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Research Article

Concept for Possible Development of Vaccine against Corona in *Escherichia coli* K-12, Yale C600 Strain

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Received: April 25, 2020; **Published:** June 29, 2020

Abstract

An AAIR (Anti-adherent Immune Response) as vaccine against n-COVID-19 could possibly be developed in support of Genetically Engineered (GE) *Escherichia coli* k-12, C600 Yale strain, if cloned with any of spike protein of infected n-COVID-19 virus and ACE-I and II receptor protein genes of lung cells. The developed antibody will suppress/block ACE- I and II (Angiotensin Converting Enzyme) of the lung cells. Antibody development against infected spike protein of n-COVID-19 would be difficult, since the n-COVID-19 viruses are changing their character rapidly. The receptor proteins of lung cells are more stable immunologically to develop antibodies. These antibodies will remain active to block the receptor of the lung cells, as soon as these antibodies will recognise that virus n-COVID-19 is entered body and is attempting to attack by changing body metabolic system. To develop hybrid genetically engineered (GE) *Escherichia coli* k-12 C600 Yale strain, the personal knowledge of the author with 026:EPEC (Enteropathogenic *Escherichia coli*) and the antibody development with GE bacteria has been highlighted. An AAIR against 026:EPEC fatal diarrhoea was possible to develop, when a GE hybrid *E. coli* k-12, MRHU (+) was possible to design and when MRHU (+) 95 MD, plasmid was possible to transfer into *E. coli* k-12 C600 Nalidixic acid (nal) and Streptomycin (sm) resistant recipient (F^-) Yale strain by Co-conjugation. At (F^+ x F^+ pRT x F^-) conjugations and at vigorous selections, *in vivo* on selective agars. A GE Hybrid *E. coli* k-12, 5405, GE *E. coli* k-12 at 10^{-3} frequency was possible to isolate and was used in AAIR on Balb/c mice. Mice were protected against fatal diarrhoea of 026:EPEC when the donors (wild types) were challenged on same Balb/c mice. The results were reviewed, and the insight of those findings are being used in the present AAIR model to develop of n-COVID-19 vaccine, as ultimate solution to protect mankind. Since SARS-COV-2, virus and n-COVID-19 infection possess very unknown nature of spreading. As per the author AAIR for n-COVIC-19 would be very complicated, complex, the combined model of coccus/bacteroid and retrovirus even in their adhering and propagating natures on the surface and the inside of the lung cells. The experimental knowledge derived from 026: EPEC AAIR on Balb/c are therefore been partially used in this concept.

Keywords: *Vaccine; Corona Virus; Escherichia coli K-12*

Introduction

During 1980 - 1986, the author was involved as doctorand fellow in Max-Planck Institute, Germany to study various EPEC (Enteropathogenic *Escherichia coli*), ETEC (Enterotoxigenic *Escherichia coli*) and UTI (Urinary tract Infective *Escherichia coli*) strains, their surface antigens (the expression of fimbriae/pili) and MRHU (+, D-Mannose Resistant Hemagglutination of Human Erythrocytes), the presence of their plasmids responsible for HA (hemagglutination). Among all studied strains, 026: EPEC (Enteropathogenic *Escherichia coli*) was most interesting. The strain was positive in vitro to MRHU (+), expressed on china clay sera-plates at 4°C. 1 - 2 micrometre (um) diametric erythrocyte. The granules were developed, if added with human blood cells (erythrocytes), extracted without serum and lymphocytes. The method was applied also for different strains and different animal (Horse, Bovine, Guinea pig) and birds (chicken) bloods. The bloods were mixed with D-mannose sugar and anticoagulant potassium oxalate). Phenotypically the strain expressed 1 - 10 cm long and 10 - 20 nm diametric fimbriae/pili in SEM (Scanning Electron Microscopy), (Figure 1). MRHU (+) strains, their outer most surface antigenic fimbriae properties were routine wise studied and reviewed. Genetics involved in expressions of MRHU (+) and fimbriae in SEM were investigated and compared to find out their pathogenic correlation ships. The pathogenic mode and colonization properties/adherence of bacteria, MRHU (+) were compared. Agarose Gel Electrophoresis (AGE) for the studies of plasmid profiles were used to establish and to correlate the genotypic relations of those investigated ETEC, EPEC and UTI strains caused serious illness among

