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Original Research Article

Effect of aqueous extract of *Eucalyptus microcorys* on *Salmonella typhi* strain adhered cells to polythene fragments in water microcosm

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ABSTRACT

This study was aimed at evaluating the effect of aqueous extract of *Eucalyptus microcorys* on the bacterial cells of *Salmonella typhi* adhered to polythene fragment at different cell growth phases in aquatic microcosm. The cell growth phases that have been considered are the lag, exponential, stationary and decline phases. The analyzes were performed in static conditions. The duration of cell adhesion process (DCAP) used to allow cell adhesion to polythene were 3h, 6h, 9h, and 24h. Polythene fragments containing adhered bacteria of known amount were then introduced into the solution containing different extract concentrations of *Eucalyptus microcorys* (10,15 and 20 $g.l^{-1}$). Fragments were removed after 1h, 2 h and 3h contact time with extract solution. The results revealed that the extract of *Eucalyptus microcorys* causes the detachment of several bacterial cells initially adhered to fragments of polythene. The degree of detachment is modulated by the phase of cell growth. The largest reduction of *Salmonella typhi* cells adhered to polythene was observed with cells coming from exponential and stationary growth phases and also after 3h exposure to fragments in the extract.

1. Introduction

Water, by its dual function "matter" and "environment", is a resource of capital importance for the maintenance of life (plants, animals and humans) on earth. It is essential for humans both for their food needs and for their agricultural and industrial activities. However, today, a third of humanity lives in a situation known as “water stress”, with less than 1.700 cubic meters of fresh water available per capita and per year.[1] Despite the small quantity of fresh water directly accessible to humans, its quantity and quality are strongly influenced by the phenomenon of global warming on the one hand and by pollution which can be physical, chemical or biological on the other hand.[2]

Water pollution, mainly due to poor management by humans of natural waters, can lead to the alteration of its physico-chemical quality and the presence in this vital fluid of viral particles and potentially pathogenic microorganisms.[3] Among the bacteria known to be responsible for water pollution are the species belonging to the genera *Salmonella, Shigella, Escherichia, Yersinia, Vibrio* and *Campylobacter* among others.[1] The different physical and chemical disinfection methods aimed at ridding water of these bacteriopollutants do not always give positive results, the latter having developed mechanisms

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providing them with increased resistance to disinfection, among which there is the formation of biofilms.[4] In developing countries and in Cameroon in particular, the supply of drinking water by the national water distribution company does not always follow the great demographic explosion and uncontrolled urbanization. In addition, plastic utensils (barrels, buckets, cans, etc.) are widely used in developing countries for water supplies in ignorance of their physicochemical properties and their capacity for microbiological adhesion. From the point of view of public health, the formation of a biofilm in the drinking water network is problematic. In fact, the partial uprooting of a biofilm developed on the internal walls of the pipes can be the cause of the resuspension of potentially pathogenic microorganisms, which constitutes a health risk for consumers, particularly by the increase in the frequency of gastro-enteric symptoms (diarrhea, vomiting).[5]

Several studies have been conducted on the adhesion of bacteria to substrates in an aquatic environment. These studies have shown that the adhesion of bacteria is a function of the surface properties of the bacteria, the physiology of the bacteria, the chemical composition of the medium and the substrate serving as support.[6]

The studies of some authors revealed that adsorption of A. hydrophila cells on polythene in water treated with NaOCl and H_2O_2 decreased with incubation duration and disinfectant concentrations.[7] The use of the bioactive compounds of extract plant can be an alternative process, therefore, in the fight against the adhesion to bacteria for support or against the presence of biofilms along the pipeline, and also in the bacterial cell inhibition.[8] The main objective of this study is to assess the effect of the aqueous extract of *Eucalyptus microcorys* on *Salmonella typhi* cells coming from different phases of cell growth, and adhered to polythene fragments in water.

