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Dataset Paper

Adaptation of *Escherichia coli* ATCC 8739 to 11% NaCl

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Escherichia coli (*E. coli*) is a nonhalophilic microbe and used to indicate faecal contamination. Salt (sodium chloride, NaCl) is a common food additive and is used in preservatives to encounter microbial growth. The effect of how *E. coli* interacts with the salt present in the human diet is unclear. Thus, it is important to investigate this relationship. In order to adapt and survive the changes in the environment, *E. coli* may undergo halophilization. In this study, we observed the genetic changes and growth kinetics of *E. coli* ATCC 8739 under 3%–11% NaCl over 80 passages. Our results suggest that *E. coli* adapted to 1% increase in NaCl every month with a successful adaptation to 11% NaCl. Gram staining and PCR/RFLP showed that the cultures are Gram negative and the DNA profiles of all 4 replicates to be similar, suggesting that the cultures had not been contaminated.

1. Introduction

Escherichia coli is a textbook example of nonhalophilic bacteria and is an indicator organism for faecal contamination of water as *E. coli* is a more consistent predictor of gastrointestinal illness than other bacterial indicators in water [1]. This corroborates Burton et al. [2] whom suggested that *E. coli* was a suitable predictor of *Salmonella enterica* serovar *Newport* in various freshwater sediments and has been observed to survive as long as or longer than *Salmonella* spp., thus, fulfilling its requirement as an indicator for pathogenic bacteria. In addition, the survival of *E. coli* is independent of the amount of organic matter [2]. This suggested that *E. coli* is a suitable indicator as it can survive in media of different nutritional richness.

Previous studies [3–6] on the adaptation and evolution of *E. coli* were carried out using antibiotics and drugs. However, common food additive such as salt, which is used to preserve food and inhibit microorganisms, is less understood in terms of *E. coli*'s adaptive mechanism although recent studies [7, 8] had suggested that *E. coli* is able to adapt to food additives over extended culture. This suggests that *E. coli* may be able to adapt to higher concentrations of food additives but this has yet to be studied. Doudoroff [9] demonstrated that the viable count of *E. coli* previously cultured in ordinary fresh water media and then transferred to saline nutrient solutions remained at a constant up to 7% NaCl concentration. The viable count dropped progressively with further increase in concentration suggesting that *E. coli* is nonhalophilic and 7% NaCl is bacteriostatic. Hrenovic and Ivankovic [10] reported that the growth of *E. coli* is optimal below 5% NaCl. The adaptation of *E. coli* to salt may suggest similar resistance to other preservatives and its ability to grow in saline environment may be underestimated.

This study aims to observe gradual adaptation of *E. coli* ATCC 8739, a fully sequenced strain, cultured in NaCl-supplemented medium up to 8% NaCl over 80 passages. Our results suggest that *E. coli* adapted to 1% increase in NaCl every month with a successful adaptation to 11% NaCl. Gram staining and DNA fingerprinting by PCR/restriction fragment length polymorphism at Passage 72 showed that the cultures are Gram negative and the PCR-RFLP profiles of all 4 replicates to be similar. This suggested that the cultures had not been contaminated with *Staphylococcus aureus*, which is

Table 1: OD600 tabulation for minimum inhibitory concentration estimation. Representative data from each salt concentration is shown.

Passage ([NaCl]% in Media)	Replicate	[NaCl]% for Minimum Inhibitory Concentration						
		0%	1%	3%	5%	7%	9%	11%
11 (3%)	A	0.684	0.672	0.866	0.464	0.118	0.006	−0.004
	B	0.616	0.613	0.863	0.355	0.075	0.006	−0.001
	C	0.637	0.657	0.706	0.376	0.089	−0.003	−0.004
	D	0.686	0.719	0.870	0.370	0.090	0.008	−0.001
30 (4%)	A	0.644	0.703	0.793	0.362	0.139	0.006	0.005
	B	0.678	0.658	0.744	0.381	0.085	0.021	0.002
	C	0.659	0.627	0.757	0.425	0.005	0.010	0.003
	D	0.670	0.639	0.784	0.363	0.035	0.007	−0.001
49 (5%)	A	0.705	0.680	0.840	0.696	0.104	0.012	0.006
	B	0.761	0.720	0.927	0.757	0.123	0.030	−0.003
	C	0.919	0.705	1.354	0.792	0.158	0.064	−0.007
	D	0.726	0.767	1.060	0.582	0.115	0.002	0.023
61 (6%)	A	0.471	0.515	0.771	0.433	0.049	−0.022	−0.020
	B	0.432	0.446	0.732	0.461	0.078	−0.018	−0.033
	C	0.54	0.561	0.628	0.444	0.085	−0.021	−0.016
	D	0.985	0.569	0.760	0.431	0.124	−0.009	−0.020
72 (7%)	A	0.549	0.746	0.610	0.401	0.418	0.289	0.259
	B	0.804	0.742	0.791	0.501	0.301	0.288	0.200
	C	0.55	1.013	0.984	0.407	0.309	0.241	0.163
	D	0.873	0.790	0.689	0.387	0.340	0.286	0.230

