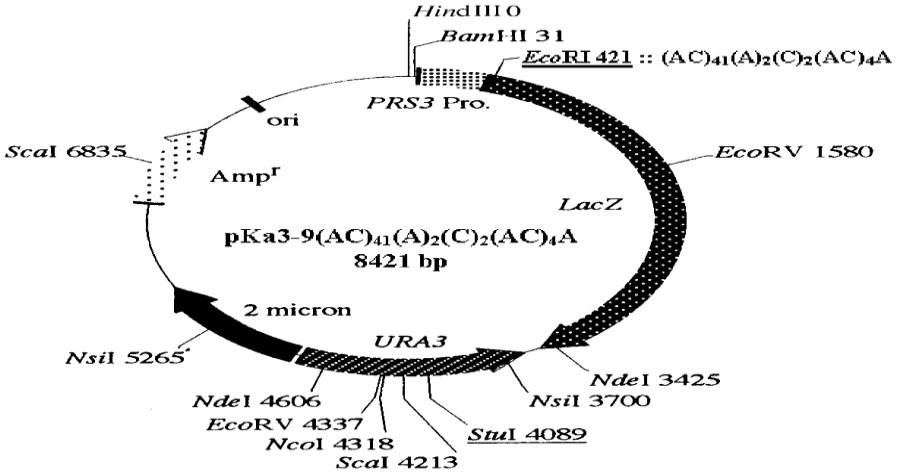
Assay Plasmids

The assay plasmids that were used were a high-copy 2-µ based vector, pKa3-9(AC)41(A)2(C)2(AC)4(A) as shown in the figure below.

Table2.3Plasmids used

Plasmid	**SelectioninS.*Cerevisiae***	**Source/Reference**
pKa3-9(AC)$_{41}$(A)$_2$(C)$_2$(AC)$_4$(A)	*URA3*	Larkin&Schweizer,1999

Figure2.1 Schematicrepresentationof thehigh-copy2µ-basedvectorpKa3-9(AC)$_{41}$ (A)$_2$(C)$_2$(AC)$_4$(A)(AdaptedfromLarkinand Schweizer,1999)



The plasmid map above indicates the construction of a high-copy 2-µ based vector. Thisis relevantto restriction endonuclease site, which is also 369-bp fragment.This fragment includes the promoter region and the first 28 codons of the PRS3 gene. At the moment, it is fused to the 8 codon of the LacZgene of E.coli. The repetitive tract (AC)$_{41}$(A)2(C)2(AC)4(A)) is found at the EcoRI site, which is sited close to the initiation of the PRS3"LacZ fusion . The high copy 2-µ based plasmid pKa3-9(AC)$_{41}$occurs episomally with a copy of 50-150 per yeast cell, whereas Co/EI origin letsE.Coli to be replicated.Both of yeast and E.coli selections are allowed by the URA gene (Larkin & Schweizer, 1999).

III. Molecular biology techniques

Transformation of yeast using PLATE approach

PLATE (PEG/Li-acetate/TE) Solution

To produce 100 ml of PLATE solution:

90 ml 45% (w/v) PEG (Polyethylene glycol) 4000

10 ml 1 M LiOAc (Lithium Acetate) (pH 7.5) (Sigma Aldrich, Germany)

1 ml 1 M Tris-HCl (pH 7.5) (Sigma Aldrich, St. Lois, USA)

0.2 ml 0.5 M EDTA (pH 7.5) (Fisher Scientific, Leicestershire, UK)

Carrier DNA: Sheared Herring Sperm Carrier DNA (10 mg/ml)

IV. Procedure

The yeast strains were initially grown on YEPD plates and incubated for 2 days at 30°C. From these plates, a glob of cells (about 107– 108) were removed and placed in a small centrifuge tube. 10 µl of the carrier DNA and 1 µg of the transforming DNA were then added and vortexed (For the control, no transforming DNA was added). Then, 0.5 ml of the PLATE solution was added. The tube was vortexed again and left to incubate overnight at room temperature on the bench.

On the other hand, a single colony was picked from the YEPD plate and incubated in 10 ml of YEPD broth in universal bottles, incubated overnight at 30°C at 170 rpm in the rotary shaker. 0.5 ml of the culture was taken and centrifuged. The supernatant is then decanted by inverting the tube. The carrier and transforming DNA and PLATE solution was added as stated above.

The tubes containing the transformants were then centrifuged at 12,000 rpm for 1 minute. The supernatants were decanted. 100 µl sterile distilled H2O was added to resuspend the cells. These were then streaked onto SC-U plates, incubated at 30°C for 2 days.

