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Phenotypic Characterization of Escherichia Coli Strains Taken from Human Intestinal and Urinary Tracts

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PHENOTYPIC CHARACTERIZATION OF *Escherichia coli* STRAINS

ISOLATED FROM HUMAN INTESTINAL & URINARY TRACTS.

A thesis submitted in the partial fulfillment

of the requirements for the degree of

Master of Science

By

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2006

Wright State University

WRIGHT STATE UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DATE _____

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY <u>Kruthi Murthy</u> **ENTITLED** <u>Phenotypic Characterization of *Escherichia coli* strains isolated from human intestinal and urinary tracts</u> **BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF** <u>Master of Science</u>.

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ABSTRACT

Murthy, Kruthi M.S., Program in Microbiology and Immunology, Wright State University, 2006. Phenotypic Characterization of *Escherichia coli* isolated from human intestinal and urinary tracts (Advisor: Dr. Oleg Paliy, Assistant Professor)

Escherichia coli (E. coli) is a gram negative bacterium commonly found as a commensal in the intestinal tract of humans and other warm blooded animals. The commensal strains of *E. coli* are non-pathogenic and do not cause an infection in the host. However, some strains of *E. coli* are pathogenic and can cause several diseases in humans that include neonatal meningitis, intestinal infections and urinary tract infections. Almost 80-85% of the uncomplicated urinary tract infections are caused by uropathogenic *E. coli.* Our project involved the characterization of 12 strains of *E. coli,* isolated from humans in health and disease. These strains were characterized since no studies had been conducted on them previously. The *E. coli* strains were isolated from patients with urinary tract infections and from the intestinal tracts of partners of these patients. Laboratory *E. coli* strain K12 served as a control. Phenotypic studies were carried out by studying the bacterial physiology in three different conditions. Motility tests were done to identify phenotypes that were similar to and different in behavior with respect to the wild type. Since antimicrobial resistance was a growing problem in urinary tract infections, susceptibility tests were done for these novel strains of *E. coli*, with the most commonly used antibiotics to treat these infections. Additionally phenotypic profiles were generated for the wild type strain as well as one uropathogenic strain using Phenotype Microarrays manufactured by Biolog., Inc which was a novel technique especially for uropathogenic strains. These plates were unique in helping us identify different phenotypes within a

limited time period. The results of our tests showed, some isolates whose phenotypic behavior differed, in terms of growth and motility when compared to the wild type. Antibiotic susceptibility tests enhanced our understanding of determinants of resistance which was crucial for the management of urinary tract infections. Phenotype micro array profiles generated for 2 strains, the wild type *E. coli* strain and one uropathogenic strain showed a total of 37 differences in carbon, nitrogen, sulfur and phosphorus metabolism in these strains. These differences indicated the likely genome variations between two strains, a fact that was shown for recently sequenced UTI strain CFT073. Therefore phenotypic characterization was useful in establishing genetic variability of the isolates and more specifically of *E. coli* strains causing urinary tract infections.

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INTRODUCTION

Escherichia coli:

Escherichia coli (*E. coli*) is a gram negative bacterium that commonly resides in the intestinal tracts of humans and other warm blooded animals and represents 1% or less of the microbial population of the gut (43). It is rod-shaped and belongs to the family *Enterobacteriaceae.* The bacteria shows specific biochemical and morphological features where they are oxidase negative, non-spore forming, possess ability to reduce nitrate to nitrite and grow rapidly in the presence or absence of oxygen. Most strains are motile and move by means of peritrichous flagella (43). The intestinal strains of *E. coli* that are not pathogenic, help in the synthesis of essential vitamins like Vitamin K and are essential for digestion. However some strains of *E. coli* are pathogenic and can cause significant morbidity and mortality in humans (1). The diseases caused by pathogenic strains of *E. coli* are classified into three main types. They are (1) enteric or intestinal infections (2) neonatal meningitis or neonatal sepsis (3) urinary tract infections. Physiological studies for *E. coli* have shown the ability of these organisms to adapt themselves to their characteristic habitats. The pathogenic strains have been further classified based on the types of antigen they possessed. The antigenic categories were of three main types such as (1) Somatic (O) (2) Capsular (K) and (3) Flagella (H). A total of 700 different types of pathogenic *E. coli* have been identified to date.

Background information:

Initial studies conducted on *E. coli* shows the following. These strains can synthesize all the essential components of the protoplasm from a single carbon and nitrogen source in the presence or absence of oxygen (1a). These strains can judiciously turn on and turn off genes and can sense changes in the environment and adjust the expression pattern of all of its genes. These gene modulations suggested the presence of highly organized networks that could be activated in presence of all nutrients or in response to deprivation of nutrients, or during threats to survival of these strains (1a). Having the basic idea about the behavior of the wild type strains of *E. coli*, we were interested to look at the physiology and behavioral responses of pathogenic strains of *E. coli.* In our project we characterized pathogenic strains of *E. coli* taken from urinary tract infections.

Urinary Tract Infections:

Urinary tract infections (UTI) have gained more attention in the past few years as they are seen in many people all over the world. These infections are more common in women than men and more common among sexually active females (10). Epidemiological studies have shown at least 11 % of women above 18 years of age having experienced at least one urinary tract infection with a 12 month period (10). More than 11.3 million women in the United States have at least one physician-diagnosed UTI that is treated with antibiotics (10). According to the data obtained in 1995 estimated costs of $1.6 billion have been lost for treating these infections that included treatment costs and other costs due to lost productivity (10). Every year urinary tract infections have accounted for 7 million doctoral visits, 1 million emergency departmental visits and 100,000

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hospitalizations (34). Urinary tract infections are broadly categorized into three main types based on the site of infection – bladder (cystitis), urethra (urethritis) and kidney (pyelonephritis). These infections may be asymptomatic or symptomatic with a wide range of symptoms and if not treated may lead to death. Recurrent infections have been reported that are caused by the same strain seen in the initial infection (10). These infections are caused by certain fungi and a wide range of bacteria that include *Escherichia coli, Klebsiella, Enterobacter* and *Proteus* species. However, 80-85% of uncomplicated urinary tract infections are caused by *E. coli* (11). The epidemiological studies suggested the wider clinical importance of uropathogenic *E. coli* as causes of urinary tract infections than previously identified.

Objective:

Our objective was to phenotypically characterize these novel uropathogenic *E. coli* strains as no studies had been done on these strains previously, and we wanted to establish the phenotypic variability of these strains. Additionally studies were also done for the non-pathogenic commensal strains to see behavioral similarities and differences, based on growth in three different kinds of conditions, motility and susceptibility to antibacterial drugs. We also generated phenotype profiles for the wildtype and one uropathogenic strain using a new technology called Phenotype Microarray technology and were able to show differences in metabolism of unique carbon, nitrogen, sulfur and phosphorus sources in the uropathogenic strain of *E. coli.*

The 12 human isolates were originally isolated at the Tang Health Center and obtained from Dr. Sidney Kustu from the University of California Berkeley. Our initial

experiments involved identification tests for the strains to confirm the strains as *E. coli*. For our further experiments the laboratory strain K12 served as control. The bacterial physiology for these strains were carried out by doing growth experiments on solid and in liquid media using three sets of conditions, that represented a wide range of variations for bacterial growth. We used Luria Bertani broth as this medium was proven to be an enriched medium and served as a best source for bacterial growth. We used minimal media for the next set of growth experiments where the medium was provided with a single source of carbon and nitrogen, where we checked for bacterial growth of the clinical isolates in such nutrient limited conditions. Glucose and ammonium chloride were added to the minimal media since glucose and ammonium chloride served as best carbon and nitrogen sources. For the next set of experiments glycerol and arginine were added to the minimal media as they were poor sources of carbon and nitrogen when compared to glucose and ammonium chloride. These carbon sources were selected as they provide a wide range of growth conditions. Growth was assessed by making growth curves for the strains and by comparing the doubling times for the strains in the above mentioned conditions. Since motility was a distinguishing feature for these strains, motility tests were done for all *E. coli* strains and we were able to identify phenotypes that were different in terms of them being non-motile unlike the wild type strain. The ability of the bacteria to develop resistance to antibiotics was a significant problem for treating urinary tract infections and antibiotic resistance was a growing problem in UTI (15). Ongoing surveillance of resistance trends and enhanced understanding of the determinants of resistance played an important role in the determination of mechanisms of resistance to antibiotics in these strains. We speculated the possibility of resistance

genes to be present on plasmids and hence all strains were tested for the presence of plasmids. Plasmids were isolated using a standard kit for plasmid isolation.

Phenotype Micro Arrays manufactured by Biolog, Inc., were used to generate phenotypic profiles for the wild type strain and one chosen pathogenic strain. Different plates were used to check the ability of the cells to use different sources of carbon (PM 1 & 2), Nitrogen (PM 3), Phosphorus and Sulfur (PM 4). The growth of the cells in each well for each plate was assessed by making growth curves for the strains using the BMG Labtech Floustar Microplate Reader. Phenotypic characterization of uropathogens was used to assess the differences in behavior of these strains with the non-pathogenic K12 strain.

MATERIALS & METHODS

1. **Bacterial Strains:** The bacterial strains were originally isolated at the Tang

Health Center and obtained from Dr. Sydney Kustu at (UC Berkeley). These strains

were isolated from human intestinal tracts and urinary tracts of patients with UTI at

(Tang health center). The isolates were identified by letters "OPE" followed by a

number for our identification purposes, where the first two letters refer to the initials

of the professor under whom this study was done and the letter "E" referred to the

initials of the strain that was studied. The strains used for study are shown in Table 1.

Paliy Lab	Type of Strain
OPE001	MG1655K12
OPE002	K12 (wild type)
OPE004	(UTI)
OPE005	(UTI)
OPE006	(UTI)
OPE007	(UTI)
OPE008	(commensal)
OPE009	(commensal)
OPE010	(commensal)
OPE011	(commensal)
OPE012	(commensal)
OPE013	(UTI)
OPE014	(commensal)
OPE015	(commensal)

Table 1: The bacterial strains used for study

2. **API 20 E Bacterial Identification Strip for Enterobacteriaceae:**

API 20 E Bacterial Identification kit for Enterobacteriaceae, manufactured by

Biomerieux was used to identify bacterial strains belonging to the family

Enteracteriaceae. We used this kit to confirm the identity of the 12 clinical isolates

and the wild type strain K12 as *E. coli.* 20 biochemical tests were provided on the test

strip and each test checked for the presence of enzymes or reactions that were specific to the bacteria tested. Identification was based on the ability of bacteria to catabolize the substrates provided in these tests (14). The results for these tests were interpreted by looking at the colors produced in small compartments called microtubes provided for each of the 20 biochemical tests placed on the strip. The color reactions produced by each strain were compared with 2 test strips provided in the API website that showed positive and negative test results for all 20 biochemical tests. The software provided by Biomereiuex was used to analyze the seven digit profile number that served to identify the strains to their respective genus and species.

A single colony of cells was taken from a freshly streaked LB agar plate and suspended in 5 ml of sterile saline solution. The test strip was then inoculated with the freshly prepared bacterial solution. For tests CIT, VP and GEL the bacterial suspension was added to both the tube (lower portion of the microtube) and the cupule (upper portion of the microtube). Anaerobic conditions were created for tests ADC, LDC, ODC, H_2S and URE by overlaying the bacterial suspension with mineral oil. For the other tests the tube was filled with the bacterial suspension only. The inoculated test strips were placed in the incubation box containing 5ml of water and incubated at 37ºC for 16-18 hrs. After incubation, 1 drop of TDA reagent, 1 drop of the James reagent (kovac's reagent) and 1 drop of reagents VP1 and VP2 were added to tubes for tests TDA, IND and VP. After incubation the colors in the microtubes where observed and recorded (15). The 20 tests provided on the strip are shown in Table 2.

#	Tests	Active Ingredients	QTY (mg/cup)	Reactions/Enzymes
1	ONPG	2-nitrophenyl-βD-galactopyranoside	0.223	β-galactosidase
2	ADH	L-arginine	1.9	Arginine DiHydrolase
3	LDC	L-lysine	1.9	Lysine DeCarboxylase
4	ODC	L-ornithine	1.9	Ornithine DeCarboxylase
5	CIT	trisodium citrate	0.756	Citrate Utilization
6	H2S	sodium thiosulfate	0.075	H_2S Production
7	URE	urea	0.76	Urease
8	TDA	L-trytophan	0.38	Tryptophan DeAminase
9	IND	Indole	0.19	Indole production
10	VP	sodium pyruvate	1.9	Acetoin Production
11	GEL	gelatin (bovine origin)	0.6	Gelatinase
12	GLU	D-glucose	1.9	fermentation/oxidation
13	MAN	D-mannitol	1.9	fermentation/oxidation
14	INO	inositol	1.9	fermentation/oxidation
15	SOR	D-sorbitol	1.9	fermentation/oxidation
16	RHA	L-rhamnose	1.9	fermentation/oxidation
17	SAC	D-sucrose	1.9	fermentation/oxidation
18	MEL	D-melibiose	1.9	fermentation/oxidation
19	AMY	amygdalin	0.57	fermentation/oxidation
20	ARA	L-arabinose	1.9	fermentation/oxidation

Table 2: The 20 Biochemical Tests Provided in API 20 E Test Strip.

3. Growth on Solid Agar (LB and minimal media):

The bacterial isolates were streaked on LB and minimal media agar ($N^-C^-(K)$) (1.5%) (containing potassium salts without any carbon and nitrogen sources). The minimal media were supplemented with a source of carbon and nitrogen in order to assess the growth of these strains in nutrient limited conditions. Two sets of conditions were chosen for minimal media where minimal media was supplemented with a 1) Good source of carbon and nitrogen and 2) Poor source of carbon and nitrogen. Glucose and ammonium chloride served as a good source of carbon and nitrogen and glycerol and arginine served as a poor source of carbon and nitrogen. The first set of N^-C^- agar plates were

supplemented with glucose (final concentration = 0.4%) and ammonium chloride (final concentration = 10 mM). The second set of $N^-C^-(K)$ agar plates were supplemented, glycerol (final concentration = 0.4%) and arginine (final concentration = 10 mM). The inoculated plates were kept for incubation at 37°C. The LB plates were incubated for 16-17 hours, N^-C^- (K) plates with glucose and ammonium chloride were incubated for a longer period of 40-48 hours. The plates with glycerol and arginine were incubated for 64-72 hours. The results recorded were based on observing the growth on the three different types of media where growth was assessed by comparing the morphology of the colonies on solid agar. The recipes for Luria Bertani broth and minimal media have been shown in the appendix sections.

3. Growth in Liquid Media:

LB

The 12 isolates were grown in separate tubes containing 10 ml of LB broth at 37°C. This was done by initially streaking the strains on LB agar and picking a single colony from each plate for each strain which was then inoculated into 10 ml of LB broth. The optical densities of the strains were measured at 600 nm using a spectrophotometer. The strains were allowed to grow in LB until they reached the mid log phase, where the optical density was between 0.5 and 1. The optical densities for the 12 strains were measured and suitable dilutions were made after which a small amount of bacterial suspension was transferred from the first set of tubes to the second set of LB tubes. This was done to maintain the same initial optical density for all the strains that corresponded to the same amount of cells for each strain. After doing suitable dilutions, the optical densities of the

12 strains were measured once every 30 minutes. The measured optical densities were used to construct growth curves for the strains in LB. The optical densities were measured until all the strains reached the stationary phase. The growth curves were produced for the 12 isolates were compared with that of the control strain and comparative studies were done by calculating the doubling times for these strains.

