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Pakistan Science Foundation Project :

S - KU/BIO(132)

Extrachromosomal Elements For In Vivo Genetic Engineering

PRINCIPAL INVESTIGATOR

Hajra Khatoon

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PAKISTAN SCIENCE FOUNDATION
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EXTRACHROMOSOMAL ELEMENTS FOR IN VIVO GENETIC ENGINEERING

FINAL RESEARCH REPORT

SEPTEMBER 1985 - AUGUST 1987

Dr. H. Khatoon
Principal Investigator

Dr. Manzooruddin Ahmad
Vice-Chancellor

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S U M M A R Y

This research was undertaken for the isolation of extra-chromosomal elements (plasmids) and transposons that can be used for in vivo genetic engineering of gram negative bacteria of plant origin.

The process of in vivo genetic engineerings, in bacteria, involves the use of transposons. The transposons can only be introduced into a bacterial cell after their insertion into a plasmid, followed by conjugal transfer of the plasmid + transposon complex into the bacterial cell. Thus, the process of in vivo genetic engineering depends on two major factors: (1) a suitable transposon that can express its function into a desired bacterium and (2) a suitable plasmid that can be used as a vehicle to carry the transposon into the bacterium. In this regard a wide-host-range plasmid (a plasmid that can be transferred to a large variety of bacteria) serves as a better vehicle.

With a view to detect/isolate plasmids and transposons from bacteria associated with plants, we have screened 194 becteria for their resistance to the following eight antibiotics: ampicillin, agrimycin, chloramphenicol, gentamycin, kanamycin, neomycin, streptomycin and tetracycline. The screened bacteria, which included species of plant pathogenic bacteria (e.g. Xanthomonas, Erwinia, Pseudomonas etc.) as well

as plant symbiotic bacteria (e.g. Rhizobium) exhibited antibiotic resistance in different patterns and combinations. These bacteria were tested for the presence of plasmids (R plasmids) by conjugating them with standard Escherichia coli recipients and observing whether the resistances borne by them were transferable. From the experiments conducted, it appears that the tested bacteria either lack plasmids or contain plasmids that are conjugally non-transferable. At times, an abortive transfer of resistance was observed indicating that the plasmid was although transmitted but could not be stably maintained in Escherichia coli. A great difficulty in detecting the conjugal transmission was ineffective donor elimination in most cases.

Some of the plant pathogenic/associated bacteria exhibited the production of bacteriocins (known to be usually associated with the presence of bacteriocinogenic plasmids.) However, none of these bacteria transferred its bacteriocinogenic plasmid by conjugation. The difficulty was again ineffective donor elimination.

As a parallel attempt, we have studied whether plasmids of other gram negative bacteria could be used for in vivo genetic engineering of plant pathogenic/associated bacteria. In this regards, twelve R plasmids were isolated from gram negative enteric bacteria, screened for resistance to seven different antibiotics. The R plasmids, that carried

different patterns of antibiotic resistance, were then studied for their conjugal transmission to plant pathogenic/associated bacteria including: Xanthomonas, Erwinia, Citrobacter, Rhizobium, Agarobacterium and Pseudomonas. Some of these R plasmids could be conjugally transmitted to as many as four different bacteria of plant origin. However, some could be transmitted to three, two or one plant pathogenic/associated bacteria. The R plasmids were not only transmitted to plant pathogenic/associated bacteria but also expressed all their resistances in these bacteria. Studies were also made to see whether these R plasmids are stably maintained in their new hosts. Most of them (R plasmids) were found to be either completely or partly stable indicating that they could be used for in vivo genetic engineering of plant pathogenic/assocaited bacteria. Retransfer of the R plasmids from plant pathogenic/associated bacteria to enteric bacteria could not be detected due to some technical difficulties. Similarly, the R plasmids were not found to carry any transposons during studies conducted so far.

Conclusively, we can say that we have isolated some broad-host-range R plasmids that could be used as vehicles for in vivo genetic engineering (by using the available transposons, such as bacteriophage Mu) of plant pathogenic/associated bacteria.

DETAILED REPORT

INTRODUCTION

In vivo genetic engineering refers to the manipulation of the genome of a cell, while it is still alive, by purely genetical means (1, 2). By these manipulations, one can introduce new characters into a cell or can change or remove the existing characters (2, 3). The necessary tools for in vivo genetic engineering include extrachromosomal elements and transposons (2, 4). The current project was planned for the isolation/detection of extrachromosomal elements and tranposons that can be used for in vivo genetic engineering of the bacteria associated with plants.

Cells of gram negative bacteria normally carry a single circular chromosome that carries all the essential genes required for survival (5, 6, 7). Certain bacteria, however, contain other self-replicating DNA structures referred to as extrachromosomal elements, plasmids or espisomes (8, 9, 10, 11) that can be exemplified as follows:

1. R plasmids: These plasmids carry genes for resistance to one or several antibiotics (8, 10).
2. Colicin plasmids (or Col plasmids): Colicins or bacteriocins are antibiotic like substances, produced by certain bacteria, that can kill other bacteria. The genes for colicin production are also located on extrachromosomal

elements (8, 10).

3. F plasmid or factor: This plasmid carries genes for fertility of bacteria (8, 10).
4. Ti plasmids: These plasmids confer the property of causing crown gall disease of dicotyledonous plants on Agarobacterium tumifaciens (9, 10).
5. Other Miscellaneous plasmids: These include nitrogen fixing plasmids of Rhizobium (9, 11), degradative plasmids of Pseudomonas (10) and pathogenicity plasmids of enteric bacteria (10).

Plasmids are an addition to the genome of a cell because they carry additional genes which confer additional properties, depending on the type of plasmid, on the host cell. Plasmids can also be either non transferable or transferable by conjugation (8). In the latter case, genes associated with a plasmid can be easily transmitted alongwith the plasmid from one bacterial cell to the other (8). Usually, the host range of plasmids is rather limited, that is, they are conjugally transferred to the species of the same genus or to the species of closely related genera. However, there are few exceptional cases where plasmids can be transferred to a wide variety of bacterial genera; such plasmids are referred to as broad-host-range plasmids (1, 12).

Occacionally, a gene from a plasmid can jump from its place and be inserted into certain other DNA strcuture

(such as another plasmid or a phage that might be present into the cell or into the chromosome), thus, causing a genetic alteration. Such a gene, referred to as a jumping gene or a transposon (2, 4), can be made use of in manipulating the genome of a bacterial cell. The genetic alterations or genetic rearrangements caused by transposons include mutation, deletions, inversions, fusions, and recombinations (3, 4). These changes can be brought about in a desired bacterial cell only if the transposon can be introduced in the cell. The only method of introducing a transposon into a cell is to insert the transposon into a broad-host-range-plasmid (in a genetically marked host bacterium such as an Escherichia coli) followed by conjugal transfer of the plasmid + transposon complex into the desired bacterial cell. If one wishes to perform in vivo genetic engineering in plant associated bacteria, one must have broad-host-range plasmids that are conjugally transferable to these bacteria and transposons that can express their functions in such bacteria.

R plasmids are more useful among all plasmids because they carry easily recognizable markers that can be easily traced in genetical studies. These plasmids, including conjugative and non conjugative both, have been studied in greater detail in enteric bacteria (8, 12). Several plant pathogenic bacteria have been found to carry plasmids that lack easily recognizable markers (9, 13). As we were interested

in studying R plasmids and transposons that could be used for in vivo genetic engineering of the bacteria associated with plants, we explored two different possibilities (i) isolation of the conjugative R plasmids from plant associated bacteria for use in the same bacteria and (ii) isolation of conjugative R plasmids from any of the gram negative bacteria for use in plant associated bacteria. Work carried out in both these directions has been described.

EXPERIMENTAL PROCEDURES

Media for growth and storage:

For broth cultivation of bacteria, L. broth (14) or L.B. broth was used. It had the following composition:

L. B. Broth

Bacto-tryptone	10	grams
Yeast extract	5	grams
NaCl	10	grams
Glucose	10	grams
Distilled water	1000	ml

The pH of L. B. broth was adjusted to 7 before autoclaving. If required, calcium chloride was added to the final concentration of 0.002 M after autoclaving the medium. L. B. agar was made by adding 1.5% agar and L. B. soft agar was made by adding 0.6% agar to L. B. broth. L. B. agar was used for the growth of plant pathogenic/associated bacteria. L. B. soft agar was used for the detection of colicinogeny. Plant pathogenic bacteria could also be grown on nutrient agar with the following composition.

Nutrient Agar

Beef extract	3	grams
Peptone	5	grams
NaCl	5	grams
Agar	2	grams
Distilled water	1000	ml
pH	6.8 - 7	

Rhizobia could be grown either on L. B. agar or on Yeast extract manitol agar, also called medium 79 (15), with the following composition:

Yeast Extract Manitol Agar (Medium 79)

K_2HPo_4	0.5	grams
$MgSo_4$, $7H_2o$	0.2	grams
NaCl	0.1	grams
$CaCo_3$	3.0	grams
Yeast extract	1.0	gram
Agar	15.0	grams
Congo red	10.0	ml (of 4% solution)
Manitol	100.0	ml (of 10% solution)
Distilled water	900.0	ml
pH	6.5 - 7.0	

(Note: Congo red and manitol are separetely sterlized at a pressure of 10 lbs and added after autoclaving the medium).