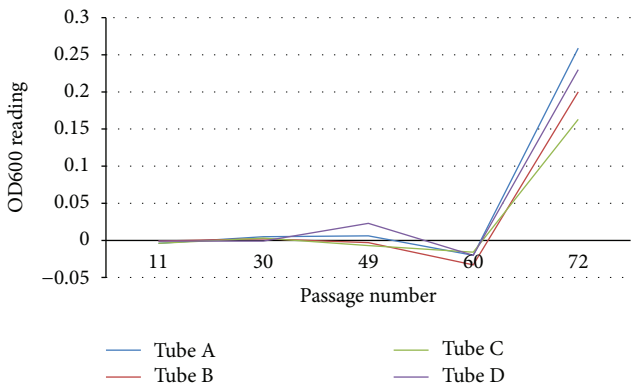

Figure 1: OD600 at 11% NaCl, 21 to 23 hours after inoculation. This graph illustrates the OD600 reading in 11% NaCl of the MIC experiment demonstrating an increased OD reading after passage 60, suggesting that the cells are able to divide at 11% NaCl.

Gram positive and the most likely contaminant as *S. aureus* is a salt-tolerant commensal found on human skin.

2. Methodology

Main culture experiment was carried out with an initial inoculum of 9.7×10^6 *E. coli* ATCC 8739 cells from Passage 70 of Lee et al. [8] as the first passage in each of the 4 replicates of 10 mL of 1X nutrient broth with a fixed concentration of NaCl (we refer to this as adaptation media) and cultured in tightly capped 15 mL conical tubes. Subculture was performed using 1% of the previous culture on every odd day except Sunday (3 subculturing per week). NaCl concentration for the passages was as follows: Passages 1 to 15 at 3% NaCl, Passages 16 to 31 at 4% NaCl, Passages 32 to 39 at 4.5% NaCl, Passages 40 to 50 at 5% NaCl, Passages 51 to 62 at 6% NaCl, Passages 63 to 74 at 7% NaCl, and Passages 75 to 80 at 8% NaCl.

Contamination monitoring was as follows. The cultures were monitored routinely for contamination using Gram staining and DNA fingerprinting. The DNA fingerprinting by PCR/restriction fragment length polymorphism was performed using the procedure in a previous similar adaptation study [8] where each of the 3 primers (Primer 5, CgCgCTggC; Primer 6, gCTggCggC; and Primer 7, CAggCggCg) was used as both forward and reverse primers. The PCR reaction was carried out (Hybaid Limited, PCR express) under the cycling condition of initial denaturation at 95°C for 10 minutes; 35 cycles of amplification at 95°C for 1 minute, 27°C for 1 minute, and 72°C for 3 minutes; followed by a final extension at 72°C for 10 minutes before digestion with 1 unit of TaqI restriction endonuclease for 16 hours at 65°C before analysis on 2% (w/v) agarose gel with 1X GelRed.

Passage monitoring was as follows. The growth conditions of each passage were monitored by optical density at 600 nm wavelength (OD600 readings) using Shimadzu UV-1601 UV/light spectrophotometer. OD600 reading of each replicate was taken prior to each subculture for estimating inoculum density. OD600 readings were taken at the fifth and seventh days after inoculation for estimating cell density at stationary phase. At every third passage, 10 μL of *E. coli* culture from each replicate (A, B, C, and D) was inoculated into 1 mL of 1X nutrient broth and OD600 readings taken at

Table 2: Colony MIC OD Readings for Passage 44 (4% NaCl).

