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ESCHERICHIA COLI ATCC 8739 ADAPTS SPECIFICALLY TO SODIUM CHLORIDE, MONOSODIUM GLUTAMATE, AND BENZOIC ACID AFTER PROLONGED STRESS

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ABSTRACT

Escherichia coli lives in the human intestine and any form of adaptation may affect the human body. The effects of food additives on *E. coli* have been less studied compared to antibiotics. A recent study has demonstrated that *E. coli* is able to adapt to food additives by demonstrating global stress response. This study continues to study the evolution of *E. coli* in different food additives (sodium chloride, benzoic acid, monosodium glutamate) in different concentrations, singly or in combination, for over 83 passages. Adaptability of the cells was estimated with generation time and cell density at the stationary phase. Polymerase Chain Reaction (PCR)/ Restriction Fragments Length Polymorphism (RFLP) were used to analyze the adaptation at genomic level. Our results show that adaptation started to slow down and the gradients of generation time against passage are less steep compared with previous study, suggesting that most adaptive mutations occurred within the first 500 generations. In the genomic level, ecological specialization is observed as we find that the cells adapted through a different mechanism and diverge from each other although the resulting effect of the medium is the same. It suggests that different concentrations of food additives cause different types of chemical stress, instead of different levels of chemical stress.

Keywords: *Escherichia coli*, ATCC 8739, food additive, chemical stress

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INTRODUCTION

Evolution can affect the behavior and characteristics of the organism [1] and may result in adaptation of the organism in the new environment. Adaptation is a process where an organism changes physiologically, structurally or changes their behavior in order for them to survive better in the environment. For example, *Biston betularia* (peppered moths) adapts to colour changes in the environment due to pollution [2]; *Escherichia coli* gains the ability to utilize citric acid as a nutrient source [3] and *Flavobacterium sp.* utilizes the by-product of nylon manufacture through two newly evolved enzymes [4]. Long-term evolutionary adaptation may result in formation of new species from the original species [5]. The process is known as speciation [6].

Two main mechanisms responsible for evolution are natural selection and genetic drift [1]. The process whereby the heritable traits are passed on to successive generations to improve the survivability or an organism is known as natural selection [7]. Through natural selection, the advantageous traits are passed on to the next generation and more offspring will be able to survive and adapt better. The second mechanism is genetic drift [8]. Genetic drift is the change in the frequency of a genetic variant (allele) occurring in the population due to random sampling. Unlike natural selection, which determines the genetic variant due to successive generations, genetic drift randomly determines the variant and it is not affected by physical, chemical or environmental stress.

Evolution occurs in all organisms but using higher organisms to study evolution is not feasible due to their long reproduction cycle. Hence, bacterium such as *Escherichia coli* has several advantages in evolutionary studies compared to multicellular organisms. Firstly, *E. coli* grows rapidly on chemically defined environment [9], generally hardy in different temperatures [10,11], and is able to survive in prolonged stressed environments [12-14]. Secondly, rapid generation time of *E. coli* allows for higher mutation rate on a per day basis [15]. Thirdly, *E. coli* is readily preserved for extended periods [16], allowing for a complete "fossilized" record throughout the entire experiment [9]. Lastly, *E. coli* is a model organism and several strains have been fully sequenced [17].

Bacterial resistance and tolerance to antibiotics are well established and widely studied [18, 19]. However, mechanisms of insusceptibility to non-antibiotic agents like food preservatives and antiseptics are less well understood. For example, citric acid inhibits the growth of proteolytic strains of *Clostridium botulinum* [20]. Sodium chloride can inhibit the growth of many bacteria such as *Listeria monocytogenes* [21], *Ochrobactrum anthropi* [22] and *Lactobacillus plantarum* [23] by lowering the water activity [24]. In addition, *E. coli* had been recently shown to be able to adapt to 11% sodium chloride, demonstrating its adaptability in prolonged stress [12, 13].

A recent study examines the growth kinetics and genetic distance of *E. coli* ATCC 8739 in 3 different food additives [25]. Among the 8 different treatments, 6 of them are high and low concentration of monosodium glutamate, benzoic acid, and sodium chloride singly. The other 2 treatments are combinations of the 3 additives in high and low concentrations. After over 70 passages, *E. coli* adapts to their individual treatments as seen from the decrease of generation time. Genetic distance analysis by PCR/RFLP shows a diverging trend, suggesting adaptive mutations. However, the genetic distance converges by the end of the study at 70

passages. Lee et al. [25] suggests that *E. coli* might be exhibiting global stress response as previously observed in *E. coli* [26].

