Chron

Neil H. Offen 4/10

NANCY –

JUST FYI... and

best regards.

Neil Offen

P.S. Copy sent to Sandy Berger as well.

N.

Printed by TYLER BUSINESS SERVICES — 202-333-6599

4-14-98

To Burkhardt for reply?

✓ yes

— no

Coordinate with NSC?

✓ yes

— no

DIRECT SELLING ASSOCIATION
1666 K Street, NW, Suite 1010, Washington, DC 20006-2808
202/293-5760 • Fax 202/463-4569

Neil H. Offen
President

April 10, 1998

Mr. Kris Engskov
The President's Aide
The White House
1600 Pennsylvania Avenue
Washington, DC

Dear Kris:

This letter is in follow-up to our previous correspondence that the President requested we send via you. Please pass it on to the President. If you have any questions, please let me know.

Sincerely,

DIRECT SELLING ASSOCIATION
1666 K Street, NW, Suite 1010, Washington, DC 20006-2808
202/293-5760 • Fax 202/463-4569

Neil H. Offen
President

April 10, 1998

President William Jefferson Clinton
The White House
1600 Pennsylvania Avenue
Washington, DC

Dear Mr. President:

Our industry's situation continues to worsen in China, with the government behind a negative propaganda campaign disparaging our industry. Perhaps the reason is that we now estimate there are as many as four million direct salespeople in China, with meetings being conducted throughout the country and our industry being very welcome at the grass roots level. My reason for writing is to ask your permission to use the video you did on our industry in 1996 (in which you specifically mention China) to show to Chinese government officials and the Chinese press to counter the negative statements being printed (see a few attached examples).

Our industry will not be silent on Chinese government violations of trade and commercial norms and libeling our method of marketing. In addition to the potential loss of the world's largest market, the ripple or domino effect of such a precedent, i.e. a country prohibiting our industry to operate, is of the greatest concern to us and our 25 million salespeople around the world.

Thanks.

Best personal regards.

Sincerely,

Neil

Enclosures

WORLDWIDE DIRECT SALES DATA

April 10, 1998

	Year		Retail Sales (in U.S. $)	Number of Salespeople
Argentina	1997	$	**1.074 billion**	429,000
Australia	1997	$	**1.2 billion**	650,000
Austria	1996	$	340 million	40,000
Belgium	1997	$	98 million	13,924
Brazil	1996	$	**3.5 billion**	887,000
Canada	1997	$	**1.6 billion**	1,300,000
Chile	1996	$	180 million	160,000
Colombia	1997	$	445 million	230,000
Costa Rica	1997	$	20 million	2,500
Czech Republic	1997	$	70.8 million	77,252
Denmark	1996	$	50 million	5,000
Finland	1997	$	180 million	60,000
France	1996	$	**1.19 billion**	166,768
Germany	1996	$	**3.6 billion**	395,000
Greece	1996	$	41 million	25,000
Hong Kong	1997	$	66 million	60,840
Hungary	1997	$	50 million	87,269
India	1997	$	80 million	125,000
Indonesia	1997	$	450 million	2,800,000
Ireland	1997	$	60 million	14,000
Israel	1996	$	80 million	14,000
Italy	1997	$	**2.1 billion**	340,000
Japan	1997	$	**30.2 billion**	2,500,000
Korea	1997	$	**2.1 billion**	909,000
Malaysia	1996	$	760 million	1,400,000
Mexico	1997	$	**1.4 billion**	1,200,000
Netherlands	1996	$	142.8 million	39,700
New Zealand	1997	$	120 million	110,000
Norway	1996	$	90 million	9,000
Peru	1996	$	295 million	177,000
Philippines	1996	$	320 million	745,644
Poland	1997	$	210 million	350,000
Portugal	1997	$	63 million	25,800
Russia	1997	$	360 million	350,000
Singapore	1997	$	78 million	24,000
Slovenia	1996	$	58 million	15,500
South Africa	1996	$	400 million	200,000
Spain	1995	$	652 million	123,656
Sweden	1997	$	120 million	50,000
Switzerland	1997	$	362 million	5,438
Taiwan	1996	$	**1.74 billion**	2,360,000
Thailand	1996	$	800 million	500,000
Turkey	1996	$	98 million	212,000
United Kingdom	1996	$	**1.396 billion**	400,000
United States	1996	$	**20.840 billion**	8,500,000
Uruguay	1996	$	35 million	22,500
TOTAL		$	**79.017 billion**	28,111,791

Note: •The People's Republic of China (PRC) Ministry of Internal Trade reported 500,000 salespeople in that country in 1995. These numbers are not included in this survey. The Ministry did not give a report of estimated retail sales for the PRC.

Sales figures exclude VAT

Direct selling branded social evil as mainland moves towards ban

MARK O'NEILL in Beijing and MATTHEW MILLER

The mainland government is moving to slam the door on all direct selling, following nearly 10 years of strong growth.

Beijing has decided the social and economic damage of direct marketing outweighs the benefit of employing millions of people.

Direct sales companies that are legally approved will be offered the alternative of selling their products through retail shops.

Such a ban would be a serious blow to foreign companies that have invested heavily in factories and sales networks in China.

They include US companies Avon, Sara Lee, Mary Kay and Amway.

Amway operates in 32 cities in 13 provinces, posting sales of US$178 million last financial year to August 31.

In 1992, the company opened a $100 million factory in Guangzhou, and in November, 1997, agreed to build a second US$30 million cosmetics and nutritional products plant in the Pudong district of Shanghai.

Amway China legal affairs director Herbert Ho said the company had yet to receive formal notification concerning a prohibition.

"All we can do is closely monitor the situation," he said.

Mary Kay has invested in a $25 million mainland plant to manufacture its cosmetics.

Beijing's dissatisfaction with direct marketers is being loudly touted through a television and newspaper campaign on the evils of direct sales.

Orchestrated by the Communist Party's propaganda department, the campaign details stories of how would-be salesmen and women have been cheated of their money, with some going mad, and of how customers have been tricked into buying high-priced, low-quality goods.

The *China Economic Times*, reported earlier this month the State Council had decided on the ban, which will be introduced before the end of the year.

Asked if there was a ban, a spokesman for the National Industrial and Commercial Bureau (NICB), which is responsible for supervising direct sales, said: "We have not received such an order."

(Cont'd Page 4, Col 6)

Mainland moves to ban direct selling

(From Page 1)

The spokesman declined to comment further.

On February 14, NICB fair trading bureau chief Li Bida told state television direct selling was bringing calamity to the nation and the people.

On January 24, Vice-Premier Li Lanqing said in Beijing that, under present market conditions, direct selling had caused many abuses and should be banned.

Senior police officials have said that, if allowed to develop, direct selling would become uncontrollable and a danger to social stability.

Direct sales firms have had a chequered history in China since they began in 1990, when Avon first began calling on homes in Guangzhou.

By the end of 1995, there were 163 such firms, employing 12 million people.

But fraud, sale of poor-quality, high-priced goods and other abuses became so widespread that the NICB imposed a seven-month moratorium on the business in September 1995.

After a nationwide investigation, it approved 41 companies to resume operations in April 1996.

The NICB issued new rules, including a ban on direct sales of medicines, jewellery and fresh food, mandatory training for direct sales staff and a ban on civil servants, serving soldiers, journalists, doctors and teachers doing such work.

South China Morning Post

March 30, 1998

Circulation: 100,000

'Social evil'

IT appears Avon will not be calling any more in China after Beijing summed up in two words what millions of Americans have known for decades – direct selling is a "social evil". Direct selling companies have invested more than US$100 million in the mainland.

BEIJING

LI LIANQING VISITED OLD PROFESSORS AND INSPECTED TEACHERS' HOUSES AND MARKETS

(Excerpt)

Li Lanqing stressed that the form of direct sales in commodity circulation has many malpractices and shall be prohibited. For direct sales activities, there are neither relevant policies, regulations nor effective management, and there exists extremely severe illegal cases such as tax evasion, tax fraud, etc. For consumers, the quality and validity of commodities cannot be guaranteed, but furthermore, the cost cannot be lowered and the price usually becomes increasingly more expensive along the line of the sale, some even more expensive than those sold in stores, damaging the consumers' interests. It is nothing new that people participating in direct sales activities are tricked. Meanwhile, in direct sales, some feudalistic superstitious and even pornographic activities have been discovered to be carried out in the name of direct sales; many cases of swindling have also appeared which must all be seriously investigated and punished according to law. He hoped that propaganda departments would expose this and educate people not to be taken in.

From People's Daily
January 25, 1998

P.S. 164Q Music Legacy

Dear President Clinton & Council On The Arts,

Thank you for recognising the great need for arts programs in education. We know at P.S. 164Q what a positive effect the arts play in the daily lives of our students, and we hope you will continue to support and encourage funding for the arts in education.

In December of 1993 it was my pleasure to come to your house, the White House, where my chorus performed in the East Room. It was truly one of the most amazing experiences of our lives. Since we were invited to visit you, we would like to return the hospitality and cordially invite you to come to P.S. 164Q and see our arts programs at work. The enclosed pictures and letters are a small example in the children's words of how the arts make school a better place for them and truly enhance all their learning. I hope you will peruse them and use them in your defense of the arts in education. All of us here at P.S. 164Q thank you for your support and belief in what we do to bring the arts to all children every day.

Sincerely,

Lucie Evens

Lucie Evens
Music Teacher

4-14-98
to Burkhardt
for repeny

THE WHITE HOUSE

WASHINGTON

4-14-98

April 13, 1998

MR. PRESIDENT:

Attached are recommended phone calls to Mark O'Meara and Jack Nicklaus regarding the Masters Golf Tournament. I have also attached congratulatory letters if you don't want to make the calls. Of course, you can make the calls and send the letters if you wish.

Phil Caplan

Phil

Please sign

copied
Currie
Hevnreich
Engskov

THE WHITE HOUSE

WASHINGTON

April 13, 1998

RECOMMENDED TELEPHONE CALL

TO: Mark O'Meara (PGA Tour Player)

DATE: Monday, April 13, 1998

RECOMMENDED BY: Gene Sperling
Rahm Emanuel

PURPOSE: If you have not already called, you should call Mark O'Meara to congratulate him on winning the 1998 Masters Tournament.