Colony	[NaCl]%						
	0%	1%	3%	5%	7%	9%	11%
AA	0.474	0.412	0.249	0.280	0.024	0.005	0.014
AB	0.463	0.441	0.317	0.466	0.042	0.006	0.010
AC	0.233	0.321	0.408	0.299	0.024	0.008	0.011
AD	0.644	0.621	0.417	0.250	0.090	0.012	0.016
AE	0.393	0.404	0.294	0.518	0.020	0.012	0.023
AF	0.592	0.596	0.480	0.270	0.027	0.007	0.010
AG	0.373	0.378	0.355	0.305	0.036	0.009	0.020
AH	0.365	0.350	0.367	0.305	0.039	0.015	0.011
AI	0.228	0.330	0.343	0.306	0.025	0.005	0.029
AJ	0.265	0.325	0.352	0.252	0.030	0.009	0.020
BA	0.382	0.383	0.230	0.309	0.021	0.006	0.017
BB	0.422	0.410	0.331	0.258	0.034	0.012	0.018
BC	0.443	0.414	0.307	0.417	0.041	0.016	0.023
BD	0.450	0.430	0.347	0.473	0.056	0.014	0.031
BE	0.475	0.465	0.341	0.288	0.029	0.030	0.020
BF	0.457	0.392	0.302	0.357	0.031	0.014	0.014
BG	0.437	0.478	0.305	0.354	0.027	0.019	0.023
BH	0.464	0.415	0.339	0.344	0.029	0.005	0.028
BI	0.460	0.466	0.354	0.330	0.039	0.026	0.023
BJ	0.430	0.477	0.350	0.344	0.040	0.036	0.023
CA	0.745	0.682	0.386	0.264	0.071	0.041	0.023
CB	0.459	0.434	0.306	0.300	0.047	0.044	0.034
CC	0.538	0.473	0.338	0.309	0.026	0.013	0.025
CD	0.447	0.475	0.295	0.330	0.038	0.008	0.016
CE	0.436	0.435	0.329	0.375	0.031	0.019	0.012
CF	0.409	0.398	0.307	0.269	0.037	0.020	0.046
CG	0.397	0.429	0.381	0.438	0.023	0.023	0.017
CH	0.766	0.784	0.696	0.377	0.119	0.038	0.038
CI	0.746	0.787	0.649	—	0.022	0.031	0.040
CJ	0.488	0.501	0.419	0.234	0.022	0.035	0.029
DA	0.547	0.595	0.694	0.099	0.041	0.019	0.028
DB	0.244	0.676	0.640	0.469	0.051	0.028	0.052
DC	0.495	0.562	0.700	0.213	0.046	0.013	0.024
DD	0.391	0.378	0.273	0.308	0.024	0.036	0.014
DE	0.486	0.558	0.538	0.412	0.032	0.018	0.025
DF	0.411	0.605	0.468	0.505	0.038	0.017	0.023
DG	0.390	0.531	0.499	0.416	0.027	0.032	0.029
DH	0.219	0.453	0.480	0.538	0.038	0.038	0.032
DI	0.475	0.431	0.453	0.499	0.042	0.017	0.018
DJ	0.496	0.506	0.575	0.314	0.028	0.026	0.036

intervals of up to 360 minutes and may be used to estimate generation time.

For culture MIC, 1% of *E. coli* culture from each replicates (A, B, C, and D) was inoculated into 1 mL of 1X nutrient broth supplemented with 0% (w/v) NaCl, 1% (w/v) NaCl, 3% (w/v) NaCl, 5% (w/v) NaCl, 7% (w/v) NaCl, 9% (w/v) NaCl, and 11% (w/v) NaCl of different salt concentrations. For colony MIC, each sample of *E. coli* was streaked on nutrient agar and incubated overnight at 37°C. Ten colonies were randomly taken from each plate and inoculated into 1 mL of 1X nutrient

Table 3: Colony MIC OD Readings for Passage 53 (5% NaCl).