Culturing yeast transformants onto SC-U plates in the absence and presence of Tea

Colonies that were transformed were selected from the original SC-U plates and restreaked onto SC-U plates and then incubated at 30°C for 2 days. Single yeast colonies were then taken (from the restreaked plates) and inoculated in 10 ml of SC-U broth in universal bottles, incubated overnight in a rotary shaker at 30°C at 170 rpm.

The culture was then diluted ten-fold. 1ml of the dilution was taken and placed in a cuvette. The number of cells/ml in the overnight culture was determined using spectrophotometer at a wavelength of 600nm (1OD = 3 x107). Serial dilutions were carried out and aliquots containing a known number of cells (~2000 cells per plate) were plated onto SC-U plates and plates with tea of different volumes. These were left at incubate at 30 °C for 2 days before the β- galactosidase overlay can be done.

β- galactosidase assay (Overlay technique)

To produce 500 ml of overlay solution:

250 ml 0.05 M Sodium phosphate buffer, pH 7.0
5 ml 10% SDS (Sodium dodecyl sulphate)
5 ml DMF (N,N-dimethylformamide) (Acros Organics, USA)
10 ml 2.5% X-Gal (Apollo Scientific) in DMF
250 ml 1% Agarose in distilled H2O

<u>0.05M Sodium phosphate buffer, pH 7.0</u>

To produce 100ml of buffer:

19 ml 0.1M Na2HPO4 (BDH, Poole, UK)
31 ml 0.1M NaH2PO4 (BDH, Poole, UK)
50 ml Distilled H2O

Adjust the pH using 0.1M NaOH.

V. Method

250 ml of a 1% agarose solution in distilled H_2O was prepared by heating in a microwave for a few minutes. It was then added to 250 ml of 0.05M phosphate buffer (pH7.0) which was pre-warmed at 65°C in a water bath. 5 ml of 10% SDS (w/v), 5 ml DMF and 10 ml 2.5% X-Gal dissolved in DMF (w/v) were added. 10 ml of this solution was poured over each plate. In order to allow colour to develop, each plates were incubated at room temperature for one to two weeks.

Measurements of frame slippage event

The changed yeast should make white colonies. When the β- galactosidase overlay assay was conducted, blue and blue-sectored colonies formed suggesting frame-slippage events. The remaining colonies that were unchanged in any way represent the 'out-of-frame' assay plasmid. The blue and blue-sectored colonies were counted. The frequency of frame slippage was determined by expressing in fraction the number of blue colonies over the total number of colonies on the plate.

Statistical analysis

Statistical assessments were carried out through the use of statistical tools and in particular Microsoft Excel program as well as PASW Statistics 17 (SPSS) for mac program. The analysis specifically focused on calculating the means of the frequency of blue colonies ± as well as standard error of means. In addition, T-tests were also conducted between sample means with the objective of finding the p value ($p<0.05$ is considered significant)

VI. Results

Strains and plasmids

As stated earlier,this study used a mutated strain known as YN97-147*msh3Δ* and a wild type strain called YN94-1WT. YN97-147 strain is mutated in mismatch pair protein msh3 and thus, it is recognized by the label *Msh3.* It is expected thatYN97-147*msh3Δ* will have a higher frequency of frame slippage compared to the

wild type strain (YN94-1). As described previously in section 2.4.1, the study utilized a β-galactosidase assay plasmid known as ppKa3-9$(AC)_{41}(A)_2(C)_2(AC)_4(A)$. This plasmid is known to provide a qualitative assay and has a long repetitive track that can be a source of DNA damage.

To begin with, single yeast transformants containing assay plasmid were selected and incubated for two days at 30°C. Three colonies were picked from the selected plates and were given labels A, B and C. They were then plated onto fresh plates containing the media synthetic complete dextrose lacking Uracil, and were thenincubated at 30°C for two days. Single yeast colonies were selected from each of the three cultures chosen and were added into two universal bottles containing 10 ml of SC-U (one for the wild type stain and the other for the mutated strain). They were then incubated in a rotary shaker for one night at 170rpm at 30°C.A spectrophotometer was used to determine the number of cells in the overnight culture at a wavelength of 600 nm(1 OD = 3 x 10^7 cells). After that, specific concentrations of dilutions were prepared and added to the SC-U control plates and in the SC-U plates containing two different volumes of tea. Each of the yeast transformants (YN94-1 WT and YN97-147) used in the pure green tea and the mint green experiments yielded between 250 and 500 colonies per plate. However, only one experiment in which the green tea containing 50 ml and 100 ml mint was used yielded fewer colonies per plate, between 100 and 200. It is not clear why the latter experiment yielded fewer plates, even though the same procedures were applied as in the other experiments. Table 3.1 presents the average number of colonies for the culture B transformant that were yielded per plate in both experiments.