This study aims to continue the long-term evolution in *E. coli* under food additives to simulate a person consuming food additives for an extended period of time, observing growth kinetics and genetic changes for another 83 passages. Continuing the experiment will allow us to observe for changes in trends of generation time, or fitness in the later part of adaptation, since some genetic changes may only occur after an extended amount of time. Lee et al. [25] suggests that the rate of adaptation is likely to decrease if the study was continued. Hence, we hypothesize that the rate of adaptation of *E. coli* in the 8 different treatments will decrease from 71st to 150th passage, as most adaptation will have occurred in the early half of the experiment. Since fewer adaptations will occur in the second half of the experiment, it is likely that the PCR/RFLP profiles will be similar.

METHODS

Main Culture Experiment

The procedure of culture and subculturing used (Figure1) were identical to Lee et al. [25]. Cells from different treatments from Passage 70 in Lee et al. [25]'s study were revived in nutrient in before inoculating into 8 different treatment supplementation in 10 mL Nutrient Broth as Passage 71 [0.025% (w/v) as high monosodium glutamate (H MSG); 0.0025% (w/v) as low monosodium glutamate (L MSG); 0.025% (w/v) as high benzoic acid (H BA); 0.0025% (w/v) as low benzoic acid (L BA); 1% (w/v) NaCl as high salt (H SALT), Nutrient Broth as low salt (L SALT); 0.025% (w/v) monosodium glutamate, 0.025% (w/v) benzoic acid and 1% (w/v) NaCl as high combination (H COMB); 0.0025% (w/v) monosodium glutamate and 0.0025% (w/v) benzoic acid as low combination (L COMB)]. Subculturing was performed by transferring 1% (100 μL) of the previous culture on every Monday, Wednesday and Friday to the next passage. Optical density (OD) readings were taken before the next subculture at 600 nm wavelength to estimate the number of generations within the current passage and to also determine the number of cells that are being inoculated into the new passage. Generation time was measured on every 3rd passage. Glycerol stocks for each treatment were made from 1% of the culture for every 12th passage after culturing on MacConkey agar. Gram staining was conducted routinely to check for contamination. This was continued and maintained until Passage 153.

Swap Experiment

Swap experiments were done fortnightly, which is equivalent to 6-7 passages interval, involving the transfer of *E. coli* cells cultured in different treatments to other treatments for measurement of generation time. A total of five swap experiments were carried out, whereby the cells were inoculated into the new treatment in a 100 times dilution. The first four sets of swap experiments were identical to Lee et al. [25]. The first set of swap involves the inoculation of basal medium (L SALT) treated cells into the six non-salt mediums.

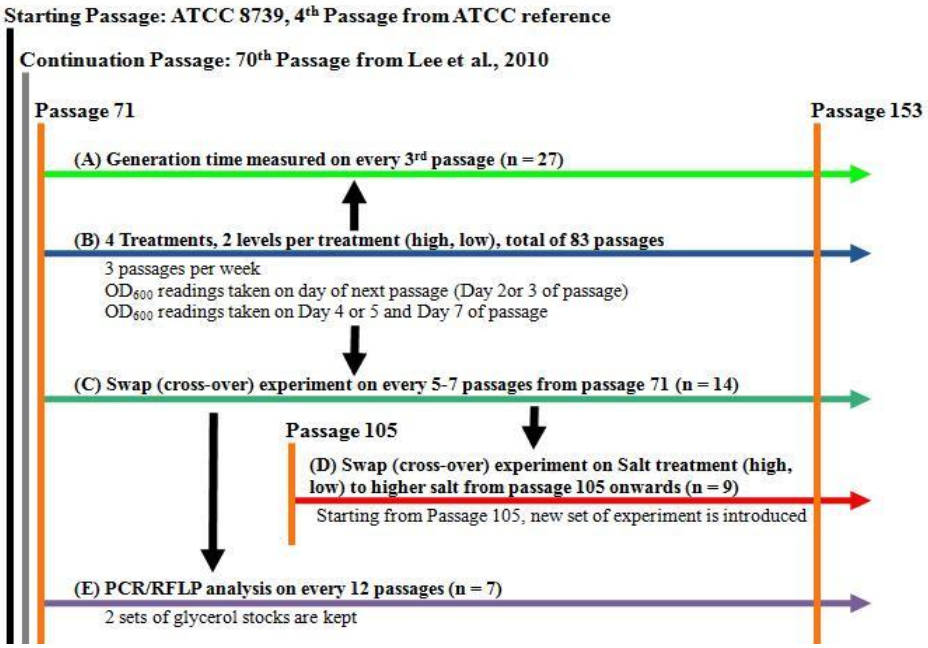

Figure 1. Complete Experimental Design.

