Identification of specific host virulence factors of enterohemorragic *Escherichia coli* strains of serogroup O26 by Subtractive Hybridization

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Introduction

Enterohemorragic *E.coli*

In humans

Infected by plant and animal foods soiled by feces from cattle/sheep

E. coli serotypes that cause hemorragic diarrhea.

In 90%

Hemorrhagic colitis

Hemolytic Uremic Syndrome

Important problem in public health in developped country

Particularly in children and elderly

Renal sequelae

Death

Enterohemorragic *E.coli*

In cattle

Several serogroups (O26, O111, O118) directly associated with diarrhea in calves

(2 weeks - 2 months)

Consequence: **Economic losses**

Enterohemorragic *E.coli*

In cattle/sheep

Healthy carrier

Consequence: **Public health hazard**

Pathogeny

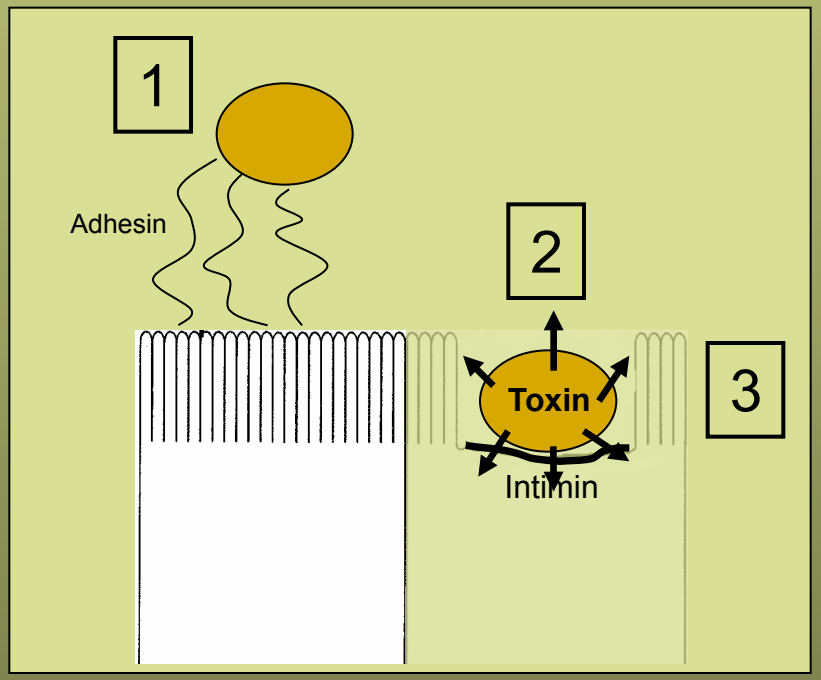
Probably 3 steps :

1) Initial attachement by adhesins

2) Secondary attachement and colonisation :

attaching and effacing lesion

3) Production of verotoxins



Pathogeny

Probably 3 steps :

1) Initial attachement by adhesins

2) Secondary attachement and colonisation :

attaching and effacing lesion

3) Production of verotoxins

Host specificity:

1) Lpf

interest for the first step

2) ToxB and efa1

- Important step

- Badly known for EHEC strains

Aim of the project

Determination of the factors implicated in :

- **initial attachment**
- **host specificity (man or cattle)**

of EHEC strains O26

Scientific stategy

1) Identification of the factors

Comparison of the genome

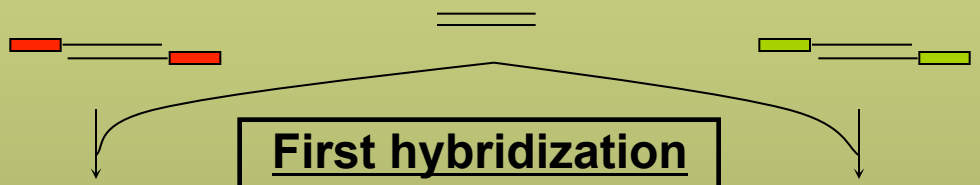
Between human/bovine pathogenic EHEC
and non-pathogenic EHEC

Suppressive substractive hybridization (SSH)

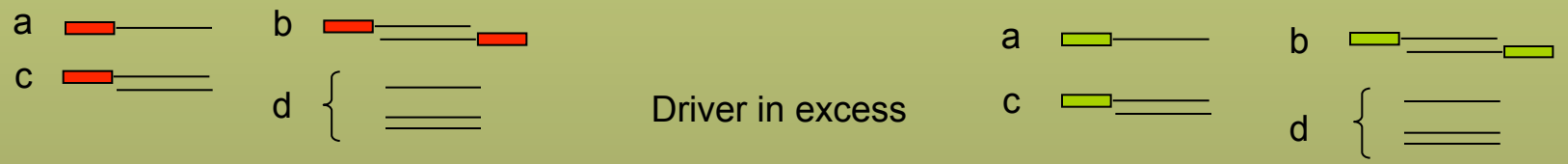
Driver in excess
(pathogen human O26 EHEC genome or
non pathogen bovin O26 genome)