2. Material and Methods

2.1. Choice of plant and preparation of aqueous extracts

The choice of *Eucalyptus microcorys* in this study was motivated by several reasons: its multiple traditional uses in households in our country to fight against respiratory diseases and muscle pain, for its bioactive molecules (Anthocyanins, Anthraquinones, Tannins) and antimicrobial properties.[9,10] Fresh leaves of *E. microcorys* were harvested in Yaounde, Centre region (Cameroon) and dried at room temperature (23+2 °C) in the laboratory for 30 days. The leaves were thereafter ground and sifted to obtain powder which was used to prepare a decoction. The latter was dried in an oven at 45–50 °C.[11]. The crystals obtained were used to prepare the crude extract. Three ranges of extract concentration: $10g.l^{-1}$, $15g.l^{-1}$ and $20g.l^{-1}$ were prepared by dissolving the different masses in sterile physiological water.

2.2. Phytochemical screening of Eucalyptus microcorys extract

In order to determine the different classes of bioactive compounds present in the extract of *Eucalyptus microcorys*, phytochemical screening was carried out using standard method.[12] These methods include researching polyphenol, triterpenoids, sterols, alkaloids, saponins, gallic and cathechic tannins, flavonoids, anthraquinones and anthocyanins.

2.3. Substrate used for the adhesion test and making of the supports

The substrate used in this study is high density polythene. This substrate has already been used in some studies.[7,8] Its frequent use in the manufacture of drinking water pipes and in household utensils for water supplies justify the choice of this substrate. In addition, the hydrophobic nature of this plastic material makes it a support favorable to the adhesion of bacteria.

2.4. Determination of growth phases of Salmonella typhi

The study focused on *Salmonella typhi*. This bacterial species was chosen because of its use as an indicator of the microbiological quality of water intended for consumption and its importance in public health.[2] On the basis of previous studies regarding the protocol of determination of different growth phases of *Aeromonas Hydrophila* and enteropathogenic *Escherichia coli*, and the cell growth phases of *Samonella typhi* were determined.[8,13] The experiment being carried out in triplicate, the average of the CFU was calculated after each incubation period as well as the log (CFU). The log curve (CFU) as a function of the incubation time has been plotted. The durations of the cell growth phases of the bacterial strain were then evaluated.

2.5. Performing adhesion tests of Salmonella typhi on polythene

The adhesion process was done according to the protocols developed by some authors. Rectangular shaped of polythene fragments with 13.28 cm^2 of the total surface were prepared and were immersed in thirty flasks water containing bacteria. The experiments were conducted in static water conditions to promote the adhesion of bacterial cells to the surfaces of the plates. The Duration cell adhesion process (DCAP) was 3 h, 6 h, 9 h, and 24 h.[8]

The supports immersed in the plant extract at different concentrations were removed after 1 h, 2 h and 3 h of exposure at the rate of 3 tubes after each contact time. The 3 tubes removed correspond to the different concentrations

of the extract (10g.l^{-1}, 15g.l^{-1}, and 20g l^{-1}). For each substrate, detachment was carried out 3 times in 10 ml of sterile physiological water in order to maximize the detachment of the adhered cells. Collection and numbering of unhooked cells was made by culture on selective agar medium (Hektoen), followed by incubation on Petridishes at 37°C for 24 hours. The results are expressed in colony forming units per square centimeter of substrate (CFU/cm^2).

2.6. Data analysis

The abundance of bacterial cells unhooked from the supports and those of the bacterial cells counted in the solutions of the plant extracts after removal of the supports were represented by histograms in logarithmic coordinates per unit area (ln (CFU/cm^2)), using Excel 2003 software. The relationships between the parameters analyzed were evaluated by the Spearman correlation test and the Kruskal Wallis comparison test using SPSS 12.0 software.

The percentages of adhered (% A) and detached (% D) cells of *S. typhi* were calculated with these formula.[11]

$$\%\,D = \frac{Nd}{No}\,X\,100 \%\,A = \frac{Na}{No}\,X\,100$$

With:

% A = Percentage of adhered cells; % D = Percentage of detached cells; Na = Abundance of adhered cells; Nd = Abundance of detached cells; N0 = Abundance of bacterial cells unhooked on the control support.

The inhibition percentages of *S. typhi* cells adhered to the polythene supports were calculated. The percentages of inhibitions calculated here are those obtained after 3 hours of contact of the polythene supports with the extract at different concentrations. The formula used is as follows:

$$PIa = \frac{No - (Na + Nd)}{No}\,X\,100$$

with:

PIa = Percentage inhibition of bacterial cells; N_0 = Abundance of bacterial cells unhooked on the control support.