BACKGROUND: O'Meara made a 20-foot birdie putt at the 405-yard 18th hole to win the 62nd Masters by a shot over playing partner Fred Couples and David Duval, solidifying his reputation as one of the greatest golfers. Mark birdied three of his last four holes, including the last two, to finish with a round of 67 and a 9-under total of 279. O'Meara started the final day of the Masters two shots off the lead and trailed, David Duval, with four holes to play.

Since turning professional, Mark has had 14 PGA Tour victories, including five at Pebble Beach, the latest in 1997. Also the 1997 Buick Invitational champion and the winner of the Trophee Lancome In Europe. Mark, a former US Amateur Champion 1979 (considered a major championship), tied for third in the 1988 US Open and tied for third in the 1985 and 1991 British Opens. Mark has also been a Member of the 1985, 1989, 1991 and 1997 Ryder Cup Teams. He also tied for fourth in the 1992 Masters, his best in 14 appearances.

TOPICS OF DISCUSSION:

- I would like to congratulate you on winning your *second* (including 1979 U.S. Amateur Championship) Championship, the 62nd Masters Tournament. I also want to congratulate you on solidifying your self as one of golf's greatest practitioners and final round closers.

- It must have been great to receive the green jacket from your close friend and last year's winner Tiger Woods.

- I am sure you are going to be in the Washington area, the week the Kemper Open is played at Avenel (June 4-7). I would like to invite you and your family to the White House when you are here.

- Hillary and I send our congratulations and our best!

CONTACT PERSON AND
TELEPHONE NUMBER: Mark O'Meara (407) 876-0848 - home
Peter Malik, Agent (IMG) 216-522-1200 - office

DATE OF SUBMISSION: April 13, 1998

THE WHITE HOUSE

WASHINGTON

April 13, 1998

RECOMMENDED TELEPHONE CALL

TO: Jack Nicklaus (PGA Tour Player)

DATE: Monday, April 13, 1998

RECOMMENDED BY: Gene Sperling
Rahm Emanuel

PURPOSE: To congratulate Jack "the Bear" Nicklaus on a tremendous showing in the 1998 Masters, his 40th Master's tournament.

BACKGROUND: Jack finished four strokes behind winner Mark O'Meara, at 5-under 283, tied for sixth place. Nicklaus, 58, became the oldest player to ever finish in the top ten.

In 1963, Jack Nicklaus, 23, won his first Masters Title and became the Tournament's Youngest Champion at the time. In 1986, Jack became a six-time Masters Champion at age 46, the oldest to win. Jack's other Masters victories were in 1963, 1965, 1966, 1972 and 1975. One of Jack's greatest Master's moments was when he successfully defended his tile in 1966 by winning in a three-way playoff. Jack is also the youngest second-time winner ,1965, where he won with a record score of 271 (tied by Raymond Floyd in 1976) and a then record nine stroke winning margin. He is also the youngest three-time and four-time winner at the Master's. Runner-up in the Masters in 1964, 1971, 1977 and 1981. Jack has won four US Opens - 1962, 1967, 1972, 1980; five PGAs - 1963, 1971, 1973, 1975, 1980; and three British Opens - 1966, 1970, 1978. Jack has also been a member of six Ryder Cup Teams, Captain in 1983 and 1987. He is also the recipient of the 1975 Bob Jones Award presented by the USGA for 'distinguished sportsmanship in golf.'

TOPICS OF DISCUSSION:

- I would like to congratulation you on completing you 40th Master Tournament and becoming the oldest player to ever finish in the top ten.

- I want to also congratulate you on being honored, April 7, 1998, with a plaque that will be permanently affixed to the fountain between holes No. 16 and 17 in recognition of his play and for his contributions at the Masters.

- Please give my best to Barbara and your family.
- Hillary and I send our congratulations and our best!

CONTACT PERSON AND
TELEPHONE NUMBER: Marilyn Keough, Agent (561) 626-3322 - office

DATE OF SUBMISSION: April 13, 1998

THE WHITE HOUSE

WASHINGTON

April 13, 1998

4.14.98

MR. PRESIDENT:

It's my understanding from Rahm that you asked for the attached.

Phil Caplan

Ce Eb

copied
Bowles

THE WHITE HOUSE

WASHINGTON

4.14.98

APRIL 13, 1998

MEMORANDUM TO: THE PRESIDENT

FROM: RAHM EMANUEL

RE: PHOENIX HOUSE

This is a memo from Dr. Rosenthal to General McCaffrey regarding the Phoenix House, which you know is one of the most respected drug treatment centers in the country.

Phoenix House Foundation, Inc./164 West 74 Street, New York, New York 10023/(212) 595-5810

Phoenix House

OFFICE OF THE PRESIDENT

April 10, 1998

General Barry R. McCaffrey
Director
Office of National Drug Control Policy
Executive Office of the President
750 17th Street, N.W.
Washington, DC 20503

Dear Barry:

The clear congressional mandate barring use of federal funds to support needle exchange programs has lapsed, and there is some question as to whether remaining statutory prohibitions now apply. So, it is possible that the Department of Health and Human Services may choose to make federal funds available to programs that provide clean needles to IV drug users, in an attempt to reduce the spread of HIV infection through the sharing of contaminated injection equipment. Certainly, the Department and Secretary Shalala will be urged to do so.

As one who has devoted his entire career to helping young men and women overcome drug dependence and build purposeful new lives, I feel justified in urging the President to discourage the Secretary from allocating any federal funds for this purpose. Strong arguments support this position.

1. There is no consistent evidence that the availability of needle exchange reduces the transmission of the HIV virus among IV drug users. (At least one recent study actually showed an <u>increased</u> rate of transmission.)

2. Nor is there evidence that needle exchange programs are <u>necessary</u> to reduce the spread of HIV infection through needle sharing, for the rate of infection has fallen among addict populations denied such programs.

3. Government "enabling" of drug use (subsidizing it, in this case) sends a dreadful message to young people and undermines prevention efforts.

4. The most effective means of combatting the spread of HIV infection by IV drug users is through treatment that addresses their characteristically antisocial, self-destructive, and reckless behavior. This behavior contributes to the spread of the disease at least as much through irresponsible sexual contact as through needle sharing.

5. By enabling drug abuse in this manner, the federal government would be helping many drug abusers to resist the treatment they need to overcome their addiction and curb their addictive behaviors.

6. Needle exchange programs are not a useful route to treatment. They are essentially noncoercive, and addicts who most need treatment are those most likely to resist it -- particularly treatment that is demanding enough to help them recover. This often makes coercion necessary, and involuntary treatment has proven to be as effective as voluntary treatment.

7. Communities rightfully resist needle exchange programs, for these are sites where addicts will concentrate, often leaving behind litter that may include infected needles.

8. Considering the shortage of treatment capacity, particularly the shortage of comprehensive, residential treatment for the hard core (the most economically and socially costly substance abusers), it would be hard to justify spending federal dollars, unavailable for treatment, to subsidize addiction.

Other arguments can be made against needle exchange, but it would not be fair to say that these programs automatically increase the incidence of drug abuse. It should be recognized, however, that even if exchange programs were an effective means of controlling infection among IV drug users, they have no capacity to limit spread of the virus among crack cocaine users. And, at Phoenix House, as at other treatment programs, we have found the highest incidence of HIV infection among users of crack cocaine.

Finally, I believe that needle exchange is more a cause than a cure, that it allows its advocates to feel that they are doing something assertive to combat AIDS. But it is, on balance, nothing that federal funds should be used to support.

Sincerely,

Mitch

Mitchell S. Rosenthal, M.D.

'98 APR 13 PM7:40

THE WHITE HOUSE
WASHINGTON

April 13, 1998 7:30 pm 4.14.98

Mr. President - OK - media
minor changes

Attached is a draft of your State dinner toast in Santiago. The Chileans hope to have a "final" copy by Tuesday night, giving them time to translate the toast into Spanish and print it in the official program (and avoiding the need for consecutive translation at the dinner.)

If you have a few moments between now and tomorrow night, please send us your edits. Of course, if you cannot get to it, we will not send anything forward to the Chileans.

Tony Blinken ATB

Copied
Blinken
Bowles

draft 4/13/98 7:14 PMM

PRESIDENT WILLIAM JEFFERSON CLINTON
STATE DINNER TOAST WITH PRESIDENT FREI
SANTIAGO, CHILE
APRIL 16, 1998

Thank you, Mr. President, and thank you, Mrs. Frei [FRAY]. Thank you to all Chileans for the warm welcome we have received here -- and thank you in advance for your patience, because I fear that the events of this week will create the biggest "taco" in your history. That means a traffic jam, for those in our delegation who don't know.

Mr. President, it was just over a year ago that Hillary and I hosted you and Martita for a state visit in Washington. On that trip you delivered a powerful address to a joint session of our Congress, and I will have the honor of addressing your Parliament tomorrow. The short time between our visits reflects the growing strength and the growing importance of the relationship between our nations.

Chile is admired around the world for its natural beauty, its brilliant writers and artists, its world-class athletes, its leadership in seeking peace in volatile regions, ~~and~~ its remarkable economic growth and stability. ~~The people of Chile also have gained the respect of the world for~~ and the people of your people their bravery in restoring long-standing democracy here after two turbulent decades.

That Chile is host to the second Summit of the Americas shows the esteem ~~with~~ in which your country is held across this hemisphere. It also demonstrates, Mr. President, the great respect you have earned among your fellow leaders.

Some see you, Mr. President, as a man of calm reserve, a civil engineer who expertly builds bridges to improve the lives of his fellow citizens. But there seems to be another side to you. It said that you love opera and the tango. When you addressed our Congress last year, the first person you quoted was not some gray-suited economist but that great political leader Don Quixote de la Mancha. The words you selected go to the core of our shared values: ~~The noble Don, through his creator, Cervantes, said~~: "Freedom ... is one of the most precious gifts heaven bestows on man. All the treasure of the Earth ... cannot equal it."

