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Effects of Beneficial Microorganisms and Mycorrhizal Fungus Colonized Rhizoplane on the Suppression of Root Rot Pathogen, *Fusarium solani*

Ki-Don Han, Sang Sun Lee[1], Sung Ho Kim and Min Woong Lee*
Dept. of Applied Biology, Dongguk University
[1]Korea Teacher College, Korea

근면 정착 유용 미생물과 균근균이 근부병원균, *Fusarium solani*의 발병억제에 미치는 영향

한기돈 · 이상선[1] · 김성호 · 이민웅*
동국대학교 응용생물학과
[1]한국교원대학교 생물학과

ABSTRACT: The survival or colonization of beneficial organsisms and suppression of root rot of ginseng (*Panax ginseng*) by two distinct bacteria, *Pseudomonas cepacia, Bacillus cereus* and three mycorrhiza in pot soil were investigated and compared with uninoculated root. In separate inoculation, colonization of roots by *P. cepacia* was maintained at 6.25 (log cfu/g root) during growth for 10 days under pot culture conditions comparing to 5.62~6.19 by mixed treatment with other organisms. Colonizations of *P. cepacia* were gradually decreased from 6.25 (log cfu/g root) in 10 days growth to 3.01 (log cfu/g root) in 270 days incubation period. This reduction was also investgated in combination treatments by *B. cereus* or *F. solani*. The numbers of *Fusarium* spp. were colonized high number in rhizosphere soil from 3.33 to 3.67 (log cfu/g root) in control within 10~60 days after treatment of pathogen *F. solani*, but it's numbers were markedly decreased in 270 days cultivation of plant from 3.33 to 1.02 (log cfu/g root) after treatment. In treatment of beneficial strains of *P. cepacia* and *B. cereus, P. cepacia* significantly suppressed the development of root rot from 4.3 in control to 1.2 in treatment, whereas *B. cereus* alone had no effect on the rate of disease suppression. The disease index (1.8~2.3) in combination of two bacteria was reduced in plants inoculated with both *P. cepacia* and *B. cereus* comparing to the index (4.3) of control. As an effect of inoculation with mycorrhiza on disease suppression, suppression of root rot by *F. solani* was reduced to 1.2~1.6 in disease index in treatment of *Glomus albidum* and *Acaulospora longular* comparing to 4.3 of control. In the treatment of bacterial strain *P. cepacia* and mycorrhizal fungus *Glomus albidum*, the disease suppression was apparent to 1.2 and 1.2 comparing to 4.3 of control in disease index respectively.

Key word: Beneficial microorganism, Disease suppression, Root rot, Colonization, Mycorrhizae, *P. cepacia*

Fusarium solani (Mart.) Sac.f.sp.*pisi* (F.R. Jones) Snyder & Hans cause root rot diseases affecting korean ginseng (*Panax ginseng* C.A. Meyer) yield reduction (Kim & Lee, 1974; Lee, 1984). The pathogen is known to persist through long intervals between susceptible hosts (Burgess, 1981). Persistence in the absence of a susceptible host has been attributed to the production of long lived chlamydospore and the colonization of non-susceptible crop plants (Nash & Snyder, 1967). This soil-borne plant pathogen, *Fusarium solani* f.sp.*pisi* can survive for long periods and is highly resistant to microbial at-

*Corresponding author

tack in the soil (Gordon & Okamoto, 1990). Hiltner mentioned already that the rhizosphere is zone of soil in which a microflora was influenced by the root (Hiltner, 1904). Since the concept of the rhizosphere was first introduced, many researchers have demonstrated that the rhizosphere provides the defense line for root against attack by soilborne plant pathogens (Rovira & Davey, 1974). Some soil microbiologists have recently succeeded in altering the native microflora of plant roots by introducing some beneficial organisms (Kloepper *et al*, 1980; Weller & Cook; Weller, 1988). Certain roots associated with introduced organisms increase nutrient availability, produce growth substance or suppress root pathogens. Although facts of root colonization are very important in rhizosphere for protection, relatively little information is available for the fate of inoculants in field soil (Parke, 1991; Harris *et al.*, 1989).