The enteric bacteria were purified on MacConkey's medium of either Difco or Oxoid, with the following composition:

MacConkey Agar

Peptone	17.0	grams
Proteose peptone	3.0	grams
Lactose	10.0	grams
Bile Salts No. 3	1.5	grams
Sodium chloride	5.0	grams
Agar	13.5	grams
Neutral red	0.03	grams
Crystal violet	0.001	grams
Distilled water	1000	ml
pH	7.1	

Conjugal crosses between standard auxotrophic strains were made on the minimal agar that had the followin composition:

Minimal Agar

NH_4 Cl	1	gram
KH_2 Po_4	3	grams
Na_2 HPo_4	6	grams
Agar	20	grams
Distilled water	1000	ml

Solutions of glucose (20%) and $MgSo_4$ (20%) are sterilized separately for 15 minutes at 15 lbs pressure. After autoclaving the medium, 2 ml of glucose and 0.1 ml of $MgSo_4$ were added. If required, amino acids and vitamins were added to the final concentration of 40 ug/ml and 2 ug/ml respectively.

All the purified cultures were picked and stored in tryptone agar stabs consisting of: Bactotryptone 17 grams, Bacto-agar 5 grams and distilled water 1000 ml, at 4°C.

Procurement of cultures:

The cultures of bacteria associated with plants were obtained from plant material of various types. This included rotten fruits and vegetables and diseased plant material. Rhizobia were isolated from nodules. Diseased plant material as well as purified cultures were also obtained from National Agriculture Research Council (Islamabad), Agriculture University (Faiselabad) and Nuclear Institute of Agriculture and Biology (Faiselabad). All the isolated cultures are listed in appendix-I at the end.

The procedure for isolating the plant pathogenic bacteria from diseased plant material was as follows. The diseased plant material (tissue of leaves, stem or skin of fruits etc.) was washed with sterilized distilled water and then cut into small bits with scissors or scalpel. After being treated with 70% alcohol for 1 or 2 minutes, to remove

contaminants, the pieces were rinsed with sterilized distilled water and stabbed onto L. B. agar or Nutrient agar plates. Bacterial growth, appearing after an incubation of 24 hours or more, at 30°C, was purified twice.

The procedure for isolating the Rhizobia was the same as described in reference 16. Nodules, with 0.5 - 1 cm of root attached, were removed from the plants. With the help of the attached root, the nodules were first washed with 70% alcohol, then immersed in mercuric chloride for four minutes and finally rinsed with distilled water for at least six times to remove mecuric chloride. Single nodules, that were separated from the roots by a sterile scalpel or forceps, were placed between two sterile glass slides and crushed by pressing. The juice that came out of the crushed nodules was streaked on medium 79 plates that were incubated at 30°C. Cream coloured growth of Rhizobia appearing on the medium was purified twice. On this medium, Agarobacterium, which absorbs the dye (congo red) strongly, produces red coloured colonies.

The cultures of other gram negative bacteria e.g. enteric bacteria, that originated from diseased animals (poultry) and human beings were obtained from Poultry Research Institute (Karachi) and hospital and clinical labs of Karachi. All the cultures were purified twice on MacConkey's agar.

Standard cultures:

The standard cultures used during the studies, as recipients of R plasmids, included the following:

1. Xanthomonas malvacearum (TR 134). Isolated from infected leaf of angular leaf spot of cotton. From Dr. M.A.R. Bhatti, Faisalabad Agriculture University.

2. Xanthomonas campestris obtained from Dr. Mohammad Aslam of N.A.R.C. (Islamabad).

3. Erwinia chrysanthemi AC 4075. From Dr. Arun K. Chatterjee, Kansas State University, U.S.A.

4. Agarobacterium tumifaciens. From Dr. R. A. Lelliott., National collection of plant pathogenic bacteria. Ministry of Agriculture, Fisheries and Food, U.K.

5. Rhizobium. From Dr. Richard Griffin, Nitrogen Fixation and Soybean Genetic Research Laboratory, United States Department of Agriculture, U.S.A.

6. Pseudomonas syringae. From R. A. Lelliott, National Collection of plant pathogenic bacteria. Ministry of Agriculture, Fisheries and Food, U.K.

7. Citrobacter freundii ATCC 8090. From American type culture collection, U.S.A.

8. Escherichia coli BU 40: (F^- Δ prolac trp^- Sm^r) from Dr. A. I. Bukhari of Cold Spring Harbor, New York, U.S.A.

9. *E. coli* AB712: (F^- lac^- leu^- thr^- pro^- Sm^r) from Dr. E. Adelberg of U.S.A.

Preparation of Antibiotic Solution:

The antibiotics used in these studies included: ampicillin trihydrate, gentamycin sulfate, chloramphenicol, kanamycin sulfate, neomycin sulfate, streptomycin sulfate, tetracycline hydrochloride and agrimycin. Stock solutions of all antibiotics, except chloramphenicol, were made in distilled water in the concentration of 10 mg/ml. Chloramphenicol was dissolved in ethanol. All the solutions were sterilized by filteration through bacteriological filters and kept refrigerated at 4°C. Solutions not in use were kept frozen.

Preparation of Amino Acid Solutions:

Stock solutions of all the amino acids were made in distilled water in the concentration of 4 mg/ml. The solutions were either autoclaved (at 15 lbs pressure for 15 minutes) or sterilized by filtration through bacteriological filters and kept frozen until used.

Screening for Antibiotic Resistance:

Prior to screening, all the cultures were purified twice on the suitable media and maintained in tryptone agar stabs at 4°C. The method of velvet replication (17) was used

for screening. For this purpose, a broth culture of the test strain was plated on a suitable medium to obtained isolated colonies. Approximately 20 individual colonies were picked up onto a master plate, grown overnight and replicated on a suitable medium (L. B. agar for plant associated bacteria and MacConkey agar for enteric bacteria) containing desired concentratin of each of the test antibiotics. The replication was performed using a sterile velveteen replica. Growth of all the replicated clones indicated resistance to a particular level of an antibiotic.

Sensitivity Levels of Recipients:

Overnight broth cultures of the recipient strains were plated in 0.1 ml amounts on L. B. agar containing known graded concentrations of each antibiotic to determine the minimal inhibitory concentration (MIC) of the latter capable of totally annihilating the recipient cells. Growth (or its absence) was recorded after proper incubation at 30°C (for plant associated bacteria) and 37°C for enteric bacteria.

Conjugation Experiments:

(a) Liquid method (tube conjugation):

The cultures of donor and recipient cells were grown overnight in L. B. broth. Next morning, the overnight culture was diluted 50 - fold in L. B. broth and grown with shaking at 30°C or 37°C (depending on the type of

organism) for ½ hour or till slight turbidity was visible. Donors were then mixed with recipients in the proportion of 1:10 and incubated at 30°C (in case of plant associated bacteria) or at 37°C (in case of enteric bacteria) for the desired length of time. If the cross involved a plant pathogenic and an enteric bacterium, the incubation was carried out at 30°C for six hours as described by Chatterjee (14). In case the donor and recipient both were enteric bacteria, the incubation was carried out a 37°C for two hours as described by Khatoon (18). Controls, which consisted of the donor or recipient cells, were treated similarly.

After proper incubation the conjugation mixture was plated on nutrient or minimal media containing appropriate antibiotic and/or nutritional markers to contraselect recipient and, in some cases, also donors. Control platings of unmated donor and recipient cells were always made. In case of a cross between two enteric bacteria, transconjugants appeared after an incubation of 24 hours at 37°C. Whereas in case of a cross between an enteric and a plant pathogenic bacterium, transconjugants appeared in 4 days following an incubation at 30°C. Transconjugant colonies arising on the plated media were purified twice on the selective medium used and replicated to check for the donated markers.

(b) Solid Method (plate conjugation):

These conjugations were performed by a similar method as described by Chatterjee (14). A 0.05 ml sample of the exponentially growing donor culture (about 5 x 10^8 cells /ml) was placed and spread on the L. B. agar. Upon this culture, a sample (0.05ml) of the recipient cell (about 10^9 cells/ml) was overlaid, and the plates were incubated for 6 hours at 30°C. L. B. agar plates containing only the recipient or the donor cells served as controls. The mating cells were then streaked on the selective media to check for the donated markers. Controls were also treated in a similar way.

(c) Combined Liquid and Solid Method:

This method was a combination of the two methods described above and was adopted to ensure conjugation if the above methods failed. The use of this method was restricted to crosses between plant pathogenic/associated bacteria and enteric bacteria.

