Interaction of *Escherichia coli* with Growing Salad Spinach Plants

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ABSTRACT

In this study, the interaction of a bioluminescence-labeled *Escherichia coli* strain with growing spinach plants was assessed. Through bioluminescence profiles, the direct visualization of *E. coli* growing around the roots of developing seedlings was accomplished. Subsequent in situ glucuronidase (GUS) staining of seedlings confirmed that *E. coli* had become internalized within root tissue and, to a limited extent, within hypocotyls. When inoculated seeds were sown in soil microcosms and cultivated for 42 days, *E. coli* was recovered from the external surfaces of spinach roots and leaves as well as from surface-sterilized roots. When 20-day-old spinach seedlings (from uninoculated seeds) were transferred to soil inoculated with *E. coli*, the bacterium became established on the plant surface, but internalization into the inner root tissue was restricted. However, for seedlings transferred to a hydroponic system containing 10^2 or 10^3 CFU of *E. coli* per ml of the circulating nutrient solution, the bacterium was recovered from surface-sterilized roots, indicating that it had been internalized. Differences between *E. coli* interactions in the soil and those in the hydroponic system may be attributed to greater accessibility of the roots in the latter model. Alternatively, the presence of a competitive microflora in soil may have restricted root colonization by *E. coli*. The implications of this study’s findings with regard to the microbiological safety of minimally processed vegetables are discussed.

The incidence of foodborne illness associated with the consumption of minimally processed ready-to-eat salad vegetables is increasing (2, 3, 19, 32, 36). To date, the majority of contamination of vegetables by human pathogenic bacteria has been considered to occur during postharvest handling (21). Nevertheless, it has been shown that salad vegetables can also be contaminated during cultivation via soil, water, animals, and harvest equipment (5, 29). Post-harvest washing, typically with the use of sodium hypochlorite (at 100 to 200 ppm), is carried out to remove field-acquired contamination, but it is now becoming established that this procedure achieves only a <2-log reduction in bacterial counts (4, 5, 7). Although a diverse range of more potent disinfectant types have been applied, log reductions in bacterial counts achieved with these disinfectants on naturally contaminated produce have not been significantly improved over those achieved with standard chlorine washes (27, 41). It is becoming evident that bacteria (including human pathogens) can be protected from the lethal effects of biocidal washes by virtue of their locations in protective areas of the plant. It has been proposed that biofilms could be responsible for this protective effect (7, 9, 33). However, it has also been reported that *Escherichia coli* O157:H7 cells inoculated onto lettuce leaves survive biocidal washes when they are located in stomata or, to a greater extent, when they gain entry into the inner part of the leaf via cut or damaged areas (28, 34). The internalization of human pathogens via natural openings has also been reported for apples inoculated with *E. coli* O157:H7 (6) and tomatoes inoculated with *Salmonella* (42).

Previous reports have suggested that human pathogens can also become internalized into the vascular systems of growing plants (14, 26), although this question remains open (22). Research has confirmed that human pathogens can become internalized within sprouted seeds such as radish (13, 15), alfalfa (18, 23, 24, 35), and bean sprouts (39). Furthermore, the internalization of *E. coli* in lettuce (37), cabbage (38), and tomato plants (10–12) has been observed. However, to date, little work on other salad vegetable types has been carried out. The difficulties encountered in the decontamination of salad spinach (24) could possibly be attributable to internalized bacterial populations (i.e., endophytes). In this study, the interaction of *E. coli* with growing spinach plants was assessed.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Bioluminescent *E. coli* P36 (nonverocytotoxix *E. coli*) was constructed from a slaughterhouse isolate by the Tn5 mini-transposon method. The mini-Tn5 plasmid (kindly donated by P. Hill, University of Nottingham), containing *lux* CDABE and a kanamycin resistance gene cassette, was maintained within *E. coli* λpir1. Competent *E. coli* S17-1 cells were transformed with a purified plasmid preparation as described by Winson et al. (40). Conjugation between *E. coli* S17-1λpir and recipient cells (*E. coli*) was carried out with...
E. coli IN GROWING SPINACH