In order to allow the blue colour to develop, β-galactosidase assay overlay was used. After the β-galactosidase assay overlay is made, the yeast colonies should turn from white colour to blue colour, as white signifies the 'out of frame' assay plasmid, while denotes instability of DNA (Marden et al. 2006). From the results, the yeast colonies in mint green plates were turned successfully from white to blue, whereas the colonies in most pure green tea plates formed a pink colour rather than white colour. This feature makes it hard to know the blue colonies.

Table 3.1: average number of colonies per plate (culture B)

Strains	**Average CFU/plates ±SEM**					
	Green tea+ mint			**Pure green tea**		
	0ml	**50ml**	**100ml**	**0ml**	**50ml**	**100ml**
YN94-1WT [pKa3-9$(AC)_{41}(A)_2(C)_2(AC)_4(A)$]	158.9± 15	132.2 ± 16	156.4 ± 20	325.8 ± 22	343.2 ± 35	305.2 ± 49
YN97-147msh3Δ [pKa3-9$(AC)_{41}(A)_2(C)_2(AC)_4(A)$]	136.9± 15	122.4 ± 8	125.6 ± 9	309.6 ± 32	396.8 ± 45	324.8 ± 29

The table above shows that the average number of colonies in both strains for the same culture transforamnts (B). It can be seen that the colonies number for green tea containing mint are too low compared to pure green tea plates.

Volumes of tea

This study used pure green tea and green tea containing mint. Both of these teasare known to provide health benefits. For the green tea with mint, the mint should increase the bioavailability of the tea as it contains antioxidant properties. Pure green tea has been proved in reducing frame slippage.

In this study, two bags of tea were put into 200 ml of hot distilled water and stirred for three minutes (water was microwaved for two minutes). This was followed by preparation of two different concentrations of tea to be poured onto SC-U bottles. 50 ml and 100 ml of tea were poured onto 450 ml and 400 ml SC-U media respectively. The two SC-U bottles containing different volumes of tea were then poured onto fresh petri dishes. 25 ml and 50 ml of the two volumes were then mixed with 475 ml and 450 ml of SC-U media correspondingly in order to achieve lower concentrations.

The plates were then inoculated with specific measures of yeast transformants and were incubated at 30°C for two days. There were good numbers of coloniesyielded on the plates. The results obtained are listed in table 7.1 and 7.2 of the appendix.

The ability of green tea with mint to reduce DNA damage

For both yeaststrains (YN94-1WT and YN97-147 msh3Δ) containing theplasmid [pKa3-9(AC)41(A)2(C)2(AC)4(A)], there wereappropriate numbers of colonies in the SC-Uplatesand theplates containing 50mland100mlgreenteawith mint. These colonies were overlaid by β-galactosidase assay to allow blue colonies to develop.Table 3.2 shows the relative frequency of blue colonies yielded.

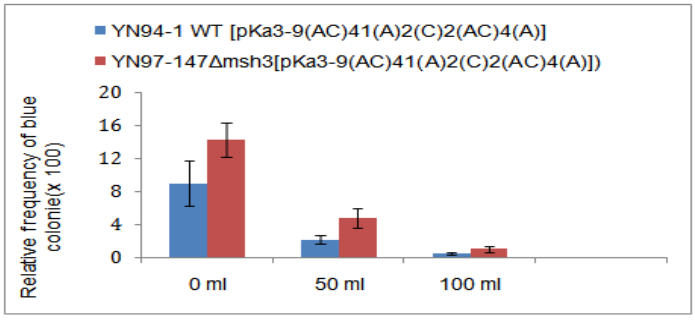
Table 3.2. The average number of the relative frequency of blue events ± SEM

Strains	The relative frequency of blue events ±SEM					
	Green tea+ mint					
	0ml	25ml	50ml	0ml	50ml	100ml
YN94-1WT [pKa3-9(AC)$_{41}$(A)$_2$(C)$_2$(AC)$_4$(A)]	$3.5x10^{-3}$ ± $8.9X10^{-2}$	$9.8x10^{-2}$ ± $2.9X10^{-2}$	$4x10^{-2}$ ± $2.7X10^{-2}$	$8.9x10^{-2}$ ± $6.9X10^{-2}$	$2.1x10^{-2}$ ± $1.4X10^{-2}$	$5.12x10^{-1}$ ± $5.9X10^{-2}$
YN97-147msh3Δ [pKa3-9(AC)$_{41}$(A)$_2$(C)$_2$(AC)$_4$(A)]	$4.7x10^{-3}$ ± $6.9x10^{-2}$	$2.1x10^{-3}$ ± $4.1x10^{-2}$	$4.7x10^{-2}$ ± $3.4X10^{-2}$	$1.4x10^{-3}$ ± $4.7x10^{-2}$	$4.7x10^{-2}$ ± $2.6x10^{-2}$	$1x10^{-2}$ ± $7x10^{-2}$