Growth in minimal media with glucose and ammonium chloride

Similar experiments were conducted for the 13 strains in minimal media. Initially the strains were allowed to grow in LB until they reached a mid log phase. The 13 strains (including control strain) in 13 separate tubes (in LB) were washed twice with phosphate buffered saline solution to remove traces of LB. This step was done when the strains were transferred from LB to minimal media in order to remove all traces of LB. The cell pellet obtained after washing were resuspended in minimal media that was used for further dilutions. The 13 strains were then transferred to 13 separate tubes containing 10 ml of minimal media in each tube supplemented with glucose (final concentration = 0.4%) and ammonium chloride (final concentration = 10mM). Dilutions were made accordingly so that the initial optical density values for all the tubes were identical or showed small differences ranging between (0.001-0.007) OD units that corresponded to approximately the same number of cells for each tube. The tubes were rotated continuously and incubated at 37°C and the optical density for the strains was measured after every 30 minutes and the time points were used to construct growth curves for each strain.

Growth in minimal media with glycerol and arginine

Similar to the previous experiment the cells were initially grown in LB broth, and transferred to minimal media but here the minimal media tubes contained glycerol (final concentration = 0.4%) and arginine (final concentration = 10mM) as sole sources of carbon and nitrogen. The tubes were kept at 37 degrees and the optical density was measured after every 60 minutes and used to construct growth curved for these strains.

The growth curves produced for the 12 isolates and the K12 strain in LB, minimal media with glucose and ammonium chloride and in minimal media with glycerol and arginine were assessed. Significant differences were identified by comparing the doubling times for the 13 strains.

The doubling time formula has been shown in the appendix section.

4. Motility Tests (LB and Minimal media with glycerol and ammonium chloride).

The motility tests were carried out on semisolid agar medium (0.4% agar). The sterilized media was poured into X section plates and allowed to cool and each section was used to inoculate one strain. A single colony was picked from a freshly streaked LB plate with a sterile toothpick or a pipette tip and the strains were inoculated in the semisolid agar medium. This was done for the 13 strains that included the 12 isolates and the wild type. Another *E. coli* strain MG1655 K12 was used as our negative control because we knew that the strain was non-motile.

Motility tests were done on LB, minimal media with glycerol and ammonium chloride and on minimal media with glycerol and arginine. After inoculation, the plates were placed in the incubator at 37ºC for 16 hrs (LB) and 18-48 hrs for minimal media.

After incubation the motility of the strains were observed and recorded based on a diffused growth that was seen from the line of inoculation, a process which was called swarming. The differences in motility were assessed by measuring the diameter in mm of the diffused growth around the stab of inoculation for each strain on the semisolid agar medium. The motility test results have been shown in the later sections.

5. Antibiotic Susceptibility Tests:

Susceptibility tests were carried out using BD BBL[TM] Sensi-Disc[TM] Antimicrobial Susceptibility Test Discs. The antibiotics that were used are shown in Table. 3.

Antibiotics	Concentration/per disk
Sulfisoxazole	0.25 mg
Ciprofloxacin	5 µg
Ceftriaxone	30 µg
Ampicillin	10 µg
Nitrofurantoin	300 µg
Trimethoprim	5 µg
Sulfisoxazole/Trimethoprim	23.75 µg/1.25 µg

Table 3: Most commonly used antibiotics to treat UTI with different concentrations per disc

The standard agar disc diffusion method was used for susceptibility testing of these strains. The antibiotics discs of Sulfisoxazole, Ciprofloxacin, Ceftriaxone, Ampicillin, Nitrofurantoin, Trimethoprim and Sulfisoxazole/Trimethoprim were used for the tests (16). Tests were conducted on Mueller Hinton Agar medium (19).

The 13 strains mentioned in Table 1 were initially grown in 13 separate tubes of LB broth until they reached a late log phase. The cells were spun down using a centrifuge at 1300 RPM for 3 minutes and washed twice with phosphate buffered saline to remove all traces of LB. Two 10 fold dilutions were performed for a total dilution of a 100 in order to obtain a turbid solution that corresponded to an optical density of 0.034. The tests were done on Mueller Hinton Agar. Two hundred µl of the prepared bacterial suspension (from the 100 fold dilution tubes) was added to the Mueller Hinton agar and swabbed evenly with a cotton swab or a sterile spreader to obtain uniform growth all over the plate. Sterile antibiotic impregnated discs were gently placed on the dried agar surface with sterile forceps. After placing the discs on the plates, these plates were incubated in the incubator at 37ºC. After 18-24 hours of incubation the results were observed and recorded by measuring the diameters of the zone of inhibition of the antibiotic.

6. Isolation of Plasmid DNA:

The 13 strains were tested for the presence of plasmids as we speculated the presence of antibiotic resistance genes on plasmids if present. Plasmids were isolated using QIAminiprep plasmid extraction kit (manufactured by Qiagen) following the protocol provided in the kit.

7. Characterization of the strains using Phenotypic Microarrays:

The phenotypic profiles for the strains were generated using BIOLOG Phenotype Microarrays (from Biolog, Inc, Hayward), that included PM plates (1-4) with 96 wells incorporating different carbon (PM 1-2), nitrogen (PM 3), Sulfur and Phosphorus (PM 4)

sources. BMG Labtech FLOUstar Microplate reader was used to measure the optical density to assess the growth of bacteria in these wells.

The phenotype microarrays made it possible to quantitatively assess many cellular phenotypes at once. The phenotype microarrays were 96 well plates with dehydrated substrates in each well. The phenotype microarrays made use of a principle of redox chemistry where the cell respiration over time was used to measure cell activity and growth. As the cells respired, the tetrazolium dye present in the well was reduced which further resulted in the formation of purple color. The color formation indicated cell respiration and the intensity of color produced was directly proportional to the rate of cell respiration.

Phenotype Micro Arrays manufactured by Biolog, Inc., were used to generate phenotypic profiles. OPE002 (K12) and OPE013 (UTI) were used for study. The strains were initially streaked on LB agar and 3-4 colonies were suspended in the inoculating fluid provided by Biolog. After an optical density of 0.034, was reached, 100µl of the bacterial suspension was added to each well. For PM plates 1 and 2 no carbon sources were added. For PM plates 3 and 4 an additional carbon source succinate was added before inoculating into the wells. After all the wells were inoculated the cells were incubated in BMG Labtech FLOUstar microplate reader for 24 hrs for PM plates 1 and 2 and 36-48 hours for PM plates 3 and 4 with continuous shaking. The optical densities of the cells were measured at 590 nm after every 15 minutes. Using these optical density values growth curves were constructed for the cells in each well. The cell respiration was assessed by looking at the color reactions in the wells. The growth curves produced in

each well for the wild type and the uropathogenic strains were studied to identify significant phenotypic differences between the uropathogenic strain and the wild type.

Calculation of Significant Differences between two Strains of *E. coli.*

Percentage Increase = [Ymax – Ymin] $_{002}$ – [Ymax- Ymin] $_{013}$

$$Z_{low} + 0.05$$

Ymax = maximum value on the growth curve.

Ymin = minimum value on the growth curve.

A= [Ymax – Ymin] $_{002}$ = Change in OD for OPE002

B= [Ymax – Ymin] $_{013}$ = Change in OD for OPE013

Z_{low} = Lower of the two values (A and B)

When initial calculations were done, Z_{low} showed values of zero. Therefore a small number such as 0.05 was added to Z_{low}. Only those values showing 100% or more difference ($\geq$ 1) on the growth curves were considered to be having a significant difference. The growth curves for the strains OPE002 and OPE013 on PM plate 1 is shown in the result sections. The growth curves for the strains on other PM plates and tables with actual numbers are shown in the appendix section.

RESULTS:

1. API Test Results:

The API® 20 E system (from Biomerieux, Inc.) uses a standard principle of assessing the enzymatic and metabolic activities that were specific for bacteria belonging to *Enterobacteriaceae.* Tests were done for *E. coli* K12, *Salmonella* and the 12 clinical isolates. We conducted tests for another species of gram negative bacteria such as *Salmonella* as we were using API test kits for the first time in our lab and wanted to assess the specificity of the test kit. Two test strips showing positive and negative test results for all 20 biochemical tests were used for reference (provided by API Web). The API test results for our strains were compared with the results seen on these two test strips. The colors produced on the API test strips for *E. coli* K12 and *Salmonella* were compared where specific differences could be identified. Differences were seen in 7 out of 20 biochemical tests between *E. coli* and *Salmonella*. *E. coli* was positive for tests ONPG, IND and MEL whereas *Salmonella* was negative. This indicated the presence of enzymes, which were present in *E. coli* and absent in *Salmonella* to metabolize these substrates. *Salmonella* was positive for tests ODC, CIT, H_2S and INO whereas *E. coli* was negative. These differences were significant on the API test strips which were observed as color differences in the microtubes seen after incubation and addition of reagents. Figures 1a and 1b show the positive and negative results for all 20 biochemical tests. Figure 1c shows the color reactions on the strip after inoculating with *Salmonella.* Figure 1d shows the colors on the strip after inoculating with *E. coli.* Figure 1e shows the colors produced on the strip for OPE006 (UTI strain).

Fig 1a: Strip showing positive reactions for all 20 biochemical tests

Fig 1b: Strip showing negative reactions for all 20 biochemical tests

Fig 1c: Strip showing color reactions for *Salmonella*
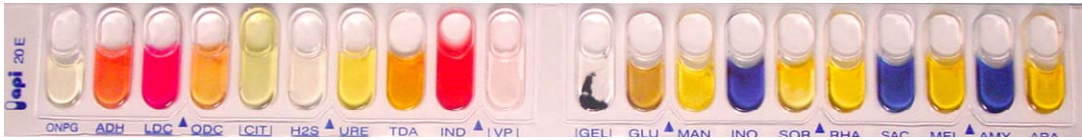
Fig 1d: Strip showing color reactions for *E. coli* K12

Fig 1e: Strip showing color reactions for OPE006 (UTI)

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The API test results showed that all 12 isolates tested were *E. coli*. The seven digit profile number was used to confirm the identity of the 12 isolates as *E. coli*. The application software provided by API Biomerieux showed the percentage similarity of the strains to *E.coli* and *Salmonella*. The percentage similarities for the 12 isolates are shown in the Table 4.

Strains	Type of Strain	% identity
OPS001	*Salmonella. Sp*	99.9
OPE002	*E. coli* K12	99.6
OPE004	(UTI)	96.3
OPE005	(UTI)	92.7
OPE006	(UTI)	99.8
OPE007	(UTI)	99.7
OPE008	(commensal)	97.9
OPE009	(commensal)	99.8
OPE010	(commensal)	99.8
OPE011	(commensal)	99.6
OPE012	(commensal)	99.8
OPE013	(UTI)	99.8
OPE014	(commensal)	97.9
OPE015	(commensal)	99.8

Table 4: Percentage identity of the strains tested

2. Growth on Solid Agar:

The 13 strains of *Escherichia coli* were streaked on LB agar plates and growth was seen for all 13 strains on LB. Eleven out of 13 strains had growth on minimal media with glucose and ammonium chloride and on minimal media with glycerol and arginine. Two strains OPE005 and OPE014 out of a total of 13 strains showed no growth on minimal media. The minimal media plates with glucose and ammonium chloride and plates with glycerol and arginine are shown in figures 2a and 2b. These plates have been streaked with two strains OPE004 and OPE005. OPE004 shows growth that can be seen on the right portion of the plate. No growth is seen on the left portion of the plate which is streaked with OPE005. Similarly OPE014 showed no growth on minimal media plates. These results suggested the inability of the strains to survive in nutrient limited conditions.

Fig 2a: OPE005/OPE004 on minimal media with glucose and ammonium chloride
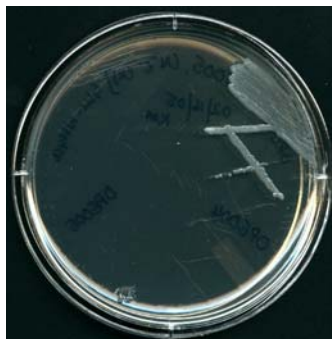
Fig 2b: OPE005/OPE004 on minimal media with glycerol and arginine

3. Growth in Liquid Media:

All 13 strains showed growth in liquid LB. Growth was assessed by measuring the optical density of the cells every 30 minutes at an absorbance of 600 nm. All the isolates reached the stationary phase at approximately 220 minutes. The 12 isolates grew similarly to the wild type strain which could be identified by looking at the growth curves. The K12 strain had a doubling time of 25 minutes in LB whereas the other strains showed a doubling time between 22 and 25 minutes which suggested no significant differences in terms of growth between the isolates and the wild type strain in liquid LB which further suggested that all strains were capable of growth in liquid LB.
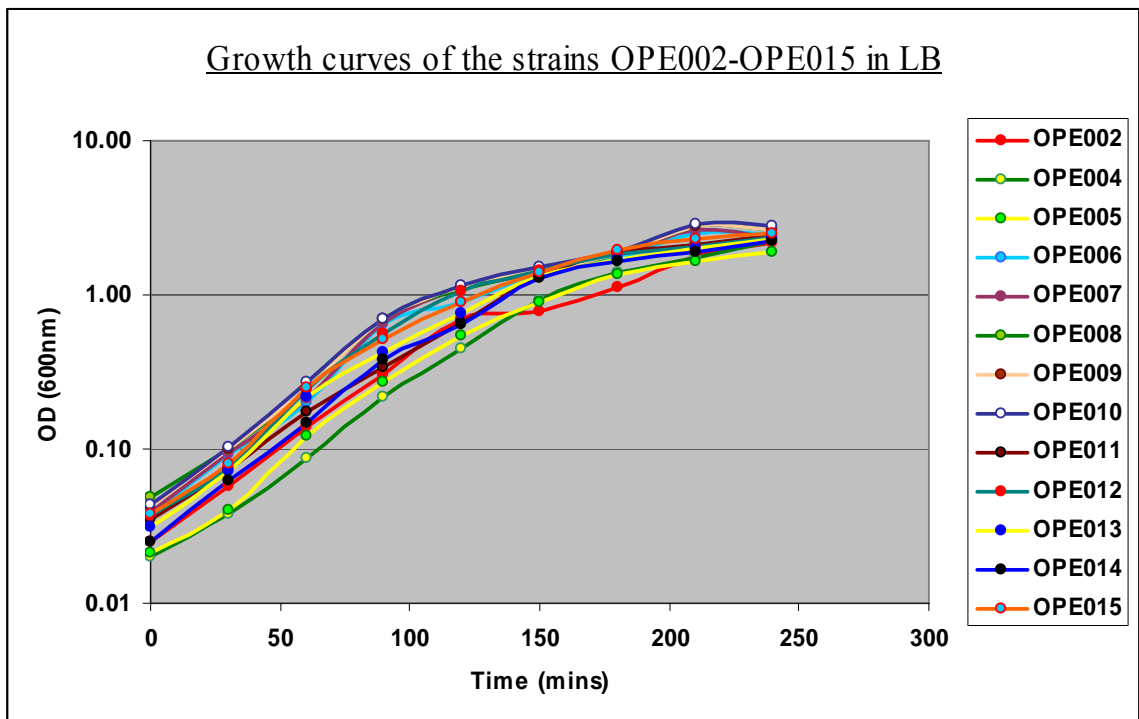
Fig 3: Growth curves obtained for the strains OPE002, OPE003, OPE004, OPE005, OPE006, OPE007, OPE008, OPE009, OPE010, OPE011, OPE012, OPE013, OPE014 and OPE015 on LB.