There was also much interests in using mycorrhizal fungi which are considered highly beneficial to the control of plant disease (Wyss *et al.*, 1992). Our study was designed to obtain more detailed knowledge of the interactions between pathogen and isolated beneficial organisms such as *Pseudomonas cepacia.* or *Bacillus cereus* and mycorrhizal fungi for reducing root rot of korean ginseng.

Materials and Methods

Microbial cultures and inocula

The root rot pathogen of ginseng, *Fusarium solani* (Mart.) Sac.f.sp.*pisi* (*F. solani*) was isolated from infected ginseng root (Kim & Lee, 1974). The fungal culture was maintained on PDA agar. The pathogen was cultured in PDA agar plate for seven days at 26C. After 7 days of growth, cultures were filtered through glass wool to remove mycelial mats. Microconidia left in filterates were pelleted by centrifugation (8000 rpm, 15 min). The number of microconidia in suspension was counted microscopically by direct observation on a hamocytometer, and adjusted to 10^5 spores/m*l*. The spore suspension was mixed with non sterile soil for pot test (Kim *et al.*, 1992). The spore density of *F. solani* as inoculum was assessed by dilution plating count on PCNB agar plates (Papavizas, 1967) before treated in soil. The inoculum density in the soil was finally adjusted to 10^4 (spores/g. soil). *Bacillus cereus* (strain, B4) was isolated from the surface top soil of Chungwang-Mt, Kanwon-do (Kim, 1993) and *Pseudomonas* sp. (strain SID2) was isolated from ginseng field soil (Han, 1995).

The strain SID2 was identified as *Pseudomonas cepacia* (Han, 1995). To get an inoculum sources of mycorrhizae, spores were collected from the natural soil infested with mycorrhizae such as *Glomus* sp., *Gigaspora* sp. and *Acaulospora* sp. (Eom & Lee., 1990). These spores were cultured with alfalfa plant in pot soil conditions for 5 months. During 5 months cultivation period, the selected mycorrhizal spores were multiplied in pot soil. One hundred grams of the soils were mixed with 1kg of ground soil as an inoculum source for pot cultivation. For the bioassays, bacterial inocula of *P. cepacia* and *B. cereus* were prepared by scraping overnight cultures (27°C) from King's agar (KB, King *et al.*, 1954) plates and nutrient agar in sterile solution respectively. These suspensions were used in the bioassay for applying on root surface for suppression and colonization assay. The bacterial inoculum density were approximately 10^8 cfu/m*l*.

Selection of antibiotic resistant mutants

Modified antibiotic resistant mutants of *P. cepacia* and *B. cereus* were obtained by

streaking wildtype of these bacteria to KB agar and nutrient agar plates supplemented with 50 μg/ml of streptomycin and tetracycline (Thompson *et al.*, 1992). The stability of this mutants was tested by subculturing the mutants on KB agar several times for 48 h at 27°C and counting the number of colony forming units (CFU) after plating of a dilution series of suspensions on the media.

Root colonization assay

Root colonization of antibiotic resistant mutants was modified as described by de Weger *et al* (1987). The mutants cells were grown on KB for 48 h, scraped from the plates, and diluted to give suspensions of 10^8 cfu/ml. Two-year- old ginseng with roots of approximately 6 ± 1 cm length was dipped in suspensions of mutants cells in sterile water (10^8 cfu/ml) for three times (Burr *et al*, 1978) and planted 5 roots in pots containing upland soil of Dongguk University in Ilsan, Kyunggi-do infested with pathogen. The pots were grown in field conditions from March to December in the farm. Fresh roots and rhizosphere soils were taken from 10, 30, 60 and 270 days period after treatments.

After harvesting the plants, adhering soil was removed from the root, and rootlet of ginseng plant was also cut off for colonizing assay. The rhizhoplane microflora in root colonization comprised the mutants cells of *P. cepacea* and *Fusarium* spp after serial dilution. Rootlet and soil samples were vigorously shaken for 10 min in glass flasks containing 50 ml sterile water (Thompson *et al*, 1992). After serial dilutions of the resulting suspension, aliquots (0.2 ml) of a dilution series were spread onto KB agar medium supplemented with 50 μg/ml of streptomycine and tetracycline (Thompson *et al*, 1992) for enumeration of introduced mutant bacteria, and PCNB medium for *Fusarium* spp. The assays were performed twice.