An example would be inoculating cells from L SALT into the H MSG medium. For the second set, cells cultured in high and low concentration of each treatment were swapped for all treatments. For example, cells growing in H BA were inoculated into L BA treatment medium and vice versa. For the third set, cells in high concentration treatments (H MSG, H BA, H SALT) were inoculated into H COMB treatment. The next set of swap is similar to the third set except that the cells of low concentration were swapped into L COMB treatment medium. Cells from L MSG, L BA, and L SALT treatment were swapped into L COMB. The last set involved the inoculation of H SALT and L SALT treated cells into a higher concentration (2%) treatment media. OD 600 readings were recorded at intervals and generation times were then calculated.

Genomic DNA Extraction

Treatment cultures of every 12th passage were used for DNA extraction. The extracted DNA was subjected to Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP). The extraction method used [27] was based on the proposed method of DNA extraction for Gram-negative bacteria, which was used in Lee et al. [25]. The pellet was air-dried and dissolved to 100ng/µL in pH 8.0 Tris/HCl buffer and stored at -20°C.

Polymerase Chain Reaction/ Restriction Fragments Length Polymorphism

PCR/RFLP was performed using the procedure described in Lee et al. [25]. Each reaction consisted of the 50µL of mixture prepared using 200ng of DNA template in 10 pmoles of DNTPs, 50 pmoles of primer, 1 unit of Taq polymerase, and 1X standard buffer (with 1.5

mM of $MgCl_2$) provided by the supplier (New England Biolabs, Inc.). Primer 5, CgCgCTggC; Primer 6, gCTggCggC; Primer 7, CAggCggCg were used separately. The PCR reaction was carried out (Hybaid Limited, PCR express) under the cycling condition of initial denaturation at 95^{o}C for 10 minutes; 35 cycles of amplification at 95^{o}C for 1 minute, 27^{o}C for 1 minute, 72^{o}C for 3 minutes, followed by a final extension at 72^{o}C for minutes before gel electrophoresis in 2% (w/v) aragose gel with 1X GelRed. 11µL of PCR product was digested with 1 unit of restriction endonuclease (TaqI, HinfI, MspI), in a reaction mixture consisting of 1X restriction digestion buffer and 100ng/µL acetylated BSA made to a total of 20µL with distilled water. HinfI and MspI reaction mixtures were incubated at 37^{o}C, while the TaqI reaction mixture was incubated at 65^{o}C. All reaction mixtures were incubated for 16 hours before analysis on 2% (w/v) agarose gel with 1X GelRed.

Data Analysis

Data analysis was performed using the procedure described in Lee et al. [25]. Cell density was calculated from OD600 readings using the correction suggested by Sezonov et al. [28]. Briefly, the cell density is directly proportional to OD600 readings when OD600 reading is below or equal to 0.3, at which the cell density is equivalent to 5 X 10^7 cells per milliliter. If OD600 reading is above 0.3, the cell density is estimated by the equation of Cell Density = 52137400 X In (OD reading) + 118718650. Generation time for all experiments was calculated from difference in cell density at intervals between 120 and 300 minutes after the inoculation of cells into fresh media, and the geometric mean was calculated. The migration distance of the bands of PCR and RFLP of different treatments within the same passage was tabulated and a Nei-Li dissimilarity index [29], where the maximum value of 1 is obtained when there are no common bands comparing between two treatments, while a minimum of 0 will be obtained when two treatments have exactly the same bands [30-32]. The correlation coefficient (CC) value between dissimilarity indices across passages statistically tested against the CC value of 0.95 (~ 1) using the Z-test for two correlation coefficients where the p-value of more than 0.05 would indicate that the null hypothesis (CC is equal to 0.95) is not rejected. Pseudo-replicates generated from data of pre-70th [25] passages using Jackknife resampling were used for statistical evaluation for differencesin trends between pre-70th [25] and post-70th passages.