FIGURE 1. Flow diagram of inoculation and spinach cultivation schemes. Spinach seeds were inoculated (A and B) and cultivated on the surface of solidified hydroponic solution or within soil microcosms. Uninoculated seeds were cultivated in soil or in hydroponic solution inoculated with E. coli P36 (C and D).

a Whatman cellulose acetate membrane (0.45-μm pore size) overlaid onto a Luria-Bertani agar plate, which was subsequently incubated for 14 h at 37°C (8). The selection of E. coli exconjugants was based on aliquots (50 to 200 μl) of the mating mixture that were plated onto chromogenic E. coli coliform selective agar medium (Oxoid, Basingstoke, UK) containing kanamycin (30 μg/ml) and incubated overnight at 37°C. The donor strain, E. coli S17-1, is deficient in glucuronidase (GUS) activity and formed pink colonies. The recipient E. coli colonies on agar plates were imaged via putative GUS activity. Bioluminescent colonies on agar plates were imaged using a Night-Owl Image analyser in conjunction with the manufacturer’s computer software (E. G. and G. Berthold, Munich, Germany). The bioluminescent phenotype was stable in the selected exconjugate, and growth characteristics were not significantly different from those of the parental strain (39).

Suspensions of E. coli P36 were prepared from overnight cultures grown aerobically at 37°C in brain heart infusion broth (Difco Laboratories, Sparks, Md.). The cells were harvested by centrifugation (3,550 × g for 10 min at 4°C) and washed once in sterile maximum recovery diluent (MRD; Oxoid). The cell pellet was finally resuspended in MRD to yield a suspension containing ca. 10^7 CFU/ml (A_600 = 0.2).

Inoculation of spinach seeds. Spinach (Spinacia oleracea L. cv. Sharan) seeds (Toza Seeds Ltd., Surrey, UK) were initially soaked in 4% (wt/vol) glycolic acid (Aldrich, Dorset, UK) for 30 min to stimulate germination. The seeds (in 20-g batches) were then washed three times in 2 liters of sterile distilled water before being submerged in E. coli suspensions (cell density, 10^7 CFU/ml) for 20 min. The inoculated seeds were allowed to dry at room temperature for 8 h on sterile filter paper. For the bacterial loading of inoculated seeds, three 1-g (ca. 150-seed) batches were withdrawn and placed in 10 ml of MRD, and bacteria were released by vortexing for 1 min. Aliquots were plated onto brain heart infusion agar supplemented with kanomycin (30 μg/ml) and incubated at 37°C for 24 h.

To capture bioluminescence profiles of E. coli during germination, seeds were individually placed in glass tubes containing 10 ml of solidified hydroponic nutrient solution (1:300 dilution hydroponic nutrient solution [Nutriculture Ormskirk, Lancashire, UK] containing 0.5% [wt/vol] agar). The tubes (n = 10) were then incubated in the dark at 15°C, and bioluminescent images were captured daily (Fig. 1A).

In situ β-glucuronidase (GUS) stain. Whole 25-day-old spinach seedlings (cultivated on solidified hydroponic media) derived from seeds inoculated with E. coli P36 were submerged for 15 min in sodium phosphate buffer (50 mM, pH 7.0) containing 0.3% (vol/vol) formaldehyde (16). The seedlings were washed three times in sterile distilled water and, finally, transferred to a sodium phosphate buffer (50 mM, pH 7) containing 1 mM 5-bromo-4-chloro-3-indoyl-β-D-glucuronide (X-GLUC; Sigma, Poole, Dorset, UK). The seedlings were incubated in the enzyme substrate solution for 8 h at 37°C and were subsequently rinsed with 70% (vol/vol) ethanol. The blue-black stained sprouts (resulting from GUS activity) were then mounted on glass slides and viewed under a microscope.