Pot bioassay of root rot

For the development of a bioassay to study suppression of *Fusasrium* spp. soft rot of ginseng by *P. cepacia* and mycorrhizal fungi, potting soil was mixed with inoculum soil of pathogen(10^5 spores/g.soil) and mycorrhiza spores as described in microbial culture and inocula (Eum & Lee, 1990). Root was treatmented by the same method as root colonization assay (de Weger *et al*, 1987). Root samples were taken from each pot soil along with incubation periods in 10, 30, 60, and 270 days after treatment. Disease severity was assessed by degree of infested portion and rotting (Shim & Lee, 1990).

Results

The colonization ability of selected bacteria such as *P. cepacia or B. cereus* for a suppression of root rot pathogens *F. solani* was tested in pot trials. Development of microcolonies of *P. cepacia* inoculated singly and combination with pathogen were observed in different harvesting time after treatment with *P. cepacia* and *B. cereus*. Large variations in the colonization density were found in Table 1. During the different sampling period, the number of *P. cepacia* was found highly in 10 days old root after planting in pot. But the number was gradually decreased with cultivation periods longer than 10 or 30 days in general (Table 1). Maximum colonization by the bacteria was observed within 10 days range from 5.62~6.25 (log cfu/g root) after treatments of organisms, but the colonization was gradually decreased and also showed significantly difference ($p=0.05$) in accordance with cultivation time.

Especially, the numbers observed in 270 days after treatment were lowest in it's

Table 1. Colonization of the ginseng root by *P. cepacia* in rhizoplane at different harvesting time after treatment of beneficial *P. cepacia* and *B. cereus* in pot test.

Treatment	Log cfu/g root			
	Incubation time (day)			
	10	30	60	270
P. cepacia	6.25a	5.56b	5.17c	3.01d
P. cepacia plus *B. cereus*	6.19a	6.19ab	5.69c	3.67d
P. Cepacia plus *F. solani*	5.85a	5.48b	5.06c	3.41d
P. cepacia plus *B. cereus* plus *F. solani*	5.62b	5.88a	5.22c	3.97

Each figure indicate the mean of three replicates. Different letters in rows are significantly different (P=0.05) in DMRT.

Table 2. Colonization of the ginseng root by *Fusarium* spp. in rhizoplane at different harvesting time after treatment of fluorescent *P. cepacia* and *B. cereus* in pot test.

Treatment	Log cfu/g root			
	Incubation time (day)			
	10	30	60	270
Fusarium solani (control)	3.33ab	3.02bc	3.67a	1.02c
F. solani plus *P. cepacia*	3.16a	2.53b	3.05ab	0.94c
F. solani plus *B. cereus*	3.26ab	3.08bc	3.55a	2.19c
F. solani plus *P. cepacia* plus *B. cereus*	3.10ab	2.64bc	3.33a	0.99c

Each figure indicate the mean of three replicates. Different letters in rows are significantly different (P=0.05) in DMRT.

number 3.01~3.97 (log cfu/g root) (Table 1). In comparison of colonizing ability of fluorescent *P. cepacia* on root within different period of culture time, a treatment of *P. cepacia* plus *B. cereus* showed a higher colonization than other treatment in accordance with cultivation time in general (Fig. 1). A development of microcolonies of *Fusarium* spp. were also observed in different harvesting time after treatment with beneficial organisms, *P. cepacia* and *B. cereus* (Table 2). The number of *Fusarium* spp. was found 3.10~3.16 (log cfu/g root) in 10 days old root rhizosphere after planting in pot. The number of *Fusarium* spp. were observed as 2.53~2.64 (log cfu/groot) in treatment with beneficial organisms as *P. cepacia* in 30 days compared to control of 3.02 (log cfu/g root). The numbers were seemed like stable within 10 days, but slightly decreased in 30 days. But the

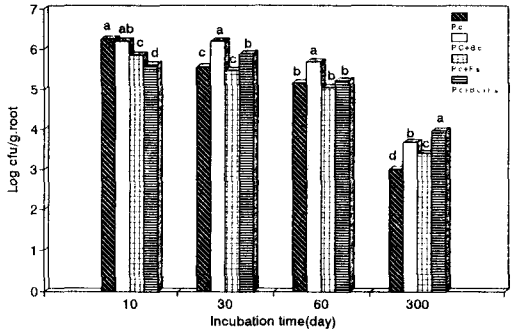

Fig. 1. Population dynamics of *P. cepacia* during different growth periods of ginseng cultivation after each treatment. Different letters are significantly (p=0.05)