Eleven out of 13 strains showed growth in minimal media with glucose and ammonium chloride. OPE002 had a doubling time of 54 minutes, whereas the other strains had a doubling time ranging between 56-61 minutes. 2 strains OPE005 and OPE014 showed no growth (Fig. 4). No growth of these two strains in minimal media suggested the possible requirement of additional factors for growth. The growth curves for the strains in minimal media with glucose and ammonium chloride are shown in Fig. 4.
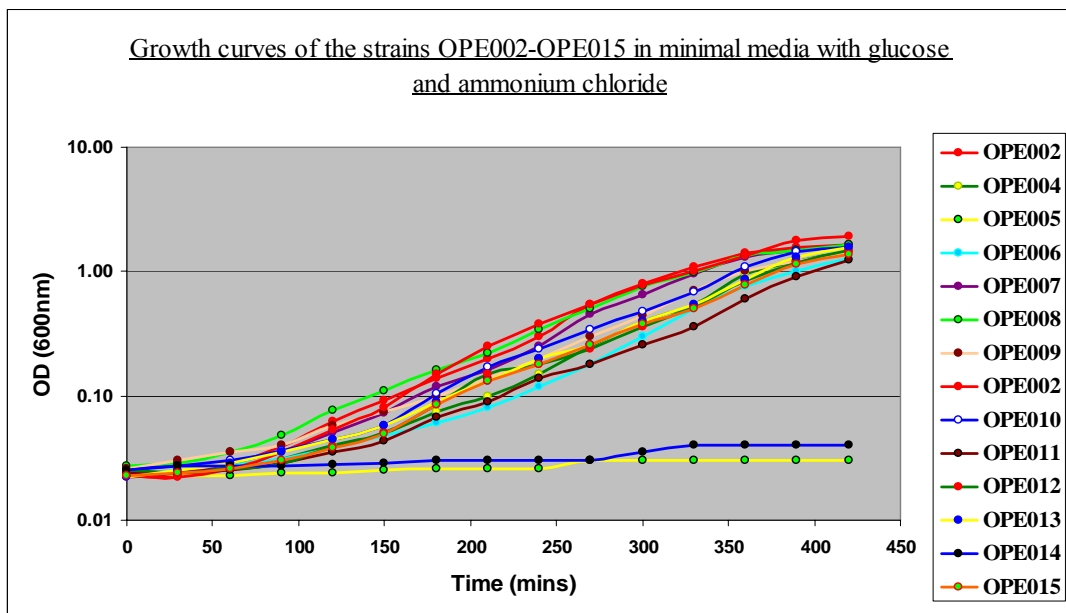
Fig 4: Growth curves for the strains OPE002, OPE004, OPE005, OPE006, OPE007, OPE008, OPE009, OPE010, OPE011, OPE012, OPE013, OPE014 and OPE015 on minimal media with glucose and ammonium chloride.

Similar growth experiments were conducted for all 13 strains in minimal media with glycerol and arginine. Two out of 13 strains showed no growth. These results also suggested that the 2 strains required additional factors for growth. The growth curves for the strains in minimal media with glycerol and arginine are shown in Figure. 5.
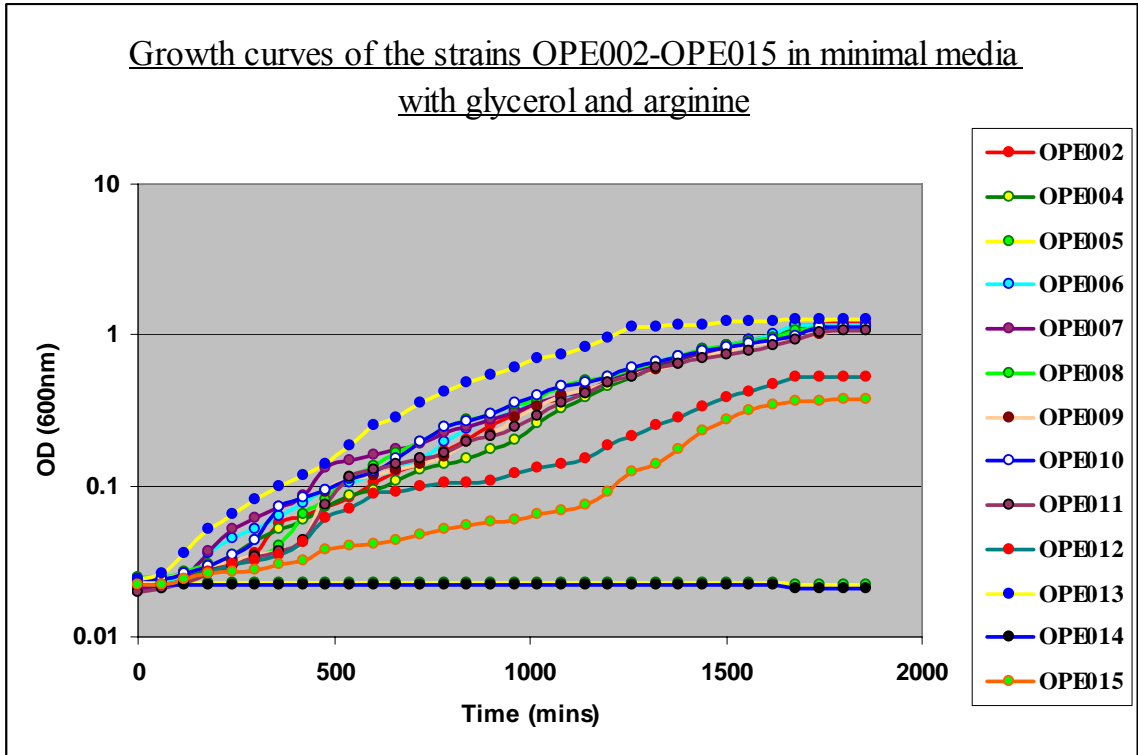
**Fig 5: The growth curves for the strains OPE002, OPE004, OPE005, OPE006, OPE007,OPE008, OPE009, OPE010, OPE011, OPE012, OPE013, OPE014 and OPE015 on minimal media with glycerol and arginine**

The doubling times for the strains in LB and the two kinds of minimal media have been shown in Table 5. The doubling times for the strains have been shown in minutes.

Strains	Type of Strain	LB (mins)	N -C-Glu+NH4Cl (mins)	N-C-Gly+Arg (mins)
OPE002	K12 (wild type)	26 ± 1.13	57 ± 5.32	267 ± 14.05
OPE004	(UTI)	24 ± 1.50	62 ± 2.40	258 ± 21.28
OPE005	(UTI)	25 ± 0.60	0	
OPE006	(UTI)	23 ± 1.50	65 ± 1.44	260 ± 23.07
OPE007	(UTI)	22 ± 0.25	59 ± 1.56	283 ± 22.07
OPE008	(commensal)	24 ± 0.35	61± 0.17	245 ± 43.50
OPE009	(commensal)	23 ± 0.29	65 ± 0.46	262 ± 36.37
OPE010	(commensal)	23 ± 0.29	56 ± 4.91	270 ± 53.13
OPE011	(commensal)	25 ± 2.08	61 ± 5.22	251± 43.14
OPE012	(commensal)	23 ± 0.40	57 ± 3.70	243 ± 31.19
OPE013	(UTI)	24 ± 0.92	52 ± 3.16	227 ± 41.40
OPE014	(commensal)	24 ± 0.92	0	0
OPE015	(commensal)	24 ± 0.92	59 ± 3.52	324 ± 5.51

Table 5: Mean doubling times for all strains ± SD in LB, minimal media with glucose and ammonium chloride and in minimal media with glycerol and arginine.
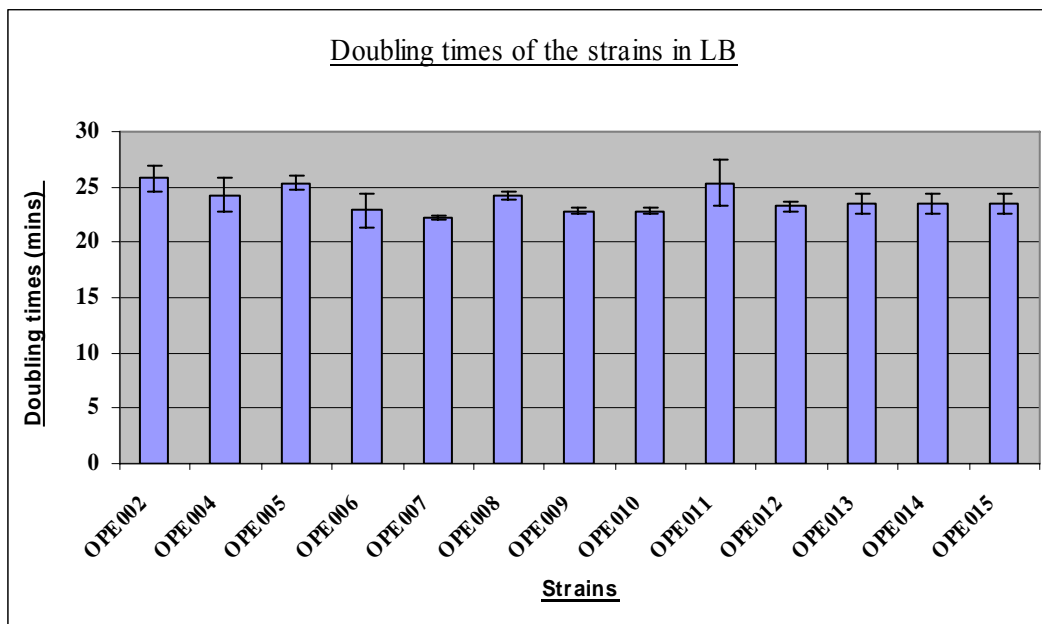
Fig 3a: Graph showing the mean doubling time values ± standard deviation for all strains in LB
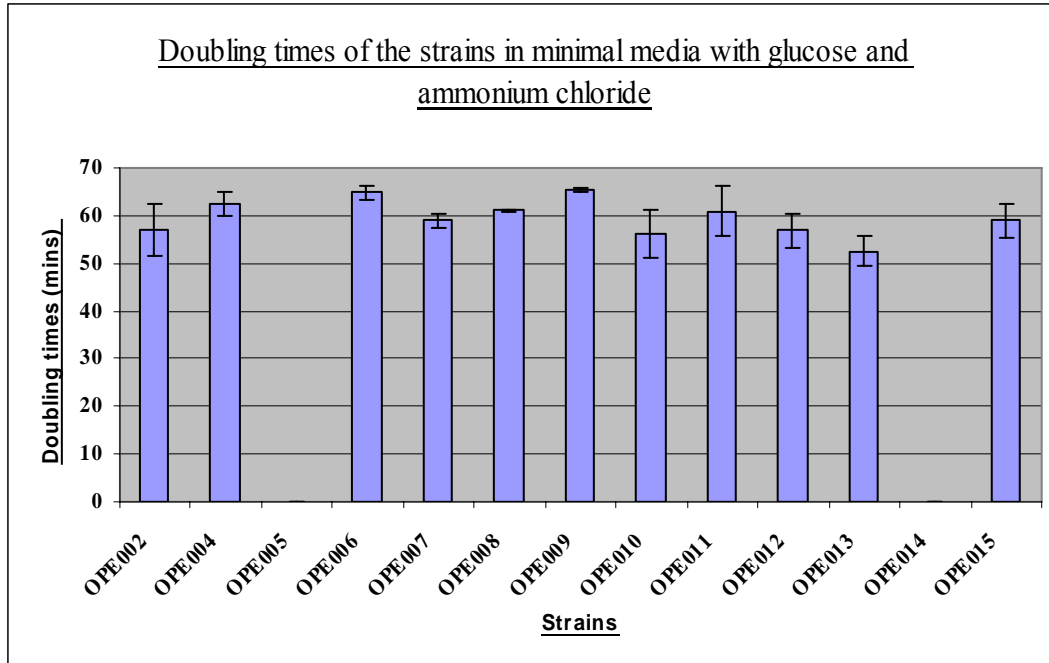
Fig 4a: Graph showing mean doubling time values ± standard deviation for all strains in minimal media with glucose and ammonium chloride
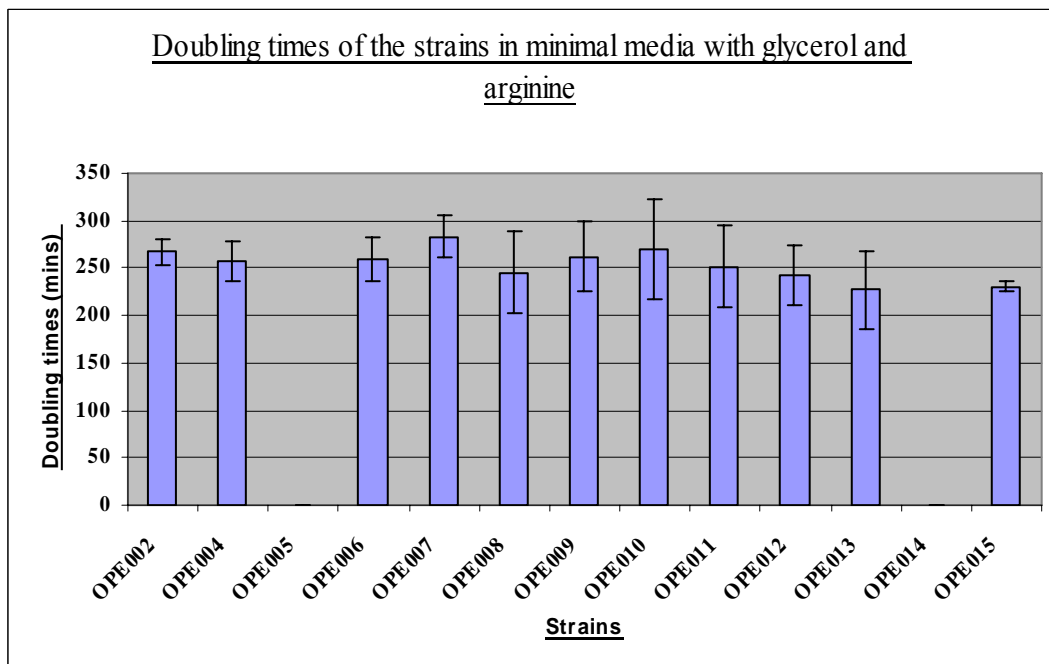
Fig 5a: Graph showing the mean doubling time values for all strains in minimal media with glycerol and arginine

Since OPE005 and OPE014 showed no growth in minimal media containing glucose and ammonium chloride and in minimal media with glycerol and arginine we needed to add certain additional growth factors to the medium. Previous data on our isolates confirmed that, some isolates failed to show growth in minimal media alone and that it could grow on addition of B vitamins as an additional nutrient. Therefore B vitamins were added to the minimal media to enhance the growth of these strains. B vitamins could normally be synthesized by *E. coli* strains in nutrient limited conditions. OPE014 showed growth on addition of vitamin mixture containing seven vitamins that included Folic Acid, Biotin, Nicotinamide, Pantothenic Acid, Pyridoxine, Riboflavin, Thiamine and Cyanacobalamine to minimal media with glucose and ammonium chloride. We tested the growth of OPE014 with 7 individual vitamin stock solutions in order to identify the specific vitamin that was responsible for growth. OPE014 showed growth in presence of the vitamin nicotinamide.