I also know, Mr. President, that like your father before you, you care passionately about the least fortunate in your society. I'm sure your father was in your thoughts when, in your first address as your nation's president, you pledged to bring hope and dignity to the poorest Chileans. You have worked hard to ensure that Chile's growing prosperity will benefit not just a few, but everyone. And Mrs. Frei deserves great credit for her active work on behalf of Chilean artisans, ~~women, children and the poor~~.

The United States wants to deepen our partnership with Chile on the many challenges we share: Strengthening democracy, improving education, protecting the environment, preserving peace, expanding trade between our prosperous nations. Our meetings today furthered these goals, and I know we will continue to expand our cooperation, so much is at stake.

Working with the Chilean people and with you, Mr. President, is a great honor for me and my fellow citizens. In the darkest days of the past here, when dissent was suppressed, when the people were denied a meaningful vote, and true leaders were denied the chance to lead, the

Chilean people never abandoned hope that one day things would be better. Now, because your ~~had~~ hope, courage and vision, things are better -- much better. Now, the United States and Chile celebrate together ~~our~~ the precious gift of freedom, ~~our~~ and prosperity, and our determination to support democracy across the Americas and around the world.

I am honored, therefore, to invite all of you to toast President Frei, Mrs. Frei , the people of Chile, and the brilliant future we are building together.

###

[illegible] tape [illegible]
[illegible]
cc: gift unit
cc: Judy [illegible]

THE WHITE HOUSE

WASHINGTON

4-14-98

April 9, 1998

Tracye Wear
1139 Waverly
Houston, Texas 77008

Dear Tracye:

Thanks so much for your letter and for sending *The Strange Demise of Jim Crow*. I'm looking forward to watching it and am certain it will be useful in preparing for my trip to Houston.

Thank you also for your offer to be of assistance for the trip. I will keep that in mind.

Best wishes to you and David.

Sincerely,

Bill

Copied
Winston
Bowles

Winston gets
original tape
& copy of ltr.

ORM gets copy
tape & original
letter

Nancy - I sent this copy again so you would remember who I am. Here is the tape I said I would send up to the Presidents about the end of Jim Crow in Houston as background for his visit - Tracye Wear

March 30, 1998

To Nancy Hernreich,

My name is Tracye Wear and three or four times a year I fax a note up to the President. I would fax Debi Schiff with the note and she would give it to Bill. I know Debi left the West Wing, so I called up and the person who answered the phone told me to fax notes to you. I am a friend who knew the Clintons in Fayetteville, Arkansas. My husband and I and the President got to visit when he was in Houston in January where we got to catch up with each other and gossip about our friends and family. I am an old friend who wants the best for Bill and Hillary.

What follows is a note to Bill.

Also, I am going to send up a tape that friends made here in Houston about "The Strange Demise of Jim Crow in Houston." The film was shown here and in large cities across the nation on the Public Broadcasting Stations during February, Black History Month.

I have the White House Residence address and will address it (the tape) in care of you (I did that with Debi once or twice when I sent a book up). If you would get it, as background, to Bill and/or the people who are going to do the Race Initiative discussion here in Houston in two weeks, I would appreciate it.

Thank you.

Tracye Wear and David Brown
1139 Waverly
Houston, TX 77008
713-869-8372

I only have a fax at work (at Jones High School) 713-732-3428.

3-30-98

Bill,

We are happy you are coming back to Houston especially to host your Race Initiative Series.

Here is the tape I mentioned - about the end of Jim Crow in Houston - and a background letter from the Producer. It had multiple viewings nationwide on PBS this February.

I hope this will be helpful for you and/or your staff for the Houston Visit.

I was so proud to see the picture of you and Nelson Mandela in the newspaper.

If David and I can help with your visit, let us know.

Yours Jean

The University of Texas Medical Branch at Galveston

School of Medicine
Graduate School of Biomedical Sciences
School of Allied Health Sciences
School of Nursing

Marine Biomedical Institute
Institute for the Medical Humanities
UTMB Hospitals and Clinics

Institute for the
Medical Humanities

President Bill Clinton
The White House
Washington, D.C.

March 26, 1998

Dear Mr. President:

I am excited to learn that you will be conducting a town meeting on race and sports here in Houston on April 16. As you and your staff prepare for that town meeting, I want to urge you to watch our enclosed documentary, *The Strange Demise of Jim Crow: How Houston Integrated Its Public Accomodations, 1959-1963.* It provides largely unknown and essential background for understanding what makes Houston a model city in its handling of race relations. Houston has a reputation as a city where "nothing happened" during the civil rights movement. **Wrong!** There was a vigorous and courageous movement here which never received its due. **And** there was also an enlightened elite of whites and blacks in the business community who made sure that Houston integrated quietly, without the violence and publicity of Little Rock, Selma, or Birmingham.

The Strange Demise of Jim Crow also touches directly on the issue of race, sports, and civil rights in Houston. As you know, Houston is the largest city and has the largest African American population in the South. I bet you don't know that Houston landed the first professional baseball franchise in the South (the Astros) and that in the summer of 1960 black leaders secured the promise of a totally integrated stadium in return for supporting a bond issue to finance the stadium. The same process of African American leadership negotiating a piece of the action took place in 1996, when Houstonians voted to build a new stadium adjacent to the old Union Station, a site of sit-in protests by local students and Freedom Riders 35 years ago.

I hope you find the time to watch our documentary, which recently aired on 60 PBS stations during Black History Month. I believe you will be moved by this untold story and informed about the history of race relations in Houston. I believe that *The Strange Demise of Jim Crow* will help you set the positive tone necessary for a successful town meeting. I would be happy to serve in any capacity to help insure that the Houston dialogue on race and sports is well-informed, respectful of differences, as well as hopeful and healing.

Thank you.

Sincerely,

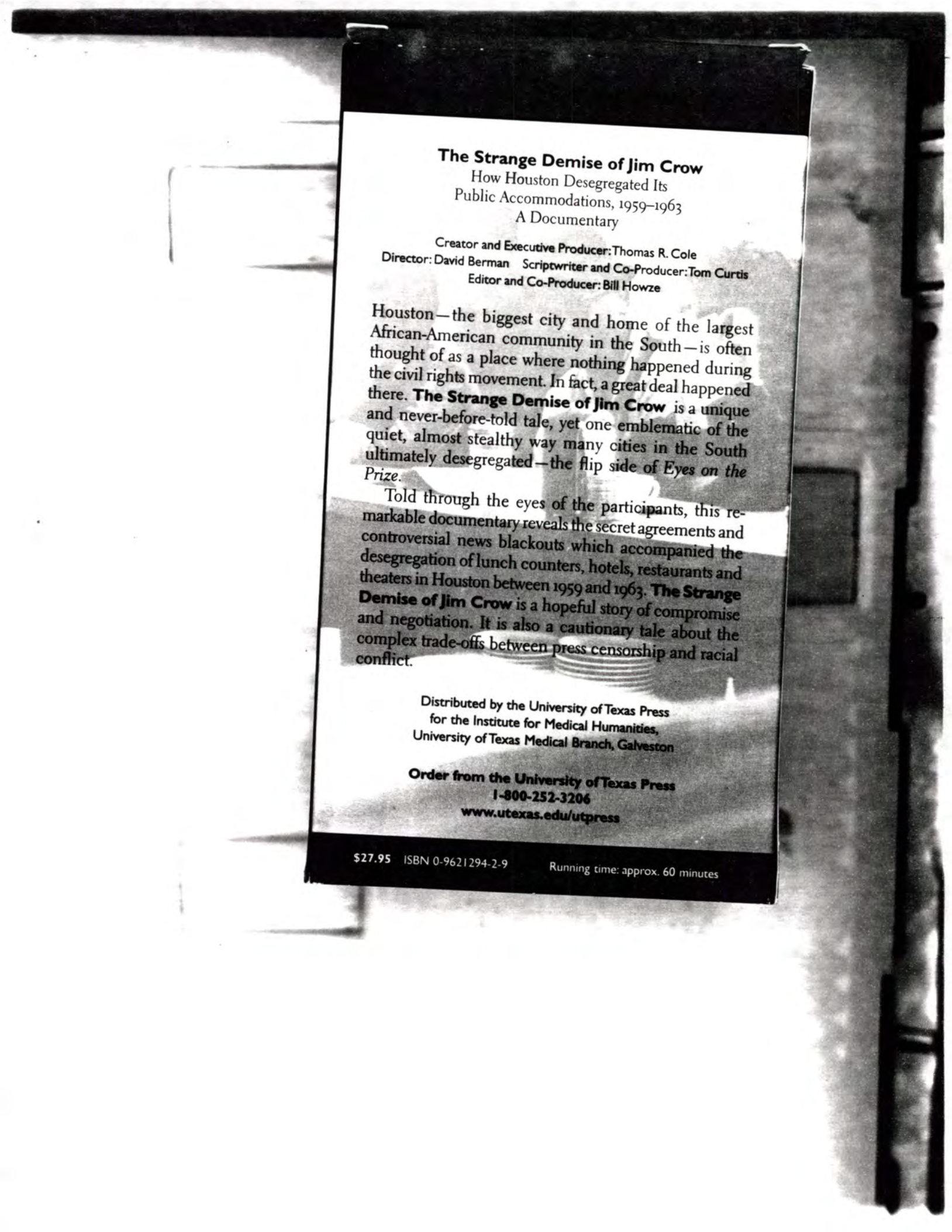
Tom Cole

Thomas R. Cole
Executive Producer, *The Strange Demise of Jim Crow*
Professor

SUITE 2.210, ASHBEL SMITH BUILDING 301 UNIVERSITY BOULEVARD GALVESTON, TEXAS 77555-1311
(409) 772-2376 FAX (409) 772-5640

THE STRANGE DEMISE OF JIM CROW

How Houston Desegregated Its Public Accommodations, 1959–1963



The Strange Demise of Jim Crow

How Houston Desegregated Its Public Accommodations, 1959–1963

A Documentary

Creator and Executive Producer: Thomas R. Cole
Director: David Berman Scriptwriter and Co-Producer: Tom Curtis
Editor and Co-Producer: Bill Howze

Houston—the biggest city and home of the largest African-American community in the South—is often thought of as a place where nothing happened during the civil rights movement. In fact, a great deal happened there. **The Strange Demise of Jim Crow** is a unique and never-before-told tale, yet one emblematic of the quiet, almost stealthy way many cities in the South ultimately desegregated—the flip side of *Eyes on the Prize*.