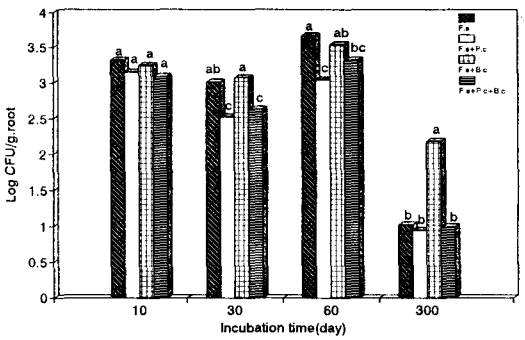

Fig. 2. Population dynamics of *Fusarium* spp. during different growth periods of ginseng cultivation after each treatment. Different letters are significantly (p=0.05)

number of *Fusarium* spp. observing in 60 days are rehabilitated to 3.0~3.55 (log cfu/g root) as the level of control 3.67 (log cfu/g root). After these periods, the numbers were sharply decreased within 270~days long cultivation piriod ranging from 0.94~2.19 (log cfu/g root) in treatment of beneficial organisms (Table 2). Colonization by *Fusarium* spp. at different harvesting time was significantly different in accordance with cultivation time (Fig. 2).

In colonization of *Fusarium* spp. in 270 days after treatmet, the number was different in each treatment. Treatment of *P. capcia* was not significantly different to control, but *B. cereus* stimulated and increased of *Fusarium* spp. on the rhizosphere (Fig. 2). A selected two potentially beneficial bacteria were treated for their disease suppressing properties against fusarium soft rot. Of the bacteria tested with separate inoculation, *P. cepacia* suppressed fusarium soft rot to 1.2 in the pot bioassay, but *B. cereus* lowered slightly it's suppression effects to 3.8 compared to control treatment by 4.3 (Table 3). To investigate co-inoculation effects in the pot bioassay, the combination of *P. cepacia* and *B. cereus* reduced disease index to 2.3 compared with non- treatment control (Table 3).

The effect of inoculation with mycorrhizal fungus by *A..longular, G. albidum* and *G. magarita* on suppression of soft rot of ginseng were observed in a pot trial. Ginseng roots were heavily infected in non-treate of mycorrhizal fungus by the pathogen, *F. solani.* showing disease index 4.3. In separately single treatment of the mycorrhizal fungus to rhizosphere, rate of root rot was significantly low. The result indicated that inoculated plant with mycorrhizal fungus to rhizosphere decreased the disease development to 1.2~3.4 comparing with 4.3 of control index. Especially the inoculation of *G. albidum* suppressed the disease development from 4.3 of control to 1.2 by the prescence of mycorrhizal fungus in disease index (Table 4).

Table 3. Diseases suppression of ginseng root rot in treatment of beneficial organisms in pot test.

Organisms	Disease index
Fusarium solani (control)	4.3a
F. solani plus *Pseudomonas cepacia*	1.2f
F. solani plus *Bacillus cereus*	2.8c
F. solani plus *P. cepacia* plus *B. cereus*	1.8e
P. cepacia	1.2f
B. cereus	3.8b
P. cepacia plus *B. cereus*	2.3d

Disease index: 0; whole plant root healthy, 1; whole plants were healthy, but rootlet weakly infected, 2; roolet of root mostly infected, 3; main roots were becoming infected, 4; half of root parts were infected, 5; most of roots part were infected.
Different letters in column are significantly different (P=0.05) in DMRT.

Table 4. Diseases suppression of ginseng root rot in treatment of mycorrhizal fungus in pot test.

Organisms	Disease index
Fusarium solani (control)	4.3a
Acaulospora longular	1.6c
Glomus albidum	1.2d
Gigaspora magarita	3.4b

Disease index: 0; whole plant root healthy, 1; whole plants were healthy, but rootlet weakly infected, 2; roolet of root mostly infected, 3; main roots were becoming infected, 4; half of root parts were infected, 5; most of roots part were infected.
Different letters in column are significantly different (P=0.05) in DMRT.