Inoculated seeds cultivated in soil microcosms. Inoculated seeds were sown into soil microcosms (6 cm², 5 cm deep) containing John Innes no. 2 compost (Wessex Horticulture Products Ltd., Salisbury, Wiltshire, UK) (Fig. 1B). Soil samples (ca. 4 g each) were withdrawn from the microcosms, and bacterial counts were determined for three 1-g batches. The plants were cultivated for 35 days, and a further 4-g sample of soil was taken for mi-
Microbiological analysis. Plants were harvested intact and separated into three batches (three plants per batch). The roots and leaves from a batch of plants were separated with a sterile scalpel and placed in sterile stomacher bags. Surface bacteria and bacteria surviving surface sterilization were recovered as described below.

Hydroponic cultivation of spinach plants. Uninoculated spinach seeds were germinated on damp filter paper in the dark at 15°C for 7 days (Fig. 1C). Germinated seeds were transferred to rockwool blocks (Growdan, Hedehusene, Denmark) predamped with a commercial hydroponic nutrient medium. Seedlings were allowed to develop in growth chambers maintained at 16°C with illumination for 12 h per day. After the seedlings had developed sufficiently (ca. 13 days), the plants were transferred to a nutrient film technique (NFT) hydroponic system within a contained greenhouse. The system consisted of three polyvinyl chloride plastic channels 3 m long and 22 cm wide holding 17 plants in each trough. The nutrient solution, held in an 80-liter tank, was continuously circulated in a closed loop via a centrifugal pump. The bases of the plants were covered with a polyethylene sheet to prevent contact of spinach leaves with the underlying rockwool base substrate.

Suspensions of *E. coli* in MRD were added to the nutrient solution in the holding tank to yield final cell densities of $10^3$ or $10^2$ CFU/ml. Control hydroponic systems containing no *E. coli* were set up in parallel. Periodically, 50 ml of hydroponic solution was withdrawn from the holding tank to determine bacterial counts. For this purpose, 0.1-ml aliquots of hydroponic solution were plated on tryptic soya agar (TSA) with and without kanamycin (30 g/ml) and incubated at either 30°C for 48 h (for the

![Figure 2. Bioluminescence profile for germinating spinach seeds inoculated with *E. coli* P36. Inoculated seeds were germinated on the surface of solidified nutrient solution, and the bioluminescence images were obtained over a 25-day period. Images taken under illumination are shown on the right.](http://meridian.allenpress.com/jfp/article-pdf/66/10/1790/1675679/0362-028x-66_10_1790.pdf)
total viable count (TVC) or 37°C for 24 h (for E. coli). When E. coli counts had decreased to below the level of detection, 10 ml of hydroponic nutrient solution was passed through a sterile filter, which was subsequently overlaid on a TSA-kanamycin plate and incubated for 24 h at 37°C.

Inoculated soil microcosms. Suspensions of E. coli were added to 3 kg of John Innes no. 2 compost (Wessex Horticulture Products) and distributed throughout the soil with manual agitation (Fig. 1D). The soil was then placed in soil microcosms (6 cm², 5 cm deep), into which three 20-day-old spinach seedlings were sown. The soil microcosms were placed on a tray containing irrigation water whose level was maintained at 3-cm depth. Cultivation of the spinach was carried out over a 42-day period in contained greenhouses maintained at 20 to 26°C under natural lighting conditions. Soil samples (ca. 4 g each) were withdrawn periodically from soil microcosms, and bacterial counts were determined for three 1-g batches.

Microbiological analysis of spinach plants. The loosely attached bacteria on the surfaces of the plants were released by rinsing with sterile MRD. The samples were then surface sterilized for 10 min in a 4,000-ppm sodium hypochlorite solution. Residual chlorine was removed by rinsing samples five times in 500 ml of sterile distilled water. Confirmation of surface sterilization efficacy was carried out by placing two treated leaves from a batch in contact with a TSA plate for 20 s. The plate was subsequently incubated at 30°C for 48 h. Surface-sterilized plant samples were macerated by stomaching in MRD for 2 min. The TVCs for the different extracts were determined with the use of TSA (with incubation at 30°C for 48 h). E. coli was enumerated on TSA supplemented with kanamycin (30 μg/ml) and incubated at 37°C for 24 h. Confirmation of E. coli was accomplished by visualizing bioluminescent colonies with a Night Owl image analyzer as described above.