Told through the eyes of the participants, this remarkable documentary reveals the secret agreements and controversial news blackouts which accompanied the desegregation of lunch counters, hotels, restaurants and theaters in Houston between 1959 and 1963. **The Strange Demise of Jim Crow** is a hopeful story of compromise and negotiation. It is also a cautionary tale about the complex trade-offs between press censorship and racial conflict.

Distributed by the University of Texas Press
for the Institute for Medical Humanities,
University of Texas Medical Branch, Galveston

Order from the University of Texas Press
1-800-252-3206
www.utexas.edu/utpress

$27.95 ISBN 0-9621294-2-9 Running time: approx. 60 minutes

4-13-98

To Burkhardt for reply?

Yes ✓ No —

B.

9712 Medical Center Drive, Rockville, Maryland 20850
(301) 838-0200
(301) 838-0208 Fax

'98 APR 13 PM4:04

April 7, 1998

The President
The White House
1600 Pennsylvania Avenue
Washington, DC 20500

Dear Mr. President:

The actions taken by you and your Administration over the past few months regarding food safety have provided the leadership and definition necessary to begin solving this growing problem. As you and I discussed at Hilton Head over the New Year's holiday, the work of The Institute for Genomic Research (TIGR) and other research organizations to determine the complete genetic code of micropathogenic genomes, plus the availability of new technologies, allow for new approaches to be employed in updating our food safety programs. We are prepared to help with this effort.

The increased visibility of outbreaks of illnesses from food-borne pathogens has heightened awareness and sensitivity to food safety issues throughout the world. As you indicated in announcing the Food Safety Initiative last October, increasing the funding for food safety at FDA in the proposed 1999 budget, and your call for food safety legislation this past week, we have reached the point where we must update our food safety standards to meet the needs of the 21st century.

Global trade has dramatically impacted all facets of our Nation's economy. The number of food items coming into the country has doubled over the last seven years, resulting in the U.S. becoming a net importer of food. While this allows all Americans to be exposed to new and culturally significant foods, it also allows a significant percentage of our population to be exposed to new, different or undetected strains of food-borne pathogens.

In your call for legislation, you note that food safety is "part of the basic contract now between the consumers of our country and their government," but indicated we must also have the tools and technology to "give Americans the extra protection they deserve." The new era of genomics provides us both the tools and technology to meet the food safety needs of the 21st century. The use of cutting edge molecular biology techniques allow for the rapid detection of new emerging pathogens that are likely to be present as food imports continue to increase. Current PCR-based technologies exist that will allow routine testing of known organisms within hours as opposed to days. In addition, automated data analysis and interpretation brings much needed objectivity to each result while minimizing errors and making the entire process easier to standardize.

As the "Initiative to Ensure the Safety of Imported and Domestic Fresh Fruits and Vegetables: Status Report" identifies, there are a number of aspects of food testing that must be taken into account. For example, it is imperative to quickly identify the existence of bacteria or other organisms. Depending upon the organism identified, it may be necessary to determine its origin. Ultimately, it will be important to have the smallest, most cost-effective, efficient, rapid detection and identification system possible to incorporate food testing into the manufacturing process.

As you know, the use of genomics has begun to permeate a number of industries in its short history. Food safety is just another of these applications. There a number of new products which are all modifications of technologies used initially to undertake sequencing the human genome. We are committed to continue the assimilation of these products into other applications that will also greatly improve human health.

Some of these new approaches have been used recently to test ground beef in the San Francisco Bay Area. In the samples tested, an inverse correlation was found between quality and quantity of *E. coli* contamination. These new techniques identified major contaminates that were not routinely identified with the standard screening process.

TIGR has been working with a commercial organization, Perkin-Elmer, which is already implementing new aspects of molecular biology to food safety issues. I have enclosed more technical descriptions of these technologies for further review. I believe that this process can be accelerated in conjunction with the initiatives you have already undertaken. I offer to meet with you, members of your domestic policy staff, and/or appropriate members of the food safety initiative to explain these technologies further and to discuss appropriate methods to introduce them into current testing practices.

I applaud the efforts that you and your Administration have taken to increase the visibility of improving our food safety measures. Clearly, the application of these and other new technologies in the new millennium will allow for an impact that will be comparable to that of President Theodore Roosevelt's in the beginning of this millennium. I stand ready to assist you in anyway that you and your staff deem appropriate.

Respectfully,

J. Craig Venter
President and Director

enclosures

Investigation of *E. coli* O157:H7 Epidemic Samples from Japan using Genomic Fingerprinting Techniques and Genetic Based Typing Methods

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Abstract

During the summer of 1996, a large outbreak of *E.coli* O157:H7 food poisoning in Japan affected more than 9,000 people and caused 11 deaths. Since the source of that outbreak was not identified many samples need to be analyzed to understand the dynamics of the outbreak. In this study we analyzed more than 50 outbreak *E.coli* O157:H7 isolates along with *E.coli* O157:H7, *E.coli* O26:H7 and *E.coli* O111:NM strains isolated in the United States, including several from food poisoning outbreaks. These isolates were analyzed using the 5' nuclease assay and amplified fragment length polymorphisms (AFLP) in addition to PFGE, RAPDs and phage typing. The 5' nuclease assay is a homogeneous PCR-based assay that enables the specific detection of amplification by fluorescence. All of the *E.coli* O157:H7 isolates were scored positive with the 5' nuclease assay while the non-*E.coli* O157:H7 scored negative. The PFGE, RAPD and toxin typing segregated the Japanese isolates into three groups. All of the isolates were characterized using AFLP™, a novel fingerprinting technique involving selective amplification of restriction fragments, the Japanese strains also segregated into two distinct but closely related groups while the US *E.coli* O157:H7 strains and the non-*E.coli* O157:H7 strains were found to segregate into groups that were clearly differentiated from the Japanese *E.coli* O157:H7 strains. This study shows the combination of 5' nuclease assay for the initial screening and AFLP for characterization of samples containing *E.coli* O157:H7 is a powerful tool for analysis of an outbreak and typing of the isolates in days instead of weeks.

Introduction

During the past year, the impact of the pathogenic *E. coli* serotype O157:H7 has become increasingly more visible throughout the world. In the US, President Clinton's Food Safety Initiative publicized in January 1997(12) illustrates the increased awareness and importance of ensuring food safety. Several outbreaks in Japan, the US, and Scotland affecting thousands of people have clearly shown the need for a simple screening technique in combination with a universal epidemiological tool. During the investigation of these recent outbreaks several techniques were utilized to trace the source of the food poisoning. Many of these techniques employed genetic analysis or fingerprinting methods such as RFLP (restriction fragment length polymorphism) and RAPD (random amplified polymorphic DNA)(7). The global aspect of these outbreaks, however, demands methods that can be easily standardized and allow comparison of data both within a lab and between labs. This requires the ability to simplify the initial screening techniques and digitize information obtained from the analysis techniques. Speed of analysis is another important requirement. During outbreak investigations, key samples need to be quickly and accurately identified for more detailed analysis. The fluorogenic 5' nuclease assay is a PCR-based assay that can be used for screening samples. It enables detection of target organisms within hours and includes automated data analysis(3, 15). The technique known as AFLP (amplified fragment length polymorphism) is a fairly new epidemiological method that employ both restriction digestion of the DNA and PCR amplification of selected restricted fragments(13). In combination with fluorescently labeled PCR primers, it yields fluorescently labeled products that can be run on an automated sequencer/genetic analysis instrument to generate a fingerprint for that sample and provide data in a standardized digital format. By running in-lane size standards along with the samples on the gel or capillary, standardization is incorporated into the method and analysis . This format promotes the exchange of information, allows direct comparison and enables the cataloguing and storage of this information. Japanese isolates were tested using the 5' nuclease assay, and then the AFLP technique was used to compare these Japanese isolates to US outbreak isolates. The AFLP results were compared to those obtained with PFGE, RAPD, and toxin typing.

Materials and Methods

Bacterial strains: The *E. coli* O157:H7 bacterial isolates obtained from Japan, a total of 49 (Table 1), and the CDC or ATCC, a total of 9 (Table 2a), were originally isolated from either foods, cattle, or human feces. In addition, 8 isolates representing other *E. coli* serotypes or different bacterial species were analyzed(Table 2b).

Bacterial DNA: The DNA from the Japanese isolates was isolated using a phenol-chloroform extraction procedure. The DNAs were precipitated with ethanol and resuspended in 500 µL TE.

SLT profiles: The presence of the SLT genes was established using the previously described method of Karch and Meyer (6).

PFGE: The PFGE analysis was as previously described (2) with minor modifications. Bacterial cells embedded in an low melting agarose plug were treated to release the DNAs for restriction digestion. The DNAs in each plug were treated with XbaI and the PFGE was run on a 1% agarose gel in 0.5x TBE at 10 °C, 200V. Switching times were used for separation of the whole genome or for separation of fragments less than 100 kb. The gels were stained with ethidium bromide and were photographed using UV transillumination.

Phage typing: The phage typing was done as previously described (2).

RAPD: The DNA was isolated s described above and 1 µL of DNA from each sample was used in a PCR performed in 25 µL reaction containing buffer, primer, and AmpliTaq™ DNA polymerase. The PCRs were amplified for 35 cycles and run on a 1.2% agarose gel in 1xTAE buffer.