The ability of green tea with mint to reduce frame slippage events is described in figure 3.1. The bar graph shows that both 50 ml and 100 ml volumes of green tea with mint were able decrease the relative frequency of blue colonies. These blue colonies represent the frame slippage events. The decrease in slippage events appeared to be dependent on the volume of tea that was added. The reduction of frame slippage in the wild type strain YN94-1 containing plasmid [pKa3-9(AC)$_{41}$(A)$_2$(C)$_2$(AC)$_4$(A)] was about 76% for the 50 ml concentration of green tea with mint, and about 94% for the 100 ml of mint green tea concentration. This reduction in slippage events was lower in the mutation strain YN97-147 [pKa3-9(AC)$_{41}$(A)$_2$(C)$_2$(AC)$_4$(A)] being about 66% and 93% for both 50 ml and 100 ml volumes of green tea with mint. The p-value ($p<0.05$) for the mutated strain was statistically significant between the control plates and the plates containing 50 ml and 100 ml green tea with mint. However, they were no significant differences in the wild strain for both concentrations of green tea with mint (50 ml/100 ml). These results can be clearly seen in figures 3.1 and 3.2 and also in tables' 7.3.1 and 7.3.2 in the appendix.

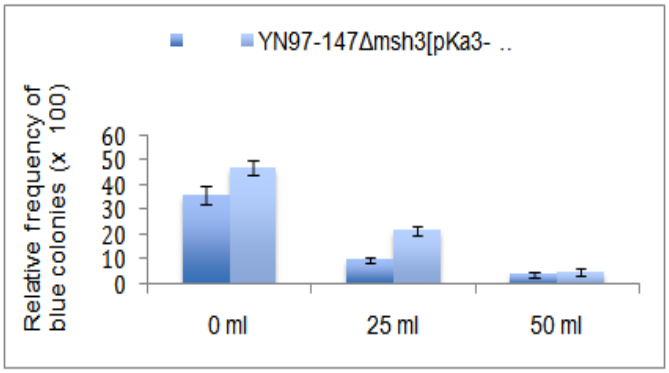
The quantity of mint green tea was reduced to 25 ml and 50 ml to confirm the effect of the tea in reducing the frequency of blue colonies. The reduction of these blue colonieswas lower than with previous concentrations (50 ml/ 100 ml). Figure 3.2 indicates that 25 ml and 50 ml volumes of green tea with mint were able to decrease the relative frequency of blue colonies for both strains (YN94-1 and YN97-147) containing plasmid [pKa3-9(AC)$_{41}$(A)$_2$(C)$_2$(AC)$_4$(A)]. From the graph, the relative frequency of blue events (multiplied by 100) in the WT strain plates containing 25 ml mint green tea was reduced by 73%, while 89% was decreased for the volume of 50 ml of green tea with mint. This reduction in frame slippage was lower in the mutated strain being about 54% for the volume of 25 ml of green tea with mint, and 90% for 50 ml mint green tea. The p-value ($p<0.05$) was calculated and we found that the differences between the control plates and the tea volume plates for both strainsare statistically significant (the p-value is less than 0.05). The results acquired are displayed in tables 7.3.3 and 7.3.4 in the appendix.

Figure3.1 Theabilityof50 ml and 100 ml of green tea with mint to reduce blue events for both wild type and mutated strains containing plasmidpKa3-9(AC)$_{41}$(A)$_2$(C)$_2$(AC)$_4$(A)



The bar graph shows the ability of the 50 ml and 100 mlof mint green tea to reduce the frequency of blue eventsforthewildtypeand mutated strainscontainingpKa3-9(AC)$_{41}$(A)$_2$(C)$_2$(AC)$_4$(A). The vertical (y) axis signifiesthe relative number of blue eventsmultipliedby 100and alsothe error barsfor the SC-U control plates and SC-U plates containing 25 ml and 50 ml tea. P value($p<0.05$) is Significant in the mutated strain,while no significant differences in the wild type strain for both volumes.