animal and human. Plasmid profiles as expressed in OC (open coil/ relaxed), CC (closely covalent) and CCC (closely covalent and circular) were also used to study the reason for expressing fimbriae, antibiotic resistance, the reason for colonization/adherence and the mode of their infective modes in spreading infections. Among all strains 026: EPEC, MRHU (+) was interesting. To establish plasmid based *E. coli* infections, the expression of fimbriae, MRHU (+) and the colonization, a hybrid genetically engineered (GE) *E. coli* k-12 strain was essential and to confirm the plasmid based *E. coli* infection and to use the said hybrid strain in serological reaction, HA typing and perhaps to use the same to generate anti adherent immune response (AAIR) for basic studies and for vaccine to protect animal and human against diarrhoea and *E. coli* infections [1-10].

Materials and Methods

The materials were purchased mainly from Sigma and partially from Merck. The instruments, like Beckman-Ultracentrifuge, Remi-Table Centrifuge, Autoclave, Microscope, Laminar Hood. UV lamp, Cantillation counter and SEM (Scanning Electron Microscopy) studies were supported by MPI-Germany, The complete microbiological studies as used for Co-conjugations and Plasmid Genetic Engineering experiments, Southern, Northern-hybridizations, Reverse transcriptase, Restriction Enzymes, as used for DNA chopping, mapping and labelling were all available in MPI-Germany and were used extensively with all satisfaction. All the selective strains, the donors 026:EPEC, their mutants and GE- Hybrids were kept in MPI. Since there was at that material time no PCR, we had to prepare DNA labelling manually and to use them in all unknown DNA hybridization to locate their presence in our hybrid recombinant DNA. Radio isotope labelling were prominent. Radio isotopic p32 alfa d ATP were purchased from Sigma and were used in addition of reverse transcriptase the probe DNA for labelling. To identify the presence of donor plasmid DNA in the hybrids, GE-*Escherichia coli* k-12 C600NS strains. Isotopic labelling was made carefully under protected environment. Balb/c mice and animal house were provided by the MPI-Germany. All the methods were being incorporated partially in result and discussion [1-5].

Results and Discussion

Figure 1 represents the SEM view of phenotypic nature of isolated fimbriae (pili) and the cell conformation of two 026:EPEC (Enteropathogenic *Escherichia coli*) as top view and 08:ETEC (Enterotoxigenic *Escherichia coli*) at the bottom. SEM view were taken at the magnification of X90,000 + 10,000 photographic enlargements. From the changing pattern of fimbriae (pili), right top and right bottom their mode of colonization on the surface of villi of the intestines would be different.

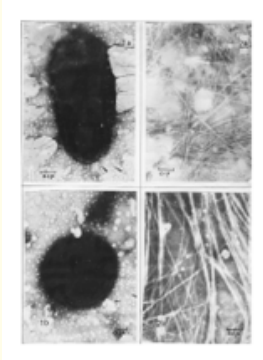


Figure 1

Figure 2 represents, MRHU (+/-) blood granules. *In vitro* HA-typing signified partially that the colonization/adherence of investigated *E. coli* are different. As per the opinion of the author, the studies of HA-typing could partially provide different colonization and pathogenic properties of *E. coli* 026:EPEC, r as 20075a; 08:ETEC, toxigenic *E. coli* as 20800, their mutants as 20890, UV, nal®, the hybrid *E. coli*, as 5405 MRHU (+) auxotroph and recipient E. as K-12, C600, non-pathogen, MRHU (-) as C600 NS, were reproduced and showed different granular and non- granular HA-complex. Serotyping with rabid serum was also performed to correlate the HA and Sera-typing (Figure 3).