Similar growth experiments were conducted for OPE014 by changing the minimal media and supplementing the minimal media with glycerol and arginine to which the vitamins were added. No growth was seen for OPE014 in minimal media with glycerol and arginine in the presence of vitamins. The carbon and nitrogen sources added to the media were switched, where the media was supplemented with glycerol and ammonium chloride and a second media that was supplemented with glucose and arginine. OPE014 showed growth in minimal media supplemented with glycerol and ammonium chloride with vitamins. The growth curves for OPE014 in minimal media with vitamins are shown in Figs. 6 and 7. OPE005 showed no growth in minimal media with glucose and ammonium chloride and in minimal media with glycerol and arginine. No growth was

seen for the strain on addition of vitamins to the media. The growth curves for the strain OPE005 is shown in Fig. 6.
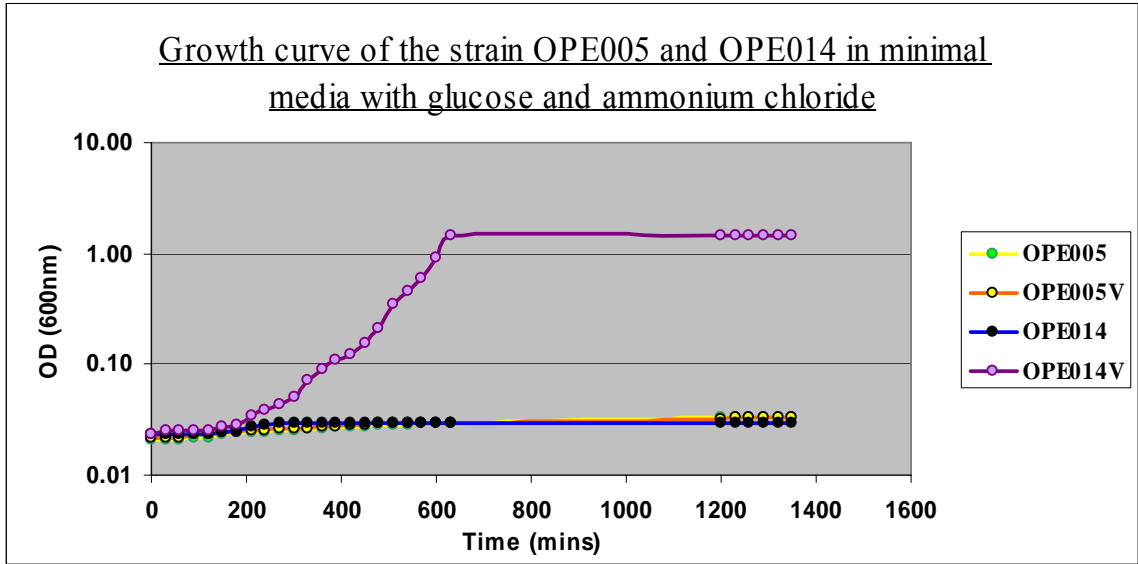
Fig 6: Growth curves of the strains OPE005 and OPE014 in minimal media with glucose and ammonium chloride and in minimal media with glucose and ammonium chloride supplemented with vitamins.
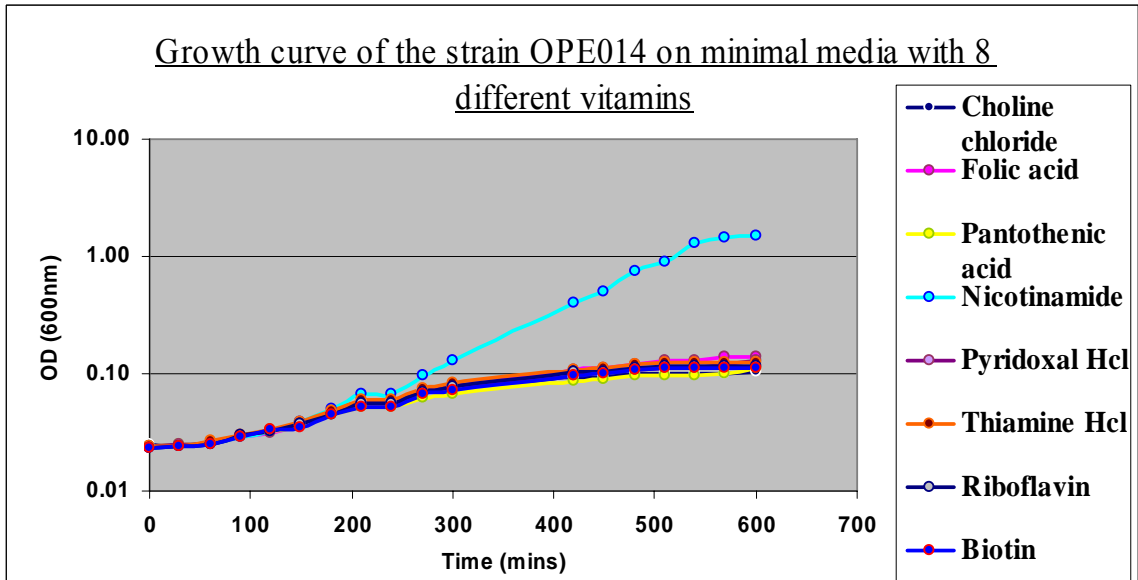
Fig 7: Individual growth curves for OPE014 on minimal media (glucose and ammonium chloride) supplemented with 7 different vitamins.

Similarly growth experiments were conducted for OPE014 in minimal media supplemented with glycerol and arginine as the carbon and nitrogen sources to which vitamins were added and no growth was seen. The media conditions were changed where Glucose and arginine and Glycerol and ammonium chloride were used. Growth was seen in the media containing glycerol and ammonium chloride and no growth was seen in the media containing arginine. These results showed a possible defect in the arginine utilization pathway for this particular strain of *E. coli.*

4. Motility tests:

Motility tests were carried out for all 13 strains of *E. coli*. An additional strain (OPE001) MG1655K12 was used as a negative control as we knew from literature studies that this strain of *E. coli* was non-motile. Motile strains showed swarming growth around the point of inoculation. The strains with diameters between 2 and 5mm were considered to be non motile as growth was seen confined to the stab of inoculation. Strains with diameters between 5 and 15mm were considered to be moderately motile and those that had diameters of more than 15mm were considered to be highly motile strains. The motility tests were conducted on semisolid agar medium having 0.4% agar. Strains that were non motile on 0.4% agar were again inoculated in 0.2% agar because, decreasing the concentration of agar increased the pore size in the agar which enabled better movement for the strains in agar. The strains were then observed for motility. These tests were conducted on LB, minimal media with glycerol and ammonium chloride and on minimal media with glycerol and arginine. The diameters of dispersion of the strains on LB are shown in Table 6. On LB agar 8 out of 13 strains were moderately motile having diameters ranging between 7 and 12mm. 5 strains were highly motile with diameters ranging between 19 and 34mm. The motility test results on LB have been shown in Table 6. The test diameters shown represent the averages of the diameters obtained for the strains on LB.

Strains	LB (0.4%) (24 hrs)
OPE002	7mm (moderate)
OPE004	7mm (moderate)
OPE005	19mm (high)
OPE006	20mm (high)
OPE007	33mm (high)
OPE008	34mm (high)
OPE009	8mm (moderate)
OPE010	11mm (moderate)
OPE011	8mm (moderate)
OPE012	9mm (moderate)
OPE013	20mm (moderate)
OPE014	9mm (moderate)
OPE015	7mm (moderate)

Table 6: Motility test results showing the diameters (in mm) of the strains in LB.

On minimal media with glycerol and ammonium chloride, 6 strains were non motile with diameters ranging between 2mm and 3mm where growth was confined to the stab point. OPE010 was moderately motile with a diameter of 10mm. Four strains were highly motile with diameters ranging between 24 and 40 mm. The 6 strains with low motility on 0.4% agar were inoculated in 0.2% agar to further establish their motility. OPE002 showed a diameter of 3mm in 0.4% agar but had a diameter of 40mm in 0.2% agar. OPE004 and OPE009 were considered to be moderately motile in 0.2% agar and showed a diameter of 15 and 10mm. OPE015 showed a similar behavior like the wild type with a diameter of 40mm. However, two strains OPE011 and OPE012 had low motility with a diameter of 3mm. OPE005 and OPE014 did not show growth in semisolid agar since these strains are unable to grow in these media. The motility test results on glycerol and ammonium chloride are shown in table 7. The test diameters shown represent the

averages of the diameters obtained on minimal media with glycerol and ammonium chloride.

Strains	MM-Gly-Amm (0.4%) (48hrs)	MM-Gly-Amm (0.2%) (48 hrs)
OPE001	Not done	No growth
OPE002	2mm (non-motile)	40mm (highly motile)
OPE004	3mm (non motile)	15mm (moderately motile)
OPE006	30mm (highly motile)	Not done
OPE007	40mm (highly motile)	Not done
OPE008	33mm (highly motile)	Not done
OPE009	2mm (non-motile)	10mm (moderately motile)
OPE010	10mm (moderately motile)	Not done
OPE011	2mm (non-motile)	3mm (non- motile)
OPE012	2mm (non-motile)	3mm (non-motile)
OPE013	24mm (highly motile)	Not done
OPE015	4mm (moderately motile)	40mm (highly motile)

Table 7: Motility test results showing the diameters (in mm) for the strains in minimal media with glycerol and ammonium chloride.

Similar experiments were done for strains in minimal media with glycerol and arginine. The six strains similar to the previous experiment showed low motility with diameters between 2mm and 3mm. OPE010 was considered to be moderately motile with a diameter of 7mm. 4 strains showed highly motility with diameters between 20 and 28mm. OPE005 and OPE014 failed to show growth. OPE004, OPE011, OPE012 and OPE015 were lowly motile having a diameter between 2mm and 4mm. OPE002 and OPE009 were moderately motile with diameters of 8mm and 10mm. The motility test results on minimal media with glycerol and arginine are shown in Table 8. The test diameters shown represent the averages of the diameters obtained on minimal media with glycerol and arginine.

Strains	MM-Gly-Arg (0.4%) (72 hrs)	MM-Gly-Arg (0.2%) (72 hrs)
OPE001	Not done	No Growth
OPE002	3mm (non-motile)	14mm (moderately motile)
OPE004	3mm (non-motile)	4mm (non motile)
OPE006	30mm (highly motile)	-
OPE007	28mm (highly motile)	-
OPE008	32mm (highly motile)	-
OPE009	3mm (non-motile)	10mm (moderately motile)
OPE010	7mm (moderately motile)	-
OPE011	3mm (non-motile)	2mm (non-motile)
OPE012	3mm (non-motile)	2mm (non-motile)
OPE013	20mm (highly motile)	-
OPE015	3mm (non-motile)	2mm (non-motile)

Table 8: Motility test results showing the diameters (in mm) for the strains in minimal media with glycerol and arginine.

The motility of few strains are shown in the figures below. Figure 8a shows motility test results for strains OPE002, OPE007, OPE008 and OPE009. OPE007 and OPE009 were found to be motile on LB and minimal media supplemented with sole sources of carbon and nitrogen.
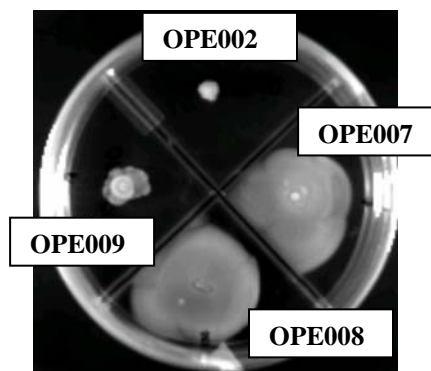
Fig 8a: OPE002, OPE007, OPE008 and OPE009 on LB

From the above picture very low motility or almost no motility was seen for strains OPE002 and OPE009 in minimal media supplemented with glycerol and ammonium chloride and minimal media supplemented with glycerol and arginine. Therefore OPE002 was again inoculated in minimal media with glycerol and ammonium chloride, containing 0.2% agar. OPE002 showed a larger diameter of about 40 mm that suggested the motility of this strain. These results also showed that the K12 strain required a larger pore size in the media to move. MG1655 K12 was also inoculated with the K12 strain as MG1655 served as a negative control. The results are shown in Fig 8b.
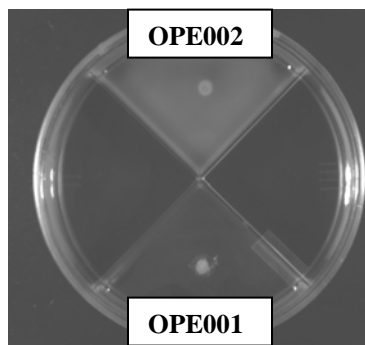
Fig 8b: OPE002 and OPE001 on minimal media with glycerol and NH4Cl (0.2%agar). OPE002 is motile, whereas OPE001 is non-motile.

OPE002 (K12) was showed low motility when inoculated in 0.4% agar but showed high motility when inoculated in a lesser concentrated agar medium with 0.2% agar. This indicated that the wild type strain required a lesser concentrated agar medium to show motility.

OPE009 that showed a diameter of 3 mm on minimal media (with 0.4% agar) had a larger diameter in the 2 kinds of minimal media. The motility of OPE009 on minimal media (with0.2% agar) is shown in figure 8c.
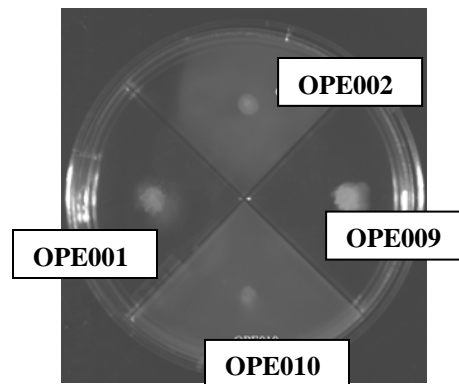
Fig. 8c: OPE002, OPE009, OPE010, OPE001 on minimal media with glycerol and NH4Cl (0.2% agar)

5. Antibiotic Susceptibility Tests:

Susceptibility tests were conducted for the 13 strains of *E. coli* with antibiotics to assess the prevalence of antibiotic resistance. Each isolate was tested with seven antibiotics that were most commonly used to treat UTI. The zones of inhibition of bacterial growth around the antibiotic disc were measured to check the susceptibility of the strains to the antibiotics. The sizes of the zones of inhibition were directly proportional to the rate of diffusion of the antibiotic, sensitivity of the bacterium and the growth rate of the bacterium. The zones of inhibition indicated the sensitivity of the organisms to the antibiotics. The strains with inhibition zones greater than 20 mm were considered to be susceptible, those between 10 and 20 were considered to be intermediately susceptible and inhibition zones that were less than 10 mm were considered to be having low susceptibility to the antibiotic. Strains with no zones of inhibition around the antibiotic disc were considered to be resistant.

The susceptibility of the strains to the different antibiotics are shown in Table 9.