Figure3.2 Theabilityof25 ml and 50 mlof greentea with mint to reduce the relative frequency of blue events for bothwild typeandmutatedstrainscontaining plasmidpKa3-9(AC)$_{41}$(A)$_2$(C)$_2$(AC)$_4$(A)



The bar graph indicates the ability of 25 ml and 50 ml of mint green tea to decrease the frame slippage for both wild type and mutated strains containing pKa3-9(AC)$_4$1(A)$_2$(C)$_2$(AC)$_4$(A). The vertical (y) axis represents the relative number of blueeventsmultipliedby 100and the error bars for the SC-U control plates and SC-U plates containing 25 ml and 50 ml tea. P value ($p<0.05$) is statistically significant for both strains.

The ability of pure green tea to reduce DNA damage

Two yeast strains (YN94-1 and YN97-147) having a plasmid [pKa3-9(AC)$_4$1(A)$_2$(C)$_2$(AC)$_4$(A)] were used with another type of tea (pure green tea) in this experiment. There were two green concentrations that were added to SC-U medium (50 ml/ 100 ml and 50 ml/ 50 ml). β-galactosidaseoverlay was made in order to permit colour development. The plates of SC-U and SC-U containing tea for both strains had a number of blue events. Table 3.3 shows the average number of blue events obtained.

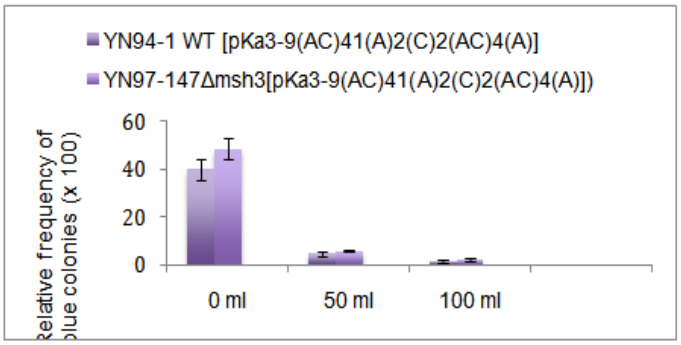
Table 3.3. The average number of the relative frequency of blue events±SEM

Strains	**The relative frequency od blue events±SEM**					
	Pure green tea					
	0ml	**25ml**	**50ml**	**0ml**	**50ml**	**100ml**
YN94-1WT [pKa3-9(AC)$_4$1(A)$_2$(C)$_2$(AC)$_4$(A)]	3.8×10^{-3} ± 7.1×10^{-2}	4×10^{-2} ± 2×10^{-2}	2.1×10^{-2} ± 1.3×10^{-2}	3.9×10^{-3} ± 1×10^{-3}	4.6×10^{-2} ± 9.4×10^{-2}	1.5×10^{-2} ± 1.2×10^{-2}
YN97-147msh3Δ [pKa3-9(AC)$_4$1(A)$_2$(C)$_2$(AC)$_4$(A)]	4.6×10^{-3} ± 4.7×10^{-2}	8.3×10^{-2} ± 4.7×10^{-2}	4.3×10^{-2} ± 1.8×10^{-2}	4.8×10^{3} ± 9.4×10^{-2}	5.7×10^{-2} ± 2.7×10^{-2}	2.3×10^{-2} ± 1.4×10^{-2}

Both volumes that were used led to a reduction in frame slippage depending on dosage. However, the relative level of frame slippage in volumes containing pure green tea was lower compared to the reduction in frame slippage in volumes containing green tea with mint. The differences can be clearly seen in tables 7.1, 7.2, 7.3and 7.4 in the appendix.Figure3.3showstheabilityof 50 ml and 100 mlgreenteato reduce frame slippage eventsfor both the wild type and the mutated strainscontaining a plasmidpKa3-9(AC)$_4$1(A)$_2$(C)$_2$(AC)$_4$(A). It shows that the reduction of frame slippage (indicated as blue colonies) in the plates containing wildly strain was about 88% for the 50 ml green tea, while there was a 96% reduction for 100 ml green tea. The reduction of blue events was almost the same in the mutation strain compared to the reduction in the wild type strain. Thisdecrease in fame slippage was 88%for 50 ml and 95% for 100 ml. The p-value ($p<0.05$) for both strains were statistically significant between the control plates and the plates containing 50 ml and 100 ml green tea. These results are scheduled in tables 7.4.1 and 7.4.2 in the appendix.