AFLP: The DNA was isolated as described above for the Japanese obtained isolates and spectrophotometrically quantitated and diluted to 10 ng/µL in TE (20 mM Tris-HCl, pH 8.0, 1 mM EDTA). Either 5 µL or 1 µL aliquots were restricted with endonucleases Eco RI and Mse I according to the AFLP Microbial Fingerprinting Kit protocol (PE Applied Biosystems). The restricted fragments were then ligated to adaptors with specified 5' sequences. These sequences then served as the targets for PCR primer sequences enabling specific amplification of the ligated restriction/adaptor fragments. Selective amplification was done using these adaptor sequences along with selective nucleotides placed on the 3' end of the primer. These added bases function to control the number of restriction fragments amplified. The EcoRI complementary PCR primers contained a fluorescent label on the 5' end which functioned as a reporters for the detection during collection and analysis on either the ABI PRISM™ 377 DNA Sequencer or 310 Genetic Analyzer. When the samples were loaded on these instruments a molecular weight standard was mixed with each sample along with the loading buffer. The sizing standard GS500 Rox was used in these experiments. The ABI PRISM 377 is a PAGE (polyacrylamide gel electrophoresis) based instrument with 36 or 50 wells while the 310 is a CE (capillary electrophoresis) based instrument.

The data was then analyzed using GeneScan® 2.1 to tabulate fragments by size and fluorescent intensity. A data matrix was then constructed and subsequently used for all non-hierarchical cluster analysis. The non-hierarchical data analysis was done using MatLab and a distance matrix constructed in MatLab for an hierarchical analysis using PHYLIP(5). The statistical analysis/independence of the clustering was confirmed in both types of cluster analysis using disjoint principle component analysis(10) and cross validation.

***E. coli* O157:H7 5' Nuclease Assay:** The PCR contained components necessary for amplification and detection such as buffer, dNTPs, PCR primers, TaqMan™ probe, and AmpliTaq Gold™ DNA polymerase. The reactions were cycled in a GeneAmp® PCR Amplification System 9600 for 40 cycles.

Results

Using both the PFGE and the RAPD analysis several groupings were made. When categorized using the SLT toxin profile, two groupings are formed, the largest group(44 isolates) was positive for both of the SLT toxins (I and II) while a smaller group(5 isolates) were positive for only SLT II. The PFGE groupings then subdivide the SLT I & II positive group. PFGE Group I consists of SLT I & II positive isolates and are characterized as PFGE groupings Ia, b, and c. All of these isolates were obtained during seven outbreaks that occurred in May and June 1996 in Okayama, Gifu, Hiroshima, Aichi, Fukuoka, and Osaka prefectures. Other SLT I & II isolates had PFGE patterns that differed significantly from PFGE Group I classification. They were classified as Group II and subdivided into II a-g. Some of the isolates were obtained from the outbreak in Sakai City, Osaka Prefecture differed significantly from the PFGE group I isolates and were classified as PFGE group II(14). These isolates appeared as though they were clonal in origin. The SLT II only isolates were grouped separately and contained isolates that had patterns distinct from the PFGE groups I and II as described above. These isolates were organized into PFGE groups IV and V.

The RAPD patterns showed two major groupings, Group A and Group B. The group A isolates also had both SLTI and II, while the group B had either both or just SLT II . All of the *E.coli* O157:H7 isolates were detected by the 5' nuclease assay while the non-*E.coli* O157:H7 organisms were not detected.

In AFLP we used PCR primers containing the adaptor sequences for EcoRI or MseI. The EcoRI primers contained the added base A(FAM) while the MseI primers contained the added bases CA. With this combination CA/A(MseI/EcoRI), the Japanese isolates fell into two major groupings, corresponding to the SLT groupings and PFGE groupings I and II(Table I). Groupings obtained from two independent experiments run on two different platforms (377 and 310) showed these AFLP groupings were consistent (Figures 1 & 2). The older US isolates fell into a separate group, more similar to the Japanese isolates of *E.coli* O157:H7 than the US isolates *E.coli* O111:NM or O26:H11 while the more recent US isolates were grouped with the Japanese isolates (Figure 3). This corresponds with the initial PFGE analysis (submitted for publication). The closely related organisms *E. coli*, *Salmonella*, *Vibrio*, and *Campylobacter* could all be clearly differentiated from *E. coli* O157:H7 as well as from each other (Figures 2 & 3).

SLT Profile	RAPD	PFGE	Origin	No. Isolates	Year Isolated
			Outbreaks		
I&II	A	Ia	Hiroshima	3	June 1996
		Ia	Fukuoka	4	June 1996
		Ib	Gifu	5	June 1996
		Ic	Okayama(Oku)	1	May 1996
		Ib	Okayama(Niimi)	4	June 1996
		Ib	Aichi	2	June 1996
		Ib	Osaka(Kawatinagano)	1	July 1996
		Ic	Sporadic cases(Shiga)	3	Aug. 1996
			Outbreaks		
	B	IIa	Osaka(Sakai)	5	July 1996
		IIa	Osaka(Habikino)	3	July 1996
		IIa	Osaka(Chuo-ku)	1	July 1996
		IIa	Osaka(Higashisumiyoshiku)	1	July 1996
		IIa	Kyoto	1	July 1996
		IIa	Wakayama(Kushimoto)	3	July 1996
		IIa	Wakayama(Hashimoto)	1	July 1996
		IIa	Wakayama(Gobo)	2	July 1996
		IIa	Sporadic cases(Osaka)	3	July 1996
II Only		B	IV Gunma	3	July 1996
		V	Sporadic cases (Kanagawa)	1	June 1996
		V	Cow liver	1	June 1996

Table 1. Description of Japanese E. coli *O157:H7 isolates. SLT profile, RAPD grouping and PFGE grouping of Japanese isolates, along with geographic origin and isolation date.*

2a

Isolate	Origin	Year of Isolation
PE 28	Human feces	1986
PE 29	Meat	1982
PE 30	Human feces	-
PE 35	Human feces	1988
PE 45	Meat	-
PE 49	Meat	-
PE 679	-	1993
PE 689	-	1996
PE 699	-	1995

2b

Strain	Bacteria
PE 25	*E.coli*
PE 26	*E.coli*
PE 40	*E.coli O157:NM*
PE 322	*Salmonella serotype typhimurium*
PE 323	*Campylobacter freundii*
PE 663	*Campylobacter jejuni*
PE 672	*E. coli O26:H11*
PE 705	*E. coli O55:H7*

Table 2. Description of US isolates. 2a. US E. coli *O157:H7 isolates origin and year of each isolation. 2b. Non-*E.coli *O157:H7 bacteria used in the AFLP and 5' nuclease assay. List of bacterial species used and corresponding strain number.*

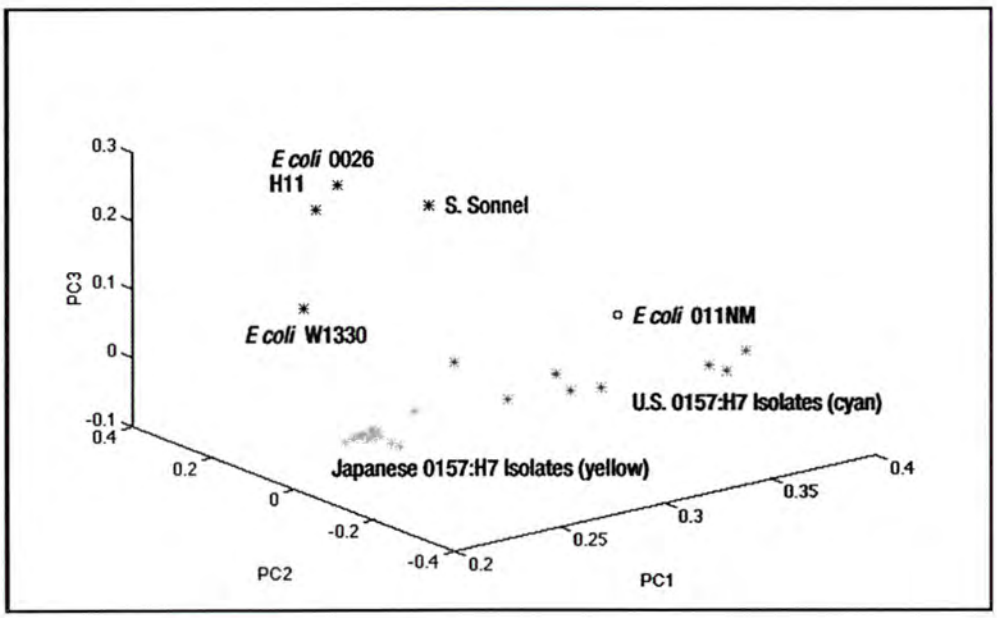

Figure 1. Clustering of the Japanese and US isolates using AFLP analysis on the ABI PRISM 377.

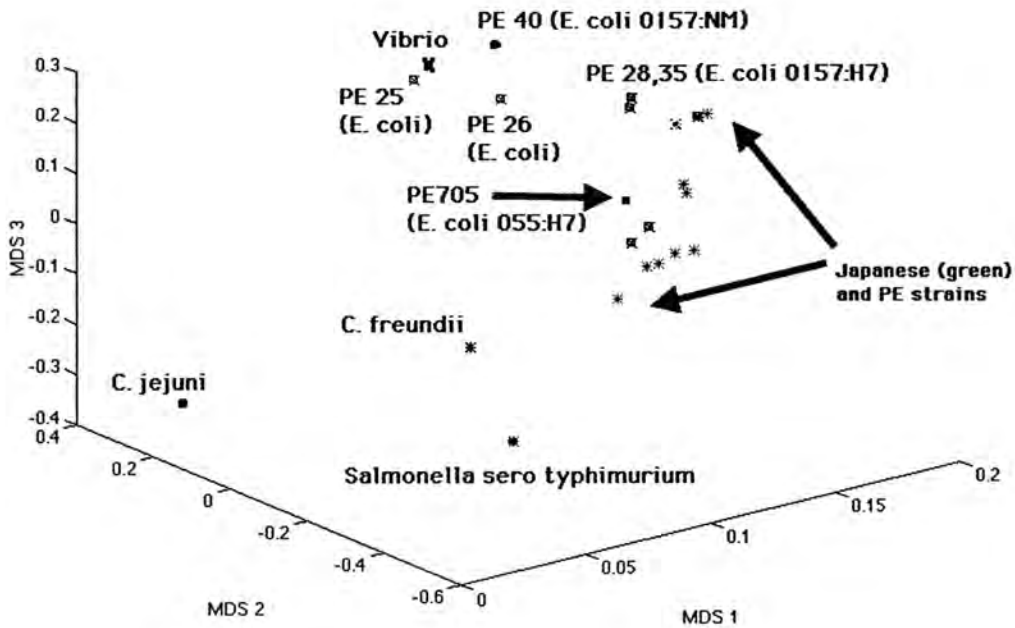

Figure 2. Clustering of the Japanese and US isolates using AFLP analysis on the ABI Prism 310.