3. Results

3.1. Abundance of the chemical constituents of the leaves of Eucalyptus microcorys

The main chemical constituents of the aqueous extract of *Eucalyptus microcorys* are the alkaloids and the anthraquinones, followed in less quantity by the flavonoids and the saponosides. Anthocyanins, gallic tannins, and polyphenols are found in very small quantities. The different chemical constituents of the leaves of *Eucalyptus microcorys* and their abundance are summarized in Table 1.

3.2. Growth curve of salmonella typhi

The growth of *Salmonella typhi* describes a hyperbolic curve with 4 phases: a Lag growth phase or adaptation phase which lasts 30 minutes, an exponential growth phase from 30 minutes to 10 hours, a stationary growth phase from 10 to 20 hours, and a decline phase from 23 to 28 hours.

3.3. Abundance of adhered and detached bacterial cells in the presence of the extract

In general, it was noted that the abundance of adhered bacterial cells decreases in the presence of the extract regardless of its concentration and the time of exposure of the supports. As for the detached cells, they generally vary with the concentration of the extract, the incubation times of the adhesion test and the time of exposure of the supports in the extract. There are also variations in the abundance of adhered and detached bacterial cells from the different phases of cell growth.

For cells stemming from lag growth phase, the number of *S. typhi* cells adhered to the supports in the absence of the extract varied between 12 and 13 units (ln (CFU /cm^2). These numbers decrease in the presence of the extract. The minimum abundance of adhered bacterial cells, 7units (ln (CFU/cm^2)) was noted at 15 g/l of extract, after 3 h of incubation of the suspension with the supports alone, this for 1 and 2 h of exposure of the supports in the extract; 20 g.l^{-1} of extract after 3, 6, and 9 hours of incubation of the bacterial suspension with the supports alone, this respectively for 1 hour; 2h and 3h of contact of the supports with the extract solution. The maximum abundance was observed at all concentrations and generally after 1 hour of exposure. At 20 g.l^{-1} of extract, there were no detached cells after 3 and 24 hours of incubation of the bacterial suspension with the supports alone, this respectively after 2 h, 3 h and 1 h, 3 h of contact of the fragments with the extract.

For cells coming from exponential growth phase, the abundance of *S. typhi* cells adhered to the supports in the absence of the extract varied little depending on the duration of incubation. They remained greater than or equal to 14 units (ln (CFU/cm^2)). These abundances drop completely in the presence of the extract. This at 10g / l of extract after 3 hours of exposure of the fragments and at 20 g.l^{-1} after 2h and 3h of exposure in the extract. The maximum abundance of adhered cells, 14.5 units (ln (CFU/cm^2)), was observed at 15 g / l of extract mainly after 1 h of exposure of the supports in the extract. The maximum of detached cells, 14 units (ln (CFU/cm^2)) was observed at 10 and 20g.l^{-1} of extract, after 1 day of incubation of the bacterial suspension with the supports alone. At 10g.l^{-1} of extract after 6 and 9 hours of incubation of the suspension with the supports alone and for respectively 1 hour, 2 hours and 1 hour of exposure, there are no detached cells. The same observation

was made at 20g.l^{-1} after 3 and 6 hours of incubation and for 3 hours and 1 hour respectively, 3 hours of exposure of the supports in the extract.

For cells harvested from stationary growth phase, the abundances of *S. typhi* cells from this cell growth phase adhered to the supports in the absence of the extract remained approximately equal to 15 units (ln (CFU/cm2)). In the presence of the extract, there was a great reduction in these abundances. At 10g.l^{-1} of extract and generally after 3 h of exposure of the supports, and at 20 g.l^{-1} of extract after 2 h and 3 h of exposure of the supports in the extract, there is a total decrease in adhered bacterial cells.

However, it was observed that, at 15 g.l^{-1} of extract and for 1 h of exposure of the supports, there was still a large number of adhered cells: 14.5 (ln (CFU/cm^2)). The maximum of detached cells, 13 units (ln (CFU/cm^2)) was mainly observed at 15 g.l^{-1} of extract practically at all the durations of exposure of contact of the fragments with the extract. At 10 and 20 g.l^{-1} of extract, after 6 and 9 h of incubation of the suspension with the supports alone, extremely low numbers of detached cells were noted.