Strains	AMP	CEF	NIT	CIP	TRI	SULF	SULM
OPE002	I	S	I	S	I	I	I
OPE004	I	S	I	S	**R**	**R**	**R**
OPE005	**R**	**R**	I	S	**R**	**R**	**R**
OPE006	I	I	I	S	I	I	I
OPE007	I	S	I	S	I	I	S
OPE008	**R**	I	I	S	I	I	I
OPE009	S	S	I	S	S	I	S
OPE010	**R**	S	I	S	**R**	**R**	**R**
OPE011	I	S	I	S	I	**R**	I
OPE012	I	S	I	S	S	I	S
OPE013	I	S	I	S	I	I	I
OPE014	I	S	I	S	S	**R**	S
OPE015	I	S	I	S	**R**	I	**R**

Table 9: Shows the antibiotic susceptibility test results, (S) – susceptible, (I) – intermediately

susceptible, (R) - resistant

Fig 9a: The figure shows the zones of inhibition of the strain OPE009 to antibiotics Sufisoxazole.

Ampicillin, Ciprofloxacin and Trimethoprim

6. Plasmid Isolation:

Since Uropathogenic *Escherichia coli* strains were most commonly treated with antibiotics, these strains had the potential of acquiring resistance to these antibiotics as mentioned before. It was important for us to understand the genetic mechanisms prevalent in these micro organisms that were responsible for the acquisition and spread of antibiotic resistance which could further facilitate the development of effective prevention and control strategies. Also the acquisition of mobile genetic elements encoding multiple antibiotic resistant genes was understood to be the main mechanism for short term accumulation of resistance determinants in bacterial genomes (38). Many plasmids contained genes that enabled the bacteria to survive and prosper in certain environments. For example, some plasmids contained one or more genes that conferred resistance to a particular antibiotic. We could only speculate that resistant genes for these antibiotics were possibly found on plasmids if present. Bacteria containing such a plasmid could live and survive in the presence of the antibiotic. Therefore experiments were performed to check for the presence of plasmids in these strains with a possibility of these plasmids having resistant genes. Figs. 10a and b display the gel pictures of plasmids.
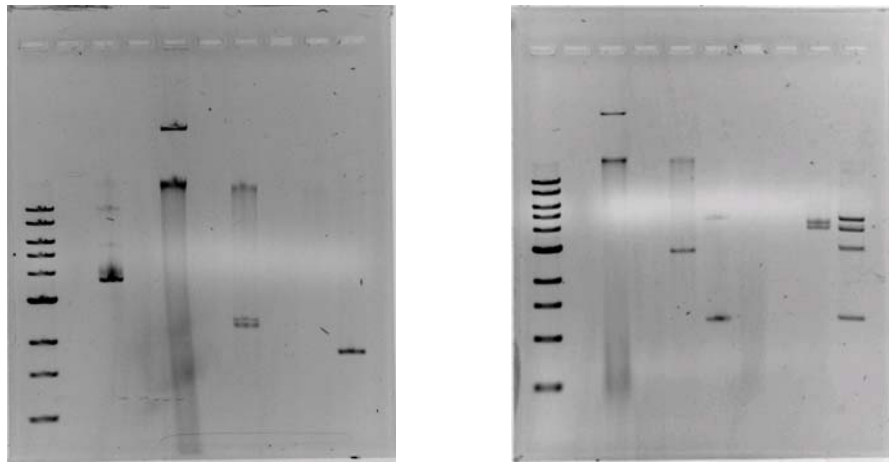
Fig. 10a: First figure: well 1 = 1 Kb ladder, well 3 = + control containing the plasmid, well 5 =

OPE002, well 6 = OPE004, well 7 = OPE005, well 8 = OPE006, well 9 = OPE007, well 10 = OPE008.

Fig 10b: Second figure: well 1 = 1 Kb ladder, well 3 = OPE002, well 4 = OPE009, well 5 = OPE010,

well 6 = OPE011, well 7 = OPE012, well 8 = OPE013, well 9 = OPE014, well 10 = OPE015.

We used a strain which contained the plasmid as the positive control as tests were initially conducted to identify the presence of plasmids in the isolates. The 1 Kb ladder was used for plasmid size estimation. It was observed that the strains OPE002, OPE005, OPE008, OPE010, OPE011, OPE014 and OPE015 contained at least one plasmid.

7. Phenotypic profiles generated for *Escherichia coli*:

High throughput assays were done using 96 well plates to analyze different phenotypes. A new technology called phenotype microarray technology was used to analyze live cells and assess the phenotypic characteristics of the strains that were tested. The different phenotypes were assessed using cell respiration and growth as the reporting system. The phenotype microarray plates we used contained unique sources of carbon, nitrogen, sulfur and phosphorus. These wells were also provided with a tetrazolium purple dye that helped us assess the growth of bacteria in these wells colorimetrically. The phenotypic microarray plates were incubated for 30-48 hours after inoculating the plates with the bacterial suspension. Cell respiration and growth over time resulted in the production of metabolites that reduced the tetrazolium dye leading to appearance of color. The amplified signal corresponded to increased respiration and growth of cells. The color changes were easily monitored, easily quantitated and highly sensitive. Since we were interested in identifying cell respiration and the actual growth of bacteria in these wells we used Floustar Optima Microplate reader which was useful in measuring the optical density of the cells in each well at 595nm.

A lengthy process of substrate transport into the cell, its catabolism, reformation, production of certain molecules and their polymerization and the formation of sub cellular components had to occur in order for cells to grow with a continuous flow of electrons from the provided substrate. These electrons finally acted upon the tetrazolium dye and changed the dye from colorless to a purple color. If no growth or respiration occurred in cells the purple color was not seen (2). The process of cell respiration is shown in Fig. 11 (2).
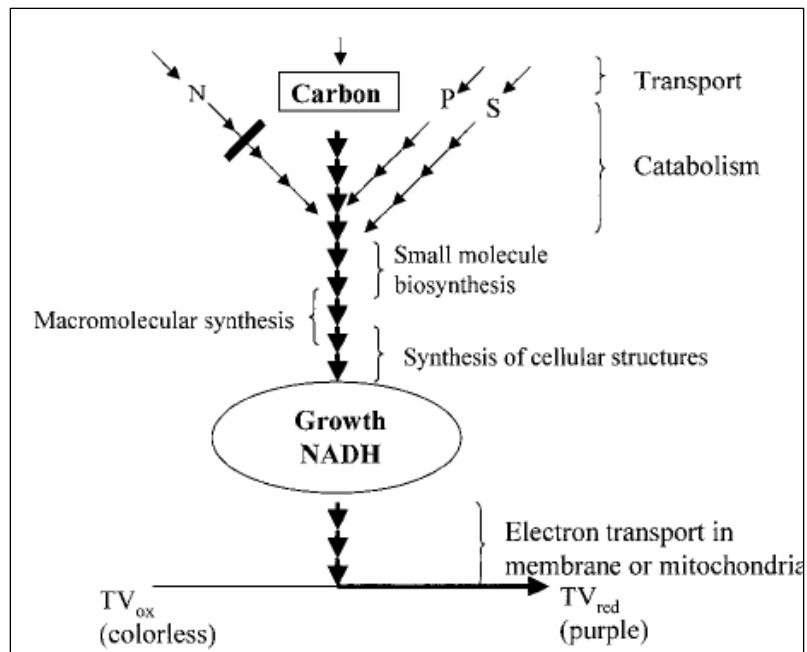
Fig. 11: Respiration Pathway coupled to cell physiology (3)

We were interested in looking at the phenotypic characteristics of the uropathogenic strain OPE013. Results from previous experiments showed faster growth and motility of OPE013 when compared to *E. coli K12* strain. Since phenotype micro array profiles were not previously generated for uropathogenic *E. coli* using 96 well plates we were interested to characterize these strains phenotypically. Phenotypic profiles of the wild type strain and uropathogenic strain were compared. Growth curves obtained for the two strains on micro plates PM 1-4 were compared and significant differences were identified. A total of these differences from 4 plates were recorded and the results were used to assess the information encoded in the genome of the strains tested (2). The color reactions obtained for PM 1 plate are shown in Figs 12a and 12b.

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Fig. 12a and 12b show the color reactions obtained on PM 1 for OPE002 (K12) and OPE013 (UTI). The Dark purple colors obtained on the plates shows optimum respiration of cells, the weak purple colors indicate the weak respiration of cells and cells that do not respire show no color in the wells. The strong color shows good growth indicating the optimum utilization of substrates provided in the wells.

The growth curves for the strains OPE002 and OPE013 are shown in figure 13 shown below.

Fig. 13. PM1 Microplate

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |

A1- Negative Control	**C1**- Glucose-6-Phosphate	**E1**- L-Glutamine	**G1**- Glycyl-L-Glutamic Acid
A2- L-Arabinose	**C2**- D-Galactonic Acid-γ-Lactone	**E2**- M-Tartaric Acid	**G2**- Tricarballylic Acid
A3- N-Acetyl-D-Glucosamine	**C3**- D,L-Malic Acid	**E3**- Glucose-1-Phosphate	**G3**- L-Serine
A4- D-Saccharic Acid	**C4**- D-Ribose	**E4**- Fructose-6-Phosphate	**G4**- L-Threonine
A5- Succinic Acid	**C5**- Tween 20	**E5**- Tween 80	**G5**- L-Alanine
A6- D- Galactose	**C6**- L-Rhamnose	**E6**- α-Hydroxy Glutaric Acid-γ-Lactone	**G6**- L-Alanyl-Glycine
A7- L-Aspartic Acid	**C7**- D-Fructose	**E7**- α-Hydroxy Butyric Acid	**G7**- Acetoacetic Acid
A8- L-Proline	**C8**- Acetic Acid	**E8**- β-Methyl-D-Glucoside	**G8**- N-Acetyl-β-D-Mannosamine
A9- D-Alanine	**C9**- α-D-Glucose	**E9**- Adonitol	**G9**- Mono Methyl Succinate
A10- D-Trehalose	**C10**- Maltose	**E10**- Maltotriose	**G10**- Methyl Pyruvate
A11- D-Mannose	**C11**- D-Melibiose	**E11**- 2-Deoxy-Adenosine	**G11**- D-Malic Acid
A12- Dulcitol	**C12**- Thymidine	**E12**- Adenosine	**G12**- L-Malic Acid
B1- D-Serine	**D1**- L-Asparagine	**F1**- Glycyl-L-Aspartic Acid	**H1**- Glycyl-L-Proline
B2- D-Sorbitol	**D2**- D-Aspartic Acid	**F2**- Citric Acid	**H2**- P-Hydoxy Phenyl Acetic Acid
B3- Glycerol	**D3**- D-Glucosaminic Acid	**F3**- M-Inositol	**H3**- M-Hydroxy Phenyl Acetic Acid
B4- L-Fucose	**D4**- 1,2-Propanediol	**F4**- D-Threonine	**H4**- Tyramine
B5- D-Glucuronic Acid	**D5**- Tween 40	**F5**- Fumaric Acid	**H5**- D-Psicose
B6- D-Gluconic Acid	**D6**- α-Keto-Glutaric Acid	**F6**- Bromo Succinic Acid	**H6**- L-Lyxose
B7- D,L-α-Glycerol Phosphate	**D7**- α-Keto-Butyric Acid	**F7**- Propionic Acid	**H7**- Glucuronamide
B8- D-Xylose	**D8**- α-Methyl-D-Galactoside	**F8**- Mucic Acid	**H8**- Pyruvic Acid
B9- L-Lactic Acid	**D9**- α-D-Lactose	**F9**- Glycolic Acid	**H9**- L-Galactonic Acid-γ-Lactone
B10- Formic Acid	**D10**- Lactulose	**F10**- Glyoxylic Acid	**H10**- D-Galacturonic Acid
B11- D-Mannitol	**D11**- Sucrose	**F11**- D-Cellobiose	**H11**- Phenylethyl-amine
B12- L-Glutamic Acid	**D12**- Uridine	**F12**- Inosine	**H12**- 2-Aminoethanol

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Results for PM 1 Microplate:

PM 1 plate was used to assess the carbon catabolic functions of *E. coli*. Studies were carried out with wild type strain (OPE002) and one of the uropathogenic strains (OPE013). We obtained a total of 8 differences on PM 1 plate. Differences were seen in wells **A8, A12, B3, E2, F1, H2, H3 and H12.** The results for PM 1 plate are shown in Fig. 13.

Results for PM 2 Microplate:

PM plate 2 was used to assess different carbon catabolic functions of K12 strain and the uropathogenic strain. 1 difference was seen between the wild type and the uropathogen in well **B1**. The results for PM 2 Microplate are shown in Fig. 17 (Appendix)

Results for PM 3 Microplate:

PM plate 3 was used to assess the ability of the strains to catabolize nitrogen. For PM plate 3 a total of **10** differences could be seen. Differences were seen in wells **C1, D1, D12, E5, E9, E12, F7, G1, G2 and G7**. The results for PM 3 Microplate are shown in Fig. 18 (Appendix)

Results for PM 4 Microplate:

The PM 4 plate was used to assess the ability of the strains to catabolize phosphorus and sulfur sources. A total of 18 differences could be seen in PM 4 plates. Differences were seen in wells **A3, A4, A12, B5, C5, D5, E1, E2, E4, E5, E11, F2, F10, G2, H2, H3, H4 and H11**. The results for PM 4 Microplate are shown in Fig. 19 (Appendix)

The differences between the wild type and the uropathogenic strains on PM 1, 2, 3 and 4 were studied and differences in their utilization of respective substrates as carbon, nitrogen, sulfur and phosphorus sources were identified. We chose to explain the utilization of few sources by either the wild type strain or the uropathogenic strain that was useful in understanding how the phenotype microarrays were useful for strain characterization. Since genome contents of OPE013 strain are not known, we use the genomic information of the completely sequenced UTI strain CFT073 to estimate OPE013 genome.

Utilization of L-Proline: (well #A8, PM1)

The wild type strain (K12) showed growth on L-Proline and could utilize this amino acid as a sole source of carbon (PM1) and nitrogen (PM3). However this was not the case for uropathogenic *E. coli.* The uropathogenic strain of *E. coli* showed growth on Proline when L-Proline only when Proline was used as a sole carbon source. The transporters for L-Proline and the genes required for the degradation of L-Proline were studied. A single Proline degradation pathway was found in both strains K12 and CFT073 shown in fig. 19 (Reference: Websites Ecocyc for K12 and Metacyc for CFT073). We did not identify defects in the genes for the degradation of L-Proline, as the single gene put A was found in both the K12 strain and the CFT073 strain. We could not see defects in transporters because the growth curves of the strains looked identical when grown on L-Proline as a sole source of nitrogen. Based on previous growth experiments conducted for *E. coli* on L-Proline, we could only speculate the probable defect in the enzyme proline dehydrogenase, when L-proline was used as a carbon source (41). This suggests a system

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that is absent or poorly expressed in the uropathogenic strains that is responsible for utilizing carbon from Proline. This system is unknown to us. The degradation pathway for L-Proline has been shown in Fig. 17.
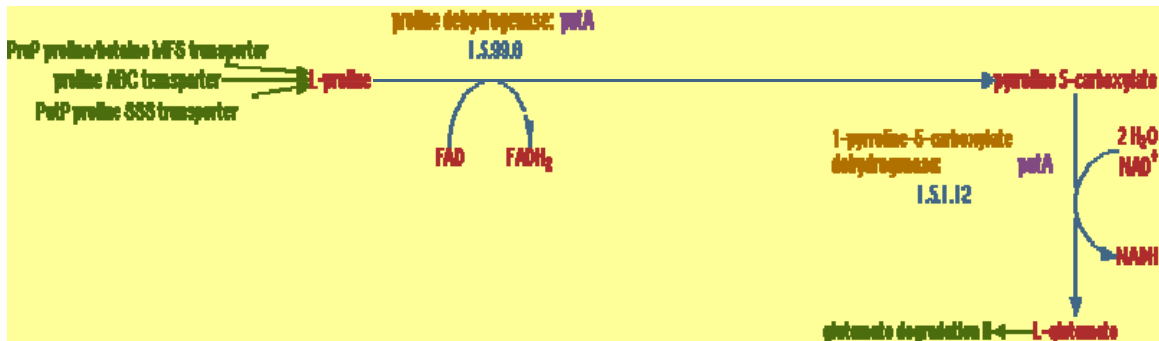
Fig. 14: Proline degradation pathway in *E. coli*. (The picture has been taken from ecocyc.org)

Utilization of D-Galactosamine and N-acetyl-D-Galactosamine. (Well# E9 and E12, PM 3).