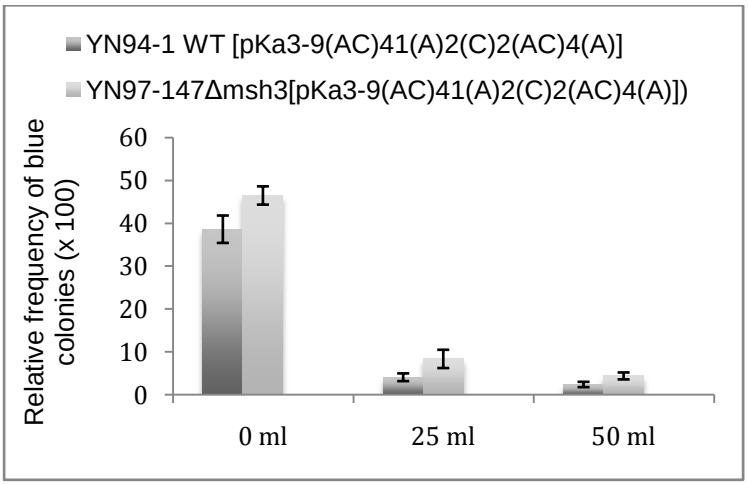
The extent of the reduction in frame slippage was determined through repetitive experiments. In the repetitive experiments, the volumes of pure green tea were reduced from 50ml and 100ml to 25ml and 50ml. Figure3.4showstheabilityof 25 ml and 50 ml of greenteatoreduce blue eventsfor both yeast strains,wildtype strainandthemutatedstrain, containing aplasmidpKa3-9(AC)$_4$1(A)$_2$(C)$_2$(AC)$_4$(A). As shown in the graph, the frame slippage reduced as the volume of pure green tea increased. In wild type strain, it decreased by about 89% for the volume of 25 ml of green tea and was reduced by about 93% for the volume of 50 ml of green tea. The reduction in blue events was lower in the mutated strain than those in the wild type strain. This decrease was 82% and 90% for both green tea concentrations (25 ml and 50 ml) respectively. The p-value ($p<0.05$) between the control plates and the green tea plates for both strains were statistically significant (the p-value is less than 0.05). The results attained are shown in tables 7.4.3 and 7.4.4 in the appendix.

Figure3.3 The ability of 50 ml and 100 ml of pure green tea to reduce DNA damage for bothwild type and mutated strain containing pKa3-9(AC)41(A)2(C)2(AC)4(A)



The bar graph shows the ability of the 50 ml and 100 ml of green tea to reduce the frequency of blue events for the wild type and mutated strainscontaining pKa3-9(AC)41(A)2(C)2(AC)4(A). The vertical (y) axis signifies the relative number of blue eventsmultipliedby 100 and also the error barsfor the SC-U control plates and SC-U plates containing 25 ml and 50 ml tea. P value ($p<0.05$) is Significant in both the wild type and the mutated strain for both volumes.

Figure3.4 The ability of 25 ml and 50 ml of green to reduce DNA damage for both wild type and mutated strainscontaining pKa3-9(AC)41(A)2(C)2(AC)4(A)



The bar graph indicates the ability of 25 ml and 50 ml of green tea to decrease the frame slippage for both wild type and mutated strains containing pKa3-9(AC)41(A)2(C)2(AC)4(A). The vertical (y) axis represents the relative number of blue eventsmultipliedby 100 and the error bars for the SC-U control plates and SC-U plates containing 25 ml and 50 ml tea. P value ($p<0.05$) is statistically significant for both strains.

VII. Discussion

Strains and plasmids

Microsatellite instability has been stated in different colon cancer types such as colorectal cancers (CRC), gastric, ovarian and sebaceous cancers. 15% of CRCs are categorized by the faulty function of the mismatch repair (MMR) system[20]. The number of repeats and the linear function of repeat units can be significant factors in determining the mutation rate of microsatellite[7]. This study aims to determine the influence of flavoured and unflavoured green tea on microsatellite. To detect that, two S.Cerevisiae strains were used as themodel system, including astrain that comprised a mutationin mismatch repair msh3 (YN97-147msh3 Δ), which was used as a source of DNA damage, and a wild type strain to contrast with the mutated strain due thethe ability of this strain to correct mismatch repair. The relative frequency of blue colonies reflects the instability of microsatellite. These blue colonies can be seen after β- galactosidases have been made. An increased number of blue colonies meant that the microsatellite instability was higher.A previous study has established that a strain distributed in either msh2 or msh1 has a higher impact on the frequency of frame slippage events than a strain distributedperformance inmsh3 or msh6. Additionally, it was found that there is

roughlya 200-850-fold increase in frame-slippage, indicated by white to blue colonies, once matched to the wild type strain while there is a 20-90-foldincreaseinmicrosatelliteinstability found in strains distributed in msh3 or msh6[21].