RESULTS

Generation Time

Generation time of cells in each treatment from passage 73 is estimated and compared to that of pre-73rd passages as reported in Lee et al. [25]. Lee et al. [25] reports that the gradients of all the eight different treatments are decreasing from passage 1 to 70. The steepest decrease in gradient are the cells in H COMB treatment with a gradient of -2.02, while the least decrease in gradient is H MSG with a gradient of -0.906. However, our results from passage 73 to 153 suggest that five treatments (H MSG, H BA, L BA, L SALT and L COMB) show

positive gradient in generation time with L COMB showing the highest increase. H COMB continues to exhibit the steepest decreasing gradient compared to the all the other treatments in passage 73 to 153, with a gradient of -0.564. Comparing gradients between pre-70th and post-70th passage using Jackknife resampling shows a significant increase in gradient in all treatments. (Table 1)

Table 1. Fitted linear regression models of generation time between pre-70th and post-70th passage

Treatment	Linear Regression Model (Passage 1-70; [25])	Linear Regression Model (Passage 71-153; This study)	P-value
H MSG	Generation time = -0.906 (Passage) + 171	Generation time = 0.03 (Passage) + 73.7	3.5E-14
L MSG	Generation time = -1.87 (Passage) + 225	Generation time = -0.058 (Passage) + 80.6	2.3E-15
H BA	Generation time = -1.15 (Passage) + 203	Generation time = 0.042 (Passage) + 63.1	1.1E-11
L BA	Generation time = -1.39 (Passage) + 196	Generation time = 0.1086 (Passage) + 67.7	1.7E-14
H SALT	Generation time = -1.12 (Passage) + 180	Generation time = -0.1492 (Passage) + 92.6	1.9E-13
L SALT	Generation time = -1.24 (Passage) + 189	Generation time = 0.175 (Passage) + 63.8	1.6E-14
H COMB	Generation time = -2.02 (Passage) + 271	Generation time = -0.564 (Passage) + 146.9	1.5E-10
L COMB	Generation time = -1.22 (Passage) + 190	Generation time = 0.223 (Passage) + 56.4	7.8E-14

Swap Experiment

Generation time of cells in each swap experiment from passage 73 is estimated and compared to that of pre-73rd passages as reported in Lee et al. [25]. Using Jackknife resampling, our results suggest no difference in the change in generation time between pre-70th and post-70th passage when low salt-treated cells (cells in nutrient broth without supplementation) are cultured to other supplemented media. There is also no difference in the change in generation time between pre-70th and post-70th passage when high salt-treated cells are cultured in nutrient media. All other comparisons with Lee et al. [25] are significant. In this study, another set of swap experiment is added. Our results show that the decline in generation time is more than twice when low salt-treated cells are cultured in 2% salt-supplemented nutrient media (total of 2.7% NaCl; HIGHER SALT) compares to when high salt-treated cells are cultured in HIGHER SALT media (gradient of -3.26 compared to -1.22). (Table 2)

Genetic distance by PCR/ RFLP

The PCR and RFLP products of all 8 treatments after agarose gel electrophoresis are used to study the differences between the genome of the *E. coli* cells. Nei-Li Dissimilarity Index

was used to calculate the dissimilarity between pair wise comparisons of the treatments, and six resulting effects obtained from the comparisons were analyzed. From the PCR/RFLP comparisons, each of the six resulting effects obtained has two originating comparisons. For example, the effects of MSG can be estimated by LMSG/LSALT and LBA/LCOMB comparisons. By plotting the two comparisons against each other and testing for significance, we can conclude whether the genomic difference in each of the 2 comparisons are actually a consequent effect from the resulting effects. While Lee et al. [25] shows that only 10BA + SALT effect is significant; our results show that all 6 resulting effects are statistically significant (Table 3). Moreover, while Lee et al. [25] report an overall trend of converging genetic distances; our results show an overall trend of divergence (Figure 2 and 3).