The cultures of donor and recipient cells were grown overnight in L. B. broth. Next morning, the cultures were diluted 50 - fold in L. B. broth and grown with shaking at 30°C or 37°C (depending on the type of organism) for ½ hour or till slight turbidity was visible.

Donors were then mixed with recipients in the proportion of 1:10 and incubated at 30°C for two hours. At the end of this incubation, a 0.1 ml sample of the conjugation mixture was placed and spread on L. B. agar and the plate was incubated for 6 - 18 hours at 30°C. Finally, the mating cells were streaked on the selective media to check for the donated markers. Controls of the unmated donor and recipient cultures were also made and treated in exactly the same way.

Derivation of Transconjugants:

Following conjugation, donor cells were eliminated from mating mixtures by (a) plating on media containing high levels or streptomycin (to which the recipient was resistant) or (b) in the case of auxotrophic strains, use of appropriate supplements in minimal medium. Recipients were contraselected with the addition of an appropriate antibiotic when the transmission of an R plasmid was being studied.

A recipient bacterium, which has received from a donor bacterium genetic material (plasmid) which codes for certain phenotypically recognizable properties, is referred to as transconjugant. A transconjugant from a bacterial mating will therefore possess all the chromosomally determined properties of the recipient in addition to the donated properties transferred to it during conjugation. In cases where donor elimination was not very effective or could not be

achieved, it was necessary to differentiate between the donors and the transconjugants. This could be done by comparing the colony morphology and colour of the colony (where possible) as well as by checking the auxotrophic markers of potential transconjugants. Transconjugants, along with controls, were replicated on L. B. agar plates (or MacConkey agar plates, in case of enteric bacteria) containing desired antibiotics to determine the pattern of resistance acquired.

Bacteriocin Production:

General techniques were the same as those described by Fredericq (19). A strain to be tested for bacteriocin production was stabbed in the middle of an L. B. plate containing 0.002 M calcium chloride. Following growth for 24 - 48 hours, the culture was killed by chloroform vapour and overlaid by a known bacteriocin sensitive indicator (E. coli AB 712), seeded in the molten L.B. soft agar. Zone of inhibition surrounding the stabbed culture, following incubation for 24 hours at 30°C, indicated bacteriocin production by the stabbed culture.

Spontaneous Segregation:

To determine the stability of R plasmids in plant pathogenic/associated bacteria, the spontaneous segregation of these R plasmids was studied as follows. A strain bearing the R plasmid to be studied was grown in antibiotic free L.B.

broth through six consecutive transfers with 24 hour intervals and a 20-fold dilution each time. Finally the culture was diluted and plated on L.B. agar to obtain isolated colonies. Some 200 colonies were then gridded on to master plates which were then replicated on the antibiotic containing L.B. agar plates to check for loss/stability of resistance determinants. Antibiotic resistant and sensitive control strains were always replicated along with the colonies of the test strain.

Detection of Transposons:

Spontaneously growing culture of E. coli BU 40 or E. coli 712 carrying individual R plasmids was diluted and plated on L.B. agar or MacConkey's agar to obtain isolated colonies. Some 200 colonies were then gridded on to master plates which were then replicated on Minimal agar containing supplements of E. coli BU 40 or E. coli AB 712. Growth of all the colonies indicated that no additional auxotrophy had occured.

R E S U L T S

Screening for Antibioitc Resistance:

Bacteria isolated from samples of plant origin (appendix - I) and some other gram negative bacteria (appendix - II) were screened for their resistance/sensitivity to the following antibiotics: ampicillin, agrimycin, chloramphenicol, gentamycin, kanamycin, neomycin, streptomycin and tetracycline. Results of the screening, that was usually performed at a level of 100 ug/ml, are listed in appendix - I and appendix - II.

Attempts to Isolate Plasmids from Plant Pathogenic/Associated Bacteria:

Attempts were made to isolate R plasmids from plant pathogenic/associated bacteria by conjugating them with standard Escherichia coli recipients, E. coli AB712 or E. coli BU 40. Of the 96 plant pathogenic/associated bacteria tested for the donation of antibiotic resistance, none transmitted any resistance(s) by conjugation. All the methods of conjugation were tested for this purpose. In some cases, an abortive transfer of some resistance was observed, however, the resistances were lost upon purification of the transconjugants.

The bacteria tested for the presence of R plasmids included the following:

TR1b, TR2, TR3a, TR3b, TR6, TR8a, TR14, TR15a, TR17, TR19,

TR24, TR26a, TR27a, TR27c, TR30a, TR33a, TR33b, TR34a, TR43b, TR36b, TR37a, TR39, TR40b, TR43b, TR45a, TR46, TR47a, TR49, TR50a, TR51, TR52a, TR52c, TR53b, TR54b, TR55b, TR57, TR59, TR60, TR62, TR63, TR66, TR67, TR70, TR72, TR74, TR75, TR80, TR82, TR83, TR84, TR87, TR89, TR90, TR91, TR94, TR95, TR96, TR99, TR100, TR103, TR104, TR106, TR111, TR112, TR115, TR118, TR123, TR125, TR130, TR135, TR137, TR139, TR140, TR141, TR142, TR143, TR144, TR147, TR148, TR151, TR152, TR153, TR154, TR155, TR156, TR158, TR159, TR160, TR161, TR162, TR163, TR164, TR165, TR166, TR168, TR169.

As the majority of the bacteria isolated from natural sources are prototrophic, their elimination in the conjugation experiments becomes difficult. We have used the method of streptomycin elimination and elimination on the basis of colony morphology for the donor cells. The apparent absence of R plasmid transmission might have been due to ineffective donor elimination in some cases. The isolation of phages, for eliminating the donor cells, did not work out.

R Plasmids from Enteric Bacteria:

Antibiotic resistant enteric bacteria (appendix-II) were used as potential donors of R plasmids in conjugations with standard E. coli recipients, E. coli AB712 or E.coli BU 40. R plasmids isolated in this manner are listed in Table - 1.

The R plasmids were freely transferable in conjugations between *E. coli* strains and also to other enteric bacteria. Transmissions were usually studied between the following conjugal crosses on the minimal medium:

E. coli AB712 + R ⟶ *E. coli* BU 40

E. coli BU 40 + R ⟶ *E. coli* AB712

TABLE - 1

R plasmid designation	Original host	Resistance pattern * of the host	Resistance Pattern of the R plasmid
R1-pFK2	Salmonella(5)	KNS	KN
R2-pFK6	Escherichia(11)	CKNST	KST
R3-pFK7	Escherichia(27)	CKNST	KNST
R4-pFK8	Klebsiella(40)	AST	AST
R5-pFK20	Escherichia(41)	AKNST	AKNS
R6-AS72	Escherichia(59)	ACGKNST	ACGKN
R7-AS34	Klebsiella(90)	AGST	AGT
R8-NJ15	Escherichia(102)	ACGKNT	ACKNT
R9-NJ18	Escherichia(135)	ACGKNT	ACKNT
R10-pFK5a	Escherichia(144)	ACKNST	A
R11-pFK17	Escherichia(166)	KNST	T
R12-pFK18	Escherichia(189)	KNST	KNT

*A = ampicillin
C = chloramphenicol
G = gentamycin
K = kanamycin
N = neomycin
S = streptomycin
T = tetracycline

N.B. The numbers in brackets refer to the numbers of appendix - II.

Transfer of R plasmids to plant pathogenic/associated bacteria:

All the R plasmids mentioned in Table 1, except R7-AS34(the host of which died during these studies), carried in E. coli BU40, were tested for their transmission to the plant pathogenic/associated recipient bacteria by conjugation. These recipient bacteria included: Xanthomonas, Erwinia, Citrobacter, Rhizobium, Agarobacterium and Pseudomonas. As the crosses were made on L.B. agar plus antibiotics, donor elimination was not possible. However, transconjugants could be differentiated from the donor on the basis of colony morphology. The results of these studies are listed in Table 2.

As can be seen from Table 2, some R plasmids could be transmitted to as many as four different plant pathogenic/associated bacteria. R1-pFK2 was transferred to Xanthomonas Rhizobium and Agarobacterium. R2-pFK6 could be transmitted to Xanthomonas, Erwinia, Rhizobium and Agarobacterium. R3-pFK7 was transferred to Xanthomonas, Erwinia, Citrobacter and Rhizobium. R4-pFK8 to Xanthomonas and Agarobacterium, R5-pFK20, R10-pFK5a and R11-pFK17 were transferred to Xanthomonas and Rhizobium. R6-AS72 was transferred to Erwinia and Agarobacterium. However its transmission to Agarobacterium was abortive, as the resistances could be lost upon purification of the transconjugants. Similarly transmission of R8-NJ15 and R9-NJ18 to Agarobacterium was abortive, however, R8-NJ15 was stably maintained in Rhizobium. R12-pFK18 was also transferable to Rhizobium.