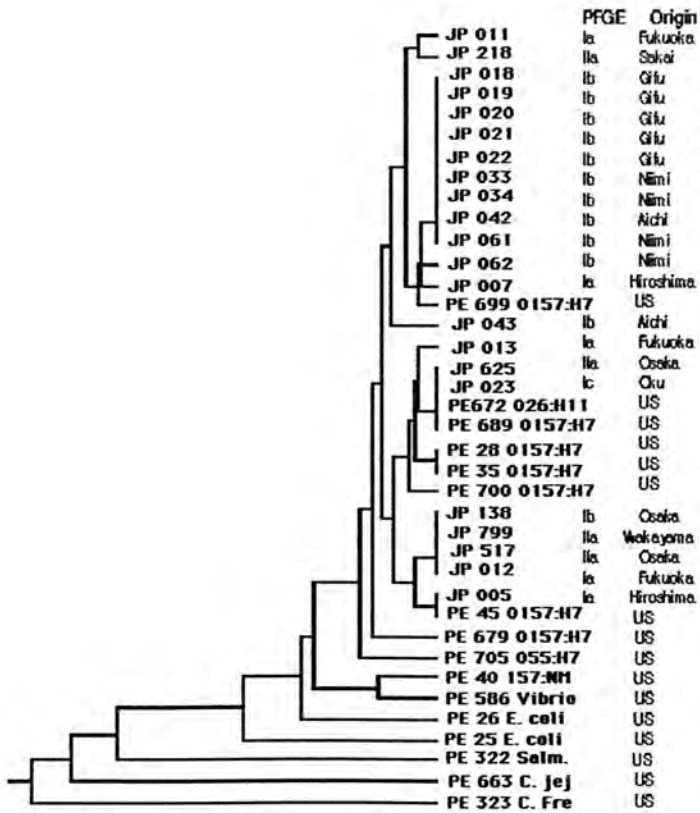

Figure 3. Included is the origin and PFGE grouping for the isolates.

Discussion

We have presented here a comprehensive genetic analysis of isolates obtained from a series of large outbreaks in Japan during the summer of 1996 as well as isolates from US outbreaks. All of these isolates contained genes for the SLT toxins, many containing the genes for both SLT I and SLTII. Using these results as well as PFGE and phage typing, the Japanese isolates were clustered into three major groups. Results obtained from AFLP analysis were consistent with this initial classification. In addition the AFLP results clearly showed that some of the recent US isolates and Japanese isolates were grouped together while the older US isolates were grouped separately (Figures 1 and 2). As has been recently reported (8), the *E.coli* O157:H7 is capable of evolving quickly, and the fact that the older US isolates actually group separately from the more recent isolates may be an indication of that evolution. The increase of travel and high amount of import/export of goods throughout the world promotes the exchange of pathogens across borders. This would reduce differences caused by geographical distances and prevent unique organisms from arising due to geographical isolation.

By using different added bases at the 3' end of the selective primers during AFLP, different restriction fragment subsets can be made and the pattern complexity can be customized depending on the amount of information and resolution needed. Since the number and size of fragments generated by the restriction digestion is regulated by the sequences of each type of bacteria, different added bases can create dramatically different patterns for generating a relatedness tree (4). This can clearly be seen in Figures 1 and 2 which shows the distinction between *E. coli* O157:H7 and other serotypes, such as O26:H11 as well as between *E. coli*, *Salmonella* and *Campylobacter*.

The reproducibility of our AFLP results was quite high even though were generated by independent researchers at separate times, locations, and on different instruments. Since the data files can be stored and sent electronically, data can be compared from different in different experiments, clearly showing the utility of digital information. Standardization is inherent in this method, and results can be obtained within 24 hours. Other methods lack this combination of standardization, digital information and quick time to result. RAPDs have been well documented for their lack of reproducibility, PFGE often requires days for results while lacking the ability to be standardized, and ERIC-PCR (rep-PCR) requires information about the bacteria being analyzed in order to determine the sequence and/or thermal cycling profile to be used(9). The DNA based methods, such as AFLP, are not affected by the health or expression of the bacteria. Phenotypic methods such as phage typing have had problems with reproducibility as well as providing enough detail for epidemiological studies or creation of a epidemiological database (1, 11).

The 5' nuclease assay and its ELISA-like format were designed for ease-of-use and can function well as an initial screening tool for selecting isolates to investigate using the more detailed analysis techniques such as AFLP. Its automated data analysis also makes it extremely compatible with an electronic database.

Conclusions

This study illustrates the utility of the 5' nuclease assay for screening and AFLP for molecular epidemiology. The throughput and short time to results inherent in these techniques are significant attributes for providing information on isolates obtained during an outbreak. The ability to accurately pinpoint the outbreak source quickly is paramount during this type of investigation. The combination of the 5' nuclease assay and AFLP provides a powerful mechanism to achieve that goal as well as a means to standardize outbreak investigations for worldwide information exchange and storage.

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We would like to thank Michiko Matsuura and JoAnne DiLeanis for their assistance in this investigation. This project would not have been possible without their contributions.

Differentiation of Pathogenic *Escherichia coli* Serotypes by Polymorphic Position Identification in the 16S Ribosomal RNA Gene Using Fluorescent DNA Sequencing

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Abstract

Foodborne outbreaks due to pathogenic *Escherichia coli* strains have become an increasingly important focus in the public health arena. Typically, these organisms have been classified according to their serotype. This study has evaluated the ability to genotype these isolates by the identification of unique polymorphic positions in the sequence of the 16S ribosomal RNA gene.

The DNAs from forty *E. coli* serotypes (0157:H7, 0157:NM, 055:H7, 026:H11 and 0111:NM) were amplified using PCR to yield a 1537 base pair product. The PCR products were sequenced using dye terminator fluorescent chemistry. Nonpathogenic *E. coli* isolates as well as other *Escherichia* species (*E. blattae, E. fergusonii, E. hermannii and E. vulneris*) and several *Shigella* species (*S. boydii, S. dysenteriae, S. flexneri and S. sonnei*) were also included in the analysis. Classification by genotype was accomplished by the identification of twenty-five polymorphic base mutations in the sequences of the 16S ribosomal RNA gene within the group studied. In some cases, the serotype and the genotype were the same ie., all isolates within a serotype showed a unique cluster in a nearest neighbor pairing tree analysis (0157:H7 and 0126:H11). There were also examples of disagreement between the serotype classification and the genotype designation (0111:NM).

The method used in this evaluation provides an additional means by which to identify pathogenic *E. coli* isolates. Genotypic classification tools will further enable molecular epidemiology investigations.

Introduction

Pathogenic strains of *Escherichia coli* have traditionally been identified using serological techniques. This study investigated the use of DNA sequencing to develop a genotyping system for several *E. coli* serotypes.

The 16S rRNA gene was sequenced for a large panel of bacteria which included *E. coli* serotypes 0157:NM, 0111:NM, 055:H7, 026:H11 and geographically distinct strains of *E. coli* 0157:H7. Nonpathogenic strains of *E. coli*, additional species of *Escherichia* and several *Shigella* species were also evaluated (Table 1).

Table 1: Strain Designations of Organisms Evaluated

Organism	Strain Designation	Serotype	Geographic Origin
Escherichia coli	Sigma (#W3110)		
Escherichia coli	ATCC 11775/MCC 3485		
Escherichia coli	PE 102/MC 1061		
Escherichia coli	ATCC 14763/PE 25		
Escherichia coli	ATCC 10798/MCC 4993		
Escherichia coli	PE 26		
Escherichia coli	PE 34	0111:NM	
Escherichia coli	PE 58	0111:NM	
Escherichia coli	PE 31	026:H11	
Escherichia coli	PE 32	026:H11	
Escherichia coli	PE 33	026:H11	
Escherichia coli	PE 27	0157:NM	
Escherichia coli	PE 56	0157:H7	USA
Escherichia coli	PE 49	0157:H7	USA
Escherichia coli	PE 29	0157:H7	USA
Escherichia coli	PE 35	0157:H7	USA
Escherichia coli	PE 28	0157:H7	USA
Escherichia coli	PE 30	0157:H7	USA
Escherichia coli	PE 46	0157:H7	USA
Escherichia coli	WA 11	0157:H7	Japan
Escherichia coli	WA 9	0157:H7	Japan
Escherichia coli	WA 10	0157:H7	Japan
Escherichia coli	WA 13	0157:H7	Japan
Escherichia coli	WA 120	0157:H7	Japan
Escherichia coli	WA 122	0157:H7	Japan
Escherichia coli	PE 676	055:H7	
Escherichia coli	PE 674	055:H7	
Escherichia coli	PE 675	055:H7	
Escherichia vulneris	ATCC 33821		
Escherichia hermannii	ATCC 33651		
Escherichia fergusonii	ATCC 35469		
Escherichia blattae	ATCC 29907		
Shigella boydii	ATCC 8700		
Shigella dysenteriae	ATCC 13313		
Shigella flexneri	ATCC 29903		
Shigella sonnei	ATCC 29930		

Materials and Methods

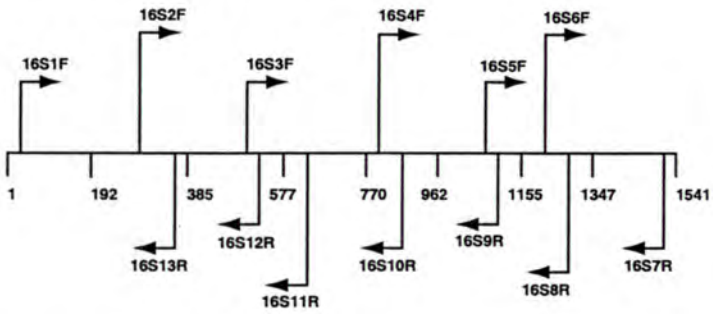


Figure 1: 16S rRNA Gene Map of PCR and Sequencing Primers

Sample Preparation

The DNA from a single loopful of bacterial cells was extracted using a classical phenol-chloroform extraction, ethanol precipitation method which has been described previously (1). Two microliters of the genomic DNA was added to a 100 µl PCR.