For cells coming from the decline growth phase, the abundances of *S. typhi* cells that adhered to the supports in the absence of the extract remained below 11 units (ln (CFU/cm 2))**.** These numbers dropped once in the presence of the extract at all concentrations. At 10 g.l^{-1} of extract, after 3 and 6 hours of incubation of the suspension with the supports only, there were no adhered cells, this respectively after 1 and 1 and 3 hours of exposure of the supports in the extract. The maximum of adhered cells, 9.8 units (ln (CFU/cm^2)), was noted at 15 g.l^{-1} of extract, after 3 h of incubation of the suspension with the supports alone and for 1 h of exposure of the fragments in the extract.

The bacterial cells of *S. typhi* present in the extract after removal of the supports vary greatly at 10 g / l and very little at 15 and 20 g.l^{-1} of extract. The maximum of detached cells, 11 units (ln (CFU /cm^2)) was observed at 20 g / l of extract, after 1 d of incubation of the suspension with the supports alone and after 3 h of contact of the supports with the extract. At 10 g.l^{-1} of extract, after 9 hours of incubation of the bacterial suspension with the supports alone, no detached cells were counted after 2 hours of exposure of the supports in the extract.

3.4. Percentages of S. typhi cells adhered (%A) and detached (%D)

The %A of *S. typhi* varied between 0.00% and 29.30%. The minimum %A was observed at all growth phases and mainly in the stationary growth phase where there was 0 %A at 20 g.l^{-1} of extract whatever the duration of incubation. The maximum cells adhered was 29.30%, observed in the exponential growth phase, at 15g.l^{-1} of extract and after 3 h of incubation (Table 2).

The %D of *Salmonella typhi* varied between 0.00% and 41.96%. The minimum % D was observed in the stationary phase, at 10 and 20 g.l^{-1} of extract after 3 h, 6 h, and 9 h of incubation, respectively. The maximum was observed in the exponential and decline phase at 15g.l^{-1} of extract after 6 and 9 hours of incubation (Table 2).

3.5. Percentage inhibition of adhered cells

The percentages of inhibition of adhered bacterial cells, after 3 hours of exposure of the supports in the extract of *Eucalyptus microcorys*, varied between 35.72% and 100% (Table 3). The minimum PI was observed in the exponential phase at 15 g.l^{-1} of extract after 24 hours of incubation. The maximum PI was observed in the exponential phase at 20g.l^{-1} of extract after 3 h and 9 h of incubation, in the stationary phase at 10 g / l of extract after 6 and 9 h of incubation and at 20g.l^{-1} l extract after 3, 6 and 9 hours of incubation (Table 3). It can be seen that the highest PIs are observed at 20g.l^{-1} of extract. These were observed in the stationary phase with an average of 92.01%. The lowest PIs were observed in the exponential growth phase with an average of 77.14%.

3.6. Relationships between considered parameters

For each growth phase, Spearman's correlation test "r" was used to assess the degree of binding between the concentrations of the extract and the abundance of adhered or detached bacteria. It was noted that the concentration of the extract was significantly and negatively correlated to the abundance of adhered or detached cells ($P < 0.01$) in the Lag phase, and very significantly and positively correlated in the decline phase (Table 4). It is also noted that, the duration of exposure of the polythene fragments in the extract has no significant influence on the abundances of adhered or detached cells. The Kruskal-Wallis test was used to compare the abundances of detached cells harvested at different cell growth stages. The abundances of adhered or detached cells were statistically significant from one cell growth stage to another ($P < 0.01$). A significant difference ($P < 0.01$) was noted between the abundances of cells adhered to the different incubation durations of the adhesion test and the various support-extract contact times. However, no significant difference ($P > 0.05$) was noted between the abundances of adhered and detached cells obtained at the different concentrations of the extract in this bacterial species. Similarly, the abundances of detached *Salmonella typhi* cells obtained at the different support-extract contact times do not differ significantly ($P > 0.05$) (Table 5)

4. Discussion

It emerges from this study that the abundances of *Salmonella typhi* cells fixed on the polythene fragments

Table 1: Chemical constituents of *Eucalyptus microcorys* leaves extract

Chemical compounds tested	Appreciation of relative abundance
Sterol and Triterpenoids	+
Polyphenols	+
Flavonoids	++
Cathechics	-
Gallics Tannins	+
Anthraquinons	+++
Anthocyanins	+
Alkaloids	+++
Saponins	++
Lipids	-

