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Antibacterial activity of kecombrang flower extract (*Nicolaia speciosa)* microencapsulation with food additive materials formulation

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Abstract. Kecombrang flower (*Nicolaia speciosa*) contains bioactive components of alkaloids, flavonoids, polyphenols, steroids, saponins, and essential oils as potential antimicrobials. The use of antibacterials in the form of essential oils has constraints; therefore microencapsulation needs to be done to prevent damage to the bioactive components. Microencapsulation can prevent degradation due to radiation or oxygen, easy-mix with foodstuffs and also slow the occurrence of evaporation. This study aimed to determine the effect of types of kecombrang extract, the concentration of microcapsules in food additives (NaCl and sucrose), and concentration of flower extract in the microcapsules. This study used Randomized Block Design (RBD) with 18 treatment combinations and two replications. Factors studied were types of kecombrang flower extract of (semi polar and polar extract), Food Additive types (sucrose and NaCl), the concentration of microcapsules in food additive (0%; 15%; 30% w /v). The results showed that polar and non-polar extract microcapsules produced antibacterial activity of 7.178 mm and 7.145 respectively of *Bacillus cereus* bacteria, while *Escherichia coli* was 7.272 mm and 7.289 mm respectively. A 30 percent microcapsule concentration provides antibacterial activity with inhibiting zone of 7, 818 mm for *B. cereus* and 8,045 for *E.coli*. Food Additive of sucrose concentrations showed that microcapsules produced tend to be more effective in inhibiting the growth of *E.coli* and *B. cereus* bacteria than that of NaCl, with each inhibition zone of 7.499 mm and 7.357 mm

Keywords : Antibacterial activity, kecombrang flower, microencapsulation,

1. Introduction

Most food ingredients are a good for the growth of various microorganisms. The organism will grow and cause food damage or changes in appearance, taste, smell and other properties of the foodstuff. The existence of food additives to be one alternative in improving the quality of food, nutritional value, taste, and appearance when used properly. According to [1], food preservatives are one of the food additives that are used to prevent or reduce chemical and food biological damage. Preservatives to prevent biological damage caused by bacteria are called antibacterials.

[2] have been extracting an antimicrobial compound (additive substance) using non-polar (hexane), semipolar (ethyl acetate) and polar (ethanol) solvents. The results are the extract of ethyl acetate flower kecombrang has large spectrum inhibition include Gram positive, Gram negative and spore-forming bacteria.

The use of antibacterials in the form of essential oils has several constraints. According to [3], antibacterials with high temperatures is easily oxidized; not easily dispersed in dry materials, and it is difficult to handle. According [4], the properties of essential oils of kecombrang is less effective, and it could be overcome by treating essential oils into microcapsules. Microcapsules are small particles containing an active substance or a core material surrounded by a coating. Microencapsulation is a process that makes the material granules wrapped by a coating material to form a microcapsule with a

diameter of 0.2-5.000 microns [5]. The coating serves as a protective against outside interference (light, temperature, air, water) and extends its shelf life.

The addition of microcapsules of flower extract of kecombrang is very suitable to be applied to process food products from meat, fish and other processed food products, where also use in other ingredients in flavor processing and either sugar or sucrose additives. Sucrose is a sweet taste disaccharide that is often also used as a preservative especially commodities that have been subjected to heat treatment. Sugar, as well as salt, also inhibits the growth and activity of bacteria that cause decay, mold, and yeast. The presence of sucrose in antibacterial food products will affect the antibacterial activity and affect the stability of antibacterial in the food. According to [6] the addition of sucrose in the manufacture of food products serves to provide a sweet taste and can also as a preservative when at high concentrations, which can inhibit the growth of microorganisms by reducing the activity of water from food.

According [7], microcapsules of flower extracts from ethanol fraction have the higher antibacterial activity against Gram-positive and Gram-negative bacteria with better physicochemical properties than microcapsules of ethyl acetate fraction, and the best encapsulation is gelatin-maltodextrin encapsulation with ratio 1: 2 (*b / b*). Antibacterial activity of microcapsules may decrease during food processing. Microencapsulation of kecombrang flower extract is expected to protect antibacterial compounds contained in the flower extract kecombrang from various environmental influences during food processing, therefore this study examines the effectiveness of microcapsule fractions of flowers as an antibacterial kecombrang; the concentration of sucrose and NaCl commonly used in food processing. Thus it is known whether or not there is the influence of antibacterial activity of microcapsule of a fraction of flower kecombrang due to the influence of sucrose and NaCl from some concentration.