Colony	[NaCl]%						
	0%	1%	3%	5%	7%	9%	11%
AA	0.561	0.402	0.368	0.321	0.026	0.006	0.012
AB	0.558	0.429	0.339	0.405	0.030	0.005	0.003
AC	0.320	0.280	0.317	0.353	0.024	0.006	0.015
AD	0.309	0.307	0.280	0.414	0.033	0.001	0.015
AE	0.353	0.322	0.315	0.394	0.016	0.045	0.018
AF	0.281	0.264	0.261	0.348	0.057	0.003	0.008
AG	0.354	0.338	0.382	0.441	0.020	0.006	0.003
AH	0.385	0.323	0.280	0.414	0.043	0.006	0.006
AI	0.352	0.320	0.380	0.421	0.022	−0.005	0.014
AJ	0.350	0.321	0.352	0.395	0.020	0.008	0.005
BA	0.330	0.302	0.319	0.447	0.021	−0.001	0.021
BB	0.322	0.299	0.314	0.456	0.060	−0.004	0.009
BC	0.342	0.255	0.221	0.374	0.069	−0.004	0.008
BD	0.365	0.314	0.288	0.417	0.085	0.008	0.023
BE	0.299	0.267	0.246	0.406	0.036	0.010	0.007
BF	0.339	0.394	0.321	0.395	0.086	−0.006	0.012
BG	0.325	0.288	0.292	0.360	0.018	0.007	0.001
BH	0.385	0.320	0.260	0.337	0.025	−0.009	0.001
BI	0.313	0.323	0.211	0.378	0.021	0.008	0.011
BJ	0.327	0.347	0.221	0.308	0.049	0.015	0.002
CA	0.384	0.366	0.284	0.406	0.022	0.005	0.010
CB	0.360	0.368	0.321	0.369	0.125	0.009	0.013
CC	0.340	0.383	0.322	0.390	0.053	0.007	0.010
CD	0.386	0.423	0.283	0.388	0.091	0.011	0.018
CE	0.306	0.372	0.301	0.349	0.083	0.033	0.002
CF	0.352	0.390	0.383	0.279	0.064	0.016	0.008
CG	0.402	0.368	0.366	0.347	0.136	0.015	0.003
CH	0.348	0.344	0.318	0.437	0.039	0.022	0.006
CI	0.377	0.380	0.323	0.414	0.089	0.016	0.015
CJ	0.282	0.280	0.231	0.466	0.048	0.024	0.005
DA	0.321	0.345	0.275	0.383	0.020	0.007	0.002
DB	0.329	0.288	0.362	0.290	0.039	0.005	0.001
DC	0.380	0.307	0.291	0.319	0.030	0.003	0.013
DD	0.325	0.307	0.286	0.335	0.047	0.019	0.013
DE	0.445	0.324	0.306	0.318	0.249	0.011	0.001
DF	0.559	0.353	0.276	0.434	0.059	−0.001	−0.001
DG	0.324	0.265	0.240	0.345	0.082	0.030	−0.001
DH	0.321	0.330	0.254	0.315	0.025	0.011	−0.004
DI	0.349	0.357	0.315	0.356	0.073	0.002	0.005
DJ	0.392	0.371	0.286	0.313	0.041	0.020	0.025

broth and incubated overnight at 37°C before inoculation into 1 mL of 1X nutrient broth supplemented with different salt concentrations. The inoculated medium was incubated for 21–23 hours at 37°C before taking OD600 readings.

3. Dataset Description

The dataset associated with this Dataset Paper consists of 5 items which are described as follows.

Dataset Item 1 (Table). OD600 readings for each replicate taken at the day of the next subculture which can be used to estimate the inoculum density of each passage and the number of generations between each culture. The table is in long format where each row has only one measured data attribute. In this case, the measured data will be the OD600 readings. The other attributes are descriptors of the measured data attribute. This is opposed to the wide format whereby the descriptors for a particular OD600 reading are given as

Table 4: Colony MIC OD Readings for Passage 72 (7% NaCl).