Tester with adaptator 1
(pathogen bovine
O26 EHEC genome)

Tester with adaptator 2
(pathogen bovine
O26 EHEC genome)



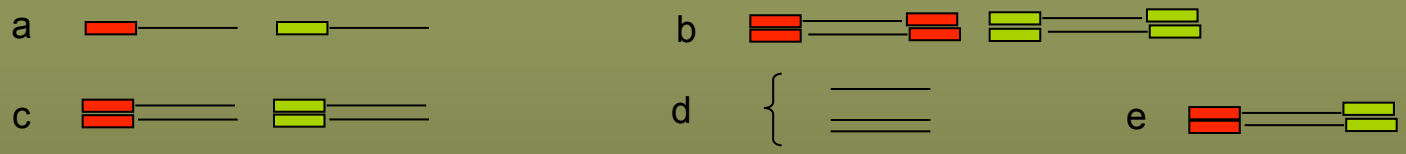
First hybridization



Second hybridization

a, b, c, d + e 

Fill in the ends



Amplification by PCR

a, d : no amplification
c : linear amplification

b : no amplification
e : exponential amplification

1) Identification of the factors

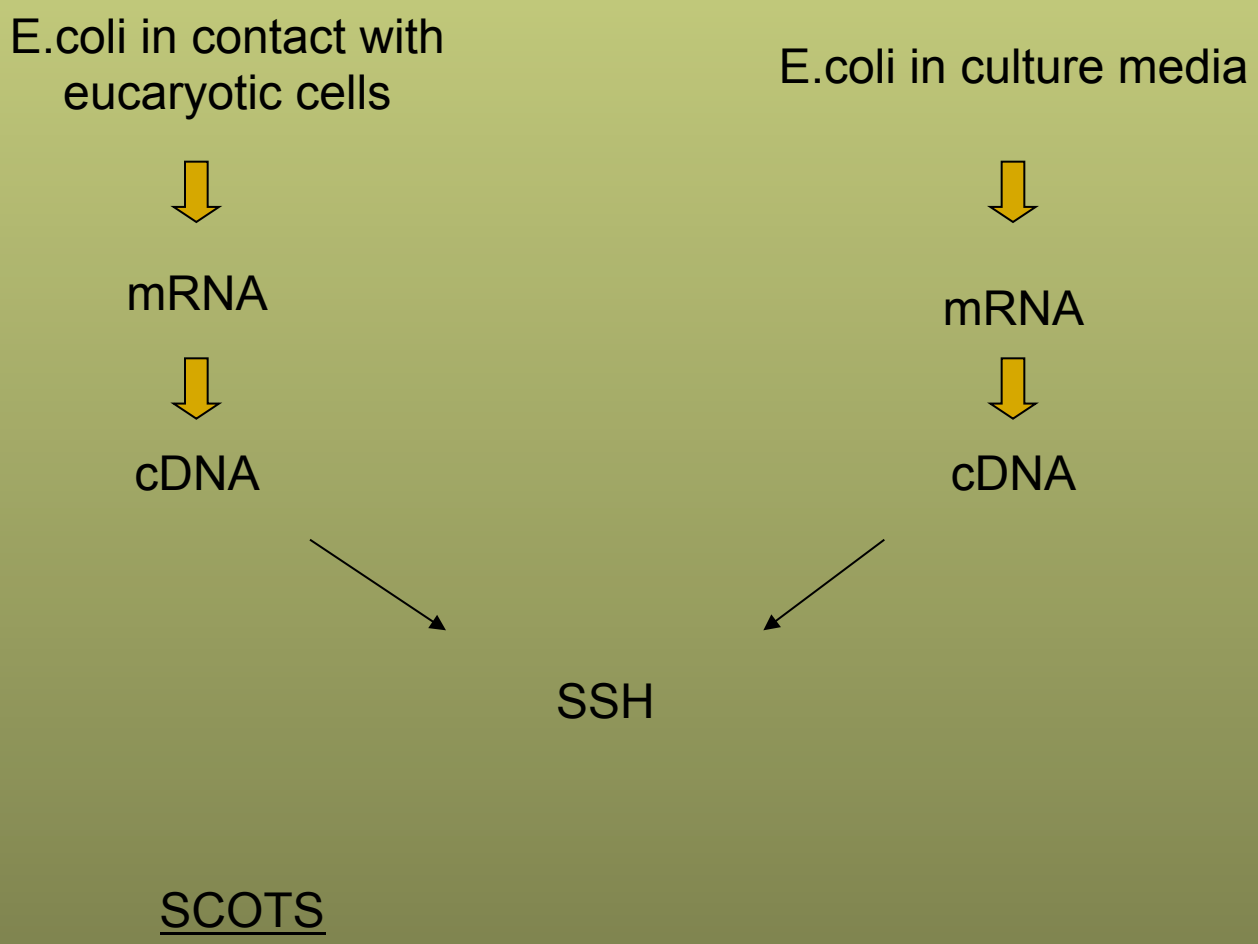
Comparison of the transcriptomes

Between human/bovine pathogenic EHEC grown in broth and in contact with intestinal cells in culture