Legend: +++: Abundant; ++: quite abundant; +: scanty; -: Non detected

Table 2: Percentage according to growth phases and incubation times, of adhered cells (%A) and detached cells (%D) after 3 hours incubation in the plant extract solution of the concentration 10 $g.^{-1}$, 15 $g.l^{-1}$ and 20 $g.l^{-1}$ after each duration of cell adhesion process (DCAP: 3h, 6h, 9h and 24h) in NaCl solution (NaCl: 0.85%)

Cell growth phase and duration of cell adhesion process		Percentage of adhered cells (%A) and detached cell (%D) after 3h incubation in the plant extract solution of the concentration 10 $g.l^{-1}$, 15 $g.l^{-1}$ and 20 $g.l^{-1}$					
Cell growth phase	Duration of cell adhesion process	10 $g.l^{-1}$		15 $g.l^{-1}$		20 $g.l^{-1}$	
		A	D	A	D	A	D
Lag	3h	06.67	26.67	01.48	02.22	02.98	0.00
	6h	15.19	29.11	27.85	0.00	01.27	01.27
	9h	18.89	20.00	20.00	05.56	01.11	04.44
	24h	09.00	08.00	07.00	01.00	05.00	0.00
Exp.	3h	0.01	0.15	29.30	0.17	0.00	0.00
	6h	0.00	01.02	20.30	41.96	0.00	0.09
	9h	0.00	01.02	20.80	41.96	0.00	0.09
	24h	03.91	23.34	26.06	38.22	16.76	23.45
Stat.	3h	02.13	0.00	0.00	07.87	0.00	0.00
	6h	0.00	0.00	0.00	06.35	0.00	0.00
	9h	0.00	0.00	0.00	0.00	0.00	0.00
	24h	0.00	0.75	0.75	20.00	0.00	10.25
Decl.	3h	0.00	02.83	01.54	39.08	24.62	31.54
	6h	0.12	0.10	06.81	24.87	09.50	54.50
	9h	0.00	0.19	3.68	38.16	3.82	17.79
	24h	0.01	0.13	1.53	23.96	3.11	13.86

*Lag.: Lag growthphase; Exp.: Exponential growth phase; Stat. stationary growth phase; Decl.:Decline growth phase

underwent relative variations in the presence of the extract of Eucalyptus microcrorys, this at the different concentrations of the extract used. Analysis of the extract solution, after removal of the fragments of solid supports and quantitative analysis of the abundances of bacterial cells adhered to the supports after contact with the extract solution, made it possible to understand the action of this extract on this bacterial species. Indeed, the results obtained indicated that the extract leads to the detachment of the bacterial cells adhered to the supports on the one hand and that bacterial inhibition increases with the contact time of the supports with the extract and on the other hand.

Salmonella typhi inhibition percentages ranged from 35.72% to 100%. The concentration of Eucalyptus microcrorys extract at 20 $g.l^{-1}$ had the strongest inhibition rates. This bacterial inhibition observed throughout the study could be due to the accumulation of certain chemicals, which would become temporarily toxic to bacterial cells.[14] Bacterial inhibition is also believed to be due to the presence of bactericidal secondary metabolites in plant extracts such as flavonoids and tannins.[8] Indeed, these compounds are known for their toxicities with respect to certain microorganisms. Tannins also have the ability to complex proteins, thereby causing enzyme inactivation, either directly by binding to active sites, or indirectly by steric hindrance created by the binding of tannin molecules to the enzyme.[15] In addition, the main characteristic of secondary metabolites is their hydrophobicity allows

Table 3: Percentage inhibition of *Salmonella typhi* cells according to growth phases, the concentration of the extract and the incubation period, 3h incubation in the plant extract solution