RESULTS

Interaction of E. coli with germinating spinach seeds. From the bioluminescence profiles obtained, it was clearly demonstrated that E. coli initially introduced onto the seed proliferated around the roots of the developing spinach plant (Fig. 2). When the spinach seedlings were removed after 25 days of cultivation, bioluminescent E. coli remained attached, suggesting a close association between the bacterial cells and the plant roots. From plate counts of seedlings (two batches of 20 plants), E. coli was shown to be present both on the exterior of the plant (7.17 ± 1.39 log CFU/g) and on inner plant tissues (4.03 ± 0.95 log CFU/g) but not on any uninoculated controls. Confirmation of the internalization of E. coli into the inner plant tissue was provided by the in situ GUS assay. For this assay, surface-sterilized seedlings were incubated in the presence of X-GLUC for 16 h at 30°C. The majority of the precipitated substrate (resulting from the action of E. coli GUS) was observed in the roots of seedlings but also occasionally within the hypocotyls (Fig. 3). It was noted that the precipitate was not continuous from the roots to the hypocotyls. This finding may imply that E. coli existed in aggregates within the plant or, alternatively, that its presence was due to the diffusion of hydrolyzed GUS substrate. No precipitate was found in control seedlings derived from uninoculated seeds, confirming that internalized E. coli was responsible for the results observed (Fig. 3).

When spinach plants derived from inoculated seeds were cultivated for 35 days in soil microcosms, E. coli could be recovered from the external (5.41 ± 0.71 log CFU/g) and internal (1.93 ± 0.08 log CFU/g) root structures but from only the exteriors of the leaves (5.20 ± 1.32 log CFU/g). The E. coli counts for soil samples increased from below the level of detection (<1.25 log CFU/g) on day 1 to 2.99 ± 0.31 log CFU/g by the end of the cultivation period.

Interaction of E. coli with spinach cultivated in soil microcosms. Spinach seedlings (20 days postgermination) were transplanted into soil inoculated with E. coli. Although E. coli was introduced at a density of 10² CFU/g, E. coli counts progressively increased over the cultivation period, while the TVC remained relatively constant (Fig. 4). The E. coli counts for the spinach plant samples paralleled the soil counts in that numbers of cells generally increased during cultivation (Table 1). The majority of E.
coli cells were present on the exterior part of the plant, although one surface-sterilized sample tested positive for *E. coli* on day 12. Toward the end of the cultivation period (day 32 onward), *E. coli* was recovered from all of the surface-sterilized spinach tested. On day 42, the remaining plants were harvested, and leaves were separated from the roots. *E. coli* was recovered from surface-sterilized root tissue (3.78 ± 0.21 log CFU/g) but not from within leaves (<1.2 log CFU/g).

**Interaction with hydroponically cultivated spinach.**

A soil microcosm represents a complex system with a high abundance of nutrients and background microflora that could potentially affect the interaction of *E. coli* with growing spinach plants. Therefore, a comparative study was carried out with hydroponically cultivated spinach. For this study, *E. coli* was introduced (at 10^2 or 10^3 CFU/ml) into the nutrient solution reservoir and circulated around rockwool blocks in which 20-day-old spinach seedlings were planted. The *E. coli* counts for the hydroponic nutrient solution declined progressively over the cultivation period, and by day 8 these counts were below the level of detection (0.1 CFU/ml). This decrease was probably partly due to the poor nutrient environment of the hydroponic solution but was also due to the filtration effect of rockwool blocks. This effect was confirmed by determining the rate of decline in *E. coli* numbers in the presence and absence of rockwool blocks (containing no spinach seedlings). The decrease in *E. coli* counts was significantly larger (*P* < 0.001) in the presence of rockwool blocks than in the absence of rockwool blocks (Fig. 5).

*E. coli* counts for spinach plants (16 days after transplanting; average plant weight, 17 g) indicated that the bacterium was transmitted to the leaves (as indicated by counts for surface-sterilized samples) at both inoculum levels used (Table 2). *E. coli* was also present on the external surfaces of (but not within) leaves cultivated in the presence of 10^3 CFU/ml (Table 2).