Discussions

There are generally common assumption

that efficient root coloniziation by beneficial organisms is critical to suppression of soil-borne diseases. A comparable relationship has been demonstrated between rhizosphere population densites. (Bull *et al.*, 1991; Xu & Gross, 1986). The population densities were influenced by rhizoshere conditions (Parke, 1991; Kim & Lee, 1994). Root colonization is defined as the proliferation of microorganisms in, on, or around root. It includes dispersal of microorganisms from a source of inoculum to the actively growing root, and multiplication or growth in the rhizosphere (Parke, 1991). van Vuurde and Schippers (1980) proved that maxima in bacterial colonization of the seminal axix of 10 day old roots were demonstrated between root tip and root base.

Lysis of root tissue were demonstrated and considered to be responsible for the maxima in bacterial colonization of the root surface (van Vuurde and Schippers, 1980). Parke *et al* observed that population density from 2~7 cm below the site decreased between 4~7 days, possibly because it could not survive competition from other rhizosphere micoorganisms developed by this time. (Parke *et al.*, 1986). On the other hand, Anderson and Guerra (1985) proved that colonization of a beneficial organisms *P. putida* was maintained in a great number at root tissue during the growth of the plants for 18 days involving complete growth conditions as well as iron and boron deficient medium, and also they found that in the plants inoculated with benefical organisms *P. putida* and pathogen, a disease development of foliar wilting by *Fusarium* and onset of lesion formation were delayed by 2~3 days. These results were interpreted for reasons by increasing lignin level (Scher & Baker, 1982; Kloepper *et al*, 1980). In our experiments, the colonizations of two bacteria were increased within 60 days, especially it was observed a great number in 10 days compared with other incubation time (Table 1).

It suggested that most bacterial growth was stimulated in short time of period after inoculation in rhizosphere, and then it was gradually decreased in accordance with culture time. This result could be possibly interpreted as a fact that they could not well survive competition from other rhizosphere microorganisms developing by this time. The bacterial strains were tested alone and in combination for the ability of colonization and suppression of the soft rot in pot soil infested with the pathogen. In general, certain combination enhanced the colonization and also showed suppression of the soft rot (Table 1, 2, 3). Overall, the best effect on colonization and suppression was observed in the treatment of *P. cepacia* singly.

Some works demonstrated the potential benefits using combination treaments to suppress diseases and suggested the importance of additive and interactive effects among introduced bacteria in biocontrol (Pierson & Weller, 1994; Leeman, 1995). Most the tested bacteria were colonized and reduced the disease development in both treatments alone and combination, although their proliferation in rhizosphere was not the same extent. This suggest that bacterial competion is responsible for the inhibition of pathogen's growth (Hartel *et al.*, 1991; 1993).

In this experiments we also found that *P. cepacia* against the pathogen showed a good competitor compared with *B. cereus*. The possible mechanisms could be interpreted as like a difference of growth pattern of microorganisms in rhizosphere. *P. cepacia* are faster generation time and greater nutritional versatility than *B. cereus* or pathogen *F. solani*, so it may reduce the amount of nutrient available for slow growing or-

ganisms (Palleroni, 1984; Hartel *et al*, 1993; Williamson & Hartel, 1991; and Li & Alexander, 1986). It is likely assumed that growth and colonization of some organisms in soil is a consequence of obtaining proper nutrients and multiplying their species. Growth rate may be important in competion for limiting nutrients (Raaijmakers, 1994; Curl & Truelove, 1986).

Anderson and Guerra (1985) pointed out that aggressive colonization of root by beneficial organisms occurred on lateral roots as well as the main root, and that it was maintained in the presence of *Fusasrium solani* f. sp. *phaseoli* and under growth condition varying in iron and boron availability. Many plants were responsive to low iron availability with acidification of the rhizosphere. The secretion of exudates by plant roots somewhat contained dihyroxyphenolic compounds. This compounds has been proposed to promote reduction of rhizosphere Fe^{+3} ion to Fe^{+2} inos(Bennett *et al*, 1982; Olsen *et al*, 1981). Our experiments on disease suppression by mycorrhizae was also tested in pot trials.