As described previously, the strains used in this project contain a plasmid known as pKa3-9(AC)41. This plasmid is identified to provide a qualitative assay and has a repetitive tract that is located at the EcoRI site near the beginning of the PRS3"LacZ fusion (figure 2.1). The high copy 2-μ based plasmid pKa3-9(AC)41exists episomally with a copy of 50-150 per yeast cell[21]. A repetitive tract provides increased insertions in the reading frame of -1 and +1 with respect to the LacZ. Moreover, the reading frame can be restored by tract instability and thus β-galactosidase activity. Thelengthoftherepetitivetract determines the frequency of the frame slippage number since a lengthier tract is more likely to get changed than a shorter tract[21].

The results obtained indicate thatthe relative frequency of microsatellite instability measured, which was represented as blue colonies,was found to be significantly lower in the wild type strain (YN94-1) than the mutated strain (YN97-147 msh3 Δ) as was expected because of MMR-proficient in the wild type strain.However, in the 50 ml and 100 mlof pure green tea experiment, the reduction of blue events was almost the same in the mutation strain compared to the reduction in the wild type strain. This is because the long repetitive in the plasmid could cause slippage in the WT 94-1 strain leading to the production of blue events.

Table 3.1 shows the 50 ml and 100 ml mint green tea experiments (culture B transformants). There were too low colonies yield for both strains containing a plasmid compared to the pure green tea experiment for the same culture transformants. However, the repeated experiments yielded sufficient amounts of colonies, thus errors could haveoccurred during the yeast transformation leading to a low yield in the number of colonies.

For the green tea with mint results, the yeast colonies turned from white to blue (the white colour signifying'out of frame' assay plasmid while the blue colour denotingframe slippage[1]. This characteristic makes them easy to distinguish. However, the colonies in most pure green tea plates produced a pink colour instead of white. The reason for this might be the amount of adenine added to the amino acid mixture during the preparation of the synthetic complete dextrose (SCD) medium -the high amount of adenine prevents the colonies from turning pink or red[6].

The ability of green tea with mint to reduce DNA damage

The consumption of green tea is possibly recognized for its anti-cancer properties. There are numerous reports in relation to the effect of green tea being anti-carcinogenic. The results acquired in section 3.3 emphasize the benefits of green tea with mint against DNA damage. It can be assumed that green tea is able to reduce the instability of microsatellite, which were distinguished as blue colonies. The reduction of blue colonies depends on the concentration of tea. It was found that the reduction of these blue colonies in the wild type stain was by about 73% and 89% for the volume of 25 ml and 50 ml of tea containing mint respectively, and this reduction was increased to about 76% and 94% for the volume of 50 ml and 100 ml of mint green tea correspondingly. As mentioned earlier, due to the mutation in mismatch repair msh3 for the strain YN97-147, the reduction of frame slippage was lower than for the wild type YN94-1. This reduction was by about 5 4 %and90% for the concentration of 25 ml and 50 ml of tea containing mint, and increased to 66% and 93% for the volume of 50 ml and 100 ml of green tea with mint respectively.

The reduction of frame slippage events is due to the greentea polyphenol properties that are considered asa good source of antioxidant nutrients. Four major polyphenol properties arehighly effective against DNA damage. EGCG is known as one of the most significant antioxidant components. In an experiment usinga yeast-based system for the positive and negative measurement upon microsatellite instability (MSI), it wasestablished that EGCG was the only one from the four tea catechins that had a positive effect on the microsatellite instability when the different green tea catechins were examined. Nevertheless, they noted that substantial reductions of 10-fold in tract reduction for the four groups of catechins joined together in which they were tested individually for wild type strain, while there was only a 2.1-fold reduction in tract alteration if the mismatch repair was faulty[1].EGCG has an ability to inhibit the growth of tumours by preventing the release of tumour necrosis factor alpha, and also to decrease a particular binding site for both 12- Otetradecanoylphorbol-1-3-acetate (TPA) type and the okadaic acid type tumour agents. Tumour necrosis factor alpha induces tumour promotion and pre-malignant cells. These effects are attained by the phospholipid bilayer interaction in the cell membranes[15].

Polyphenols in green tea are able to decrease breast cancer cell reproduction in vitro and in vivo. In vitro, they established that an EGCG and tamoxifen mixtureis synergistically cytotoxic to breast cancer cells; their results make possible the use of EGCG as breast cancer treatment. Using EGCG as a treatment reduced cell viability by about 80% inbreast carcinoma MCF-7 cells, but with noantagonistic influence on the growth of regular mammary cells[16].