In the second case results obtained in wells E9 and E12, PM-3 for the utilization of two sources D-galactosamine and N-acetyl-D-galactosamine were observed. In both the cases OPE013 showed growth whereas no growth was observed for the K12 strain. The obtained results could be explained as follows. Evidences showed that *E. coli* K12 could not catabolize D-galactosamine and N-Acetyl- D-galactosamine (5). The strains that were capable of catabolizing these two compounds had the complete *aga/kba* gene cluster that encoded for the Aga-specific phosphotransferase system (PTS) II^Aga (*agaVWE*) and a GalN-specific PTS or II^Gam (*agaBCD*). The strains also possessed genes that were required for the complete degradation and transport of both amino sugars (6). However

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K12 strains did not grow as they possessed a deletion of genes *agaW* and *agaEFA* which were vital for a specific phosphotransferase system that was essential for the uptake of the substrate into the cell. Subsequently the K12 strains lacked active II^{Aga} and II^{Gam} phosphotransferase system that played an important role in the uptake of these substrates into the cell. This could explain their inability to grow on these specific sources (6).

Utilization of L-Amino acids as a nitrogen source: (Well # A7, A8, A9, A10, A11, A12, PM 3).

The 20 L amino acids were used as sole nitrogen sources for the growth of bacterial strains. The wild type and the uropathogenic strains showed growth on L-Alanine, L-Arginine, L-Asparagine, L-Aspartic Acid, L-Cysteine, L-Glutamic acid and L-glutamine. Moderate growth was seen on L-Proline, L-Serine and L-Tryptophan. Very little growth was seen on L-Glycine, L-Lysine, L-Methionine and L-Phenyl Alanine. No growth was seen on L-Histidine, L-Isoleucine and L-Leucine. We were interested to look at growth patters of the wild type and the uropathogenic strains on L-Alanine, L-Arginine, L-Asparagine, L-Aspartic Acid, L-Cysteine and L-Glutamic acid because the growth curves appeared different and a significant lag phase was seen for the uropathogenic strains, that could not be identified in the wild type strains. We initially looked at the amino acid degradation pathways for both strains. More than one pathway could be identified for most of these amino acids, and all genes coding for all the degradative enzymes could be found in both the strains. When transporters of these amino acids were studied, it was identified that the K12 strain showed specific transporters for these amino acids whereas the uropathogenic strain showed general transporters for the transport of any amino acid.

This data was based on looking at the specific data provided in the databases for K12 (MG1655) and CFT073 in EcoCyc and MetaCyc. However this did not explain the lag phase of uropathogenic strains. When growth curves on glutamine were observed, although the wild type showed specific transporters for glutamine unlike the uropathogenic strain instead of the lag phase the uropathogenic strain had a growth curve that was very identical to that of the wild type strain. Therefore our reasoning of possible weak transporters was not the right explanation. We then looked at the growth curves obtained for the strains on ammonia and the dipeptides. The two strains had identical growth curves on the dipeptides and grew well, indicating that there was no defect in the transport system, as identical transporters were used by the strains for the transport of single or two amino acids. However, on Ammonia we were able to identify a lag in the growth curve of the uropathogenic strain. Since ammonia served to be the best source of nitrogen for growing these strains, we would not normally expect a lag in the growth curves. Since we found similar lag phases for growth curves of the uropathogenic strain on ammonia, and the above mentioned six L-amino acids, after looking at all possible explanations we decided that our observations were in fact artifacts of our experiment (4, 13, 20, 23, 28, 29, 30, 31, 35, 36, 38, and 39).

Fig. 17. PM 3 Microplate

Growth curves of the strains OPE002 and OPE013 on minimal media with succinate and arginine
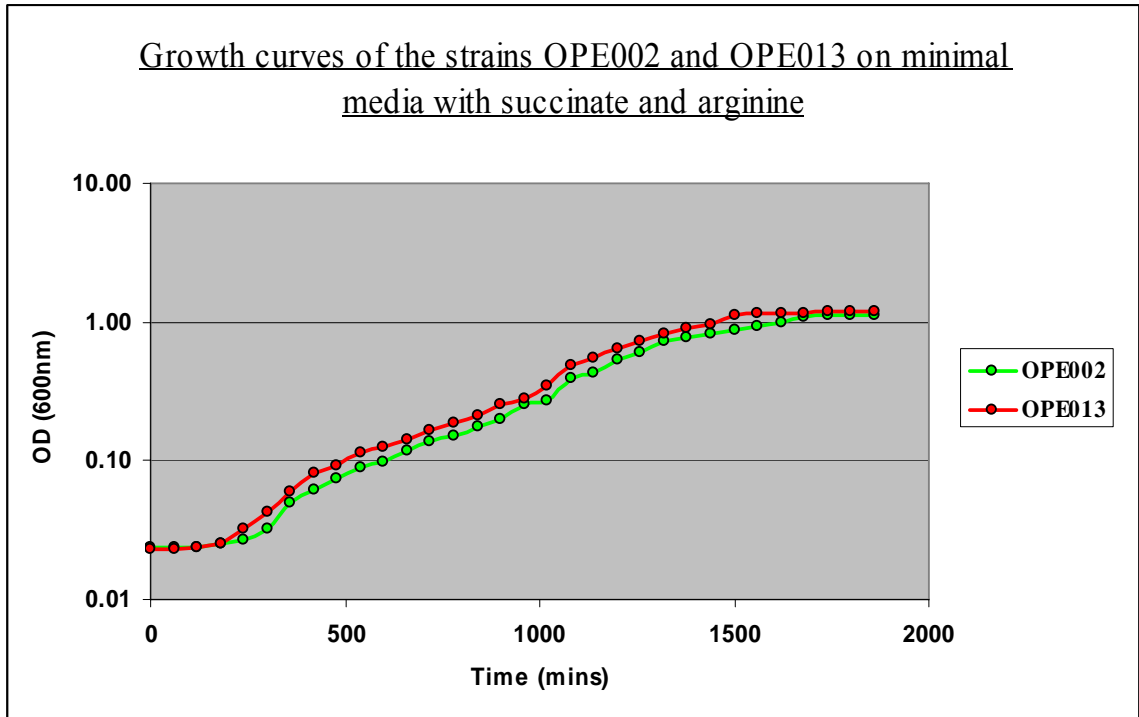
Fig.15: Growth curves obtained for strains OPE002 and OPE013 on succinate and arginine. Since growth curves are identical, this suggests that the difference in growth curves between OPE002 and

We conducted tests with OPE002 and OPE013 and grew these strains on L-Arginine. When growth curves of the strains were observed, the growth curves looked identical and OPE013 grew better than the wild type. Based on these results we speculated that the growth curves obtained for the two strains on L-Arginine on PM 3 as artifacts of the experiment.

CONCLUSION:

Phenotypic characterization of *E. coli* strains from humans in health and disease were useful in establishing the fact that uropathogenic strains of *E. coli* were different in behavior when compared to the laboratory strain K12. Studying the phenotypes was easier as suitable differences could be visualized and these differences were used to identify changes in the genetic makeup of the strains. We were able to identify phenotypes that were different from the K12 strain which were assessed by growth experiments and motility tests. We were also able to see phenotypic similarities and differences between the intestinal strains and the uropathogenic strains of *E. coli* that suggested that even though the commensal strains were non pathogenic, they showed some similarities to pathogenic strains. Phenotypic characterization of uropathogenic strains was useful in confirming the phenotypic variability of these strains and results of this study was used to study genomic variations.

API identification tests were used to confirm the genus and species of the clinical isolates. The UTI strain OPE006 showed green color in the microtube provided with citrate. The green color represented a weak positive result for the citrate utilization test. Since green was the result of a combination of two colors such as yellow and blue, we were not able to classify our results as truly positive or truly negative. The test was repeated more than once and identical results were obtained. There was a larger possibility that the strain was *E. coli,* but if we considered the results for the utilization of citrate as positive, then the API website showed the strain to be *Salmonella choleraesuis ssp arizona.*

We identified 2 strains OPE005 and OPE014 that were not able to grow in minimal media supplemented with a sole source of carbon and nitrogen, which suggested

a defect in the mechanism for the utilization of glucose and ammonium chloride and for the utilization of glycerol and arginine as respective carbon and nitrogen sources. OPE014 showed growth in presence of B vitamins and mostly due to nicotinamide with glucose and ammonium chloride. However, we were not able to identify why OPE005 (UTI) did not show any growth. We also observed that OPE013 showed better growth than the wild type in LB and in minimal media with sole carbon and nitrogen sources that suggested that this strain used a mechanism that was more effective that the wild type for the utilization of these sources of carbon and nitrogen. Some uropathogenic strains showed more motility than the wild type strain in 0.4% agar which was another significant difference that we noted. The motility test results showed that the motility of uropathogenic *E. coli* differed with respect to that of the wild type. Since we wanted to see the susceptibility of these strains to the most commonly used antibiotics antibiotic sensitivity tests were done. The isolates showed resistance to some antibiotics unlike the wild type strain that showed us that these strains had effective mechanisms by which they could overrule the activity of the antibiotic. This feature that was absent in the wild type. Tests were conducted with a panel of 7 antibiotics that belonged to three main categories. Ampicillin and Ceftriaxone were β-lactam antibiotics, Nitrofurantoin and Ciprofloxacin were nucleic acid synthesis inhibitors and Trimethoprim, Sulfamethoxazole and Sulfisoxazole were Sulfa drugs that inhibited the folic acid biosynthetic pathway. The mechanism of action of sulfonamides is shown in Fig. 19.

dihydropteroate diphosphate + p-aminobenzoic acid (PABA)

dihydropteroate
synthetase **x** ← sulfonamides

dihydropteroic acid

dihydrofolic acid

dihydrofolate
reductase **x** ← trimethoprim

tetrahydrofolic acid
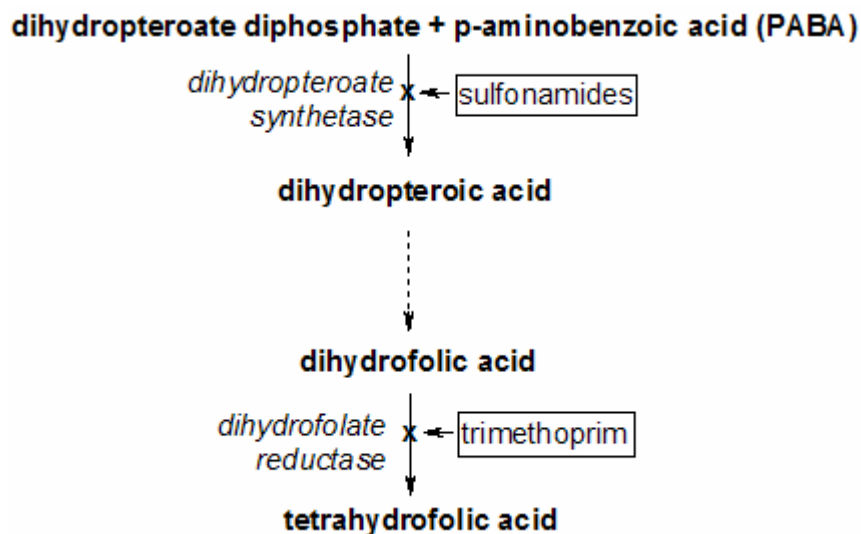
Fig 16: Mechanism of action of Sulfonamides.

All strains showed susceptibility to Ciprofloxacin and this was found to be the most effective antibiotic that inhibited bacterial growth. All strains were intermediately susceptible to Nitrofurantoin. OPE005 was resistant to both Ampicillin and Ceftriaxone (β-lactam inhibitors). The other two strains OPE008 and OPE010 were resistant to only one (Ampicillin) of the β-Lactam inhibitors. These results suggest more than one possible mechanisms of resistance by OPE005. OPE004, OPE005 and OPE010 were three strains that were resistant to all three sulfa drugs. OPE011 was resistant to one of the sulfa drugs and OPE015 showed resistance to two out of three sulfa drugs. However, we were not able to clearly provide reasons as to why one sulfa drug was better than the other for the same strain.

Phenotypic micro array profiles were generated for one of the uropathogenic strains where we chose to do tests with OPE013 because this strain showed better growth than the wild type on solid and in liquid media and was showed

high motility and for the wild type and results obtained for both these strains were compared. A total of 37 differences were identified in the 4 Plates that suggested significant differences in the utilization and metabolism of carbon, nitrogen, sulfur and phosphorus sources. Differences in growth curves seen between the two strains suggested metabolic pathways that were different in these strains. Phenotype micro arrays were useful in identifying genes and pathways that were unique to the wild type and the uropathogenic strains of *E. coli.*

From these studies we were able to establish and confirm that uropathogenic strains possessed different genetic profiles which supported the differences in the phenotypic behavior of these strains. These experiments were useful in delineating differences in phenotypic behavior of uropathogenic *E. coli* strains. Based on previous studies we knew that uropathogenic strains possessed more number of genes than the wild type *E. coli* strain, whose functions were unknown to us. From these experiments we were only able to prove the differences but were not able to provide reasons for behavioral differences of uropathogenic strains since the uropathogenic strains we worked with were not sequenced. Phenotypic profiling using 96 well micro plates with unique carbon, nitrogen phosphorus and sulfur sources however served as a valuable tool to assess at least 30 different phenotypes of uropathogenic strains, at once which was not evidenced to have been done previously.