This study aimed to determine the effect of types of kecombrang extract, the concentration of microcapsules in food additives (NaCl and sucrose), and concentration of flower extract in the microcapsules.

2. Methodology

2.1. Kecombrang flower powder processing [7].

The flower was cut and spread on trays and dried with a blower dryer at a temperature of 50^{o}C until dry. Kecombrang flower which has been dried crushed in a blender until a homogeneous powder and ready to be extracted.

2.2. The extraction process of kecombrang flower powder [7].

The extraction process is carried out by extraction multilevel, using the two consecutive solvents with ethyl acetate and ethanol as follows: A total of powdered flowers of kecombrang dissolved in ethyl acetate (1:4 w/v), then shaken with a rotation speed of 150 rpm shaker for 2 hours. Then it filtered with filter paper to obtain extract 1 and pulp. Then the extract 1 solvent was evaporated with a rotary evaporator and obtained ethyl acetate fraction. Furthermore, Dregs 1 kecombrang flower extracted again using ethanol solvent and worked the same way as using the ethyl acetate solvent and result in the extract 2 and pulp 2. The kecombrang flower extracts then flowed with N2 gases.

2.3 Microencapsulation of kecombrang flower extract [7].

Microencapsulation process began with making encapsulant in the ratio of distilled water with filler materials (1:1) which is the ratio filler materials are gelatin and maltodextrin (1:1), stirred thoroughly and left at room temperature for 12 hours. The next process is Kecombrang extract was added to the encapsulant and thoroughly stirred for 5 minutes and spread on trays and dried in a cabinet dryer at 40˚C for 10 hours.

2.4 Experimental Design

The experimental design used was Randomized Block Design (RBD) with 18 treatment combinations and 2 replications, so that 36 experimental units were obtained. The treatments include the type of coated fraction consisting of fractions of ethanol (polar) and ethyl acetate fraction (semi-polar); concentration of microcapsules, i.e., 0 percent, 15 percent, 30 percent; sucrose concentration, i.e., 0 percent, 5 percent, 10 percent.

2.5 Microorganism preparation [7]

The tested bacteria were *Bacillus cereus* (FNCC 057), *Pseudomonas aeruginosa* (FNCC 063) obtained from The Center for Food and Nutrients, Gadjah Mada University, and *E. coli* (ATCC 25922). The bacterial stock cultures were maintained on nutrient agar slants and stored at 4°C. The bacterial strains were grown in the Nutrient Broth at 37°C for 24 hr before being used for the antimicrobial activity tests.

2.6 Antibacterial activities analysis [8].

The *N. Speciosa* fruit extract antibacterial activity against *B. cereus, P. aeruginosa* and *E. coli* were carried out using the agar well-diffusion method. The antibacterial activity was expressed as the diameter of the zone of inhibition (mm) formed by the bacteria

2.7 Statistical Analysis

Data obtained from the results of the study were analyzed by using variance analysis (F test). If the results of the analysis show a significant difference, then proceed with Duncans Multiple Range Test (DMRT).

3. Result and Discussion

3.1 Effect of coated fraction type on antibacterial activity of microcapsules in B.cereus and E.coli bacteria

The result of variance analysis showed that fraction type did not have a significant effect on the inhibition zone diameter of *B.cereus* and *E.coli* bacteria. The mean diameter of the inhibitory zone against *B. cereus* on the semi-polar fraction and the polar fractions respectively 7.178 mm; 7.145 mm, while *E. coli* respectively 7.272 mm and 7.289 mm. Microcapsules of ethyl acetate and ethanol fractions are both able to inhibit the growth of *E.coli* and *B.cereus*. For ethyl acetate fraction tends to be more effective in inhibiting the growth of *B. cereus* whereas ethanol fraction tends to more effectively inhibit *E.coli* growth. Figure 1 shows the effect of fraction type on antibacterial activity of microcapsule fraction of flower kecombrang.