Cell growth phase and duration of cell adhesion process		**Percentage inhibition of *S. typhi* cells after 3h incubation in the plant extract solution of the concentration 10 g.l^{-1},15$g.l^{-1}$and20$g.l^{-1}$**		
Cell growth phase	**Duration of cell adhesion process**	10 g.l^{-1}	15 g.l^{-1}	20 g.l^{-1}
Lag	3h	66.57	96.30	97.04
	6h	53.70	72.15	97.47
	9h	61.11	74.44	94.44
	24h	83.00	92.00	95.00
Exp.	3h	99.84	79.52	100.00
	6h	99.87	42.00	100.00
	9h	98.98	37.24	99.91
	24h	72.75	35.72	59.79
Stat.	3h	97.87	91.06	100.00
	6h	100.00	84.24	100.00
	9h	100.00	87.97	100.00
	24h	99.25	54.00	89.75
Decl.	3h	97.16	59.38	43.85
	6h	99.78	68.32	36.00
	9h	99.81	50.16	78.39
	24h	99.86	74.51	83.03

*Lag.: Lag growth phase; Exp.: Exponential growth phase; Stat. stationary growth phase; Decl.: Decline growth phase

Table 4: Spearman correlation coefficients "r" between the concentrations of the extract and the abundances of adhered or detached bacteria, for each phase of cell growth.

Growth Phase	**Adhered**	**Detached**
Lag	-0,562**	-0,579**
Exponential	-0,219	0,061
Stationary	-0,221	-0,077
Decline	0,651**	0,729**

**: Incredibly significant correlation P < 0.01 dll = 35

Table 5: Overall comparison between abundances of adhered or cells *Salmonella typhi* obtained at the different growth phases, at the different incubation times, at the different concentrations of the extract and at the different contact times

Consider Parameters	**Adhered**	**Detached**	**ddl**
Growth phases	0.00**	0.023*	3
Incubation Period	0.14**	0.098^{ns}	3
Concentration	0.796^{ns}	0.751^{ns}	2

** Incredibly significant difference (p <0.01); ns: no significative difference

their solubilization in the membranes, which causes destabilization of the structure and an increase in membrane permeability.[16] If the loss of material is too great or if the released cytoplasmic elements are essential for the survival of the bacteria, this leads to cell death. This result could be explained by the bactericidal effect of the aqueous extract. In general, it has been observed that the inhibition of the growth of bacteria in aquatic environments is linked to the presence of bactericidal and bacteriostatic compounds in plants.[17] The best known for *Eucalyptus microcorys* are quinones and anthraquinones. It has also been shown in vitro that the essential oils of *Eucalyptus microcorys* have strong antibacterial activity against Enterococcus faecalis and *Salmonella typhi* murium.[18]

The passage of the strains from the adhered to planktonic state further exposes the bacterial strains to the antibacterial effect of the flavonoids and the alkaloids contained in the extract.[10] Alkaloids are hydrophobic cations with antibacterial properties targeting DNA as a cellular target.[19] This inhibitory effect is modulated by the support-extract contact time, the long contact time acting on targets not reached by relatively short contact times.[11] This is what is said to be the cause of recorded and abundant cell death after 3 hours of contact of the supports with the extract. The extremely high percentages of inhibition, the percentages of adhered cells and the percentages of detached cells,

which were very low in the stationary phase of *Salmonella typhi* would be due to the properties of this stage of cell growth. In fact, in the stationary phase, the growth rate is zero, there is accumulation of toxic products and the ionic balance can be unfavorable to cells. The adhesion potentials in this physiological state would therefore be reduced and the properties of flavonoids and alkaloids more effective.

The detachment of the bacterial cells, initially fixed on the polythene fragments, in the presence of the extract of *Eucalyptus microcorys* could be justified by the fact that the secondary metabolites present in the extract cause the breaking of the hydrogen bonds within the exopolysacharide, secreted by bacterial cells as a protective matrix. In bacteria, the permeabilization of membranes by these compounds is associated with loss of ions and degradation of the ATP potential, the aromatic molecules having the highest antibacterial coefficient being the phenols.[20] In addition, despite the protective and structural properties determining the biofilm conformation, exopolysaccharides may also exhibit temporal variations in response to environmental stress.[21,22] The polyphenols contained in this extract constituting a form of stress, have probably deprived the bacteria of their protective glycocalyx, thus causing a disorganization of the biofilm and the dislodging of the bacteria from the surface of the polythene supports.