APPENDIX

Media used for growth experiments:

a. LB Broth:

Tryptone – 10 grams

Yeast extract – 5 grams

NaCl – 5 grams

Distilled water – 1 liter

b. Minimal media (N⁻C⁻(K))

K_2SO_4 – 1 gram

K_2HPO_4 – 13.5 grams

KH_2PO_4 – 4.7grams

NaCl – 2.5grams

$MgSO_4.7H_2O$ – 100miligrams

Distilled water – 1 liter

c. 40% Glucose Stock

D- Glucose – 40 grams

Distilled water – 60 ml

40 grams of glucose was dissolved slowly in 60 ml of distilled water and the volume was

increased to 100 ml and the solution was sterilized by autoclaving.

d. 50% Glycerol stock

Glycerol – 50 ml

Distilled water – 60 ml

50 ml of glycerol was dissolved slowly in 60 ml of distilled water and the volume was increased to 100 ml and the solution was sterilized by autoclaving.

e. 1 M NH4Cl stock

NH_4Cl (Mw 53.5) – 2.68 gms

Distilled water – 50 ml

2.68 grams of NH_4Cl was added to 40 ml of distilled water and mixed thoroughly to dissolve ammonium chloride and the volume of the solution was increased to 50 ml. The solution was then filter sterilized (size of the filter) and stored in 25 ml centrifuge tubes (manufactured by corning)

f. 250 mM L-arginine stock

L- Arginine (Mw 211) – 2.64 grams

Distilled water – 50 ml

2.64 grams of L- arginine was taken and dissolved in 40 ml of distilled water and mixed thoroughly to dissolve arginine. The volume of the solution was increased to 50 ml. the solution was then filter sterilized (size of the filter) and stored in 25 ml centrifuge tubes (manufactured by Corning)

Dilution Formula Used: (Measured O.D.)x = (x+10) Initial O.D.

x measures the amount of liquid that needs to be added to a 10 ml solution (from the above formula) in order to get a diluted solution that corresponds to the initial OD.

Doubling time Formula:

$$DT = \frac{0.301 * DT}{Log_{10}[OD_T/OD_o]}$$

Table 10: PM Plate 1

Well#	OPE002	OPE013	0.05	K12	UTI	
A1	Blank	Blank	-	-	-	
A2	0.376	0.656	0.657	+	+	
A3	0.465	0.651	0.361	+	+	
A4	0.554	0.697	0.237	+	+	
A5	0.264	0.532	0.854	+	+	
A6	0.360	0.586	0.551	+	+	
A7	0.398	0.524	0.281	+	+	
A8	0.343	0.146	1.005	+	-	A8
A9	0.391	0.600	0.474	+	+	
A10	0.419	0.586	0.356	+	+	
A11	0.578	0.698	0.191	+	+	
A12	0.558	0.207	1.366	+	w	
B1	0.335	0.438	0.268	+	+	
B2	0.182	0.298	0.500	+	w	
B3	0.106	0.284	1.141	w	w	
B4	0.213	0.287	0.281	+	w	
B5	0.301	0.328	0.077	+	w	
B6	0.268	0.309	0.129	+	+	
B7	0.098	0.238	0.946	w	w	
B8	0.209	0.276	0.259	+	w	
B9	0.130	0.239	0.606	w	w	
B10	0.024	0.095	0.959	-	-	
B11	0.194	0.304	0.451	+	w	
B12	0.241	0.145	0.492	+	-	B12
C1	0.344	0.407	0.160	+	+	
C2	0.250	0.343	0.310	+	+	
C3	0.215	0.312	0.366	+	w	
C4	0.219	0.277	0.216	+	w	
C5	0.041	0.063	0.242	-	-	
C6	0.230	0.363	0.475	+	+	
C7	0.288	0.351	0.186	+	+	
C8	0.114	0.158	0.268	w	-	C8
C9	0.239	0.286	0.163	+	w	
C10	0.221	0.314	0.343	+	w	
C11	0.181	0.279	0.424	+	w	
C12	0.302	0.373	0.202	+	+	
D1	0.302	0.388	0.244	+	+	
D2	0.021	0.059	0.535	-	-	
D3	0.042	0.075	0.359	-	-	
D4	0.052	0.063	0.108	-	-	
D5	0.067	0.045	0.232	-	-	
D6	0.050	0.059	0.090	-	-	
D7	0.050	0.104	0.540	-	-	
D8	0.208	0.333	0.484	+	w	
D9	0.210	0.288	0.300	+	w	
D10	0.120	0.069	0.429	w	-	D10
D11	0.019	0.052	0.478	-	-	
D12	0.282	0.434	0.458	+	+	

Well#	OPE002	OPE013	0.05	K12	UTI	
E1	0.232	0.118	0.679	+	-	E1
E2	0.213	0.076	1.087	+	-	E2
E3	0.243	0.343	0.341	+	+	
E4	0.265	0.314	0.156	+	w	
E5	0.048	0.069	0.214	-	-	
E6	0.048	0.101	0.541	-	-	
E7	0.042	0.089	0.511	-	-	
E8	0.044	0.070	0.277	-	-	
E9	0.039	0.066	0.303	-	-	
E10	0.223	0.293	0.256	+	w	
E11	0.217	0.254	0.139	+	w	
E12	0.327	0.549	0.589	+	+	
F1	0.054	0.282	2.192	w	w	
F2	0.012	0.055	0.694	-	-	
F3	0.034	0.047	0.155	-	-	
F4	0.050	0.057	0.070	-	-	
F5	0.155	0.283	0.624	w	w	
F6	0.111	0.246	0.839	w	w	
F7	0.173	0.156	0.083	+	-	F7
F8	0.241	0.332	0.313	+	w	
F9	0.208	0.324	0.450	+	w	
F10	0.139	0.260	0.640	w	w	
F11	0.035	0.082	0.553	-	-	
F12	0.434	0.621	0.386	+	+	
G1	0.051	0.172	1.198	w	-	G1
G2	0.012	0.075	1.016	-	-	
G3	0.313	0.394	0.223	+	+	
G4	0.188	0.081	0.817	+	-	G4
G5	0.279	0.259	0.065	+	w	
G6	0.172	0.192	0.090	+	-	G6
G7	0.028	0.086	0.744	-	-	
G8	0.093	0.083	0.075	w	-	G8
G9	0.033	0.095	0.747	-	-	
G10	0.252	0.295	0.142	+	w	
G11	0.224	0.320	0.350	+	w	
G12	0.373	0.656	0.669	+	+	
H1	0.083	0.210	0.955	w	w	
H2	0.021	0.458	6.155	-	+	H2
H3	0.038	0.503	5.284	-	+	H3
H4	0.025	0.093	0.907	-	-	
H5	0.122	0.211	0.517	w	-	H5
H6	0.299	0.170	0.586	+	-	H6
H7	0.021	0.129	1.521	-	-	
H8	0.470	0.495	0.048	+	+	
H9	0.429	0.519	0.188	+	+	
H10	0.449	0.515	0.132	+	+	
H11	0.029	0.074	0.570	-	-	
H12	0.034	0.142	1.286	-	-	

Fig. 17. PM2 Microplate

	A1- Negative Control	C1- Gentiobiose	E1- Capric Acid	G1- Acetamide
	A2- Chondritin Sulfate C	C2- L-Glucose	E2- Caproic Acid	G2- L-Alaninamide
	A3- α-Cyclodextrin	C3- Lactitol	E3- Citraconic Acid	G3- N-Acetyl-L-Glutamic Acid
	A4- β Cyclodextrin	C4- D-Melezitose	E4- Citramalic Acid	G4- L-Arginine
	A5- γ-Cyclodextrin	C5- Maltitol	E5- D-Glucosamine	G5- Glycine
	A6- Dextrin	C6- α-Methyl-D-Glucoside	E6- 2-Hydroxy Benzoic Acid	G6- L-Histidine
	A7- Gelatin	C7- β-Methyl-D-Galactoside	E7- 4-Hydroxy Benzoic Acid	G7- L-Homoserine
	A8- Glycogen	C8- 3-Methyl Glucose	E8- β-Hydroxy Butyric Acid	G8- Hydroxy-L-Proline
	A9- Inulin	C9- β-Methyl-D-Glucuronic Acid	E9- γ-Hydroxy Butyric Acid	G9- L-Isoleucine
	A10- Laminarin	C10- α-Methyl-D-Mannoside	E10- α-Keto Valeric Acid	G10- L-Leucine
	A11- Mannan	C11- β-Methyl-D-Xyloside	E11- Itaconic Acid	G11- L-Lysine
	A12- Pectin	C12- Palatinose	E12- 5-Keto-D-Gluconic Acid	G12- L-Methionine
	B1- N-Acetyl-D-Galactosamine	D1- D-Raffinose	F1- D-Lactic Acid Methyl Ester	H1- L-Ornithine
	B2- N-Acetyl-Neuraminic Acid	D2- Salicin	F2- Malonic Acid	H2- L-Phenylalanine
	B3- β-D-Allose	D3- Seduheptulosan	F3- Melibionic Acid	H3- L-Pyroglutamic Acid
	B4- Amygdalin	D4- L-Sorbose	F4- Oxalic Acid	H4- L-Valine
	B5- D-Arabinose	D5- Stachyose	F5- Oxalomalic Acid	H5- D,L-Carnitine
	B6- D-Arabitol	D6- D-Tagatose	F6- Quinic Acid	H6- Sec-Butylamine
	B7- L-Arabitol	D7- Turanose	F7- D-Ribono-1,4-Lactone	H7- D,L-Octopamine
	B8- Arbutin	D8- Xylitol	F8- Sebacic Acid	H8- Putrescine
	B9- 2-Deoxy-D-Ribose	D9- N-Acetyl-D-Glucosaminitol	F9- Sorbic Acid	H9- Dihydroxy Acetone
	B10- l-Erythritol	D10- γ Amino Butyric Acid	F10- Succinamic Acid	H10- 2,3-Butenediol
	B11- D-Fucose	D11- δ-Amino Valeric Acid	F11- D-Tartaric Acid	H11- 2,3-Butanone
	B12- 3-0-β-D-Galactopyranosyl-D	D12- Butyric Acid	F12- L-Tartaric Acid	H12- 3-Hydroxy 2-Butanone

Table 11: PM Plate 2

Well#	OPE002	OPE013		0.05	K12	UTI	
A1	Blank	Blank		-	-	-	
A2	0.091	0.117		0.184	-	-	
A3	0.088	0.148		0.435	-	w	A3
A4	0.115	0.149		0.206	-	w	A4
A5	0.143	0.149		0.031	w	w	
A6	0.598	0.598		0.000	+	+	
A7	0.111	0.048		0.643	-	-	
A8	0.175	0.158		0.082	w	w	
A9	0.124	0.163		0.224	-	w	A9
A10	0.114	0.120		0.037	-	-	
A11	0.133	0.127		0.034	-	-	
A12	0.178	0.198		0.088	w	w	
B1	0.092	0.441		2.458	-	+	B1
B2	0.297	0.313		0.046	+	+	
B3	0.322	0.296		0.075	+	+	
B4	0.037	0.043		0.069	-	-	
B5	0.166	0.052	Ignore	1.118	w	-	B5
B6	0.051	0.031		0.247	-	-	
B7	0.048	0.031		0.210	-	-	
B8	0.051	0.017		0.507	-	-	
B9	0.074	0.027		0.610	-	-	
B10	0.038	0.040		0.023	-	-	
B11	0.050	0.080		0.300	-	-	
B12	0.484	0.474		0.019	+	+	
C1	0.064	0.088		0.211	-	-	
C2	0.055	0.065		0.095	-	-	
C3	0.046	0.047		0.010	-	-	
C4	0.041	0.055		0.154	-	-	
C5	0.073	0.083		0.081	-	-	
C6	0.040	0.024		0.216	-	-	
C7	0.301	0.201		0.398	+	w	
C8	0.075	0.059		0.147	-	-	
C9	0.222	0.217		0.019	w	w	
C10	0.054	0.043		0.118	-	-	
C11	0.043	0.044		0.011	-	-	
C12	0.073	0.116		0.350	-	-	
D1	0.083	0.096		0.098	-	-	
D2	0.051	0.046		0.052	-	-	
D3	0.054	0.050		0.040	-	-	
D4	0.057	0.066		0.084	-	-	
D5	0.048	0.052		0.041	-	-	
D6	0.052	0.123		0.696	-	-	
D7	0.056	0.039		0.191	-	-	
D8	0.054	0.042		0.130	-	-	
D9	0.039	0.034		0.060	-	-	
D10	0.057	0.034		0.274	-	-	
D11	0.049	0.046		0.031	-	-	
D12	0.097	0.092		0.035	-	-	

Well#	OPE002	OPE013		0.05	K12	UTI	
E1	0.040	0.033		0.084	-	-	
E2	0.053	0.057		0.039	-	-	
E3	0.043	0.038		0.057	-	-	
E4	0.040	0.055		0.167	-	-	
E5	0.354	0.332		0.058	+	+	
E6	0.035	0.031		0.049	-	-	
E7	0.042	0.047		0.054	-	-	
E8	0.034	0.022		0.167	-	-	
E9	0.064	0.034		0.357	-	-	
E10	0.026	0.039		0.171	-	-	
E11	0.055	0.030		0.313	-	-	
E12	0.118	0.134		0.095	-	-	
F1	0.194	0.210		0.066	w	w	
F2	0.042	0.059		0.185	-	-	
F3	0.327	0.345		0.048	+	+	
F4	0.044	0.038		0.068	-	-	
F5	0.052	0.073		0.206	-	-	
F6	0.046	0.040		0.067	-	-	
F7	0.038	0.040		0.023	-	-	
F8	0.034	0.028		0.077	-	-	
F9	0.039	0.042		0.034	-	-	
F10	0.054	0.060		0.058	-	-	
F11	0.060	0.048		0.122	-	-	
F12	0.103	0.106		0.020	-	-	
G1	0.059	0.082		0.211	-	-	
G2	0.058	0.072		0.130	-	-	
G3	0.029	0.174	Ignore	1.835	-	w	G3
G4	0.074	0.045		0.305	-	-	
G5	0.066	0.065		0.009	-	-	
G6	0.036	0.026		0.132	-	-	
G7	0.036	0.022		0.194	-	-	
G8	0.064	0.021		0.606	-	-	
G9	0.033	0.028		0.064	-	-	
G10	0.040	0.022		0.250	-	-	
G11	0.069	0.050		0.190	-	-	
G12	0.093	0.082		0.083	-	-	
H1	0.118	0.108		0.063	-	-	
H2	0.085	0.099		0.104	-	-	
H3	0.074	0.089		0.121	-	-	
H4	0.066	0.048		0.184	-	-	
H5	0.084	0.105		0.157	-	-	
H6	0.066	0.062		0.036	-	-	
H7	0.085	0.080		0.038	-	-	
H8	0.094	0.091		0.021	-	-	
H9	0.279	0.254		0.082	+	+	
H10	0.068	0.087		0.161	-	-	
H11	0.070	0.057		0.121	-	-	
H12	0.110	0.149		0.244	-	w	H12