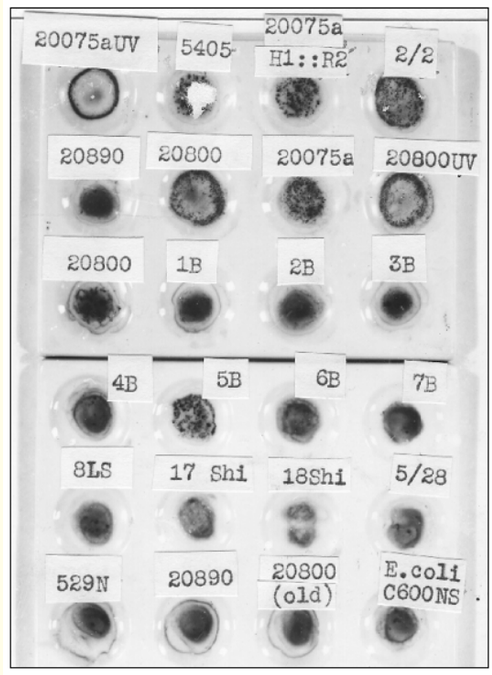


Figure 2

Strains and Serotyping	20075a 026:	20075aUV NT	5405 NT	5406 NT	20075a NTG,NT	20800 08:	20890 UV,NTG conjugate of *E.coli* K-12 and nal® 08:
AS:026:	+++	+++	---	+++	+++	---	---
AS:026 # 026: UV NTG	+++	+++	---	---	---	---	---
AS:08:(#) AS K88	+++	---	---	---	---	---	---
AS:08:	---	---	---	+++	---	+++	+++
AS:08:(#) 08: nal® mutant	---	---	---	---	---	+++	---
AS:0:K17	---	---	---	---	---	---	---
AS:018:(#) 018:K(-): pil(-)	---	---	++	++	---	++	---
AS:K-12	---	---	++	++	---	---	+++
AS CFA-I	---	---	---	++	---	+++	----
AS CFA-II	---	---	+++	++	---	+++	----
MRHU	+++	+	+++	+	---	+++	----
MRBO	+	---	---	---	---	+++	+++
MRCH	NT	NT	NT	NT	NT	NT	NT
Fimbriae=BNT =pili	---	----	+/-	+++	---	+++	+++
lac(-)	+++	+++	+++	---	+++	+++	+++
gal(-)	+++	+++	+++	+++	+++	+++	+++
IS1	NT	NT	+++	++	NT	++	++
Lambda (λ)-RT	---	---	++	+++	---	---	---

Figure 3

Figure 4 represents the immunological profiles, as conceptualised by the author after mice experiments. The negative, slow and rapid mortality of mice supported to design this insight. MRHU (+) hybrid 5405 and *E. coli* K-12 recipient were all non-pathogen to mice. Balb/c,18 weeks old male mice were used. Intra-peritoneal infection to mice. 02 mg/0.2 ml (i.e. 100 cells/ml) were used to inoculate mice in series. The derived isolate strains were used in mice experiment and to design the model (Figure 4). The mortality of mice was characterised by: (a) Wild type 026:EPEC killed mice overnight in 12 hours, (b) 026EPEC nal (r), killed mice in 48 hours. (b) 5404 hybrid GE *E. coli* K-12, sm®, nal®, tet®, leu-, meth-, vit12 positive hybrid did not kill any mice [1,2]. MRHU (+) plasmids were used to generate hybrid *E. coli* k-12, GE MRHU (+) auxotrophic strains for future uses. Molecular biology, Plasmid Genetic Engineering and Microbiology work were carried out, reproduced and studied [1-3]. The plasmid genetic properties of 026:EPEC, the presence of IS1 (Insertion Sequence

1) in hybrids, mutants and donors wild type was also carried out to observe, whether the presence of antibiotics could influence the expression of fimbriae (pili) and their colonization in corresponding to MRHU (+/-) expression (Figure 5a-5d).