B.cereus and *E.coli* bacteria because ethyl acetate or ethanol fractions have similar ability to inhibit bacterial growth. This can be seen from the antibacterial activity of microcapsules in the presence of clear zone formed on media that is not much different. The clear zone is a zone that is not overgrown with bacteria, which means that bacterial growth can be inhibited by antibacterial microcapsules. From these data on *B.cereus* bacteria, there was a tendency that semi-polar fraction resulted in higher inhibition activity than a polar fraction. With the presence of flavonoid compounds cause the ethyl acetate fraction more easily diffuses and able to penetrate the cell wall of *B.cereus* consisting of peptidoglycan and phospholipid. Polar compounds of the ethyl acetate fraction are thought to inhibit bacterial activity by diffusing the protein layer, whereas the non-polar compound diffuses through the lipid layer of phospholipids in the plasma membrane. [9] states that ethnic acetate extract of flower kecombrang can inhibit the growth of Gram-positive and Gram-negative bacteria. [10] reported that hexane-acyl acetate extract could inhibit the growth of Gram-positive bacteria (*B. subtilis*) and Gram-negative (*E.coli*). The dominant antibacterial component of the semi-polar fraction is flavonoids while the polar fraction is phenol [11]. According to [1] flavonoids contained in the flower extract kecombrang have varying solubility according to class and substitution that occurred.

The presence of sugar bound causes the flavonoid to tend to dissolve in water, whereas less polar aglikons such as isoflavones, flavones, flavones, and flavonols tend to be more soluble in semi-polar solvents. In the presence of such compounds, ethyl acetate fractions are easier to diffuse and can easily penetrate the *B.cereus* cell wall consisting of proteins (polar), phospholipids and lipoproteins (nonpolar). According [13], antibacterial substances from flavonoid compounds are lipophilic or easily soluble in lipids. The mechanism of action of flavonoid compounds is not fully known but is thought to be involved in the destruction of cytoplasmic membranes by binding to lipophilic parts [14]. To reach the bacterial cytoplasmic membrane, the flavonoid compound will first pass through the cell wall or the outer membrane to *E.coli*. In the *E.coli* cell wall there are many lipophilic parts that allow the occurrence of bonding with flavonoid compounds so that the flavonoid will be bound to the lipophilic cell wall will likely not reach the cytoplasmic membrane because it is suspected not to bind to the bacterial cell wall and directly bind to the lipophilic portion of the cytoplasmic membrane, so the cytoplasmic membrane is damaged. According to [15], bacterial cell wall serves as a protective component in bacterial cells. Therefore, differences in Gram positive and Gram-negative bacterial cell composition cause differences in both bacterial endurance against various treatments.

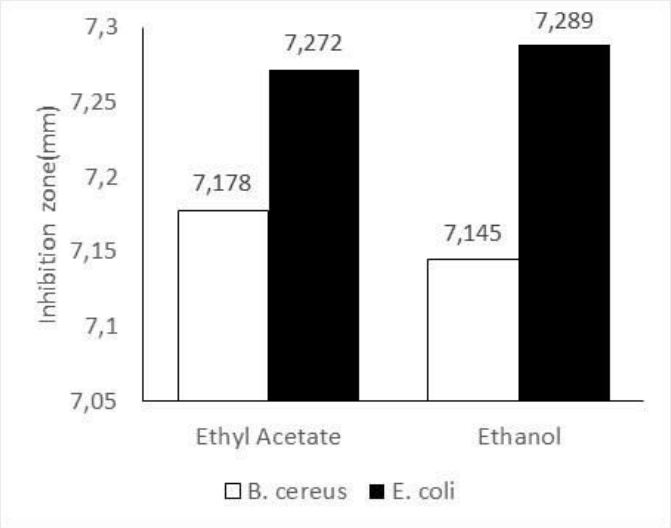

Figure. 1 Effect of coated fraction type on antibacterial activity of microcapsules in *B.cereus* and *E.coli* bacteria

In *E.coli* bacteria, there is a tendency that the polar fraction results in higher inhibition than the semi-polar fraction. It is presumed that the compounds in polar fractions contain a lot of hydroxyl groups which facilitate the water-soluble antibacterial compounds that become bacterial habitats. The polar fraction (ethanol) is thought to have an optimum polarity so that it is easier to diffuse and can inhibit *E.coli* growth. A compound having optimum polarity will have maximum antibacterial activity because for the interaction of an antibacterial compound with bacteria is required a hydrophilic-lipophilic balance [16]. Gram-negative bacteria are more resistant to antibacterial and alkaline dyes; their nutrient requirements are relatively simple but less resistant to physical treatment than Gram-positive [17]. The compounds contained in flowers kecombrang according to [18] include alkaloids, flavonoids, polyphenols, terpenoids, steroids, saponins and essential oils. [19] mentions that the phenolic component found in berry extract can inhibit some Gram-negative bacteria.