The results showed that treatment of mycorrhizae in rhizosphere as single or combination with pathogens *F. solani* reduced the disease development of soft rot to control. Especially *Glomus albidum* showed suppression of disease development among the tested mycorrhizal fungus (Table 4). Most recent studies of interaction between mycorrhizal fungus and rhizospherial fungi have been focused on microorganisms that can be used to enhance mycorrhizae formation (De Oliveira & Garbuye, 1989). But McAllister *et al* found that *T. koningii* and *F. solani* had no positive effect on mycorrhizal formation, indicating a negative effect of the saprophytes on the extramatrical phase of the endophyte (McAllister *et al.*, 1994). They also proved that mycorrhizae *G. mosseae* decreases the saprophytic fungal population through it's effect on the plant. However, *F. solani* had no effect on mycorrhizal development in the treatements tested, wheras *T. konigii* inhibited *G. mosseae* in it's extramatrical stage.

The population of *F. solani* was significantly reduced. This fact may be due not only to the changes produced by the VAM fungus on the root physiology, but also to the ability to colonize primary roots shown by same species of *Fusarium* which make it difficult to establish whether any other fungus has previously colonized the substrate (Cook & Bruehl, 1968). On the other hand, Calvet *et al* (1993) reported a contrast results with McAllister *et al* (1994). Wyss *et al* (1992) observed that the presence of biocontrol agents hindered the formation of mycorrhiza, and also infection by the root pathogen *Rhizoctonia solani* was not altered by these agents (Wyss *et al*, 1992).

In general, noteworthy fact is that the effects of interaction between *G. mosseae* and microorganisms may be very different between different species of the sam genus. Some experiments with soil microcosms in laboratory have reported a lack correlation between inoculum density on the one hand and soil or rhizoshpere colonization on the other (Bennet & Lynch, 1981; Elliott-Juhnke *et al.*, 1989), whereas other work has suggested that the final densities of bacteria are higher in soil or rhizosphere when a large number of inoculum are applied (Iswandi *et al.*, 1987). A better understanding of the rhizosphere would also allow effective management of microorganisms that could solve a number of disease problems.

Further our works is needed on the effect how could the introduced beneficial organisms will be strongly colonize on the root

zone and survive longer in soil condition in competing with established population of indigenous microorgansisms which may prevent or alter interactions of introduced organisms in field. Other work is also needed on the effects of introductions on the composition and balance of the other soil microbial populations within the natural community and the expression of the inoculant beneficial properties. Possible mechanisms of interactions between beneficial bacteria and mycorrhizal fungus also have to be pursure in future studies. We could conclude that the enhanced level associated with colonization by beneficial organisms as *P. cepacia* may contribute to an impaired root-pathogens interaction and also in addition to direct effects upon pathogen by beneficial mycorrhiza as *Glomus* sp. may also contribute or strenthen the defensive potential of the plant health.

적 요

유용 미생물로 선발한 *Pseudomonas cepacia, Bacillus cereus* 및 균근균을 인삼 (*Panax ginseng*) 근권에 처리한 후 이들 미생물의 근권내 정착 및 생존이 근부병의 발생억제에 미치는 영향을 폿트 배양으로 실험하였다. *P. cepacia*의 단독 처리에서 처리 10일후에 근권에서 조사된 세균수는 6.26 (log cfu/g.soil)이나 *B. cereus* 등과 혼합 처리한 경우 5.25~6.19 (log cfu/g.soil)으로 분포수가 적었고 처리기간이 270일 후에는 3.01 (log cfu/g.soil)으로 크게 감소하였다. *Fusarium* spp.의 근권내 분포수는 처리 10~60일 후에는 3.33~3.67 (log cfu/g.soil)이 었으나 처리 270일 경과 후에는 3.33~1.02 (log cfu/g.soil)으로 크게 감소 되었다. 근부병발생율에 있어서 대조가 4.3의 이병지수를 나타내나 *P. cepacia* 처리는 1.2로 발생이 크게 억제되었다. *P. cepacia*와 *B. cereus*의 혼합처리는 1.8~2.3의 이병지수로 대조 4.3보다는 병발생율이 낮아졌다. 균근균으로 *Glomus albidum*과 *Acaulospora longular* 처리는 병발생율 1.2~1.6으로 대조 4.3보다 감소하였다. 세균으로 *P.cepacia*와 균근균으로 *G. albidum* 처리는 근부율을 각각 1.2로 낮추는 발병억제 효과가 있다.

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