However, the calculation of the percentages of detached cells shows that these rates do not exceed 40%. This could be justified by the fact that the exopolymer covering the bacteria creates a concentration gradient so that the permeabilization of the protective layer is not total. Thus, only the bacterial cells from a certain distance from the support are reached and detached. Certain bacteria carry specific genes in their plasmids.[23] These genes, which code for virulence factors (type IV pilis, adhesins, toxins) play an important role in the cell adhesion process. They allow the interconnection of bacteria in micro-colonies promoting their stabilization, which could lead to resistance to the effect of the extract.

The Kruskal Wallis H test showed that the number of adhered and detached cells varied from one growth phase to another. This could be due to the adhesion rate which varies depending on the growth phase from which the tested cell originated. The exponential phase results in a strong cellular activity while the stationary phase presents a slowing down of this activity, involving chemical modifications on the surface of the cell.[24]

The presence of bacterial strains still alive in the planktonic state in the extract could be justified by the phenomenon of resistance to anti-bacterial agents. Bacteria can synthesize enzymes capable of destroying or modifying anti-bacterial agents such as antibiotics.[25] The enzymatic reactions leading to the inactivation of antibiotics can be carried out by hydrolysis, transfer of chemical groups or redox, this resistance varying with the bacterial strain. Analyzes have shown that the abundances of adhered and detached cells do not vary significantly with the concentration of the extract. This result would be justified by the fact that, at the chosen extract concentrations, the secondary metabolites contained in the extract would act in the same way on the adhered cells. Spearman's correlation test revealed that increasing the concentration of the extract leads to a decrease in the adherent and detached cells of latent Salmonella. typhi. This explains the increase in PI of the cells of this bacterial species depending on the concentration of the extract at this stage of growth.

5. Conclusion

The present study revealed that bacterial cells in different growth phases, of *Salmonella typhi*, adhered to polythene supports are sensitive to the secondary metabolites contained in the extract of *Eucalyptus microcorys*. The polyphenols in the extract act by permeabilization of the secreted exopolymer during the formation of the biofilm, thus causing it to be disorganized. Subsequently, the alkaloid and flavonoid molecules contribute on the one hand to detachment by action on adhered cells and on the other hand, to the inhibition of cells by actions on bacterial DNA. The bacterial inhibition is a function of the time of support-extract contact, and of the growth phase in which the cells are taken. The use of bioactive compounds from the extract of *Eucalyptus microcorys* would therefore be advantageous in the fight against biofilms and the adhesion of bacteria to supports submerged in an aquatic environment.

6. Conflict of Interest

None.

7. Source of Funding

None.

References

1. Vilaginès R. Eau, environnement et santé publique. vol. 236. Paris: Lavoisier éd; 2003.
2. Rodier J. In Dunod Paris. L'analyse de l'eau. In: 8th Edn.; 1996. p. 1384.
3. WHO. Guidelines for drinking water quality. vol. 1. Recommandation, Geneva; 2004. p. 1050.
4. Hamadi F, Latrache H, Mliji E, Mallouki B, Mabrouki M, Ellouali M, et al. Adhésion de Staphylococcus aureus au verre et au téflon. Revues Microbiologie Industrielle. *Revues Microbiol Industrielle, Sanitaire et Environnementale* . 2009;12:1–16.
5. Boutaleb N. Etude de la formation des biofilms sur les surfaces de matériaux couramment utilisés dans les canalisations d'eau potable. Thèse de Doctorat, Université de Bretagne-Sud; 2007.
6. Ewoti OVN, Nola M, Moungang L, Nougang M, Krier F, Nour-Eddine C, et al. Nour-Eddine Chihib. Adhesion of Escherichia coli and Pseudomonas aeruginosa on Rock surface in aquatic Microcosm: Assessment of the influence of Dissolved Magnesium Sulfate and Monosodium Phosphate. *Res J Environ Earth Sci*. 2011;2011(4):364–74.