PCR Amplification

All PCRs were performed in a Perkin-Elmer TC 9600 using the following cycling parameters:

95 °C	8 minutes	
95 °C	30 seconds	
60 °C	30 seconds	30 cycles
72 °C	45 seconds	
72 °C	10 minutes	
4 °C	Hold	

16S rRNA PCR Master Mix

50 µl 2X MicroSeq™ 16S rRNA Gene Kit Master Mix :
- 0.2 µM 16S1F forward PCR primer
- 0.2 µM 16S7R reverse PCR primer
- 2.5 Units AmpliTaq Gold™ DNA polymerase
- 2.0 mM $MgCl_2$
- 350 µM total dNTPs
- 25 mM KCl
- 50 µl Bacterial Genomic DNA Solution

Completed reactions were stored at -20 °C. PCR amplification products were purified and concentrated two-fold in 10 mM Tris-HCl, pH 8.0; 0.1 mM EDTA using a Microcon-100 (Amicon) microconcentrator.

DNA Cycle Sequencing

DNA cycle sequencing reactions had a total volume of 20 µl and were carried out in a Perkin-Elmer TC 9600 using the following reaction conditions:

96 °C	10 seconds	
58 °C	60 seconds	30 cycles
4 °C	Hold	

16S rRNA Cycle Sequencing Mix

8 µl ABI PRISM™ Dye Terminator Ready Reaction Mix, FS
3 µl purified PCR product
3.2 pmoles 16S sequencing primer

Dye terminator cycle sequencing reaction products were purified using a Centri-Sep (Princeton Separations) column. The column filtrate was evaporated in a vacuum centrifuge and then resuspended in 4 µl Blue Dextran/EDTA:deionized formamide(1:5). An ABI PRISM™ 377 DNA sequencer and a 5% Long Ranger (AMERESCO) DNA sequencing gel was used for the analysis.

Results

The detection of polymorphic positions in the 16S rRNA gene allowed discrimination of the *E. coli* strains to the subspecies level.

The sequences of the nonpathogenic group of *E. coli* strains (Sigma Type B, ATCC 11775, PE 102, ATCC 14763, ATCC 10798 and PE 26) were highly variable, i.e. all of the sequences were different from one another. The pathogenic serotypes of *E. coli* could be differentiated from each other and in some cases, could be separated further within a given serotype (Figure 2).

The *E. coli* 0157:H7 strains from the U.S. could be separated into 3 groups based on sequence variability. The polymorphisms in the gene which distinguished this group were at positions 75, 76, 85, 86, 89, 133, 141, 470, 1002, 1018, 1034 and 1418. The Japanese *E. coli* 0157:H7 strains all had exactly the same sequence with a single polymorphism at position 1418 (Figure 2).

The sequence for two out of the three *E. coli* 026:H7 strains evaluated were the same (PE 32 and PE 33). The sequence of the third strain, PE 31, differed from the others by a single base at position 76. The sequences of the three strains of *E. coli* 055:H7 were identical. The sequences of the two *E. coli* 0111:NM strains studied were different in several polymorphic positions (75, 76, 85, 86, 89, 998, 1002, 1006, 1015, 1016, 1017, 1018, and 1019) (Figure 2).

The nearest neighbor pairing tree analysis (UPGMA) showed several discreet clusters (2). The *E. coli* 0157:H7 and the *E. coli* 055:H7 strains formed a single out group. The *E. coli* 0157:NM strain did not cluster with the *E. coli* 0157:H7 group. The *E. coli* 026:H11 strains formed a distinct out group. The *E. coli* 0111:NM strains segregated out into different clusters on the tree (Figure 4).

Several different species of *Escherichia* and *Shigella* were also included in the study. The sequences of *E. fergusonii* and *S. flexneri* were most similar to the sequences of the *E. coli* strains evaluated (Figure 4).

Figure 2: Polymorphic Positions Detected in the 16S rRNA Gene of* Escherichia coli *Strains Evaluated

	75	76	85	85	89	133	141	157	206	246	249	269	470	998	1002	1006	1015	1016	1017	1018	1019	1034	1418	1523
01 E coli Sigma Type B	R	C	G	Y	C	T	G	A	M	A	A	T	G	R	S	Y	R	R	W	W	K	Y	G	Y
02 E coli ATCC11775 MCC3485	G	C	G	C	T	Y	G	A	M	A	A	T	G	G	G	T	A	G	A	A	T	C	G	C
03 E coli MC1061 PE102	R	M	K	Y	Y	T	G	A	C	A	A	W	G	G	G	T	A	G	A	A	T	C	G	C
04 E coli ATCC14763 PE25	R	C	G	Y	C	T	G	A	C	A	A	T	G	R	S	Y	R	R	W	W	K	Y	G	C
05 E coli ATCC10798 MCC4993	R	M	K	Y	Y	T	G	M	C	A	A	T	G	G	G	T	A	G	A	A	T	C	G	Y
06 E coli ATCC5190 PE26	R	M	K	Y	Y	T	G	A	C	A	A	T	G	G	G	T	A	G	A	A	T	C	G	C
07 E coli 0111NM PE34	R	M	K	Y	T	T	G	A	C	A	A	T	G	R	S	Y	R	R	W	W	K	T	G	C
08 E coli 0111NM PE58	G	A	T	C	T	T	G	A	C	A	A	T	G	G	G	T	A	G	A	A	T	C	G	C
09 E coli 026H11 PE31	R	C	K	Y	Y	T	G	A	C	W	W	W	R	R	S	Y	R	R	W	A	K	Y	G	C
10 E coli 026H11 PE32	R	M	K	Y	Y	T	G	A	C	W	W	W	R	R	S	Y	R	R	W	A	K	Y	G	C
11 E coli 026H11 PE33	R	M	K	Y	Y	T	G	A	C	W	W	W	R	R	S	Y	R	R	W	A	K	Y	G	C
12 E coli 0157NM PE27	R	M	K	Y	Y	T	G	A	C	A	A	T	R	G	G	T	A	G	A	A	T	C	G	C
13 E coli 0157H7 PE56	G	A	T	C	T	T	G	A	C	A	A	T	G	A	C	C	G	A	T	T	G	T	R	C
14 E coli 0157H7 PE49	G	A	T	C	T	T	R	A	C	A	A	T	G	A	C	C	G	A	T	T	G	T	R	C
15 E coli 0157H7 PE29	G	A	T	C	T	T	R	A	C	A	A	T	G	A	C	C	G	A	T	T	G	T	R	C
16 E coli 0157H7 PE35	G	A	T	C	T	T	R	A	C	A	A	T	G	A	C	C	G	A	T	T	G	T	R	C
17 E coli 0157H7 PE28	G	A	T	C	T	T	R	A	C	A	A	T	G	A	C	C	G	A	T	T	G	T	R	C
18 E coli 0157H7 PE30	G	A	T	C	T	T	R	A	C	A	A	T	G	A	C	C	G	A	T	T	G	T	R	C
19 E coli 0157H7 PE46	G	A	T	C	T	T	G	A	C	A	A	T	G	A	S	C	G	A	T	W	G	Y	A	C
20 E coli 0157H7 WA10	G	A	T	C	T	T	G	A	C	A	A	T	G	A	C	C	G	A	T	T	G	T	R	C
21 E coli 0157H7 WA09	G	A	T	C	T	T	G	A	C	A	A	T	G	A	C	C	G	A	T	T	G	T	R	C
22 E coli 0157H7 WA11	G	A	T	C	T	T	G	A	C	A	A	T	G	A	C	C	G	A	T	T	G	T	R	C
24 E coli 0157H7 WA13	G	A	T	C	T	T	G	A	C	A	A	T	G	A	C	C	G	A	T	T	G	T	R	C
25 E coli 0157H7 WA120	G	A	T	C	T	T	G	A	C	A	A	T	G	A	C	C	G	A	T	T	G	T	R	C
26 E coli 0157H7 WA122	G	A	T	C	T	T	G	A	C	A	A	T	G	A	C	C	G	A	T	T	G	T	R	C
27 E coli 055H7 PE676	G	A	T	C	T	T	G	A	C	A	A	T	G	A	C	C	G	A	T	T	G	T	G	Y
28 E coli 055H7 PE674	G	A	T	C	T	T	G	A	C	A	A	T	G	A	C	C	G	A	T	T	G	T	G	Y
29 E coli 055H7 PE675	G	A	T	C	T	T	G	A	C	A	A	T	G	A	C	C	G	A	T	T	G	T	G	Y