R2-pFK6	+	+	-
R3-pFK7	+	+	+
R4-pFK8	+	-	-
R5-pFK20	+	-	-
R6-AS72	-	+	-
R7-AS34	- - - - - - - - - - - - - (Died) - - - - -		
R8-NJ15	-	-	-
R9-NJ18	-	-	-
R10-pFK5a	+	-	-
R11-pFK17	+	-	-
R12-pFK18	-	-	-

+ = presence of conjugal transfer

- = absence of conjugal transfer

(+) = abortive transfer

Stability of R plasmids in plant pathogenic/ associated bacteria:

For performing in vivo genetic engineering in plant pathogenic/associated bacteria, it is necessary that the plasmids be stably maintained inside these hosts. A study was therefore made to see whether the plasmids were stably maintained or any loss occured due to spontaneious segregation, as might occur in case of some plasmids(8). The results of this study are presented in Table 3.

As can be seen from Table 3, most of the R plasmids were either completely or partly stable in the plant pathogenic/associated bacteria. R1-pFK2 was completely stable in Xanthomonas, Rhizobium and Agarobacterium. R2-pFK6 was stable in Xanthomonas and Agarobacterium and partly stable in Rhizobium.R11-pFK17 was stable in Xanthomonas and Rhizobium. R5-pFK20, R8-NJ15, R10-pFK5a and R12-pFK8 were stable in Rhizobium. R4-pFK8 was stable in Agarobacterium. From these results it appears that there exists a possibility of using these R plasmids for in vivo genetic engineering of the plant pathogenic/associated bacteria in which they are stably maintained.

Retransfer of R plasmids from plant pathogenic/associated bacteria to enteric bacteria

To see whether the R plasmids (Those mentioned in Table 2) were transferred back from plant pathogenic/associated bacteria to enteric bacteria, the plant pathogenic/associated bacteria were conjugated to enteric bacteria. E. coli

TABLE - 3

Study of the stability of R plasmids in plant pathogenic/associated bacteria

Host bacterium	Plasmid	Resistance markers on the plasmid	Percent loss of resistance*
Xanthomonas	R1-pFK2	KN	No loss of any resistance
"	R2-pFK6	KST	No loss of any resistance
"	R3-pFK7	KNST	K(73), N(69), S(16.5), No loss of T
"	R4-pFK8	AST	A(3.5), S(3), T(6)
"	R5-pFK20	AKNS	No loss of any resistance
"	R10-pFK5a	A	A(5.5)
"	R11-pFK17	T	No loss
Erwinia	R6-AS72	ACGKN	C(36), G(56), No loss of A, K, N
Citrobacter	R3-pFK7	KNST	S(100), No loss of K, N, T.
Rhizobium	R1-pFK2	KN	No loss of any resistance
"	R2-pFK6	KST	T(30.5), No loss of K, S.
"	R3-pFK7	KNST	T(18.5), No loss of K, N, S.
"	R5-pFK20	AKNS	No loss of any resistance
"	R8-NJ15	ACKNT	No loss of any resistance
"	R10-pFK5a	A	No loss
"	R11-pFK17	T	No loss
"	R12-pFK18	KNT	No loss of any resistance
Agarobacterium	R1-pFK2	KN	No loss of any resistance
"	R2-pFK6	KST	No loss of any resistance
"	R4-pFK8	AST	No loss of any resistance

* The number of colonies tested was 200 in each case.

Percent loss is indicated by numbers in brackets

Note: Two cultures of Erwinia (carrying R2-pKF6 and R3-pFK7) died and could not be studied.

BU40 and E. coli AB712 were used as recipients for this purpose. However no transfer of any R plasmid was observed from plant pathogenic/associated bacteria. This could have been because of two reasons (1) ineffective elimination of donor in some conjugal crosses and (2) very low frequency of transmission that was not detectable by the methods used.

Bacteriocin Production:

The plant pathogenic/associated bacteria listed in Appendix-I were tested for bacteriocin production against E. coli AB712 as the indicator strain. Some of the bacteria showed slight clearing indicating the presence of some sort of bacteriocin. However, these bacteriocins were not conjugally transferable from the plant pathogenic/associated bacteria to other gram negative bacteria such as E. coli BU40 or E. coli AB 712.

Absence of Transposons:

During the studies conducted so far, the R plasmids listed in Table 1 did not show the presence of any transposons. None of the resistance markers appeared to have jumped from the plasmid to the chromosome as no additional auxotrophy was developed by E. coli BU40 or AB712. However, prolonged studies are required to observe the presence of transposons.

DISCUSSION

The purpose of this study was to isolate plasmids (specially R plasmids) and transposons for in vivo genetic engineering of plant pathogenic/associated bacteria. To achieve this end, we have worked in two directions: (I) Screened plant pathogenic/associated bacteria for antibiotic resistance to isolate R plasmids from such bacteria and (II) Isolated R plasmids from other gram negative bacteria for use in plant pathogenic/associated bacteria. The first approach was taken with a view that R plasmids isolated from plant pathogenic/associated bacteria would have a greater chance of transmission and expression in bacteria of plant origin as compared to the plasmids isolated from other bacteria. The second approach, on the other hand, was taken to examine whether R plasmids of other gram negative bacteria could be transmitted and expressed in bacteria of plant origin.

Our attempts in the first direction have not been successful for various reasons which include the following: (I) all bacteria isolated were prototrophic and hence it was difficult to eliminate the donors in conjugation experiments. (II) fewer plant pathogenic/associated bacteria exhibited resistance to a large number of antibiotics and (III) bacteria kept dying during storage (we had a shortage of refrigeration space for storage). Inspite of these problems, we have tested

96 bacteria for transfer of R plasmids. Although no transmission was observed in most cases, in some cases an abortive transmission was observed indicating the possibility of an R plasmid. As the resistances were lost during purification of the transconjugants, it seems that the R plasmid was not stably inherited. In other cases, where no transmission was observed, three possibilities could be considered (I) The resistances were carried on the chromosome (II) there were plasmids but nontransferable or (III) There were transferable plasmids but could not be detected due to ineffective donor elimination in conjugation experiments. Plant pathogenic/associated bacteria usually carry nontransferable plasmids(8), however, conjugative or transferable plasmids have also been reported in such bacteria (9, 20, 21). If, there were any transferable plasmids in the bacteria tested by us, the failure to detect them could have been due to the third reason.

Besides R plasmids, plasmids with bacteriocin determinants may also be observed in bacteria of plant origin(22). We, therefore, also tested our bacteria for bacteriocinogeny. Slight clearing of E. coli AB712 was observed in some cases indicating the presence of bacteriocin determinants. However, no conjugal transfer of these determinants was observed. This might have been due to a low frequency of transmission of such plasmids combined with the ineffective donor elimination.

As far as our second approach is concerned, we have

isolated some R plasmids from gram negative enteric bacteria for testing their transfer and expression in plant pathogenic/associated bacteria. The isolation of R plasmids from enteric bacteria is relatively easier bacause firstly antibiotic resistance is quite common among these bacteria and secondly transfer frequency of conjugative plasmids is higher. Transconjugants can therefore be observed even in the absence of donor elimination and separated on the basis of colony morphology. In some cases, however, streptomycin elimination of the donor could be accomplished.

Eleven of the R plasmids isolated from enteric bacteria were transferred to plant pathogenic/associated bacteria (Table 2). All the R plasmids that were transmitted to plant pathogenic/associated bacteria expressed all their resistance markers indicating that the new hosts had no adverse effect on their expression/replication. Besides Agarobacterium, where inheritance of three plasmids was abortive (Table 2), inheritance was stable in other cases.

To further check the stability of the inherited R plasmids, their spontaneous segregation was studied in plant pathogenic/associated bacteria (Table 3). No complete loss of any plasmid occured even by repeated cultivation in antibiotic free broth for six consecutive days. A number of plasmids were completely stable whereas some were partly stable in their new hosts after this treatment. Cases of complete sta-

bility could be observed in all hosts except for Erwinia and Citrobacter were partial stability was observed. As no complete loss of any plasmid occured, there exists a possibility that the plasmids, particularly those that are completely stable, can be used as vehicles for in vivo genetic engineering of the plant pathogenic/associated bacteria.

Conjugal retransfer of the R plasmids could not be observed from plant pathogenic/associated bacteria to E. coli recipients. This could have been because of two reasons (I) the transfer frequently of the R plasmids, in the reverse crosses, might have been too low to be detected by the methods used and (II) ineffective donor elimination in some cases. In cases, the transfer frequency of the R plasmids is very low, membrane filter technique (14) could be used to detect conjugal transmission.

During the current studies, no transposons could be detected on any of the R plasmids. Transposons are known to produce genetic rearrangements such as mutations and deletions (2, 3, 23, 24) that can be detected in the form of auxotrophy of some markers in a genetically defined strain. However, during our current studies no additional auxotrophy was introduced into the two genetically defined strains, E. coli BU40 and E. coli AB712. More detailed studies might be necessary to detect the presence of transposons on these plasmids.