Colony	[NaCl]%						
	0%	1%	3%	5%	7%	9%	11%
AA	0.547	0.659	0.160	0.279	0.082	0.099	0.137
AB	0.593	0.675	0.238	0.174	0.194	0.166	0.104
AC	0.538	0.562	0.095	0.312	0.040	0.095	0.129
AD	0.239	0.278	0.240	0.217	0.164	0.172	0.105
AE	0.358	0.553	0.247	0.231	0.168	0.097	0.158
AF	0.318	0.320	0.246	0.207	0.016	0.011	0.005
AG	0.565	0.566	0.163	0.186	0.172	0.132	0.069
AH	0.137	0.162	0.118	0.201	0.042	0.060	0.121
AI	0.574	0.615	0.214	0.136	0.102	0.093	0.091
AJ	0.167	0.180	0.195	0.132	0.010	0.000	0.009
BA	0.445	0.499	0.192	0.225	0.117	0.103	0.111
BB	0.416	0.574	0.163	0.236	0.082	0.051	0.090
BC	0.409	0.465	0.286	0.195	0.035	0.008	0.052
BD	0.473	0.587	0.192	0.131	0.040	0.054	0.123
BE	0.628	0.529	0.145	0.176	0.075	0.088	0.071
BF	0.495	0.616	0.253	0.238	0.132	0.099	0.036
BG	0.187	0.148	0.052	0.120	0.136	0.071	0.139
BH	0.530	0.534	0.136	0.155	0.022	0.073	0.066
BI	0.164	0.132	0.076	0.193	0.072	0.064	0.101
BJ	0.593	0.600	0.150	0.150	0.031	0.075	0.068
CA	0.274	0.290	0.266	0.240	0.129	0.137	0.093
CB	0.502	0.598	0.189	0.198	0.112	0.132	0.086
CC	0.334	0.293	0.264	0.255	0.090	0.110	0.118
CD	0.374	0.350	0.332	0.282	0.110	0.061	0.072
CE	0.309	0.304	0.282	0.294	0.087	0.150	0.075
CF	0.391	0.308	0.248	0.308	0.057	0.112	0.088
CG	0.292	0.327	0.210	0.262	0.124	0.067	0.065
CH	0.250	0.290	0.215	0.241	0.069	0.092	0.094
CI	0.384	0.317	0.377	0.058	0.096	0.167	0.103
CJ	0.072	0.107	0.167	0.116	0.051	0.027	0.110
DA	0.494	0.535	0.250	0.185	0.150	0.165	0.111
DB	0.341	0.441	0.241	0.188	0.127	0.147	0.111
DC	0.510	0.617	0.396	0.254	0.101	0.124	0.113
DD	0.401	0.352	0.393	0.175	0.065	0.054	0.069
DE	0.334	0.289	0.177	0.229	0.125	0.152	0.086
DF	0.442	0.490	0.281	0.281	0.082	0.070	0.103
DG	0.413	0.451	0.298	0.234	0.081	0.061	0.126
DH	0.574	0.554	0.261	0.237	0.075	0.154	0.105
DI	0.537	0.536	0.257	0.189	0.116	0.143	0.054
DJ	0.334	0.274	0.176	0.248	0.087	0.115	0.101

row and column labels. The table contains only 3 attributes: Passage, Replicate, and OD600 Reading denoting the passage number, the replicates labeled from "A" to "D", and the OD600 measurement, respectively.

Column 1: Passage

Column 2: Replicate

Column 3: OD600 Reading

Dataset Item 2 (Table). OD600 readings for each replicate taken at the fifth and seventh days after inoculation for stationary phase cell density estimation. The table is in long format where each row has only one measured data attribute. In this case, the measured data will be the OD600 readings. The other attributes are descriptors of the measured data attribute. This is opposed to the wide format whereby the descriptors for a particular OD600 reading are given as row and column labels. The table contains 4 attributes: Passage,