Figure 3: Electropherogram of Sequence Assembly with Polymorphic Base Calls

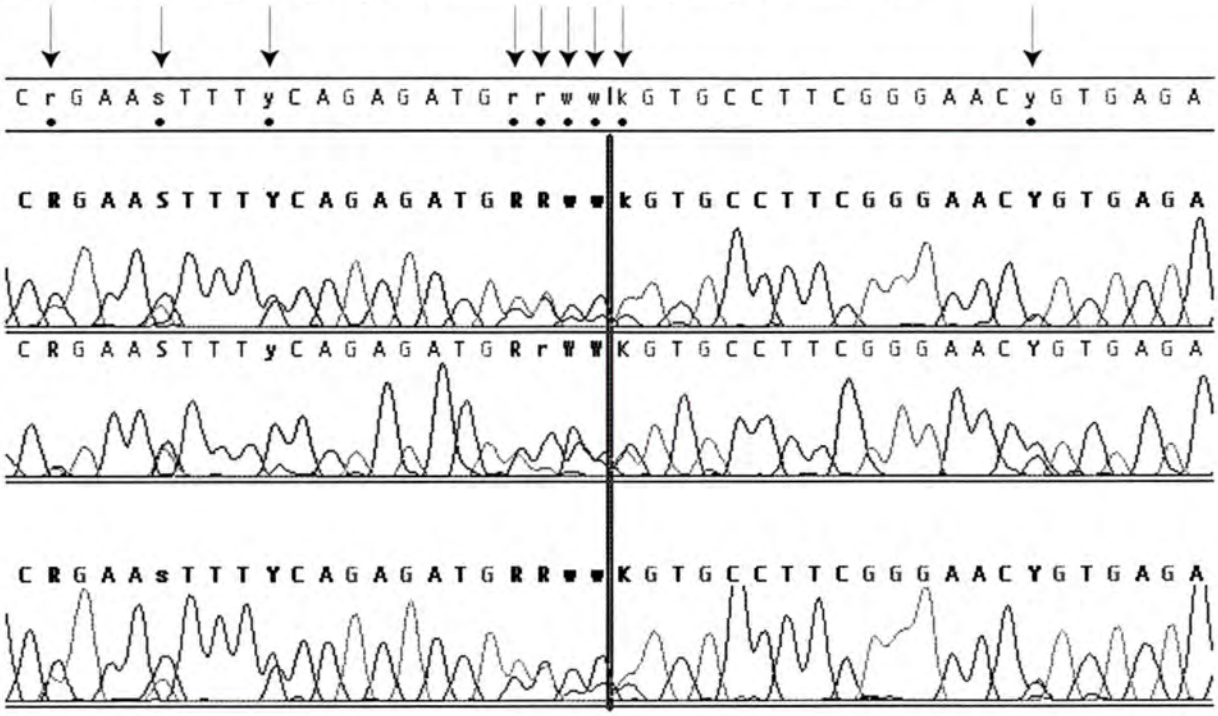
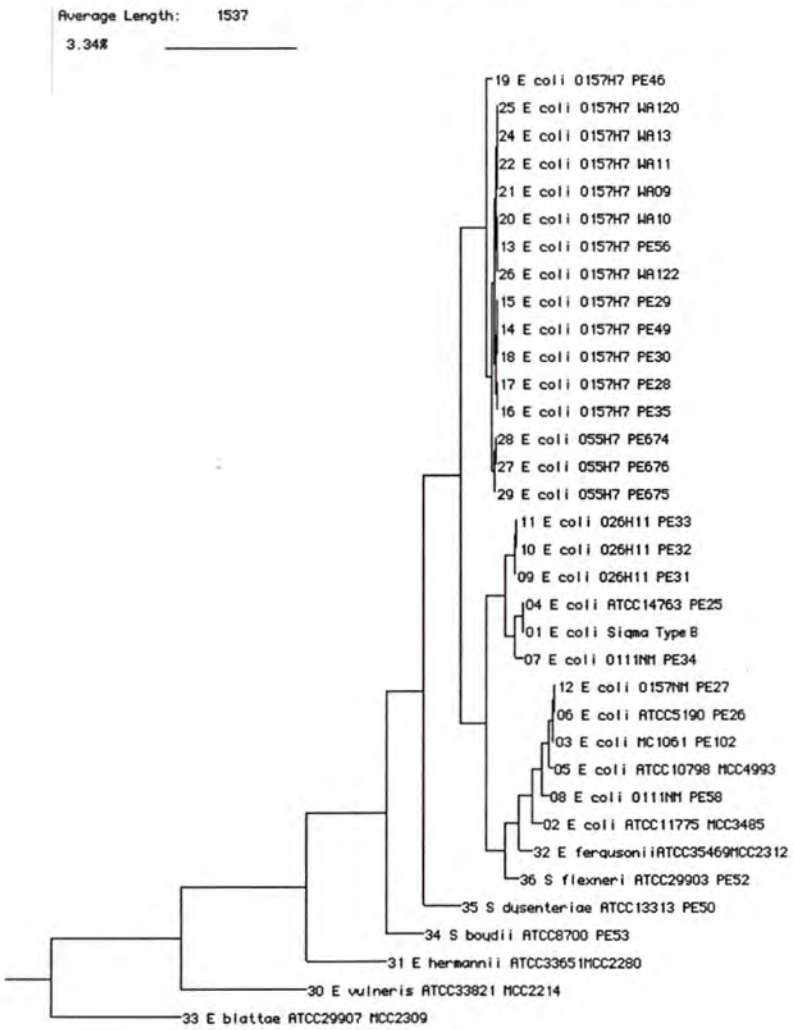


Figure 4: Nearest Neighbor Pairing Tree (UPGMA) of Strains Evaluated (2)



Conclusions

This investigation has shown that by using the polymorphic position information present in the 16S rRNA genes in those organisms possessing multiple copies it is possible to separate the closely related genera *Escherichia* and *Shigella*. Closely related species within the genus *Escherichia* (*E. coli*, *E. blattae*, *E. fergusonii*, *E. hermannii* and *E. vulneris*) can also be differentiated.

Subspeciation of the pathogenic and nonpathogenic members of the *E. coli* group was also achieved. Using serotype as a defining characteristic for the pathogenic *E. coli* , the 16S rDNA sequences of some members within a serotype were very similar to one another (026:H11, 0157:H7 and 055:H7 groups). Some of the *E. coli* serotypes showed increased sequence variability in the 16S rRNA gene within the members of the group which were studied (0111:NM and 0157:NM). The motile *E. coli* 0157:H7 strains appear to be genetically distinct from the non-motile *E. coli* 0157:NM isolate.

The identification of polymorphisms in the 16S rRNA gene enabled subspeciation and typing within the pathogenic *E. coli* strains which were evaluated. To achieve meaningful epidemiological information with respect to outbreak source it will be necessary to do a comparative analysis of the sequence information contained within genes which are more closely aligned to the organism's pathogenic capabilities such as toxin, drug resistance or surface protein coding genes.

References

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2. Waterman. 1995. *Introduction to Computational Biology*. Chapman and Hall. pp. 343–361.

Acknowledgments

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- Dr. Watanabe and Dr. Wada from the National Institute of Infectious Diseases, Department of Bacteriology, Tokyo, Japan.

THE WHITE HOUSE

WASHINGTON

April 13, 1998

INFORMATION

'98 APR 13 PM 7:08

MEMORANDUM FOR PHIL CAPLAN

FROM: DONALD KERRICK (DK)

SUBJECT: Request for President to Write a Foreword

I recommend you decline Mr. Chacon's request that the President write a foreword for the West Point class of 1951 reunion book. I am concerned that a positive reply to this request would set a precedent that we would not want to commit the President to upholding for other service academy classes.

Attachment
Tab A Incoming correspondence

Dan-

Can you do a nice turn-down letter.

Thanks

Phil

TAB A

2500

THE WHITE HOUSE
WASHINGTON

Date 4/6

To: Don Kerrick

From: The Staff Secretary

Any view on this?

POTUS generally does not write forewords for books. Please advise

Phil

2500

THE WHITE HOUSE
WASHINGTON

Date 4/6

To: Don Kerrick

From: The Staff Secretary

Any view on this?

POTUS generally does not write forewords for books. Please advise

Phil

25010

Saguaro Publications / Consultants
13118 Cedarbrook Ave. NE
Albuquerque, New Mexico 87111
505-291-0744/9

98 APR 6 [illegible]

April 1, 1998

Ms. Nancy Hernreich, Manager
Oval Office Operations
1600 Pennsylvania Ave. NW
Washington, DC 20500

Dear Ms. Hernreich:

I have written two letters to the President which have gone unanswered and I seek your assistance in at least acknowledging their receipt.

I am enclosing a copy of the enclosure to last letter for your reference. It is the proposed FOREWORD for a reunion book I am writing for my West Point Class.

Please advise whether the President is interested in signing the Foreword to the book I am writing on the West Point Class of 1951 for our 50th reunion. I would think that as Commander-in-Chief he would.

Of course, feel free to have the Foreword re-written as appropriate, perhaps the use of White House stationary is called for.

Sincerely,

José Andrés Chacón, Publisher &
Adjunct Professor
Anderson Schools of Management
University of New Mexico

APR 6 16:12

Foreword

By the Commander-in-Chief
1993 to 2001

This book is about the accomplishments of the Class of 1951 from the United States Military Academy at West Point. There were 475 who graduated on June 5, 1951 and almost immediately were tested in combat. Eight made the extreme sacrifice in Korea and two later in Vietnam. Five were decorated with *the Distinguished Service Cross for heroism and valor, involving extraordinary risk of life in connection with military operations against an enemy.*

The class of 1951 has distinguished itself not only in military service but in many walks of life. Shy Meyer served as Chief of Staff of the Army, Roscoe Robinson was the first black USMA graduate to become a four-star general, Buzz Aldrin was the first to land on the moon; Dave Abshire helped establish the Center for Strategic Studies at my alma mater Georgetown University; the list goes on and on. Members of the class continue to serve their Country and embody the spirit of West Point.

The *1947 Bugle Notes of the United States Corps of Cadets*, was originally entitled the West Point Handbook, was dedicated to those who joined the Corps in 1947 and urged that the new members to; "Preserve the traditions compiled therein, they are your heritage." I want to quote two items from the 1947 Bugle Notes that in my mind comprise the spirit of West Point as I have come to know it. **First**, the Missions of the Military Academy; (1) To instill discipline and a high sense of honor. (2) To develop the powers of analysis so that the mind may reason to a logical conclusion, and (3) To instruct and train the Corps of Cadets so that each graduate shall have the qualities and attributes essential to his progress and continued development throughout a lifetime career as an officer in the regular army. **Second**, a letter to the new class from then Secretary of War, Robert P. Patterson; he said: "With the perils of another war behind us, all Americans have renewed reason to be thankful for the spirit of West Point. We should never forget that in this imperfect world military strength still determines the destiny of nations. It is the work of West Point to give us military leadership in time of need. It also gives us, by the conduct of its graduates, a standard of valorous devotion to the nation's cause. West Point is the existing embodiment of the patriotism of its spiritual founder, the Army's first and foremost leader, George Washington. Sincerely yours, Robert P. Patterson, Secretary of War"

The Class of 1951 at West Point has served America well!

William Jefferson Clinton
President of the United States

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Albuquerque, New Mexico 87111

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PM
APR

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20500-0003