3.2. Effect of microcapsule concentration on microbacterial antibacterial activity on B.cereus and E.coli bacteria

The concentration of microcapsules has a very significant effect on the inhibition activity of *B.cereus* and *E.coli* bacteria. The mean values of the diameter of the inhibition zone to *B.cereus* at

concentrations of 0 percent, 15 percent, and 30 percent were respectively 6.142 mm; 7,525 mm; 7.818 mm, while *E. coli* respectively 6.141 mm; 7,657 mm; 8,045 mm. This proves that the microencapsulated study can protect extractable bioactive compounds. The effect of microcapsule concentration on the inhibition zone of *B.cereus* and *E.coli* can be seen in Figure 2.

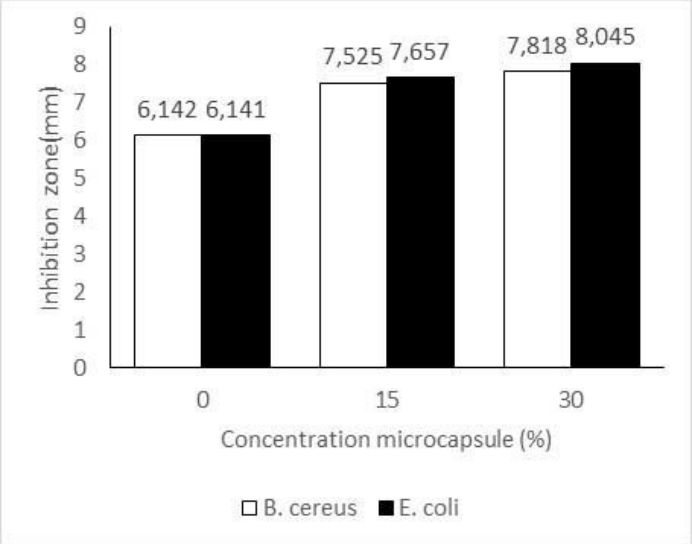

Figure 2. Effect of microcapsule concentration on microbacterial antibacterial activity on *B.cereus* and *E.coli* bacteria

It showed that the higher concentration of microcapsules added, then the higher the antibacterial activity. This is consistent with the research report of [20] that the higher concentration of beluntas leaf extract indicates the greater the inhibition zone formed. [21] adds that the higher concentration of extract kedawung, the number of antibacterial compounds released the greater, thus facilitating the penetration of these compounds into the cell.

3.3. Effect of sucrose concentration on microbacterial antibacterial activity on B. cereus and E.coli bacteria

The result of the analysis of the effect variety of sucrose concentration gave very real effect to the inhibition zone diameter of *B.cereus* and *E.coli* bacteria. The mean diameter of the inhibitory zone against *B. cereus* at concentrations of 0 percent, 5 percent and 10 percent respectively 7.003 mm; 7,124 mm; 7.357 mm, while *E. coli* respectively 7.096 mm; 7,247 mm; 7,499 mm. This proves that the microencapsulated study can protect extracted bioactive compounds in sucrose solution. The effect of sucrose concentration on antibacterial activity of microcapsule fraction of flower kecombrang can be seen in Figure 3.

Based on the results showed that the higher concentration of sucrose added, the higher the antibacterial activity. The increased sugar solution will cause the osmotic pressure outside the high cell to cause water in the bacterial cells out of the membrane so that the bacterial cell will undergo lysis (Hendritomo, 2003)[21]. If the osmotic value in the outer cell medium is increased beyond the osmotic value of the cell contents with the addition of sucrose, the water in the cell is sucked out and the protoplast contracts, causing the cytoplasmic membrane to escape from the cell wall. When occurring in this hypertonic medium are called plasmolysis, the fluid in the cells flows out resulting in dehydration and cell frowning. The mechanism of preservation by sucrose due to sucrose has high osmotic pressure, which can lead to the occurrence of plasmolysis from microbial cells and hygroscopic sucrose that can absorb water from materials and environment so that the activity of food water is low and microbial growth is hampered. According to [22], microbial can only grow in the

range of certain water activities therefore to prevent microbial growth of the value of the water activity of the material should be regulated.