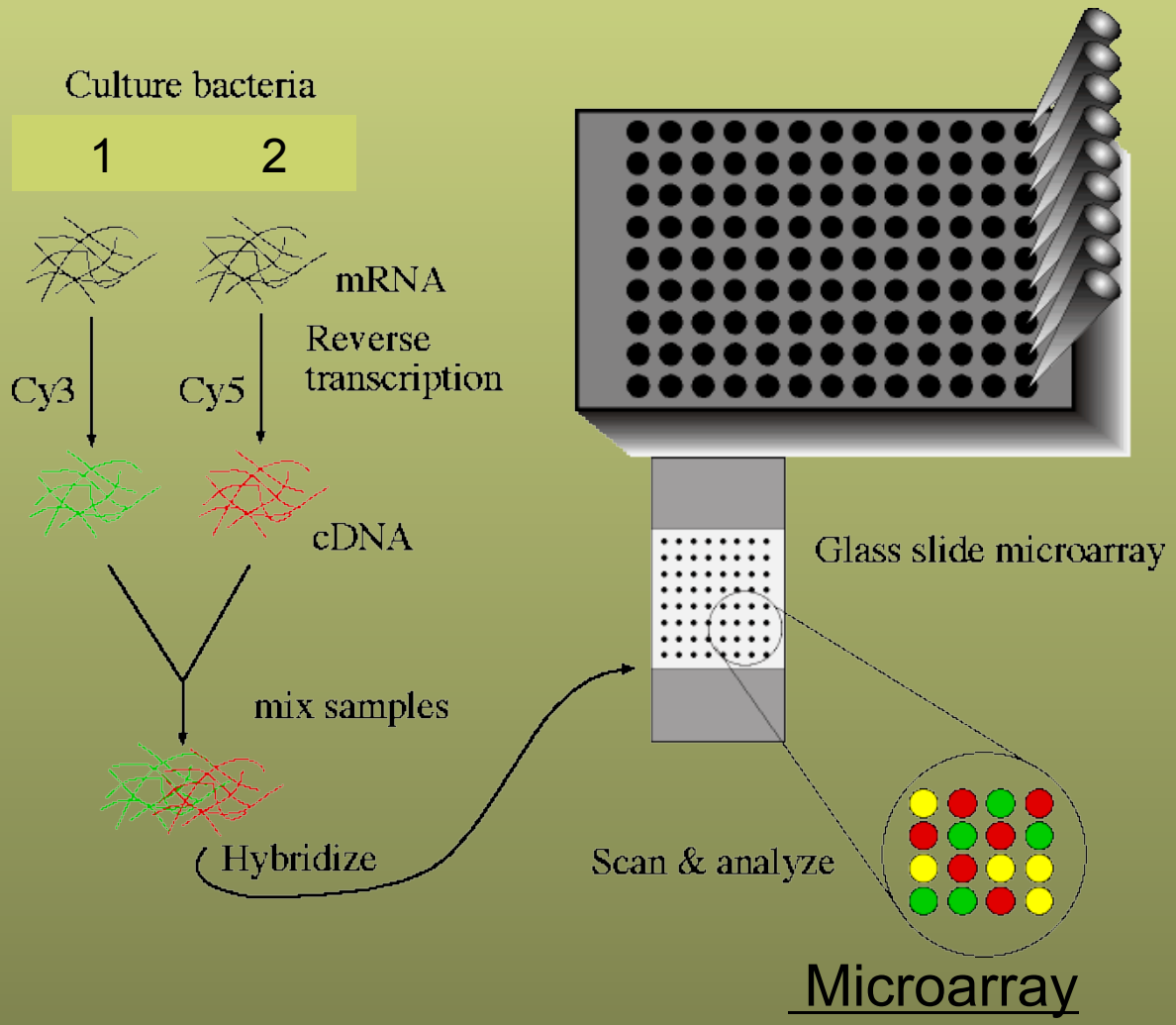
SCOTS

Microarray

1) Identification of the factors



1) Identification of the factors



2) Study of the isolated DNA fragments sequences

1) Sequencing and analysis by **computer** (BLAST etc)

2) **Mutagenesis**

Study of the mutants in adherence tests on intestinal bovine and human cells in culture

3) **Hybridization DNA-DNA**

Presence of the genes in a collection of strains isolated from bovines, sheep, humans and foods, and belonging to different serogroups

Thanks for your attention