CONCLUSION

The current studies have indicated that out of 96 plant pathogenic/associated bacteria tested, none carried conjugally transferable R plasmid(s) or else these R plasmids were not detectable due to technical difficulties. Similarly no bacteriocin producing plasmids could be detected.

Of the twelve R plasmids isolated from enteric bacteria, at least ten were stably inherited by plant pathogenic/associated bacteria and could be used as vehicles for in vivo genetic engineering of these bacteria. Two of these R plasmids could be used for as many as four plant pathogenic/associated bacteria whereas others could be used for one, two or three plant pathogenic/associated bacteria. Some of these R plasmids (at least those that are transferred to three or four bacteria) could well be regarded as broad-host-range plasmids.

None of the R plasmids was found to carry any transposons by the method employed. However, more detailed studies are needed to detect their presence. For performing genetic engineering, therefore, one could use the above R plasmids as vehicles employing the already existing transposons (e.g. bacteriophage Mu)

LIST OF SCIENTISTS

1. Dr. Hajra Khatoon (Principal Investigator): supervised the research and devoted more than 50% of her time to the project. She also prepared the final research report.

2. Mr. Muhammad Tauseef-ur-Rehman (Research Officer): carried out the research and devoted his full time to the project.

3. Mr. Fasihuddin Ahmad Ansari (Lecturer Department of Microbiology): Although not officially associated with the project, Mr. Ansari provided some technical assistance during research on the project.

4. Miss Sabiha Shahid (Co-operative lecturer, department of Microbiology): performed some honorary research work at the end of the project.

LITERATURE CITED

1. Denarie, J., C. Rosenberg, B. Bergeron, C. Boucher, M. Michel and M. Barate de Bertalmio. 1977. Potential of RP4 :: Mu plasmids for in vivo genetic engineering of gram negative bacteria. pp. 507. In DNA insertion elements, plasmids and episomes by A.I. Bukhari, J.A. Shapiro and S.L. Adhya (Eds). Cold Spring Harbor Laboratory, New York (Publishers).

2. Bukhari, A.I., J.A. Shapiro and S.L. Adhya. 1977. DNA insertion elements plasmids and episomes. Cold Spring Harbor Laboratory, New York (Publishers).

3. Howe, M.M. and E.G. Bade. 1975. Molecular biology of bacteriophage Mu. Schience 190: 624 - 632.

4. Bukhari, A.I. 1979. Transposable and invertible genetic elements. In new researches in biology and genetic. pp 131. Hamdard Academy, Karachi. (Publishers).

5. Bachmann, B.J. 1983. Linkage map of Escherichia coli K-12, Edition 7. Microbiological Reviews. 47: 180 - 230.

6. Sanderson, K.E. and J.R. Roth. 1983. Linkage map of Salmonella typhimurium, Edition VI, Microbiological Reviews. 47: 410 - 453.

7. Leary, J.V. and D.W. Fulbright. 1982. Chromosomal genetics of Pseudomonas spp. and Erwinia spp. In phytopathogenic prokaryotes. pp. 229. M.S. Mount and G.H. Lacy (Eds). Academic Press.

8. Novick, R.P. 1969. Extrachromosomal inheritance in bacteria. Bacteriol. Rev. 33: 210 - 263.

9. Coplin, D.L. 1982. Plasmids in plant pathogenic bacteria. In phytopathogenic prokaryotes. Vol 2. pp. 255. M.S. Mount and G.H. Lacy (Eds), Academic Press.

10. Williams, P.A. 1978. The biology of plasmids. In companion to microbiology. A.T. Bull and P.M. Meadow (Eds). pp. 77 - 107. Longman (Publishers) New York and London.

11. Nuti, M.P., Lepidi, A.A., Prakash, R.R., Shilperoort, R.A., and Cannon, C.E. 1979. Evidence for nitrogen fixation (nif) genes on indigenous Rhizobium plasmids. Nature, London. 282: 533 - 535.

12. Old, R.W. and S.B. Primrose. 1980. Plasmids as cloning vehicles. pp. 44. In principles of gene manipulation. Blackwell Scientific Publications (Publishers).

13. Civerolo, E.L. 1985. Indigenous plasmids in Xanthomonas campestris pv. citri. Phytopathology. 75: 524 - 528.

14. Chatterjee, A.K. 1980. Acceptance by Erwinia spp. of R plasmids R 68.45 and its ability to mobilize the chromosome of Erwinia chrysanthemi. J. Bacteriol. 142: 111 - 119.

15. Allen, O.N. 1959. Experiments in soil bacteriology. pp. 54 - 55. Burgess Publishing Co., Mineapolis 15, Minnesota, U.S.A.

16. Laboratory manual for post graduate training course on isotopic and advanced techniques in biological nitrogen fixation. Nuclear Institute of Agriculture and Biology, Faisalabad, January 8 - 21, 1983.

17. Khursheed, B. and H. Khatoon. 1984. Multiple antibiotic resistance among clinical Pseudomonads. Kar. Univ. J. Science. 12 (2): 223 - 230.

18. Khatoon, H. 1976. Genetic properties of RM 98, an R plasmid of Salmonella which determines sensitivity to the phage IKe. Zbl. Bakt. Hyg., I. Abt. Orig. A.234: 294 - 304.

19. Fredericq, P. 1957. Colicins. Ann. Rev. Microbiol. 11: 7-22.

20. Thiry, G. 1984. Plasmids of the epiphytic bacterium Erwinia uredovora. J. gen. Microbiol. 130: 1623-1631.

21. Thiry, G. and M. Faelen. 1984. Erwinia uredovora 20D3 contains an IncM plasmid. J. gen. Microbiol. 130: 1633 - 1640.

22. Hirsch, P.R. 1979. Plasmid-determined bacteriocin production by Rhizobium leguminosarum. J. gen. Microbiol. 113: 219 - 228.

23. Khatoon, H. and A.I. Bukhari. 1981. DNA rearrangements associated with reversion of bacteriophage Mu-induced mutations. Genetics 98: 1 - 24.

24. Emery, A.E.H. 1984. An introduction to recombinant DNA pp. 70 - 71. John Wiley and Sons (Publishers).

APPENDIX - I

"BACTERIA ISOLATED FROM SAMPLES OF PLANT ORIGIN"

Note:

Ap	= ampicillin	Ag	= agrimycin
Cm	= chloramphenicol	Gm	= gentamycin
Km	= kanamycin	Nm	= neomycin
Sm	= streptomycin	Tc	= tetracycline

(Numbers indicate micrograms/ml)

antibiotic sensitive = sensitive to all the above antibiotics.

1. TR1a — Rhizobium from Lab lab purpureus sweet sub-Sp. bengalensis (Jacq) Verda. Gram-ve rods. Antibiotic Resistance Pattern: Ap50.

2. TR1b — Rhizobium from Lab lab purpureus. Gram-ve rods. Antibiotic Resistance Pattern: Ap100 Cm100.

3. TR2 — Rhizobium from Lab lab purpureus. Gram-ve rods. Antibiotic Resistance Pattern: Cm100.

4. TR3a — Rhizobium from Lab lab purpureus. Gram-ve rods. Antibiotic Resistance Pattern: Ag 50 Ap100 Gm50 Nm100.

5. TR3b — Rhizobium from Lab lab purpureus. Gram-ve rods. Antibiotic Resistance Pattern: Ag100 Ap100 Nm100 Sm100.

6. TR4 — Rhizobium from Lab lab purpureus. Gram-ve bacilli. Antibiotic Resistance Pattern: Ag50 Ap100 Nm50 Sm50.

7. TR5 — Isolated from Lab lab purpureus. Gram-ve rods. Antibiotic Resistance Pattern: Ag100 Ap100 (Partial) Cm50 Km100 Nm100 Sm100.

8. TR6 — Rhizobium from Lab lab purpureus. Gram-ve rods. Antibiotic Resistance Pattern: Ag100 (Partial) Cm100 Gm100 Nm100 Sm100.

9. TR7 — Rhizobium from Lab lab purpureus. Gram-ve bacilli. Antibiotic Resistance Pattern: Ag100 Ap100 Gm50 Nm50 Sm100.

10. TR8a — Rhizobium from Lab lab purpureus. Gram-ve rods. Antibiotic Resistance Pattern: Ag50 Ap100 Nm50.

11. TR11a — Rhizobium from Lab lab purpureus. Gram-ve rods. Antibiotic sensitive.

12. TR13b — Rhizobium from Lab lab purpureus. Gram-ve rods. Antibiotic Sensitive.

13. TR14 — Rhizobium from Lab lab purpureus. Gram-ve rods. Antibiotic Resistance Pattern: Ap100 (Partial) Cm100.

14. TR15a — Rhizobium from Lab lab purpureus. Gram-ve plump bacilli. Antibiotic Resistance Pattern: Ap100 Cm100 Nm100 Sm50.

15. TR16a — Rhizobium from Lab lab purpureus. Gram-ve rods. Antibiotic Resistance Pattern: Ag100 Cm100 Gm100 Nm50 Sm100.

16. TR16b — Rhizobium from Lab lab purpureus. Gram-ve rods. Antiboitic Resistance Pattern: Ag100 (Partial) Ap100 Gm100 (Partial) Nm100 Sm100.