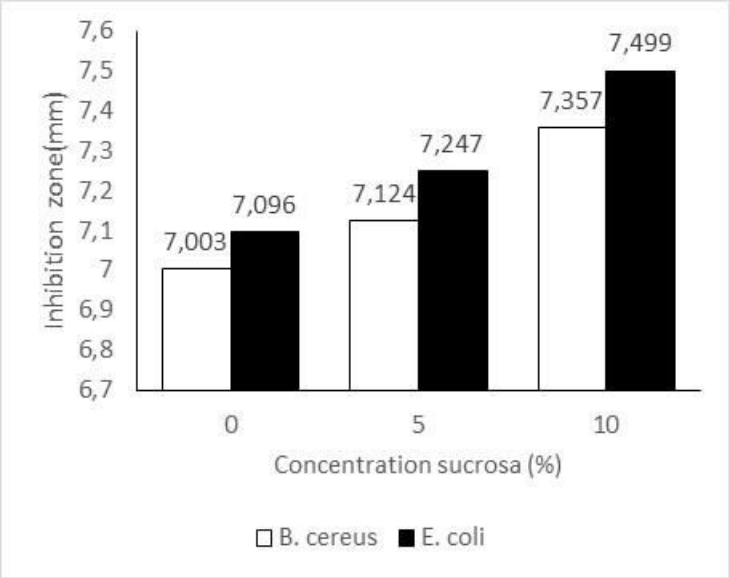

Figure 3. Effect of sucrose concentration on microbacterial antibacterial activity on *B. cereus* and *E.coli* bacteria

3.4. Effect of NaCl Concentration on Antibacterial Activity of Microcapsules

The result showed that NaCl had a significant effect on the inhibition zone diameter of *B. cereus* and *E. coli*. The mean diameter of the inhibition zone against *B. cereus* at a 0 percent NaCl concentration; 5 percent and 10 percent were 8.08 mm, respectively; 8.99 mm; and 9.10 mm. The mean diameter of the inhibitory zone against *E. coli* at a 0 percent NaCl concentration; 2.5 percent and 5 percent were 7.83 mm, respectively; 8.62 mm; and 8.93 mm (Figure 4). The higher the NaCl concentration indicates, the greater the inhibition zone formed.

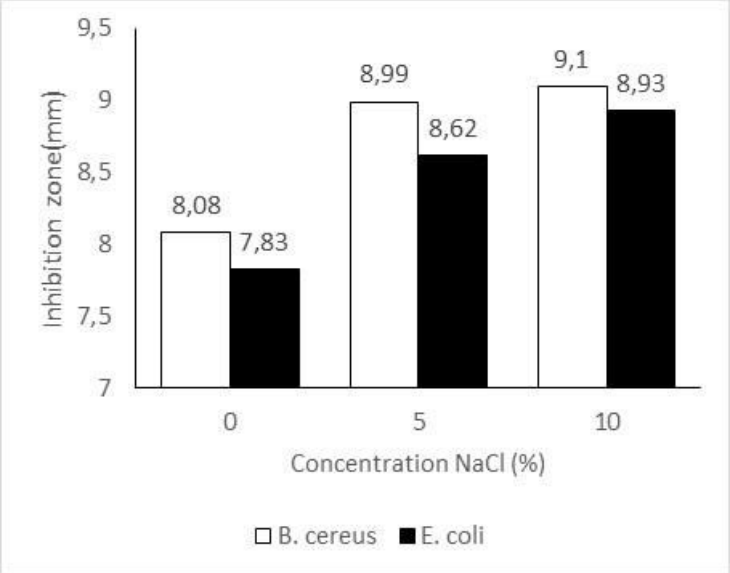

Figure 4. Effect of NaCl Concentration on Antibacterial Activity of Microcapsules