**DISCUSSION**

The interaction of *E. coli* with spinach was found to be dependent on the stage of introduction. When *E. coli*

<table>
<thead>
<tr>
<th>Days after introduction</th>
<th>Total viable count (log CFU/g)</th>
<th><em>E. coli</em> count (log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wash</td>
<td>Extract</td>
</tr>
<tr>
<td>12</td>
<td>6.26 ± 0.65</td>
<td>5.93 ± 0.70</td>
</tr>
<tr>
<td>14</td>
<td>5.44 ± 1.34</td>
<td>5.79 ± 0.20</td>
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<tr>
<td>16</td>
<td>6.65 ± 1.50</td>
<td>3.90 ± 0.45</td>
</tr>
<tr>
<td>20</td>
<td>5.92 ± 0.17</td>
<td>4.76 ± 0.63</td>
</tr>
<tr>
<td>23</td>
<td>6.40 ± 0.42</td>
<td>5.27 ± 0.51</td>
</tr>
<tr>
<td>25</td>
<td>6.82 ± 0.12</td>
<td>5.17 ± 0.20</td>
</tr>
<tr>
<td>32</td>
<td>6.49 ± 0.04</td>
<td>4.32 ± 1.10</td>
</tr>
<tr>
<td>35</td>
<td>7.54 ± 0.81</td>
<td>3.99 ± 0.15</td>
</tr>
</tbody>
</table>

^a Twenty-day-old spinach seedlings were transplanted into soil microcosms inoculated with *E. coli* P36 (10^2 CFU/g). Whole spinach plants (roots and leaves) were withdrawn from the soil microcosm periodically to assess the bacterial counts for surface samples (wash) and surface-sterilized samples (extract). Values presented are means ± standard deviations for three plant samples. ND, not detected (<1.2 log CFU/g).

^b *E. coli* was recovered from one of three samples.
was initially inoculated onto spinach seeds, its growth was sustained by the seed exudates released from germinating seeds. The exudates contained nutrients that initially attract bacteria and provide sources of energy and carbon for the colonization of roots. The NOI model would suggest that the bacterium may compete successfully in the rhizosphere. The growth of *E. coli* and *Salmonella* on sprouted seeds (radish, alfalfa, and bean sprouts) is relatively well established (13, 15, 18, 23, 25, 35, 39). In addition, the growth of human pathogens on tomato and lettuce seedlings has also been reported (10–12, 30, 37). From all these examples, it can be concluded that the internalization of *E. coli* into a diverse range of different seedling types is relatively common.

Once established within the roots of spinach plants, *E. coli* might have been expected to migrate into the edible leaf portions of plants. Although the results of the in situ GUS assay indicated the presence of *E. coli* within hypocotyls, it is possible that X-GLUC hydrolyzed by cells in the root may have migrated into the vascular system prior to polymerization. Results obtained from the soil microcosm and hydroponic studies support the view that *E. coli* is restricted to colonization of the roots of plants, at least in mature plants. A similar pattern has been observed for cabbage crops accidentally irrigated with creek water contaminated with *E. coli*. In this case, *E. coli* was recovered from the roots of plants but not from the edible leaves (38).

*E. coli* cell density in the plant environment has been reported to be a factor in determining the extent of plant-cell interactions (30, 37). Solomon et al. (30) and Wachtel et al. (37) studied the extent to which *E. coli* O157:H7 interacted with lettuce when introduced at different cell densities. The conclusions of these authors were that at high cell densities (10^8 CFU/ml), *E. coli* O157:H7 became established on the roots and hypocotyls of plants. Conversely, when *E. coli* O157:H7 was introduced at lower cell densities (10^2 to 10^4 CFU/ml), the extent of colonization was comparatively low, although the pathogen was recovered from lettuce roots after a 10-day cultivation period (37). In the present study, the introduction of *E. coli* (at cell densities of 10^2 or 10^3 CFU/ml) into the hydroponic solution reservoir restricted colonization to the plant roots (Table 1). It is possible that the introduction of *E. coli* at higher cell densities may have resulted in a greater distribution of bacterial cells within the edible leaf portion of the plant. However, considering that such high cell densities would not occur in the natural environment, the current results may reflect a more accurate representation of the extent of the interaction of *E. coli* with growing spinach.