17. TR17 — Xanthomonas malvecerum from cotton. Gram-ve rods. Antibiotic Resistance Pattern: Nm50.

18. TR18 — Xanthomonas from Lemon Plant: Gram-ve rods. Antibiotic Resistance Pattern: Ag50 Ap100 Nm50.

19. TR19 — Isolated from Chico Plant. Gram-ve rods. Light yellow pigmentation. Antibiotic Resistance Pattern: Ap100.

20. TR20 — Isolated from Guava. Gram-ve rods. Yellow colour colonies. Antibiotic Resistance Pattern: Sm100.

21. TR21 — Isolated from Lemon fruit. Gram-ve short rods. Dirty white colony. Antibiotic Resistance Pattern: Ap100 Cm50.

22. TR22 — Isolated from Lemon. Gram-ve Rods. Dirty white colony with dark centres. Antibiotic Resistance Pattern: Ag50 Ap100 Gm100 Nm100 Sm100.

23. TR23 — Isolated from Mango leaf. Gram-ve short rods. White colonies with dark centres. Antibiotic Resistance Pattern: Ag100 Gm100 Nm100 Sm100.

24. TR24 — Isolated from Lemon leaf. Gram-ve rods. White colonies with dark centres. Antibiotic Resistance Pattern: Ag100 Ap100 Cm50 Gm100 Nm100.

25. TR25a — Isolated from Spinach. Gram-ve rods scattered. Antibiotic Resistance Pattern: Ap100.

26. TR25b Isolated from Spinach. Gram-ve rods. Latose fermenting. Antibiotic Resistance Pattern: Km100 Nm100.

27. TR26a Isolated from Bringel. Gram-ve rods. Lactose fermenting. Antibiotic Resistance Pattern: Ap100.

28. TR26b Isolated from Bringel. Gram-ve rods scattered non lactose fermenting. Antibiotic Resistance Pattern: Ap100.

29. TR26c Isolated from Bringel. Gram-ve rods. Lactose fermenting. Antibiotic Resistance Pattern: Ag100 Ap100 Sm100.

30. TR27a Isolated from Same bean. Gram-ve thick rods. Lactose fermenting. Antibiotic Resistance Pattern: Ap100.

31. TR27b Isolated from Same bean. Gram-ve rods. Non lactose fermenting. Antibiotic Resistance Pattern: Ap100.

32. TR27c Isolated from Same bean. Gram-ve rods. Lactose fermenting. Antibiotic Resistance Pattern: Ap100 Cm100 Km100.

33. TR28c Isolated from Cucumber. Gram-ve thick rods. Lactose fermenting. Antibiotic Resistance Pattern: Ag100 Ap100 Sm100.

34. TR30a Isolated from Karilla. Gram-ve cocco bacilli. Lactose fermenting. Antibiotic Resistance Pattern: Ag100 Ap100 Km100.

35. TR30b Isolated from Karilla. Gram-ve short rods. Antibiotic Resistance Pattern: Ag100 Cm100 Sm100.

36. TR31a Isolated from Cucumber. Gram-ve cocco bacilli. Lactose fermenting. Antibiotic Resistance Pattern: Antibiotic sensitive.

37. TR31b Isolated from Cucumber. Gram-ve cocco bacilli. Non lactose fermenting. Antibiotic Resistance Pattern: Ag100 Cm100.

38. TR32a Isolated from Spinach. Gram-ve rods. Lactose fermenting. Antibiotic Resistance Pattern: Ag100 Ap100.

39. TR32b Isolated from Spinach. Gram-ve thick rods. Lactose fermenting. Antibiotic Resistance Pattern: Ag100 Ap100 Cm100 Sm100.

40. TR33a Isolated from Chilli. Gram-ve cocco bacilli. Lactose fermenting. Antibiotic Resistance Pattern: Ag100 Ap100 (Partial) Cm100 Km100.

41. TR33b Isolated from Chilli. Gram-ve cocco bacilli. Lactose fermenting. Antibiotic Resistance Pattern: Ag100 Ap100.

42. TR34a Isolated from Potato. Gram-ve thick rods. Lactose fermenting. Antibiotic Resistance Pattern: Ag100 Ap100 Gm100.

43. TR34b Isolated from Potato. Gram-ve cocco bacilli. Lactose fermenting. Antibiotic Resistance Pattern: Ap100.

44. TR35a Isolated from Karilla. Gram-ve rods. Lactose fermenting. Antibiotic Resistance Pattern: Ag100 Ap100 (Partial) Cm100 Gm100 Km100 Sm100.

45. TR35b Isolated from Karilla. Gram-ve rods. Lactose fermenting. Antibiotic Resistance Pattern: Gm100.

46. TR36a Isolated from Lady finger. Gram-ve rods. Non lactose fermenting. Antibiotic Resistance Pattern: Ag100 (Partial) Ap100.

47. TR36b Isolated from Lady finger. Gram-ve cocco bacilli. Lactose fermenting. Antibiotic Resistance Pattern: Ag100 Ap100.

48. TR37a Isolated from Mango. Gram-ve cocco bacilli. Lactose fermenting. Antibiotic Resistance Pattern: Ag100 Ap100.

49. TR37b Isolated from Mango. Gram-ve cocco bacilli. Non lactose fermenting. Antibiotic Resistance Pattern: (Partial) Ap100.

50. TR38a Isolated from Cucumber. Gram-ve cocco bacilli. Lactose fermenting. Antibiotic Resistance Pattern: Antibiotic sensitive.

51. TR38b Isolated from Cucumber. Gram-ve cocco bacilli. Non lactose fermenting. Antibiotic Resistance Pattern: Ag100 Ap100 Cm100.

52. TR39 Isolated from Karilla. Gram-ve large rods. Lactose fermenting. Antibiotic Resistance Pattern: Ag100 Ap100.

53. TR40b Isolated from Mango. Gram-ve cocco bacilli. Lactose fermenting. Antibiotic Resistance Pattern: Ap100.

54. TR41 Isolated from Lemon. Gram-ve rods. Lactose fermenting. Antibiotic Resistance Pattern: Ag100 (Partial) Ap100.

55. TR42 Isolated from Same bean. Gram-ve cocco bacilli. Lactose fermenting. Antibiotics Resistance Pattern: Ag100 Ap100.

56. TR43a Isolated from Cucumber. Gram-ve cocco bacilli. Lactose fermenting. Antibiotic Resistance Pattern: Ag100 (Partial) Ap100 Cm100.

57. TR43b Isolated from Cucumber. Gram-ve cocco bacilli. Lactose fermenting. Antibiotic Resistance Pattern: Ap100.

58. TR45a Isolated from Cucumber. Gram-ve cocco bacilli. Lactose fermenting. Antibiotic Resistance Pattern: Ag100 Ap100.

59. TR45b Isolated from Cucumber. Gram-ve cocco bacilli. Non lactose fermenting. Antibiotic Resistance Pattern: Ag100 Ap100.

60. TR46 Isolated from Pumpkim. Gram-ve cocco bacilli. Lactose fermenting. Antibiotic Resistance Pattern: Ap100.

61. TR47a Isolated from Lemon. Gram-ve cocco bacilli, dirty while colonies. Antibiotic Resistance Pattern: (Partial) Ap100.

62. TR47b Isolated from Lemon. Gram-ve rods, slight blue color colonies. Antibiotic Resistance Pattern: (Partial) Ap100.

63. TR47c Isolated from Lemon. Gram-ve rods mucoid colonies. Antibiotic Resistance Pattern: Ag100 Ap100.

64. TR48 Isolated from Lemon. Gram-ve rods dirty while colonies. Antibiotic Resistance Pattern: (Partial) Ap100 (Partial) Cm100.

65. TR49 Isolated from Lemon. Gram-ve cocco bacilli dirty white colonies. Antibiotic Resistance Pattern: Ap100.

66. TR50a Isolated from Coriander. Gram-ve rods, blue color colonies. Antibiotic Resistance Pattern: Ag100 Ap100 Cm100.

67. TR50b Isolated from Coriander. Gram-ve rods, dirty white colonies. Antibiotic sensitive.

68. TR51 Isolated from Tomato. Gram-ve rods, dirty white colonies. Antibiotic Resistance Pattern: Ag100 Ap100.

69. TR52a Isolated from Tomato. Gram-ve cocco bacilli, dirty white colonies. Antibiotic Resistance Pattern: Ap100.

70. TR52b Isolated from Tomato. Gram-ve cocco bacilli, light bluish grey colonies. Antibiotic Resistance Pattern: Ag100 Ap100.

71. TR52c Isolated from Tomato. Gram-ve cocco bacilli, shining colonies. Antibiotic Resistance Pattern: Ap100.

72. TR53a Isolated from Spinach. Gram-ve cocco bacilli, red mucoid colonies. Antibiotic Resistance Pattern: Ag100 Ap100 Km100 Tc100.