From the ingredients in section 2.3.1, the tea used contains peppermint leaves at 10% and natural mint flavouring at 7%. Mint is known to be rich in vitamin C (ascorbic acid) and polyphenols, which are identified as

having antioxidant components. Vitamin C acts as an electron donor for eight different enzymesand has two electrons between the 2nd and 3rd carbons of the six-carbon molecule. It is known asan antioxidant because of theelectron donating leading to inhibition of other compounds from oxidation Ascorbic acid has an ability to decrease free radicals, whichresult from oxidative stress. The oxidative process can occur directly or indirectly. The direct way is associated with DNA oxidation, while indirectly it is related to the oxidation of either proteins or lipids. This oxidation can be associated with several medical problems including cancer. Antioxidants play a significant role in decreasing radical species and lipid hydroperoxides directly, and in inhibitingradical doses on proteins[19]. In summary, mint is able to increase the bioavailability of green tea polyphenol as mint contains a sufficient amount of polyphenols.

The ability of pure green tea to reduce DNA damage

The second type of tea used was pure green tea. It was used in order to find the differences with green tea with mint. From the results obtained in section 3.4, there was a significant reduction of frame slippage for both volumes of tea. For the 50 ml and 100 mlvolumes in the WT strain, green tea was able to decrease the blue colonies, which signified microsatellite instability, by about 88% and 96% respectively. The reduction of mutation strain should be the lower than wild type strain. Thus, the 50 ml and 100 ml volumes of pure green tea decreased the frame slippage by about 88% and 95% correspondingly.

The volume of green tea was reduced to confirm the effect of green tea on DNA. As the volume of tea decreased, the reduction of blue colonies should be lower. As can be seen from the results shown in figure 3.4,the decrease of SC-U containing a 25 ml volume of green tea was 89%, while the reductionof50 ml volume of green tea was higher (93%). Additionally,blue colonies were significantly decreasedfor SC-U containing 25 ml concentration of green tea in the mutation strain (82%), whereas there was a 90% decreased in the 50 ml volume of green tea.

As mentioned previously in section 4.2, the reduction of frame slippage is due to green tea catechins. These catechins are classified as (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG), (-)-epicatechin (EC) and (-)-epicatechin gallate (ECG) (Peltomaki, 2001). It has been noted that EGCG is the most significant catechin in determining the effect of green tea. Various studies in vitro and in vivo have revealed that EGCG has a substantial effect in which it inhibits photo-carcinogenesis after numerous mechanisms including many molecular aims, as shown in figure 4.1.

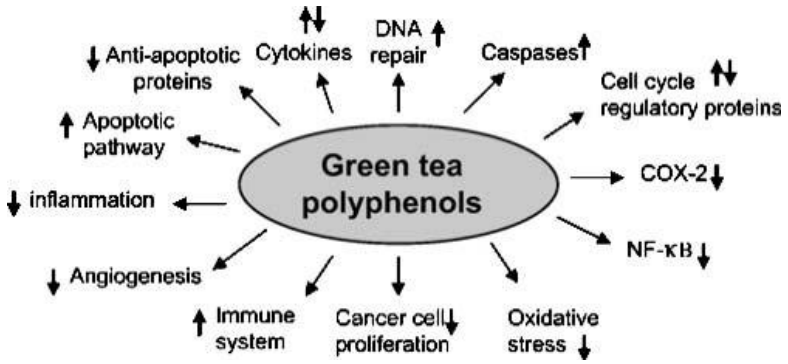


Figure 4.1. Molecularaims of EGCG.**Source:** Sinija 2008

From the results, the reduction of frame slippage for pure green tea is higher than thatfor green tea with mint. This means that pure green tea is more effective than green tea containing mint. However, after a β-galactosidase assay overlaywasmade on the plates, the colonies in most pure green tea plates produced a pink colour instead of white, making it them difficult to distinguish the blue colonies. The low amount of adenine, which were added with SC-U medium, enhanced the colonies to produce a pink or red colour[6].

VIII. Conclusion

In general, both flavoured and unflavoured green tea are able to reduce blue colonies, which signifiesthe instability of microsatellite. The reduction of blue colonies depends on the concentration of tea. The frame slippage events will decrease as the volume of green tea increase. It can be concluded that green tea is useful to treat some diseases association with the instability of DNA, like HNPCC (hereditary non-polyposis colorectal cancer).

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