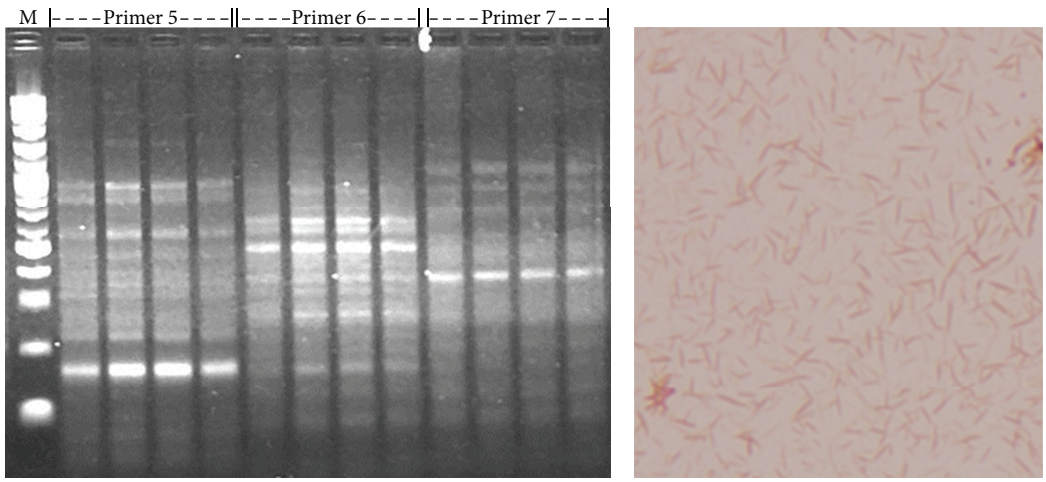

Figure 2: PCR-RFLP and Gram staining of Passage 72. (a) Genomic DNA extraction and PCR using Lee et al. [8] and 16-hour *TaqI* restriction endonuclease digestion showed similar profile in all 4 replicates. M is Log2 DNA marker. Three sets of PCR-RFLP were performed where the same primer (Primers 5, 6, and 7) was used as forward and reverse primer. (b) Gram staining showed predominately Gram-negative cultures. These suggest that the cultures were uncontaminated.

Replicate, Day, and OD600 Reading denoting the passage number, the replicates labeled from "A" to "D", whether the OD600 reading was performed on the fifth or seventh day after inoculation, and the OD600 measurement, respectively.

Column 1: Passage

Column 2: Replicate

Column 3: Day

Column 4: OD600 Reading

Dataset Item 3 (Table). OD600 readings taken at inoculation (time zero or zero minutes), 2 hours after inoculation, and at regular intervals after the second hour. This data can be used for generation time estimation. The table is in long format where each row has only one measured data attribute. In this case, the measured data will be the OD600 readings. The other attributes are descriptors of the measured data attribute. This is opposed to the wide format whereby the descriptors for a particular OD600 reading are given as row and column labels. The table contains 4 attributes: Passage, Replicate, Minutes, and OD600 Reading denoting the passage number, the replicates labeled from "A" to "D", the amount of time in minutes after inoculation when the OD600 readings were taken, and the OD600 measurement, respectively.

Column 1: Passage

Column 2: Replicate

Column 3: Minutes

Column 4: OD600 Reading

Dataset Item 4 (Table). OD600 readings from the MIC experiment of each replicate. The table is in long format where each row has only one measured data attribute. In this case, the measured data will be the OD600 readings. The other attributes are descriptors of the measured data attribute. This is opposed to the wide format whereby the descriptors for a particular OD600 reading are given as row and column labels. The table contains 4 attributes: Passage, Replicate, [NaCl], and OD600 Reading denoting the passage number, the replicates labeled from "A" to "D", the concentration of NaCl for MIC estimation, and the OD600 measurement, respectively (see Table 1 containing replicate data given in wide format for easy reference).

Column 1: Passage

Column 2: Replicate

Column 3: [NaCl]

Column 4: OD600 Reading

Dataset Item 5 (Table). OD600 readings from MIC experiment of each colony whereby 10 random colonies were selected for each replicate (total of 40 colonies were chosen) after single colony isolation from each of the 4 replicates. In this case, the measured data will be the OD600 readings. The other attributes are descriptors of the measured data attribute. This is opposed to the wide format whereby the descriptors for a particular OD600 reading are given as row and column labels. The table contains 5 attributes: Passage, Replicate, Colony, [NaCl], and OD600 Reading denoting the passage number, the replicates labeled from "A" to "D", the colony in 2 alphabets where the first alphabet denotes the replicate and the second alphabet denotes the colony, the concentration of NaCl for MIC estimation, and the OD600 measurement, respectively. For example, the colony labeled as "DA" represents colony "A" of replicate "D"

(see Tables 2, 3, and 4 containing colony MIC data given in wide format for easy reference).

Column 1: Passage

Column 2: Replicate

Column 3: Colony

Column 4: [NaCl]

Column 5: OD600 Reading

4. Concluding Remarks

Our MIC results showed an increase in OD600 readings from 11% (w/v) NaCl-supplemented nutrient media after Passage 60 (Figure 1) suggesting that *E. coli* had adapted to 11% (w/v) NaCl. This is further verified by MIC on randomly selected colonies at Passage 72 (Table 4). Thus, this study provides a set of data which may be used as benchmark for other adaptation studies. Gram staining and PCR-RFLP based on previous studies [7, 8] at Passage 72 (Figure 2) showed that the cultures are Gram negative and the PCR-RFLP profiles of all 4 replicates to be similar. This suggested that the cultures had not been contaminated.

Dataset Availability

The dataset associated with this Dataset Paper is dedicated to the public domain using the CC0 waiver and is available at http://dx.doi.org/10.7167/2013/219095/dataset.

Conflict of Interests

The authors declare that they have no conflict of interests.

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