73. TR53b Isolated from Spinach. Gram-ve cocco bacilli, dirty white colonies. Antibiotic Resistance Pattern: Ag100 (Partial) Ap100.

74. TR53c Isolated from Spinach. Gram-ve rods, dirty white colonies. Antibiotic sensitive.

75. TR53d Isolated from Sinach. Gram-ve rods, mucoid colonies. Antibiotic Resistance Pattern: (Partial) Ap100.

76. TR53e Isolated from Spinach. Gram-ve thick rods mucoid colonies. Antibiotic Resistance Pattern: Ap100.

77. TR54a Isolated from Fenugreek. Gram-ve cocco bacilli, dirty white colonies. Antibiotic Resistance Pattern: Ap100.

78. TR54b Isolated from Fenugreek. Gram-ve cocco bacilli, dirty white colonies. Antibiotic Resistance Pattern: Ag100 Ap100.

79. TR55b Isolated from Sagh Sarsoon Sesame. Gram-ve short rods, slight bluish colonies. Antibiotic Resistance Pattern: Ap100.

80. TR56 Isolated from Sagh Mako Cape goose berry. Gram-ve cocco bacilli, dirty white colonies. Antibiotic Resistance Pattern: Ap100.

81. TR57 Isolated from Podinah Mint. Gram-ve short bacilli, blue color colony. Antibiotic Resistance Pattern: Ap100.

82. TR58a Isolated from Potato. Gram-ve short rods, yellow shining colonies. Antibiotic Resistance: Ap100.

83. TR58b Isolated from Potato. Gram-ve cocco bacilli, slight bluish colonies. Antibiotic Resistance Pattern: Ap100.

84. TR59 Isolated from Bottle gourd. Gram-ve rods, dirty white colonies. Antibiotic Resistance Pattern: Ap100.

85. TR60 Isolated from Bottle gourd. Gram-ve rods, yellow pigmentation. Antibiotic Resistance Pattern: Ag100 Ap100.

86. TR61 Isolated from Bottle gourd. Gram-ve short rods, yellow diffusable pigment. Antibiotic Resistance Pattern: Cm100.

87. TR62 Isolated from Onion. Gram-ve cocco bacilli, bluish white colonies. Antibiotic Resistance Pattern: Ag100 (Partial) Ap100 (Partial) Cm100

88. TR63 Isolated from Onion. Gram-ve cocco bacilli, yellow color colonies. Antibiotic Resistance Pattern: Ag100 Ap100.

89. TR64 Isolated from Onion. Gram-ve cocco bacilli in pairs. Antibiotic sensitive.

90. TR65 Isolated from Onion. Gram-ve cocco bacilli, dirty white colonies. Antibiotic Resistance Pattern: Ag100 Ap100.

91. TR66 Isolated from Onion leaf. Gram-ve cocco bacilli, mucoid shining colonies. Antibiotic Resistance Pattern: Ap100.

92. TR67 Isolated from Onion leaf. Gram-ve cocco bacilli, Scattered red colonies. Antibiotic Resistance Pattern: Ap100 Ag100.

93. TR68 Isolated from Onion leaf. Gram-ve cocco bacilli, slight yellow pigmented colonies. Antibiotic Resistance Pattern: Ag100 Ap100 Sm100.

94. TR69 Isolated from Onion leaf. Gram-ve cocco bacilli, yellow diffusable pigment. Antibiotic Resistance Pattern: Ap100.

95. TR70 Isolated from Cauli-flower. Gram -ve cocco bacilli, bluish white pigment. Antibiotic Resistance Pattern: Ap100 Nm100.

96. TR71 Isolated from Cauli-flower. Gram -ve cocco bacilli. Antibiotic Resistance Pattern: Ap100.

97. TR72 Isolated from Potato. Gram -ve cocco bacilli in pairs, yellow diffusable pigment. Antibiotic Resistance Pattern: Ap100 Nm100.

98. TR73 Isolated from Potato. Gram -ve rods, bluish white color colonies. Antibiotic Resistance Pattern: Ap100.

99. TR74 Isolated from Potato. Gram -ve cocco bacilli, yellow pigmented. Antibiotic Resistance Pattern: Ag100 Ap100 Gm100 Sm100.

100. TR75 Isolated from Potato. Gram -ve cocco bacilli, dirty white colonies. Antibiotic Resistance Pattern: Ag100 Ap100.

101. TR76 Isolated from Peas. Gram -ve thick rods, white colonies with dark centres. Antibiotic sensitive.

102. TR77 Isolated from Peas. Gram -ve cocco bacilli, dirty white colonies. Antibiotic Resistance Pattern: Ap100.

103. TR78 Isolated from Peas. Gram -ve cocco bacilli. Antibiotic sensitive.

104. TR79 Isolated from Tomato. Gram -ve cocco bacilli, bluish white colonies. Antibiotic sensitive.

105. TR80 Isolated from Chilli. Gram -ve cocco bacilli, red color colonies. Antibiotic Resistance Pattern: Ag100 Ap100 Sm100.

106. TR81 Isolated from Chilli. Gram -ve cocco bacilli, yellow diffusable pigment. Antibiotic Resistance Pattern: Ag100 Ap100.

107. TR82 Isolated from Chilli. Gram -ve cocco bacilli, blue color colonies. Antibiotic Resistance Pattern: Cm100.

108. TR83 Isolated from Chilli. Gram -ve short bacilli, dirty while colonies. Antibiotic Resistance Pattern: Ag100.

109. TR84 Isolated from Carrot. Gram -ve short rods, yellow diffusable pigment. Antibiotic Resistance Pattern: Ag100 Ap100.

110. TR85 Isolated from Carrot. Gram -ve cocco bacilli, yellow diffusable pigment. Antibiotic sensitive.

111. TR86 Isolated from Podinah Mint. Gram -ve short rods. Antibiotic Resistance Pattern: Ap100.

112. TR87 Isolated from Podinah Mint. Gram -ve cocco bacilli, blue color colonies with dark centres. Antibiotic Resistance Pattern: Ag100 (Partial) Ap100 Cm100.

113. TR88 Isolated from Podinah Mint. Gram -ve cocco bacilli, dirty white colonies. Antibiotic sensitive.

114. TR89 Isolated from Podinah Mint. Gram -ve cocco bacilli, red color colonies. Antibiotic Resistance Pattern: Ag100 Ap100.

115. TR90 Isolated from Podinah Mint. Gram -ve bacilli white colonies. Antibiotic Resistance Pattern: (Partial) Ap100.

116. TR91 Isolated from Coriander. Gram -ve large rods, mucoid yellow pigmented. Antibiotic Resistance Pattern: Ap100.

117. TR92 Isolated from Lady finger. Gram -ve short rods, yellow colonies with dark centres. Antibiotic Resistance Pattern: Ag100 Ap100.

118. TR93 Isolated from Lady finger. Gram -ve rods mucoid colonies. Antibiotic sensitive.

119. TR94 Isolated from Lady finger. Gram -ve cocco bacilli, yellow diffusable pigment. Antibiotic Resistance Pattern: Ag100 Ap100.

120. TR95 Isolated from Fenugreek. Gram -ve cocco bacilli, red in color. Antibiotic Resistance Pattern: Ag100 Ap100 Sm100.

121. TR96 Isolated from Fenugreek. Gram -ve short rods, dark centres colonies. Antibiotic Resistance Pattern: (Partial) Ag100 Ap100 Cm100.

122. TR97 Isolated from Fenugreek. Gram -ve short rods, bluish colonies with dark centres. Antibiotic Resistance Pattern: Ag100.

123. TR98 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

124. TR99 Xanthomonas citri from citrus canker. Gram -ve, straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100.

125. TR100 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100.

126. TR101 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

127. TR102 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

128. TR103 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ag100 Ap100.

129. TR104 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Anbiotic Resistance Pattern: Ap100.

130. TR105 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

131. TR106 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern; Ap100.

132. TR107 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

133. TR108 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

134. TR109 Xanthomonas citri form citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ag100.

135. TR110 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

136. TR111 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ag100 Ap100.

137. TR112 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100.

138. TR113 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic senstive.

139. TR114 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

140. TR115 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100.

141. TR116 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

142. TR117 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

143. TR118 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100.

144. TR119 Xanthomonas citri from citrus canker. Gram -ve straight rods, Yellow pigmented colonies. Antibiotic sensitive.

145. TR120 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

146. TR121 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

147. TR122 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

148. TR123 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ag100 Ap100.

149. TR124 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmentedcolonies. Antibiotic sensitive.

150. TR125 Xanthomonas citri from citrus canker. Gram -ve rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100.

151. TR126 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

152. TR127 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

153. TR128 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

154. TR129 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

155. TR130 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ag100 Ap100 Km100 Nm100 Sm100.

156. TR131 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

157. TR132 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

158. TR133 Xamthomonas campestris pv citri from N.A.R.C. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

159. TR134 Xanthomonas malvecearum from angular leaf spot of cotton. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

160. TR135 Xanthomonas malvecearum from angular leaf spot of cotton. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100.