Table 2. Fitted linear regression models of generation time from swap experiments between pre-70th and post-70th passage

Swap Experiment	Linear Regression Model (Passage 1-70; [25])	Linear Regression Model (Passage 71-153; This study)	P-value
L SALT to H MSG	Generation Time = 0.90 (Swap Count) + 151	Generation Time = 2.70 (Swap Count) + 56.5	0.551
L SALT to L MSG	Generation Time = -5.87 (Swap Count) + 230	Generation Time = 2.70 (Swap Count) + 56.5	0.533
L SALT to H BA	Generation Time = -6.68 (Swap Count) + 385	Generation Time = 2.52 (Swap Count) + 54.0	0.535
L SALT to L BA	Generation Time = 1.29 (Swap Count) + 151	Generation Time = 1.12 (Swap Count) + 62.6	0.524
L SALT to H COMB	Generation Time = 2.34 (Swap Count) + 312	Generation Time = -1.59 (Swap Count) + 104	0.509
L SALT to L COMB	Generation Time = 3.44 (Swap Count) + 138	Generation Time = 1.25 (Swap Count) + 71.8	0.519
H MSG to L MSG	Generation Time = -6.91 (Swap Count) + 214	Generation Time = 3.08 (Swap Count) + 55.9	0.00106
H BA to L BA	Generation Time = -5.25 Swap Count + 182	Generation Time = 3.01 (Swap Count) + 53.0	0.000144
H SALT to L SALT	Generation Time = -1.53 (Swap Count) + 193	Generation Time = 2.56 (Swap Count) + 52.2	0.07
H COMB to L COMB	Generation Time = -4.20 Swap Count + 192	Generation Time = 1.82 (Swap Count) + 58.5	0.0143
L MSG to H MSG	Generation Time = -4.37 (Swap Count) + 237	Generation Time = -2.19 (Swap Count) + 107.0	0.00285
L BA to H BA	Generation time = -15.0 (Swap Count) + 398	Generation Time = 2.78 (Swap Count) + 59.3	0.00081
L SALT to H SALT	Generation Time = -10.7 (Swap Count) + 290	Generation Time = -0.335 (Swap Count) + 77.5	0.0132
L COMB to H COMB	Generation Time = -17.2 (Swap Count) + 348	Generation Time = 3.66 (Swap Count) + 54.1	2.78E-15
L SALT to HIGHER SALT		Generation Time = -3.26 (Swap Count) + 80.9	
H SALT to HIGHER SALT		Generation Time = -1.22 (Swap Count) + 70.6	

Table 3. Tabulation of *P*-value for the resulting effects of PCR/RFLP comparisons

	PCR-RFLP Comparison	Resulting Effects	Correlation Coefficient	Z Statistic	P-value	Significant
Passage 1 to 70	LMSG/LS, LBA/LC	MSG	0.786	-0.944	0.173	No
	LMSG/LC, LBA/LS	BA	0.934	-0.175	0.431	No
	LMSG/LBA, LS/LC	BA + MSG	0.764	-1.012	0.156	No
	HMSG/HS, HBA/HC	10MSG + S	0.631	-1.333	0.091	No
	HMSG/HC, HBA/HS	10BA + S	0.142	-2.068	0.019	Yes
	HMSG/H BA, HS/HC	10MSG + 10BA	0.437	-0.167	0.434	No
Passage 73 to153	LMSG/LS, LBA/LC	MSG	0.078	-3.508	2.3×10^{-04}	Yes
	LMSG/LC, LBA/LS	BA	0.006	-3.651	1.3×10^{-04}	Yes
	LMSG/LBA, LS/LC	BA + MSG	0.022	-3.619	1.5×10^{-04}	Yes
	HMSG/HS, HBA/HC	10MSG + S	0.144	-3.373	3.7×10^{-04}	Yes
	HMSG/HC, HBA/HS	10BA + S	0.341	-2.954	1.6×10^{-03}	Yes
	HMSG/H BA, HS/HC	10MSG + 10BA	0.067	-3.529	2.1×10^{-04}	Yes