7. Djimeli CL, Arfao AT, Ewoti ON, Rossi V, Lango-Yaya E, Raspal V, et al. Assessment of the adsorption of Aeromonas hydrophila on polythene in sodium hypochlorite and hydrogen peroxide treated water. *J Water Environ Sci*;2018(1):270–87.
8. Arfao AT, Djimeli CL, Ewoti ON, Bricheux G, Nola M, Sime-Ngando T, et al. Detachment of adhered enteropathogenic Escherichia coli cells from polythene fragments immersed in aquatic microcosm using Eucalyptus microcorys extract (Myrtaceae). *Curr Res Microbiol Biotechnol*. 2016;4(3):847–57.
9. Mpondo E, Siegfried D, Priso RJ, Ngoye A, Ladoh CF. Etat actuel de la médicine traditionnelle dans le système de santé des populations rurales et urbaines de Douala (Cameroun). *J Appl Biosci*. 2012;55:4036–45.
10. Donadieu Y. La Propolis Editions Dangles. Paris; 2008. p. 90.
11. Arfao AT. Effet de l'extrait aqueux des feuilles d'Eucalyptus microcorys Müller, 1860 sur la cultivabilité de quelques bactéries d'importance sanitaire. Thèse de Doctorat PhD, Université de Yaoundé 1 (Cameroun), Faculté des Sciences, Département de Biologie et Physiologie Animales, Laboratoire d'Hydrobiologie et Environnement . *Faculté des Sci*. 2017;1:237.
12. Odebiyi O, Sofowora EA. Phytochemical screening. Nigeria medicinal plants. *Loydia*. 1978;41:234–5.
13. Djimeli CL, Nola M, Arfao AT, Nguéfack RN, Ewoti ON, Nougang M, et al. Effect of disinfectants on adhered Aeromonas hydrophila to polyethylene immersed in water under static and dynamic conditions. *Int J Res BioSci*;2(2):33–48.
14. Nola M, Nlep RR, Servais P, Kemka N, Togouet SHZ, Krier F, et al. Assessment of the effects of sulfate and nitrate on the temporal evolution of Klebsiella oxytoca and Staphylococcus aureus abundance under shaking conditions, in aquatic microcosm. *J Water Sci*. 2010;23(3):197–212. doi:10.7202/044685ar.
15. Zimmer N, Cordesse R. Influence des tanins sur la valeur nutritive des aliments des ruminants. *INRAE Prod Anim*. 1996;9(3):167–79. doi:10.20870/productions-animales.1996.9.3.4044.
16. Sikkema J, de Bont JA, Poolman B. Interactions of cyclic hydrocarbons with biological membranes. *J Biological Chem*. 1994;269(11):8022–8. doi:10.1016/s0021-9258(17)37154-5.
17. Antoine TA, Chretien LD, Vivien NEO, Djaouda M, Aoudou Y, Roger T, et al. Use of the aqueous extract of Eucalyptus microcorys for the treatment in microcosm, of water containing Enterococcus faecalis: hierarchisation of cells' inhibition factors. *H2 Open J*. 2018;1(1):47–56. doi:10.2166/h2oj.2018.002.
18. Younes K, Merghache S, Djabou N, Merghache D, Muselli A, Tabti B, et al. Chemical composition, antibacterial and antioxidant activities of a new essential oil chemotype of Algerian Artemisia arborescens L. *Afr J Pharm Pharmacol*. 2012;6(42):2912–21.
19. Amin AH, Subbaiah TV, Abbasi KM. Berberine sulfate : antimicrobial activity, bioassay, and mode of action. *Can J Microbiol*. 1996;15:1067–76.
20. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils – A review. *Food Chem Toxicol*. 2008;46(2):446–75. doi:10.1016/j.fct.2007.09.106.
21. Sutherland IW. Biofilm exopolysaccharides: a strong and sticky framework. *Microbiol*. 2001;147(1):3–9. doi:10.1099/00221287-147-1-3.
22. Chen H, Fujita M, Feng Q, Clardy J, Fink GR. Tyrosol is a quorum-sensing molecule in Candida albicans. *Proce Natl Acad Sci*. 2004;101(14):5048–52. doi:10.1073/pnas.0401416101.
23. Trabulsi LR, Keller R, Gomes TAT. Typical and Atypical EnteropathogenicEscherichia coli. *Emerg Infect Dis*. 2002;8:508–13. doi:10.3201/eid0805.010385.
24. Nora H. Interactions des bactéries marines responsables de la formation des biossalissures avec les matériaux biospécifiques. Thèse, Institut Galilée-Université Paris, XIII. vol. 235; 2003.
25. Wright GD. Bacterial resistance to antibiotics: Enzymatic degradation and modification. *Adv Drug Del Rev*. 2005;57(10):1451–70. doi:10.1016/j.addr.2005.04.002.

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