It is interesting to note the differences in the interactions of *E. coli* with spinach plants cultivated in soil and with spinach plants cultivated in hydroponic systems. Although *E. coli* readily grew in the soil environment, the internalization into the roots of plants was delayed until the cultivation period was nearly complete. In contrast, although *E. coli* counts were lower for the hydroponic system, the bacterium readily became established on the interiors and exteriors of plant roots (Table 1). It is possible that the introduction of *E. coli* at higher cell densities may have resulted in a greater distribution of bacterial cells within the edible leaf portion of the plant. However, considering that such high cell densities would not occur in the natural environment, the current results may reflect a more accurate representation of the extent of the interaction of *E. coli* with growing spinach.

### TABLE 2. Counts for spinach plants after 16 days of hydroponic cultivation in nutrient solution inoculated with *E. coli* P36

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Total viable count (log CFU/g)</th>
<th><em>E. coli</em> count (log CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (uninoculated)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roots</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wash</td>
<td>6.35 ± 2.53</td>
<td>ND</td>
</tr>
<tr>
<td>Extract</td>
<td>4.59 ± 1.46</td>
<td>ND</td>
</tr>
<tr>
<td>Leaves</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wash</td>
<td>4.35 ± 0.36</td>
<td>ND</td>
</tr>
<tr>
<td>Extract</td>
<td>2.05 ± 0.40</td>
<td>ND</td>
</tr>
<tr>
<td>Initial inoculum of 10^2 CFU/g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roots</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wash</td>
<td>6.64 ± 0.63</td>
<td>2.97 ± 1.40</td>
</tr>
<tr>
<td>Extract</td>
<td>5.33 ± 0.99</td>
<td>1.43 ± 1.00</td>
</tr>
<tr>
<td>Leaves</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wash</td>
<td>2.89 ± 0.28</td>
<td>ND</td>
</tr>
<tr>
<td>Extract</td>
<td>3.96 ± 2.80</td>
<td>ND</td>
</tr>
<tr>
<td>Initial inoculum of 10^3 CFU/g</td>
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<td></td>
</tr>
<tr>
<td>Roots</td>
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<td></td>
</tr>
<tr>
<td>Wash</td>
<td>7.16 ± 0.63</td>
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<tr>
<td>Extract</td>
<td>5.35 ± 1.41</td>
<td>1.64 ± 0.71</td>
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<tr>
<td>Leaves</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wash</td>
<td>3.03 ± 0.21</td>
<td>1.91 ± 0.50</td>
</tr>
<tr>
<td>Extract</td>
<td>2.79 ± 0.25</td>
<td>ND</td>
</tr>
</tbody>
</table>

*a Twenty-day-old spinach seedlings were transplanted into a NFT hydroponic system. Nutrient solution with and without inoculated *E. coli* P36 was circulated around the blocks throughout. After 16 days of cultivation, the spinach plants were harvested and the roots were separated from the leaves with a sterile knife. Surface bacteria (wash) and bacteria derived from surface-sterilized samples (extract) were obtained as described in “Materials and Methods.” Values presented are means ± standard deviations for three samples each containing two plants. ND, not detected (<1.2 log CFU/g).
soil environment, it would be relatively easily outcompeted. Alternatively, it is possible that in the hydroponic system, the roots of spinach were more accessible to *E. coli* for colonization.

Although *E. coli* can become established in germinated seedlings, it is restricted to only the roots in mature spinach plants. Therefore, there is a low risk that the edible portion of the spinach leaves will harbor *E. coli* in the inner tissue. Nevertheless, it is possible that *E. coli* on the root maybe transferred to the inner leaf during harvesting. This possibility should be considered in the development of a hazard analysis critical control point scheme for the production of safe minimally processed salad vegetables.

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**REFERENCES**