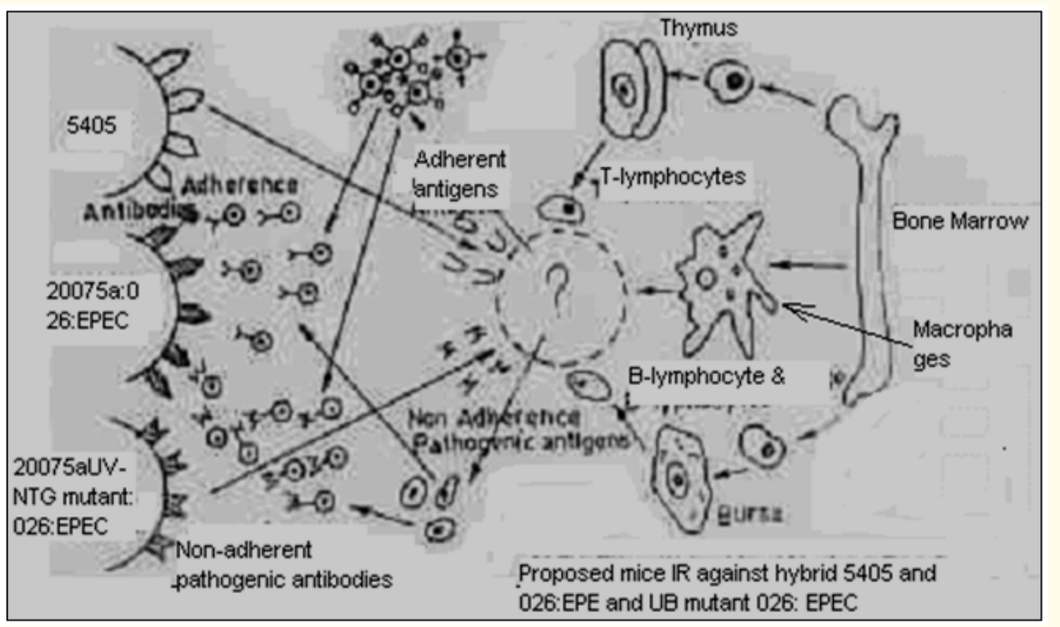


Figure 4

Figure 5a-5d show the (a) plasmid profile, slot1: ETEC, slot 2: its Nal mutant; slot 3. The slot 4 the 026:EPEC UV resistant Mutant 26:EPEC; slot 5 the hybrid *E. coli* k-12 5405. (b) SDS-PAGE of SA proteins of hybrid, *E. coli* k-12 recipient and the donor 026:EPEC, surface antigenic fimbriae (pili) protein. (c) IS1 hybridization and SEM figures of 5405 *E. coli* K-12. Their Fimbriae (pili) expression on their outmost surface were slightly varied (Figure 4c). SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel electrophoresis of 5405 *E. coli* K-12 hybrid. The presence of MRHU (+) were compared corresponding to HA-typing and the statistics for the presence of OC plasmid.

