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Role of Cell Surface Structures in Biofilm Formation by *Escherichia coli*

Hafida Zahir[1*], Hamadi Fatima[2], Lekchiri Souad[1], Mliji El Mostafa[3], Ellouali Mostafa[1], Latrache Hassan[1]

[1]Bioprocess and Biointerface Laboratory, Faculty of Science and Techniques, Sultan Moulay Slimane University, Beni Mellal, Morocco
[2]Laboratory of Biotechnology and Valorisation of Natural Resources, Department of Biology, Faculty of Science, University Ibn Zohr, Agadir, Morocco
[3]Food Safety & Environmental Department, Institute Pasteur, Casablanca, Morocco
Email: [*]hafzahir@yahoo.fr

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Abstract

This study aims to understand the relationship between capabilities of *Escherichia coli* strains to form biofilm and serotype groups expressed on cell surface. Sixteen strains of *E. coli* were originally isolated from different food processing lines in different Moroccan cities. Strains serotyped based on their O (somatic), H (flagellar), and K (capsular) surface antigen profiles using different antiserums. Biofilm assays carried out in 96-well microtiter dishes using the method of O'Toole *et al*. Our results show that no clear relation observed between origin and serotype groups. In the other hand, we observed that not all studied strains were able to form biofilm. Furthermore, combination of antigens H40 and K11 appears to be involved in biofilm formation. In fact, the H antigen seems to be implicated in the placement of the bacterial cells near the surface and the K antigen may play a role in physicochemical interactions between bacteria and inert surface.

Keywords

Biofilm, *Escherichia coli*, Cell Surface, Serotype, Antigen, Bacterial Adhesion

1. Introduction

Contamination of surfaces due to microbial attachment occurs in many environments and may create serious economic and health problems associated with food spoilage and disease transmission [1]. Rather than being

[*]Corresponding author.

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uniform, biofilms are extremely complex structures containing micro colonies separated by water-filled channels [2] [3], that may render antibiotics and biocides ineffective and may become a constant source of contamination in the food-processing industry [1] [4]. Biofilm-derived cells are also often more resistant to adverse environmental conditions, such as desiccation [4] and extreme temperatures [5], than those planktonic (free-living) cells grown in batch culture. Biofilm formation is, in addition, a dynamic process that occurs in several phases. Initially, a bare surface is covered with a conditioning film, where organic molecules are deposited from the liquid phase onto the surface, the organism must be brought into close proximity of the surface, propelled either randomly or in a directed fashion via chemotaxis and mobility [6]. Initial bacterial colonization of surfaces is reversible and may be mediated by surface-expressed appendages such as fimbriae [7] and flagella [7] [8]. Other surface characteristics including hydrophobicity, acid-base properties and surface functional groups' composition are implicated [9]-[16]. Irreversible adhesion to surfaces is often associated with the expression of extracellular material and the formation of biofilms, three-dimensional matrix-enclosed populations adherent to each other and to surfaces that have often many microns thick [17], the bacteria produce a extracellular polymeric substances (EPS) as a protective layer [18] and is microenvironment-conservative [19]. The association becomes stable for micro-colonies formation [20]-[22] and to create the glycocalyx [23]. The maturation of biofilm generate quorum sensing [24], gene transfer [25], persisted development [26] etc.

E. coli was the earliest organism to have its genome sequenced; the complete genome of *E. coli* K-12 was published in 1997 [27] [28]. For *E. coli* K-12 genes, 38% of genes were expressed differentially inbiofilm [29] [30] have shown that fimbriae play a role in adherence and biofilm formation of Salmonella enteric species. *E. coli* K-12 transformed with the 60-megadalton plasmid produced fimbriae and was able to adhere to intestinal cells [31]. It also has been demonstrated that the normal Lom protein participates directly in adhesion or regulates the synthesis of other protein (s), which may be involved in adhesion [32]. The Escherichia coli OmpR/EnvZ two-component regulatory system, which senses environmental osmolarity, also regulates biofilm formation [33]. The influence of bacterial surface lipopolysaccharides (LPS) on cell transport and adhesion has been examined by use of three mutants of *Escherichia coli* K12, it is further suggested that bacterial deposition behavior is determined by the combined influence of DLVO interactions, LPS-associated chemical interactions, and the hydrodynamics of the deposition system [34].

In order to determine ways in which medical, industrial and ecological biofilm contamination may be prevented, it is important to understand the factors that promote bacterial adhesion and the formation of multicellular communities on abiotic surfaces Thus, the purpose of this study is to understand the relationship between capabilities of 16 *E. coli* strains to form biofilm and serotype groups expressed on cell surface.

2. Materiel and Methods

2.1. *Escherichia coli* Strains, Media and Culture Conditions

16 strains of *E. coli* were originally isolated from biofilm taken from different food processing lines in different Moroccan cities. The strains have been previously identified using the API 20E system. Frozen cells have been transferred in LB broth at 37˚C for one night and subculture don solid LB medium.

2.2. Serotyping

According to the modified Kauffman scheme [35], *E. coli* are serotyped on the basis of their O (somatic), H (flagellar), and K (capsular) surface antigen profiles (185, 394). A total of 170 different O antigens, each defining a serogroup, are recognized currently. The presence of K antigens was determined originally by means of bacterial agglutination tests: an *E. coli* strain that was inagglutinable by O antiserum but became agglutinable when the culture heated was considered to have a K antigen. Antiserum types used in this study were presented in **Table 1**.