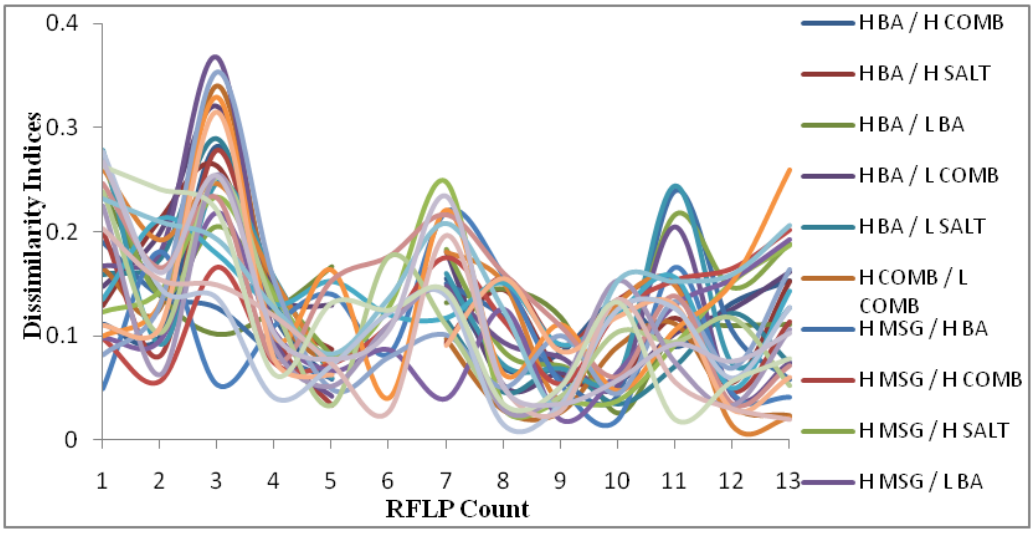

Figure 2. Dissimilarity index of the 28 pair-wise comparisons for the 13 PCR/RFLP counts. RFLP counts 1 to 6 are from Lee et al. [25] while RFLP counts 7 to 13 are from this study. The data points of comparisons with H COMB or L COMB of PCR/RFLP number 6 are excluded due to unusually high dissimilarity index which is caused by an error in the PCR of H COMB and L COMB of PCR/RFLP number 6 as reported in Lee et al. [25].

LC, HC, LS and HS referred to low combination (L COMB), high combination (H COMB), low salt (L SALT) and high salt (H SALT) respectively. As the additive concentration of the high treatment is 10 times that of low treatment for BA and MSG, BA

refers to L BA additive, while 10BA refers to H BA additive. The resulting effects represent the difference in the treatments. For example, MSG is the difference between L MSG and L SALT.

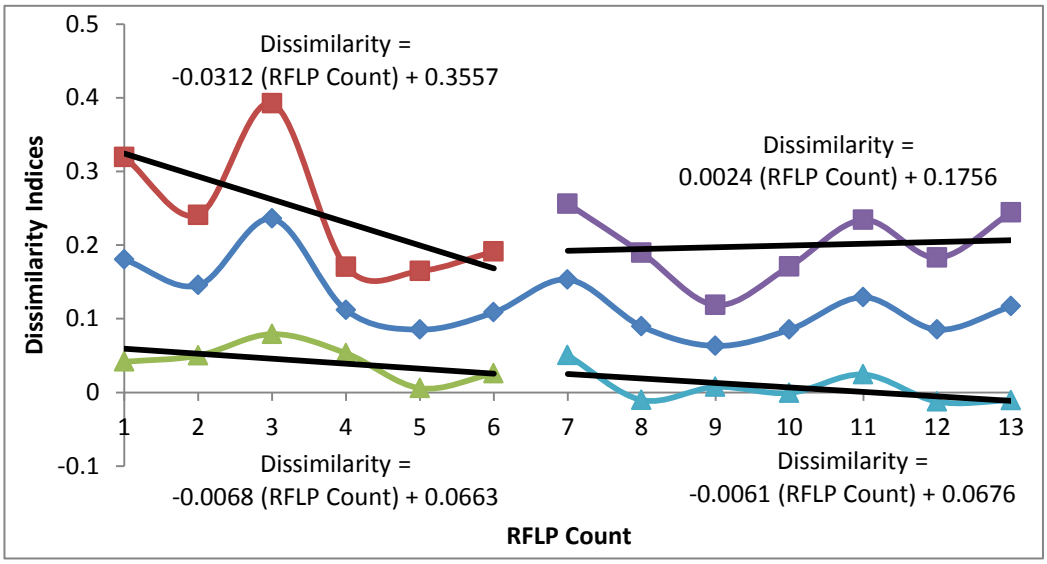

Figure 3. Estimation of the dissimilarity indices for each PCR/RFLP count. RFLP counts 1 to 6 are from Lee et al. [25] while RFLP counts 7 to 13 are from this study. The mean and ±2 SD (standard deviations) are calculated from Nei and Li distances of the 28 pair-wise comparisons. The linear regression lines are calculated from ±2 SD values in order to indicate whether the genetic distances are converging or diverging.