Fig. 18. PM3 Microplate

	A1- Negative Control	C1- L-Tyrosine	E1- Histamine	G1- Xanthine
	A2- Ammonia	C2- L-Valine	E2- β-Phenylethyl-amine	G2- Xanthosine
	A3- Nitrite	C3- D-Alanine	E3- Tyramine	G3- Uric Acid
	A4- Nitrate	C4- D-Asparagine	E4- Acetamide	G4- Alloxan
	A5- Urea	C5- D-Aspartic Acid	E5- Formamide	G5- Allantoin
	A6- Biuret	C6- D-Glutamic Acid	E6- Glucuronamide	G6- Parabanic Acid
	A7- L-Alanine	C7- D-Lysine	E7- D,L-Lactamide	G7- D,L-α-Amino-N-Butyric Acid
	A8- L-Arginine	C8- D-Serine	E8- D-Glucosamine	G8- γ-Amino-N-Butyric Acid
	A9- L-Asparagine	C9- D-Valine	E9- D-Galactosamine	G9- δ-Amino-N-Caproic Acid
	A10- L-Aspartic Acid	C10- L-Citrulline	E10- D-Mannosamine	G10- D,L-α-Amino-Caprylic Acid
	A11- L-Cysteine	C11- L-Homoserine	E11- N-Acetyl-D-Glucosamine	G11- δ-Amino-N-Valeric Acid
	A12- L-Glutamic Acid	C12- L-Ornithine	E12- N-Acetyl-D-Galactosamine	G12- α-Amino-N-Valeric Acid
	B1- L-Glutamine	D1- N-Acetyl-D,L-Glutamic Acid	F1- N-Acetyl-D-Mannosmine	H1- Ala-Asp
	B2- Glycine	D2- N-Phthaloyl-L-Glutamic Acid	F2- Adenine	H2- Ala-Gln
	B3- L-Histidine	D3- L-Pyroglutamic Acid	F3- Adenosine	H3- Ala-Glu
	B4- L-Isoleucine	D4- Hydroxylamine	F4- Cytidine	H4- Ala-Gly
	B5- L-Leucine	D5- Methylamine	F5- Cytosine	H5- Ala-His
	B6- L-Lysine	D6- N-Amylamine	F6- Guanine	H6- Ala-Leu
	B7- L-Methionine	D7- N-Butylamine	F7- Guanosine	H7- Ala-Thr
	B8- L-Phenylalanine	D8- Ethylamine	F8- Thymine	H8- Gly-Asn
	B9- L-Proline	D9- Ethanolamine	F9- Thymidine	H9- Gly-Gln
	B10- L-Serine	D10- Ethylenediamine	F10- Uracil	H10- Gly-Glu
	B11- L-Threonine	D11- Putrescine	F11- Uridine	H11- Gly-Met
	B12- L-Tryptophan	D12- Agmatine	F12- Inosine	H12- Met-Ala

Table 12: PM Plate 3

Well#	OPE002	OPE013		0.05	K12	UTI	
A1	Blank	Blank		-	-	-	
A2	0.530	0.561		0.053	+	+	
A3	0.119	0.082		0.280	-	-	
A4	0.141	0.098		0.291	w	-	A4
A5	0.121	0.093		0.196	-	-	
A6	0.101	0.102		0.007	-	-	
A7	0.577	0.600		0.037	+	+	
A8	0.591	0.641		0.078	+	+	
A9	0.546	0.755		0.351	+	+	
A10	0.549	0.651		0.170	+	+	
A11	0.803	0.776		0.033	+	+	
A12	0.677	0.469		0.401	+	+	
B1	0.444	0.425		0.040	+	+	
B2	0.249	0.252		0.010	w	w	
B3	0.085	0.059		0.239	-	-	
B4	0.115	0.050		0.650	-	-	
B5	0.138	0.029	Ignore	1.380	-	-	
B6	0.220	0.141		0.414	w	w	B7
B7	0.165	0.154		0.054	w	-	
B8	0.197	0.172		0.113	w	w	
B9	0.291	0.320		0.085	+	+	
B10	0.291	0.337		0.135	+	+	
B11	0.304	0.132		0.945	+	-	B11
B12	0.377	0.297		0.231	+	+	
C1	0.159	0.043		1.247	-	-	
C2	0.076	0.068		0.068	-	-	
C3	0.307	0.352		0.126	+	+	
C4	0.189	0.050	Ignore	1.390	w	-	C4
C5	0.045	0.059		0.147	-	-	
C6	0.052	0.061		0.088	-	-	
C7	0.081	0.050		0.310	-	-	
C8	0.392	0.367		0.060	+	+	
C9	0.061	0.043		0.194	-	-	
C10	0.122	0.061		0.550	-	-	
C11	0.108	0.041		0.736	-	-	
C12	0.486	0.451		0.070	+	+	
D1	0.076	0.424		2.762	-	+	D1
D2	0.039	0.049		0.112	-	-	
D3	0.037	0.040		0.034	-	-	
D4	0.021	0.023		0.028	-	-	
D5	0.046	0.089		0.448	-	-	
D6	0.051	0.059		0.079	-	-	
D7	0.042	0.048		0.065	-	-	
D8	0.041	0.049		0.088	-	-	
D9	0.078	0.030		0.600	-	-	
D10	0.026	0.037		0.145	-	-	
D11	0.323	0.162		0.759	+	w	
D12	0.511	0.104		2.643	+	-	D12

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Well#	OPE002	OPE013		0.05	K12	UTI	
E1	0.046	0.060		0.146	-	-	
E2	0.189	0.053	Ignore	1.320	w	-	E2
E3	0.202	0.045	Ignore	1.653	w	-	E3
E4	0.090	0.074		0.129	-	-	
E5	0.332	0.119		1.260	+	-	E5
E6	0.373	0.434		0.144	+	+	
E7	0.175	0.267		0.409	w	w	
E8	0.346	0.360		0.035	+	+	
E9	0.024	0.351		4.419	-	+	E9
E10	0.052	0.052		0.000	-	-	
E11	0.320	0.335		0.041	+	+	
E12	0.078	0.635		4.352	-	+	E12
F1	0.173	0.225		0.233	w	w	
F2	0.283	0.299		0.048	+	+	
F3	0.338	0.368		0.077	+	+	
F4	0.385	0.396		0.025	+	+	
F5	0.330	0.347		0.045	+	+	
F6	0.100	0.092		0.056	-	-	
F7	0.332	0.072		2.131	+	-	F7
F8	0.036	0.057		0.244	-	-	
F9	0.024	0.061		0.500	-	-	
F10	0.042	0.055		0.141	-	-	
F11	0.040	0.103		0.700	-	-	
F12	0.072	0.067		0.043	-	-	
G1	0.177	0.039		1.551	w	-	G1
G2	0.145	0.341		1.005	-	+	G2
G3	0.187	0.221		0.143	w	w	
G4	0.085	0.056		0.274	-	-	
G5	0.055	0.061		0.057	-	-	
G6	0.082	0.063		0.168	-	-	
G7	0.324	0.073		2.041	+	-	G7
G8	0.330	0.295		0.101	+	+	
G9	0.130	0.229		0.550	-	w	G9
G10	0.148	0.053		0.922	-	-	
G11	0.323	0.283		0.120	+	+	
G12	0.117	0.077		0.315	-	-	
H1	0.564	0.679		0.187	+	+	
H2	0.466	0.540		0.143	+	+	
H3	0.494	0.597		0.189	+	+	
H4	0.501	0.558		0.103	+	+	
H5	0.497	0.548		0.093	+	+	
H6	0.490	0.543		0.098	+	+	
H7	0.544	0.600		0.094	+	+	
H8	0.445	0.577		0.267	+	+	
H9	0.445	0.541		0.194	+	+	
H10	0.449	0.499		0.100	+	+	
H11	0.398	0.509		0.248	+	+	
H12	0.616	0.749		0.200	+	+	

Fig. 19. PM4 Microplate

A1-Negative Control	C1-Phosphoenol Pyruvate	E1-O-Phospho-D-Tyrosine	G1-N-Acetyl-L-Cysteine
A2-Phosphate	C2-Phospho-Glycolic Acid	E2-O-Phospho-L-Tyrosine	G2-S-Methyl-L-Cysteine
A3-Pyrophosphate	C3-D-Glucose-1-Phosphate	E3-Phosphocreatinine	G3-Cystathionine
A4-Trimeta-phosphate	C4-D-Glucose-6-Phospate	E4-Phosphoryl Choline	G4-Lanthionine
A5-Tripoly-phosphate	C5-2-Deoxy-D-Glucose 6-Phosphate	E5-O-Phosphoryl-Ethanolamine	G5-Glutathione
A6-Triethyl Phosphate	C6-D-Glucosamine-6-Phosphate	E6-Phosphono Acetic Acid	G6-D,L-Ethionine
A7-Hypophosphite	C7-6-Phospho-Gluconic Acid	E7-2-Aminoethyl Phosphonic Acid	G7-L-Methionine
A8-Adenosine-2'- monophosphate	C8-Cytidine-2'-monophosphate	E8-Methylene Diphosphonic Acid	G8-D-Methinine
A9-Adenosine-3'-monophosphate	C9-Cytidine-3'-monophosphate	E9-Thymidine-3'-monophosphate	G9-Glycyl-L-Methionine
A10-Adenosine-5'-monophosphate	C10-Cytidine-5'-monophoasphate	E10-Thymidine-5'-monophosphate	G10-N-Acetyl-D,L-Methionine
A11-Adenosine23'cyclic monoP	C11-Cytidine-2',3'-cyclic monoP	E11-Inositol Hexaphosphate	G11-L-Methonine Sulfoxide
A12-Adenosine35'cyclic monoP	C12-Cytidine-3',5'-cyclic monoP	E12-Thymidine-3',5'-cyclic monoP	G12-L-Methionine Sulfone
B1-Thiophosphate	D1-D-Mannose-1-Phosphate	F1-Negative Control	H1-L-Djenkolic Acid
B2-Dithiophosphate	D2-D-Mannose-6-Phosphate	F2-Sulfate	H2-Thiourea
B3-D,L-α-Glycerol Phosphate	D3-Cysteamine-S-Phosphate	F3-ThioSulfate	H3-1-Thio-β-D-Glucose
B4-β-Glycerol Phosphate	D4-Phospho-L-Arginine	F4-Tetrathionate	H4-D,L-Lipoamide
B5-Carbamyl Phosphate	D5-O-Phospho-D-Serine	F5-Thiophosphate	H5-Taurocholic Acid
B6-D-2-Phospho-Glyceric Acid	D6-O-Phospho-L-Serine	F6-Dithiophosphate	H6-Taurine
B7-D-3-Phospho-Glyceric Acid	D7-O-Phospho-L-Threonine	F7-L-Cysteine	H7-Hypotaurine
B8-Guanosine-2'-monophosphate	D8-Uridine-2'-monophosphate	F8-D-Cysteine	H8-p-Amino Benzene Sulfonic Acid
B9-Guanosine-3'-monophosphate	D9-Uridine-3'-monophosphate	F9-L-Cysteinyl-Glycine	H9-Butane Sulfonic Acid
B10-Guanosine-5'-monophosphate	D10-Uridine-5'-monophosphate	F10-L-Cysteic Acid	H10-2-Hydroxyethane Sulfonic Acid
B11-Guanosine-2',3'cyclic mono P	D11-Uridine-2'3'-cyclic monophosphate	F11-Cysteamine	H11-Methane Sulfonic Acid
B12-Guanosine-3',5'cyclic monoP	D12-Uridine-3'5'cyclic monophosphate	F12-L-Cysteine Sulfinic Acid	H12-Tetramethylene Sulfone

Table 13: PM Plate 4

Well #	OPE002	OPE013		0.05	K12	UTI	
A1	Control	Control			-	-	
A2	0.548	0.475		0.139	+	+	
A3	0.331	0.105		1.455	w	-	A3
A4	0.551	0.185		1.560	+	-	A4
A5	0.457	0.215		0.915	+	w	
A6	0.199	0.159		0.192	-	-	
A7	0.160	0.087		0.534	-	-	
A8	0.523	0.526		0.006	+	+	
A9	0.564	0.807		0.396	+	+	
A10	0.523	0.758		0.411	+	+	
A11	0.583	0.830		0.390	+	+	
A12	0.715	0.276		1.348	+	w	
B1	0.371	0.449		0.185	+	+	
B2	0.290	0.321		0.090	w	+	
B3	0.275	0.337		0.191	w	+	
B4	0.279	0.304		0.075	w	w	
B5	0.270	0.061		1.889	-	-	
B6	0.286	0.130		0.864	w	-	B6
B7	0.278	0.178		0.438	w	-	B7
B8	0.277	0.317		0.125	w	w	
B9	0.266	0.307		0.130	w	w	
B10	0.291	0.310		0.057	w	w	
B11	0.287	0.225		0.226	w	w	
B12	0.169	0.086		0.607	-	-	
C1	0.327	0.207		0.466	w	w	
C2	0.294	0.133		0.881	w	-	C2
C3	0.282	0.335		0.159	w	+	
C4	0.271	0.330		0.183	w	+	
C5	0.262	0.061		1.804	-	-	
C6	0.358	0.420		0.152	+	+	
C7	0.334	0.332		0.007	w	+	
C8	0.319	0.380		0.166	w	+	
C9	0.295	0.347		0.149	w	+	
C10	0.292	0.351		0.172	w	+	
C11	0.256	0.334		0.254	w	+	
C12	0.207	0.098		0.734	-	-	
D1	0.339	0.486		0.380	w	+	
D2	0.290	0.333		0.125	w	+	
D3	0.308	0.311		0.010	w	w	
D4	0.324	0.202		0.481	w	-	D4
D5	0.345	0.085		1.919	w	-	D5
D6	0.312	0.292		0.057	w	w	
D7	0.351	0.163		0.881	+	-	D7
D8	0.300	0.387		0.248	w	+	
D9	0.268	0.347		0.250	-	+	D9
D10	0.271	0.351		0.248	-	+	D10
D11	0.307	0.384		0.214	w	+	
D12	0.175	0.178		0.014	-	-	

Well #	OPE002	OPE013		0.05	K12	UTI	
E1	0.343	0.071		2.257	w	-	E1
E2	0.308	0.117		1.139	w	-	E2
E3	0.299	0.129		0.944	w	-	E3
E4	0.308	0.062		2.193	w	-	E4
E5	0.343	0.144		1.024	w	-	E5
E6	0.159	0.132		0.146	-	-	
E7	0.148	0.174		0.135	-	-	
E8	0.129	0.080		0.378	-	-	
E9	0.294	0.267		0.087	w	w	
E10	0.311	0.248		0.209	w	w	
E11	0.269	0.078		1.501	-	-	
E12	0.259	0.193		0.270	-	-	
F1	Blank	Blank			-	-	
F2	0.271	0.050		2.228	w	-	F2
F3	0.300	0.357		0.166	+	+	
F4	0.310	0.384		0.206	+	+	
F5	0.290	0.346		0.168	w	+	
F6	0.292	0.373		0.236	w	+	
F7	0.327	0.391		0.169	+	+	
F8	0.292	0.372		0.236	+	+	
F9	0.293	0.339		0.134	w	+	
F10	0.285	0.059		2.072	w	-	F10
F11	0.160	0.056		0.974	-	-	
F12	0.439	0.621		0.373	+	+	
G1	0.194	0.101		0.616	-	-	
G2	0.215	0.067		1.275	w	-	G2
G3	0.334	0.365		0.080	+	+	
G4	0.324	0.388		0.171	+	+	
G5	0.346	0.257		0.293	w	+	
G6	0.153	0.055		0.931	-	-	
G7	0.244	0.284		0.134	w	+	
G8	0.264	0.216		0.183	w	+	
G9	0.257	0.305		0.156	w	+	
G10	0.272	0.189		0.348	w	w	
G11	0.234	0.116		0.711	w	w	
G12	0.198	0.119		0.465	-	w	G12
H1	0.569	0.681		0.180	+	+	
H2	0.275	0.089		1.338	w	-	H2
H3	0.248	0.085		1.203	w	-	H3
H4	0.234	0.080		1.181	w	-	H4
H5	0.227	0.193		0.139	w	w	
H6	0.398	0.502		0.233	+	+	
H7	0.408	0.343		0.166	+	+	
H8	0.174	0.091		0.590	-	-	
H9	0.380	0.241		0.479	+	+	
H10	0.413	0.264		0.473	+	+	
H11	0.426	0.178		1.086	+	w	
H12	0.201	0.094		0.740	w	w	

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