2.3. Biofilm Assay

Biofilm assays were carried out in 96-well microtiter dishes using the method of O'Toole [36]. 180 µl of cells culture were placed in each well of plate and 20 µl of sterile distilled water (SDW) were added. After incubation at 37˚C, the medium was removed and the plats were washed with sterile distilled water to remove loosely attached bacteria. The wells were stained with 200 µl of 1% of crystal violet for 15 min. After staining, plates

rinsed gently by SDW until disappearance of stain and left to dry at ambient temperature.

3. Results and Discussion

The *E. coli* strains were isolated from different food processing lines. No clear relation was obtained between origin and serotype groups. The O126 and O127 seem to be expressed in a majority of isolated strains. Particularly, the H serotype is not expressed by *E. coli* isolated from meat powder and these 4 strains expressed the K88ac serotype (**Table 2**).

We observed that not all these strains were able to form biofilm. 8 *E. coli* strains, E2, E4, E6, E7, E13, E14, E15, and E16 able to form biofilm are presented in **Table 3**, they have all both Ag H40 and K11.

Table 1. Antiserums used to characterize cell surface structures.

Somatic antiserum (O):	O_{111}, O_{26}, O_{55}, O_{86}, O_{119}, O_{127}, O_{125}, O_{126}, O_{128}, O_{114}, O_{124}, O_{142}.
Flagella antiserum (H):	H_7, H_8, H_{10}, H_{11}, H_{14}, H_{19}, H_{25}, H_{26}, H_{40}, H_{49}.
Capsular antiserum (K):	K_3, K_{11}, K_{12}, K_{24}, K_{25}, K_{82}, K_{88ac}, K_{97}, K_{99}.

Table 2. Bacterial strains origins and surface serotype groups.

Code	Strain origin	Somatic O	Flagellar H	Capsular K
E1	Turkey cheese ball	O 126	H 14	_
E2	Tajine of meat	O 111	H 40	K 11
E3	Raw Asianwac	O 114	H 8	K 11
E4	Moroccan Salad	O 114	H 40	K 11
E5	Turkey sausage	O 126	_	_
E6	Carrot salad	O126	H 40	K 11
E7	Salad “crudité”	O126	H 40	K 11
E8	Meat powder	O 127	_	K 88ac
E9	Meat powder	O 127	_	K 88ac
E10	Meat powder	O 127	_	K 88ac
E11	Meat powder	O 127	_	K 88ac
E12	Cheeseburger	O 124	H 8	K 25
E13	Salad with salmon, H.2	O 127	H 40	K 11
E14	Salad with salmon, H.5	O 111	H 40	K 11
E15	Salad with corn; H.3	O 124	H 40	K 11
E16	Salad with corn, H.4	O 126	H 40	K 11

Table 3. Relation of capabilities to form biofilm and antigens expression on cell surfaces.

Code	Somatic O	Flagellar H	Capsular K	Biofilm assay
E2	O 111	H 40	K 11	+
E4	O 114	H 40	K 11	+
E6	O 126	H 40	K 11	+
E7	O 126	H 40	K 11	+
E13	O 127	H 40	K 11	+
E14	O 111	H 40	K 11	+
E15	O 124	H 40	K 11	+
E16	O 126	H 40	K 11	+

+: Ability to biofilmformation.

Antigens H40 and K11 appear to be involved in biofilm formation. This may be explained by the fact that the flagella antigens are involved in the first stage of biofilm formation. In D.L.V.O theory, the curvature of the particle play a role in the attraction to the surface, indeed over the angle of the curvature; the smaller contact is easy. The extremity flagella are relatively far from the surface of the cell, so they easily cross energy barrier and enhance the attraction of the cells to the support. Moreover, it is not possible to generalize this interpretation for all types of flagella. E3 strain has the K11 antigen but does not form a biofilm. This strain has H8 but does not H40 antigen. This indicates that presence of both antigens K11 and H40 is necessary for adsorption to surface. So, the K11 capsular antigen alone is insufficient to induce the formation of biofilm.

The adsorption operation may then be completed by the role of the capsular and somatic antigens.In our laboratory [37], we have showed that the relative hydrophobicity of *E. coli* varies between strains expressing different surface structures. The strains expressing PAP fimbriae and/or O-antigen showed a higher surface hydrophobicity than strains which express only type 1 fimbriae and/or R-antigen. Otherwise, the polysaccharide capsules of AL 213 and AL 499 strains generated a high negative surface charge but for non-capsulated *E. coli* the surface charge of rough strains is higher than smooth strain.

4. Conclusions

In this work, we have studied the relationship between capabilities of 16 *E. coli* strains to form biofilm and serotype groups expressed on cell surface.

Results showed that: No clear relation between origin and serotype groups. The O126 and O127 seem to be expressed in a majority of isolated strains. Particularly, the H serotype is not expressed by *E. coli* isolated from meat powder and these strains expressed the K88ac serotype. Not all these strains were able to form biofilm, strains able to form biofilm have all both Ag H40 and K11.

The H antigen seems to be implicated in the placement of the bacterial cells near the surface and the K antigen may play a role in physicochemical interactions between bacteria and inert surface. This worker presented a new perspective in biofilm and opened a way to fully understand the mechanisms underlying different aspects of biofilm development. Studies with a more strains of *E. coli* and other bacteria would be developed in the future.

Compliance with Ethical Standards

Conflict of Interest: The authors declare that they have no conflict of interest.

This article does not contain any studies with human participants or animals performed by any of the authors.

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