DISCUSSION

A recent study examining the adaptation of *E. coli* in 3 different food additives [25] demonstrates the ability of *E. coli* to adapt to the additives by reduction of generation time across passages. These adaptations are shown to have a genetic basis as illustrated by PCR/RFLP-based DNA fingerprinting. Lee et al. [25] suggests that the rate of adaptation is likely to decrease if the study is to be continued. This study continues the culture for another 80 passages and hypothesizes that the rate of adaptation of *E. coli* in the 8 different treatments will decrease from 71st to 153rd passage, as most adaptation will have occurred in the early half of the experiment.

Nutrient Broth Does Not Prime Cells for Adaptation in Other Treatments

E. coli cells are grown in NB with various supplementations of treatments. Thus, it is important that the adaptations of the cells are resulting from the treatments rather than from the NB. Lee et al. [25] reports that nutrient broth (L SALT media) does not prime cells for adaptation in other treatments. Our results show that the changes in generation time of low salt-treated cells inoculated into 6 non-salt treatments are not significantly different to that of

Lee et al. [25]. This suggests that NB does not assist the cells to grow better in the various treatment media; thus, not affecting the adaptability of cells in the various treatments through the 153 passages.

Decreased Adaptation Rates of Cells in Later Passages

Comparing the generation time of cells of pre-70th [25] and post-70th passage, there is either a slower decrease (L MSG, H SALT, H COMB), or slight increase (H MSG, H BA, L BA, L SALT, L COMB) in generation time. This result is significant. This suggests a deceleration of rate of adaptation of the cells to their individual treatment. This may suggest that most selection and beneficial mutations have occurred in the earlier generations to facilitate a faster rate of adaptation to the individual treatments in earlier passages, with adaptation in the later generations relying on beneficial mutations which are fewer, or smaller in effect, or both [33]. This is consistent with previous long-term experimental evolution experiments with *E. coli* [15, 34] showing that more than half of the improvements in fitness through adaptive mutation occurred in the first 500 generations of cells. Using an estimated number of 6.68 generations per passage [33], 500 generations are estimated to have occurred by 75th passage.

Ecological Specialization towards Individual Treatments

Comparing generation time trends of High to Low treatment swaps of pre-70th to post-70th passages, our results show that generation time trends of the earlier passages demonstrates a decreasing trend, while later passages show increasing trend for all 4 individual treatments. This can suggest that the cells, which had adapted to their high individual treatments, have lost their ability to adapt to low treatment media.

When organism adapt genetically to one environment, they may lose fitness in other environments through ecological specialization processes such as mutation accumulation and antagonistic pleiotropy [10, 11, 35]. Mutation accumulation refers to the process whereby mutations become fixed by genetic drifts in genes that are not maintained by selection; adaptation in one environment and loss to another are caused by different mutations. However, antagonistic pleiotropy is a process where adaptation to one environment and loss of adaptation to another arise from a trade-off, meaning the same mutation that is beneficial to one environment will be detrimental to another. Therefore, in the case of antagonistic pleiotropy, it can be expected that if adaptation to a selective environment is initially fast but decelerates over time, the functional decay should initially be more rapid. Functional decay in mutation accumulation should be at a constant rate, since it only depends on the mutation rates of neutral alleles through genetic drift [10, 11].

Our results suggest that such ecological specialization may be caused by mutation accumulation. This is due to the fact that high treatment cells of earlier passages does not show a rapid rate of functional decay, which refers to the rapid loss of ability of the cells to adapt in the media other than their individual treatments through the mutation of unused functions [35]. Generation time trends comparison of high single treatment to high combination treatment between earlier and later passages also show similar results, where

decreasing generation time in earlier passages differ to a larger extent of increasing generation time trends in later passages. In low to high treatments swaps, it is observed that only L MSG and L COMB showed increasing generation time trends, which supports the possibility of mutation accumulation.