161. TR136 Xanthomonas malvecearum from angular leaf spot of cotton. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

162. TR137 Xanthomonas citri from citrus canker. Gram -ve straight rods, yelllow pigmented colonies. Antibiotic Resistance Pattern: Ag100 Ap100 Nm100 (Partial).

163. TR138 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100.

164. TR139 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ag100 Ap100.

165. TR140 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ag100 Ap100.

166. TR141 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ag100 Ap100.

167. TR142 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100.

168. TR143 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100.

169. TR144 Xanthomonas malvecearum from angular leaf spot of cotton. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100.

170. TR145 Xanthomonas malvecearum from angular leaf spot of cotton. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100 (Partial).

171. TR146 Xanthomonas malvecearum from angular leaf spot of cotton. Gram -ve straight rods, yellow pigmented colonies. Antibiotic sensitive.

172. TR147 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100 Cm100.

173. TR148 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ag100 Ap100 Nm100 (Partial).

174. TR149 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100 (Partial) Cm100 (Partial).

175. TR150 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100.

176. TR151 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ag100 (Partial) Ap100.

177. TR152 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ag100 Ap100.

178. TR153 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ag100 Ap100.

179. TR154 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100 Cm100 (Partial).

180. TR155 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100.

181. TR156 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ag100 Ap100 Nm100.

182. TR157 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100.

183. TR158 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ag100(Partial) Ap100.

184. TR159 Xanthomonas citri form citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ag100 Ap100.

185. TR160 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ag100 Ap100.

186. TR161 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Cm100.

187. TR162 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100.

188. TR163 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100.

189. TR164 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ag100 Ap100.

190. TR165 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100.

191. TR166 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100.

192. TR167 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ap100 (Partial) Cm100 (Partial).

193. TR168 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antbiotic Resistance Pattern: Ag100 (Partial) Ap100.

194. TR169 Xanthomonas citri from citrus canker. Gram -ve straight rods, yellow pigmented colonies. Antibiotic Resistance Pattern: Ag100 Ap100.

"Enteric Bacteria and their Antibiotic Resistance Patterns"

Note: A = ampicillin C = chloramphenicol
G = gentamycin K = kanamycin
N = neomycin S = streptomycin
and T = tetracycline

All antibiotics were used at a concentration of 100 ug/ml.

1.	Escherichia	AGNST
2.	Escherichia	ANS
3.	Escherichia	ACNT
4.	Escherichia	ACST
5.	Salmonella	KNS
6.	Escherichia	AT
7.	Escherichia	ACKNST
8.	Escherichia	AGT
9.	Escherichia	AS
10.	Escherichia	Sensitive to all antibiotics.
11.	Escherichia	CKNST
12.	Klebsiella	ACST
13.	Escherichia	ACNST
14.	Escherichia	T
15.	Escherichia	AGKN
16.	Escherichia	CT
17.	Escherichia	ACKST
18.	Escherichia	N
19.	Escherichia	KST

20.	Escherichia	Sensitive to all antibiotics.
21.	Escherichia	Sensitive to all antibiotics.
22.	Escherichia	ST
23.	Escherichia	ACKNT
24.	Escherichia	AKS
25.	Pseudomonas	AKNST
26.	Klebsiella	AKNST
27.	Escherichia	CKNST
28.	Escherichia	ACNT
29.	Escherichia	ACKNT
30.	Escherichia	AGNST
31.	Escherichia	ACST
32.	Pseudomonas	AKNT
33.	Escherichia	AK
34.	Escherichia	AKS
35.	Proteus	ACKN
36.	Klebsiella	AGKNST
37.	Salmonella	ACKST
38.	Escherichia	AN
39.	Escherichia	Sensitive to all antibiotics.
40.	Klebsiella	AST
41.	Escherichia	AKNST
42.	Escherichia	CST
43.	Escherichia	T
44.	Escherichia	ST
45.	Escherichia	AKNS
46.	Escherichia	A

47.	Escherichia	KST
48.	Klebsiella	AGKNST
49.	Escherichia	AGNS
50.	Pseudomonas	AKN
51.	Pseudomonas	ACNT
52.	Escherichia	ACGKNT
53.	Escherichia	Sensitive to all antibiotics.
54.	Salmonella	AKNS
55.	Escherichia	ACKNST
56.	Proteus	ACGKNS
57.	Escherichia	AGKST
58.	Escherichia	ACGKN
59.	Escherichia	ACGKNST
60.	Escherichia	KN
61.	Pseudomonas	AKS
62.	Escehrichia	ST
63.	Escherichia	AGKST
64.	Escherichia	Sensitive to all antibiotics.
65.	Escherichia	ACKNT
66.	Klebsiella	AGKNS
67.	Escherichia	ACST
68.	Escherichia	AT
69.	Klebsiella	Sensitive to all antibiotics
70.	Escherichia	ACGKNST
71.	Salmonella	Sensitive to all antibiotics.
72.	Escherichia	ACKNST
73.	Escherichia	AGT

74.	Escherichia	CT
75.	Escherichia	Sensitive to all antibiotics.
76.	Escherichia	Sensitive to all antibiotics.
77.	Escherichia	KNT
78.	Escherichia	AKT
79.	Escherichia	ACST
80.	Escherichia	AKNST
81.	Escherichia	ACGKST
82.	Escherichia	ACKST
83.	Proteus	Sensitive to all antibiotics.
84.	Proteus	ACGKN
85.	Escherichia	AKS
86.	Escherichia	AT
87.	Escherichia	Sensitive to all antibiotics.
88.	Escherichia	AKNST
89.	Pseudomonas	ACKNT
90.	Klebsiella	AGST
91.	Pseudomonas	ACKNS
92.	Pseudomonas	AGKNS
93.	Escherichia	ACKST
94.	Escherichia	KNST
95.	Escherichia	ST
96.	Salmonella	AT
97.	Escherichia	ACT
98.	Klebsiella	ST
99.	Escherichia	ACGKNST
100.	Escherichia	ACST

101.	Escherichia	KN
102.	Escherichia	ACGKNT
103.	Pseudomonas	AKN
104.	Pseudomonas	ACKN
105.	Escherichia	GS
106.	Escherichia	KNST
107.	Escherichia	ST
108.	Escherichia	A
109.	Escherichia	ACKNST
110.	Escherichia	S
111.	Escherichia	T
112.	Klebsiella	KNST
113.	Escherichia	AST
114.	Escherichia	CKNST
115.	Escherichia	ST
116.	Escherichia	KNT
117.	Escherichia	AST
118.	Pseudomonas	ACNT
119.	Pseudomonas	AK
120.	Escherichia	ACT
121.	Escherichia	KNST
122.	Escherichia	ST
123.	Escherichia	A
124.	Escherichia	T
125.	Salmonella	KNST
126.	Escherichia	KNT
127.	Escherichia	AG
128.	Escherichia	KN
129.	Escherichia	ACGKNST

130.	Escherichia	ACGT
131.	Shigella	ST
132.	Escherichia	AKN
133.	Escherichia	A
134.	Escherichia	GS
135.	Escherichia	ACGKNT
136.	Escherichia	AGKN
137.	Escherichia	ACGT
138.	Escherichia	T
139.	Escherichia	KNST
140.	Escherichia	AST
141.	Escherichia	ACKN
142.	Escherichia	ACGKST
143.	Klebsiella	ACKT
144.	Escherichia	ACKNST
145.	Pseudomonas	AS
146.	Escherichia	ST
147.	Escherichia	T
148.	Proteus	ACST
149.	Escherichia	ACKNT
150.	Escherichia	ACGKST
151.	Escherichia	ACKNT
152.	Escherichia	GKNST
153.	Escherichia	ACKT
154.	Escherichia	AK
155.	Escherichia	GS
156.	Proteus	AST
157.	Escherichia	KNST
158.	Escherichia	ACKN

159.	Escherichia	AG
160.	Escherichia	CT
161.	Escherichia	A
162.	Escherichia	KN
163.	Escherichia	AT
164.	Escherichia	G
165.	Escherichia	KNT
166.	Escherichia	KNST
167.	Proteus	ACGKN
168.	Shigela	CST
169.	Escherichia	AST
170.	Escherichia	KNST
171.	Escherichia	ACKNT
172.	Escherichia	ACKT
173.	Escherichia	AT
174.	Escherichia	ANST
175.	Escherichia	GKNST
176.	Escherichia	ACST
177.	Pseudomonas	GST
178.	Escherichia	S
179.	Escherichia	K
180.	Escherichia	AN
181.	Escherichia	KNT
182.	Escherichia	CT
183.	Escherichia	T
184.	Escherichia	A
185.	Escherichia	KNST
186.	Escherichia	S
187.	Escherichia	KNS

188.	Escherichia	AG
189.	Escherichia	KNST
190.	Escherichia	S