According to [9] the addition of 1 to 4 percent NaCl in ethyl acetate extract and ethanol still showed antibacterial activity. NaCl can inhibit microbial growth. The mechanism of antimicrobial NaCl is by inducing plasmolysis or dehydration of microbial cells. The high concentration of NaCl outside the cell will result in water in the cell out through the membrane to the solution to achieve balance [23]

[23] states although NaCl can not kill all types of microbes, in general most microbes that cause decay can be inhibited its growth. The mechanism of preservation with NaCl is NaCl has high osmotic pressure, so it can cause plasmolysis from microbial cells and hygroscopic NaCl that can absorb water from material and environment, so that the activity of a food, water will be low and microbial growth can be inhibited. First, NaCl will act as a selective inhibitor of certain pollutant microorganisms. Proteolytic microorganisms are the most susceptible, although with a salt content of 6%. Also, NaCl also affects the water activity of the ingredients.

3.5. Interaction Effect Concentration of sucrose, NaCl concentration, and Type of fraction on Antibacterial Activity

The interaction between sucrose concentration, NaCl concentration and coated fraction type (SxMxF) had no significant effect on the antibacterial activity of microcapsules; this was thought to be related to the microencapsulation effectiveness. Microencapsulation may protect the active compounds contained within the fractions of the extracted flowers of the flowers. [3] added that the contents or flavor in the microcapsule could be released at a controlled rate under certain conditions.

The largest inhibitory activity in *B. cereus* was a combination of treatment (10% sucrose concentration, 30% microcapsule concentration and ethyl acetate fraction) of 8.041 mm and for *E. coli* the largest inhibitory activity also in the treatment combination (10% sucrose concentration, 30% microcapsule concentration and ethyl acetate fraction of 8,343 mm. It is predicted that the semi-polar fraction has polar components and semi-polar components. In the presence of such compounds cause the fraction of ethyl acetate more easily diffuses and able to penetrate the cell wall of bacteria *E.coli* and *B.cereus*. [9] stated that ethyl acetate extract has better antibacterial activity than ethanol extract. It is assumed that the semi-polar fraction has an optimum polarity so that it is easier to diffuse and able to inhibit the growth of Gram positive and Gram-negative bacteria. The higher the concentration is expected to be more and more bioactive components against bacteria that can damage the cell wall, thus causing the inhibition zone produced greater. [7] states the higher the concentration of microcapsules, the concentration of extracts coated will be higher so that the antibacterial activity is increasing. Similarly, higher concentrations of sucrose result in large inhibitory zones. The existence of sucrose in the extract will be related each other. This can be seen by the higher concentration of sucrose will result in a larger inhibition zone. Solid sugar solution up to 10% can cause high osmotic pressure so that plasma water of the cell is absorbed by the solution outside the cell resulting in the cell shortage of water and eventually die due to lysis or rupture. All organisms need water for their lives. Water plays a role in the process of cell metabolism in liquid form when the water is bound in a sugar solution; then water can not be used by microbial cells. According to [22] microbes can only grow in the range of certain water activities therefore to prevent the microbial growth of the value of the water activity of the material should be regulated.

4 Conclusions

The fraction type of flower kecombrang has no significant effect on the diameter of inhibition zone of *B.cereus* and *E.coli* bacteria. Micro polar and polar fraction microcapsules resulted in antibacterial activity of 7.178 mm and 7.145 mm in *B.cereus* bacteria, whereas *E.coli* was 7.272 mm and 7.289 mm, respectively. The concentration of microcapsules of flower extract of kecombrang had very significant effect on antibacterial activity against *E.coli* and *B.cereus* bacteria. A 30 percent microcapsule concentration provides antibacterial activity with a drag zone diameter of 7, 818 mm for *B. cereus* and 8,045 mm for *E.coli*. The concentration of sucrose has a very significant effect on the inhibitory zone diameter formed on *B.cereus* and *E.coli* bacteria. A 10 percent effective concentration

inhibits the growth of *E.coli* and *B.cereus* bacteria, on *E.coli* inhibit zone diameter of 7.499 mm while *B.cereus* is 7.357 mm. NaCl concentration affects antibacterial activity of microcapsules against *B. cereus* and *E. coli* bacteria. NaCl concentration 5 percent provides antibacterial activity with an inhibitory zone of 9.1 mm in *B. cereus* and 8.93 mm in *E. coli*.

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