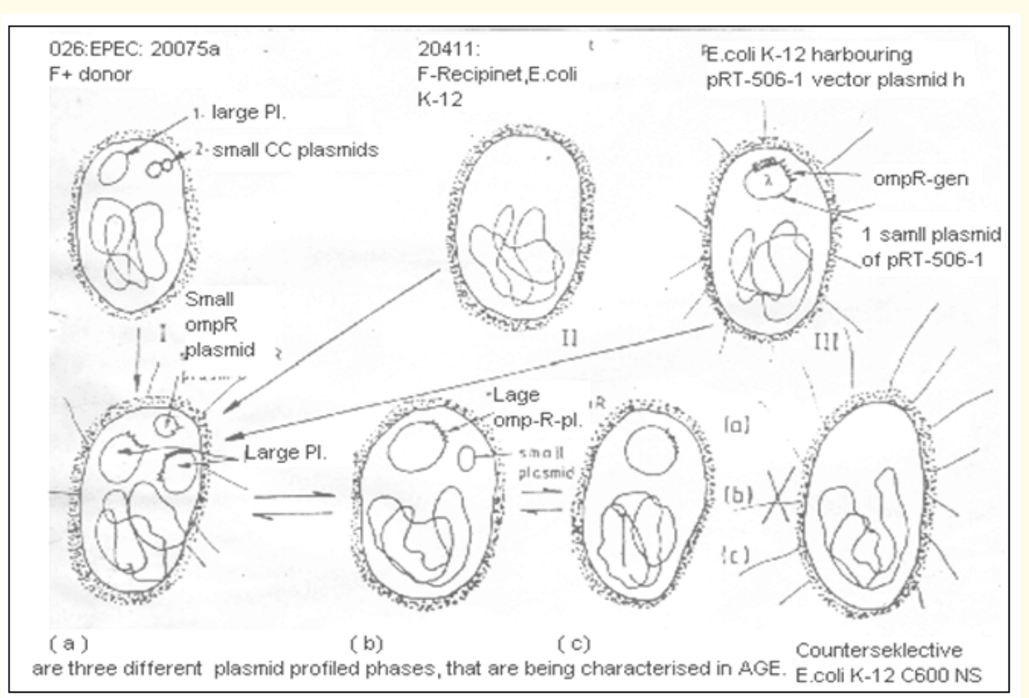
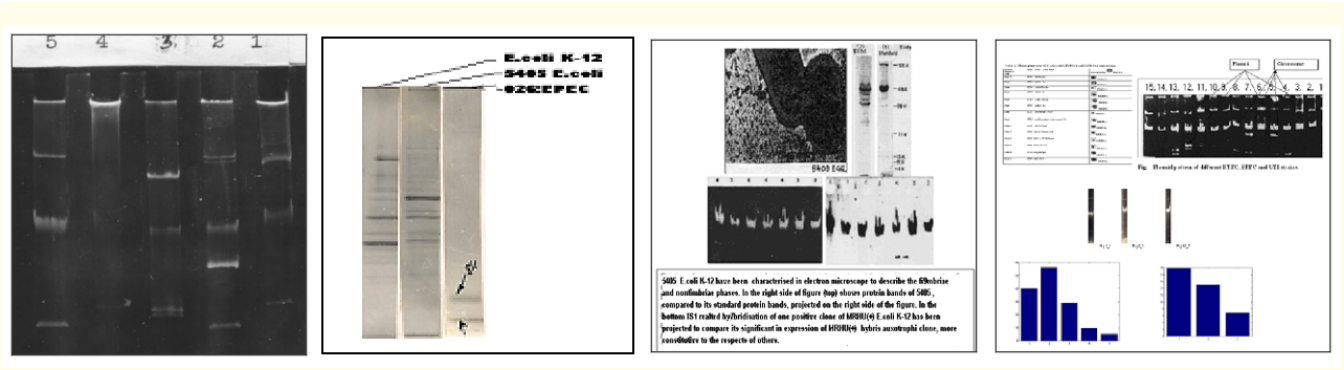


Figure 5

To continue genetic engineering and molecular biology of MRHU (+), large scale plasmid isolation was essential The donor/wild type 026:EPEC MRHU (+) plasmid was isolated and purified in CsCl (Caesium Chloride) density gradient at 75,000 rpm in Beckman Ultracentrifuge. Ethidium bromide (Et-Br) intercalated DNA from the gradient under UV was isolated by the needle from the nitrocellulose centrifuge tubes. Et-Br was isolated by CCL_4 (Tetra Chlorine Carbon) mixed and centrifuged at 8000 rpm for 2 minutes. Red Et-Br mixed CCL_4 at the bottom was removed from the bottom of the centrifuge tube. The remaining Et-Br free plasmid in water phase of the donor

026:EPEC and hybrid *E. coli* were dialysed by TES (Tris EDTA saline buffer pH 7.8), putting DNA into parchment membrane tube sealed, overnight at cold, 4°C sterile in a glass pot, immersed and in presence of small magnetic stirrer to separate salt ion from DNA, removed through parchment membrane. *In vitro*, the hybrid, positive to donor plasmid, as identified by southern hybridization, were all MRHU (-) negatives, at auxotrophic phenol-genotypes. MRHU (+) hybrids, isolated, were all prototroph, and were discarded. Finally, a co-conjugation model and their process was innovative and was initiated to transfer MRHU (+) plasmid into *E. coli* auxotroph (Figure 6). The recipient *E. coli* K-12 (F^-) and one *E. coli* K-12, vector strain, carrying pRT (Plasmid Ronald Taylor) of Yale University, USA, and *E. coli* k-12 C600 Yale strain were mixed in 50 ml conical flasks. Co-conjugations were carried out at various overnight at 30 - 35°C.