Different Concentrations of Food Additives Render Different Types of Chemical Stress Instead Of Intensity

In observation of generation time trends of Low/High salt-treated cells swapped into Higher (2.7%) salt treatment. Our results show that the rate of decline in generation time is faster in Low salt cells compared to High salt cells. This is in contrast to our hypothesis, whereby it is expected that High Salt cells are able to adapt better to Higher Salt treatment than Low Salt treatment cells, due to the assumed, lesser difference in chemical stress. This is similar to High/Low treatment swaps of the earlier passages [25], whereby Low treatment cells swapped into high treatment media had a faster rate of decrease in generation time compared to high treatment cells swapped into low treatment media. This suggests that different concentration of food additives may render different type of chemical stress, instead of different levels of stress. This may suggest that adaptive mechanisms for all 3 salt concentrations are different. Ray and Peters [36] studies the adaptation of *Pseudomonas aeruginosa* cells to dinitrophenol (DNP) and other chemical stressors suggest that different concentration of DNP caused different types of stress, rather than different levels.

Cells from Different Treatment Showed Genetic Divergence

Our results from the PCR/RFLP show genomic divergence (Figures 2 and 3) indicating that the *E. coli* of different treatments is getting genetically different. This result contradicts with our hypothesis, which suggests that the rate of adaptation will slow down after 150^{th} passage and genetic variation will decrease. This decrease will have resulted in a convergence but experimental results have shown otherwise. This suggests that the cells may have evolved specialized adaptive mechanisms to their own type of stress environment [37], also known as niche adaptation. One example of such adaptation reported is *Pseudomonas putida* [38]. *Pseudomonas putida* is a Gram-negative bacterium that can be found in various environments. Members of different species demonstrates a wide spectrum of metabolic activities, which includes the ability to live in soil contaminated with high concentrations of heavy metal to other strains found as plant-growth promoting rhizospheric and endophytic bacteria. Results have shown that horizontal gene transfer played a key role in the bacterium adaptation process. Another such bacterium is *Lactobacillus plantarum* [39], which are found in many different ecological niches such as vegetables, meat and fish, and dairy products, as well as the gastro-intestinal tract.

E. coli exposed to stress will respond to counter the effects. A study has shown that *E. coli* will utilize its ArcAB global regulatory system in aerobic conditions to counter the effects caused by reactive oxygen stress (ROS) [40]. In another study [41], *E. coli* are able to utilize the Poly-β-hydroxybutyrate (PHB) mobilization mechanism to increase their survivability under stress conditions.

Our results show that the correlation coefficient of the dissimilarity indices of the 6 selected treatment pairs shows that all pairs are statistically significant (Table 3). Each pair has a common resulting effect; such as L MSG/L SALT and L BA/L COMB have MSG as the common resulting effect. The statistical significance suggests that although both adaptations are towards MSG, due to the different media the cells are originally grown in, they might have chosen different types of adaptation pathways to adapt to the same source of stress, resulting in divergence of their genetic material across all 8 treatments maybe due to ecological specialization. A study on *Listeria monocytogenes* shows that it can change its membrane fluidity based on the temperature of the environment it is residing in [42]. Lenormard [6] suggests that prolonged local adaptation may lead to reinforcement of adaptive traits, which may result in speciation. In a study on *Pseudomonas aeruginosa*, Friman et al. [43] suggests that long-term adaptation may lead to loss of function, which has been reported by Cooper and Lenski [35] in their adaptation studies with *E. coli.* Collectively, this may suggest a 2-stage adaptation response which may result in speciation.

However, the adaptation responses and divergence of dissimilarity indices cannot be applied for the whole *E. coli* genome. This is due to the use of only 3 primers during the study, which only amplified 0.37% of the total *E. coli* genome as estimated by Lee et al. [25]. Hence, only the area amplified by the 3 primers can account for the divergence. Global stress response genes that are activated may or may not lie within the amplified region. It is likely that the adapted cells will be a genetically mixed population as suggested by Lee et al. [25]. Hence, it will be interesting to examine the combinations of genetic changes leading to adaptation to food additives. Future work may examine these genetic changes using transcriptomics studies, such as microarrays and RNA-seq, or deep sequencing technologies, such as DNA-seq.

CONCLUSION

Our results suggest that the rate of adaptation after initial culture. Apart from adapting to their individual treatment, ecological specialization are observed, whereby there is a loss of ability of cells to adapt to other environment other than their own. This is demonstrated in the results of swap experiments where cells are introduced into another environment apart from their own.

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