(a) *(b)* *(c)* *(d)*

Figure 6

Figure 6 revealed the concept of Co-conjugation in following three *E. coli*, donor x p RT vector plasmid carrying *E. coli* K-12 and recipient F^- C600 Yale strains, involved in Co-conjugation [2].

The revealing results pushed the author to understand as curiosity, whether both the wild type and mutant nal (r) would be same or different. All those three strains were inoculated into mice (Figure 4), to observe their pathogenic and non-pathogenic activities in mice.at low intraperitoneal inoculum. Isolated Genetically Engineered (GE) *E. coli* K-12, MRHU (+) was non-pathogen to mice. Restriction Enzyme cuts in hybrid plasmid was also made to draw a map as per the protocol (Sigma) plasmid cloning and mapping. coR1 (Tet resistant Cite), pST1 (Amp resistant cite) of pBR322 vector plasmid was also identified and reviewed, that recipient received the donor MRHU (+) plasmid as cloned DNA. After long trial and error experimentation, at 10^{-3} frequency, a hybrid, auxotrophic 5405 *E. coli* K-12, MRHU (+) was isolated, that confirmed, that the background is *E. coli* K-12, nal®, sm®, tet®, leu-, meth-,vit-12. The granular properties of MRHU (+) among hybrid, mutant and the wild type/donor 026:EPEC were same. GE-Hybrid. *E. coli* K-12 strain was positive to MRHU (+) and the adhering nature of 026:EPEC MRHU (+), but not their pathogenic nature. Model for possible co-conjugation was reproduced in figure 6. To develop and to confirm AAIR, the whole cells and their isolated fimbriae were also inoculated separately into Balb/C mice. Isolated fimbriae immobilised in araldite membrane were also used to study the microfluidic activities of fimbriae. As BNT (Bio-nano-tube of *Escherichia coli* K-12), GE engineered *E. coli*, with genetic information of cancer cells could be used in future for human cancer treatments [8-10]. Systematically the method was repeated to establish AAIR against 026:EPEC. The positive response of AAIR in mice, protected mice against wild type/donor, at increasing titre, inspired the author to publish and to communicate the said knowledge to you, as possible corona vaccine model with *Escherichia coli* k-12 C600 [4-10].

Conclusion

Today the author thinks whether the same method could be applied to generate AAIR against n-COVID-19 spike protein, expressed at its infective phase and if possible there genes to clone into *E. coli* k-12 to generate hybrid strains. The problem would be in this case to know and to isolate exactly the infective phase of n-COVID-19 with their spikes, like fimbriae (pili). Another possibility would be to block ACE (Angiotensin Converting Enzyme ACE I and II, metabolic activities during n-COVID-19 infections, when SARS COV-2 virus attempts to attach on the surface on lung cell membrane for proliferation and propagation and to damage lung cell to kill patient. After attachment to the lung cells, n-COVID-19 blocks the converting enzymes of ACE-I and II, involved to maintain the lung cell expanding tension. If the same gets success, then we will be in position to generate large scale n-COVID-19 vaccine at global demand through microbial root, *Escherichia coli* k-12 C600 yale strain. It is to be understood, that the isolated hybrid must be in position at auxotrophic, non-pathogenic pheno-genotypes. to develop AAIR in Balb/c mice n-COVID-19 infection. If the same get success, then the same could easily be applied in human, since the immune responsive MHC (Major Histocompatibility Complex/HLA (Histocompatibility Link Antigen) in chromosome 6 in mice and 17 in human, shows the major similarities, responsible for IR (Immune response). *E. coli* is belonging in our metabolic system in GI gastrointestinal tract. AAIR would perhaps be supported by *E. coli*. The author remains confirmed for such possibility. The author is scared to recognise the real infective phase of n-COVID-19 infection, their spike formation. Alternative possibility for cloning spike protein and their involved gens into *E. coli* K-12, bacteriophage technology could be used. To select positive clone on selective antibiotic and amino acid mixed agar plates could be used, where new grown hybrid bacteriophage carrying spike protein could grow. GOD is kind enough to provide us *E. coli* in our GI tract and bacteriophage to clone hybrid strains, that may support AAIR vaccine against Corona. Without vaccine there is no remedy from corona.

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Volume 3 Issue 7 July 2020