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Effect of seed coating with biological agents on seed quality of rice

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Abstract. *Palupi T, Ilyas S, Machmud M, Widajati E. 2017. Effect of seed coating with biological agents on seed quality of rice. Biodiversitas 18: 727-732.* The research that consisted of two activities was performed at the Laboratory. Experiment 1 the aim to obtain biological agents that have high antagonistic potential against *Xanthomonas oryzae* pv. *oryzae (Xoo)* and two biological agents that are compatible to one another. Experiments 2 to determine the effect of rice (*Oryza sativa* L.) seeds coated with biological agents to improve seed quality and reduce levels of *Xoo* infection. In the first experiment, four isolates of bacterial antagonists, i.e: isolate *Pseudomonas diminuta* A6; isolate *P. aeruginosa* A54; isolate *Bacillus subtilis* 11/C, and isolate *B. subtilis* 5/B, were tested for their antagonism activities against *Xoo* on PSA plates using the method of growth inhibition zone with filter paper. Furthermore, the four antagonists were tested for their compatibility with each other on PSA plates. In the second experiment used a completely randomized design with a single factor (seed coating) consisting of seven levels, namely: negative control, healthy seed; positive control, the seeds contaminated with *Xoo*; *P. diminuta* A6 and *B. subtilis* 5/B; alginate 3% + 1% peat + *P. diminuta* A6 and *B. subtilis* % 5/B; arabic gum 3% + 1% gypsum + *P. diminuta* A6 and *B. subtilis* 5/B; CMC 1.5% + 1% talc + *P. diminuta* A6 and *B. subtilis* 5/B, and bactericide streptomycin sulfate 20%. Results of Experiment 1 showed that isolates *P. diminuta* A6 has the highest antagonistic potential against *Xoo* based on the diameter of inhibition zone on *Xoo*, followed by isolate *B. subtilis* 5/B. Isolate *P. diminuta* A6 and *B. subtilis* 5/B had a good growth compatibility with no antagonism. Therefore, isolates *P. diminuta* A6 and *B. subtilis* 5/B were selected and used as biocontrol agents in further studies. Results of Experiment 2 showed that treatment with *P. diminuta* A6 + *B. subtilis* 5/B gave the best of the increase of seed vigor, and the decrease of *Xoo* infection level in the seeds.

Keywords: Antagonistic, *Bacillus subtilis* 5/B, growth compatibility, inhibition zone, *Pseudomonas diminuta* A6, rice seeds coated

INTRODUCTION

The quality seed plays an important role in increasing the productivity of rice (*Oryza sativa* L.). One of the bacteria infects rice seed is *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) causes Bacterial Leaf Blight (BLB) disease (Agarwal & Sinclair 1996; Veena et al. 1996). It reduces the rice production up to 50 percent. (Vikal et al. 2007).

One of an alternative for controlling BLB diseases which are not damaging of environmental by using antagonist microbe as a biological agent which naturally is associate and synergism with the host. The characteristic of the biological agent in order to control the disease and the pathogen population can be used in many ways; those are the production of the antibiotic substances; the space and or nutrition competition occur, the competition of iron (Fe) element used by the siderophore production; the induction of the resistant mechanism to the bacteria diseases; and inactivation of factor germinates for pathogen; to degradation of pathogenic factor, such as toxin; and also parasitism which induced to produce an extracellular enzyme to degradation cell wall, for example an enzyme chitinase, β-1.3 glucanase (Van Loon 2007).

The genus bacteria have been studied extensively and used as a biological agent especially in the rice crops. The two important biological agents are *Pseudomonas* spp. and the *Bacillus* spp. The *Bacillus* spp. can be applied to control *Xoo* which causes the Bacterial Leaf Blight disease in rice (Gnanamanickam. et al. 1999). Furthermore, Velusamy et al. (2006) reported that an antibiotic 2.4 diacetyl-phloroglucinol (DAPG) produced by *P. fluorescens* is able to block the growth of BLB disease by *Xoo* pathogen. *Bacillus* spp. and *Pseudomonas* is able to block the growth *Xoo* colonies originally isolated from the rice seed which is a test in vitro (Agustiansyah 2011).

The control of the BLB diseases of the rice crops using a combination of the *Pseudomonas* spp and the *Bacillus* spp. using a coating treatments have not been reported by others researchers, especially in Indonesia. Base on theses facts, thus the antagonism test between the *Xoo* and the biological agents (*Pseudomonas* spp. and *Bacillus* spp.) then the compatibility test conducted between the *Pseudomonas* spp. and the *Bacillus* spp. to find out a suitable biological agent for rice. The biological agent is able to depress the growth of the *Xoo* and have compatible treats among them.

The objectives of the present investigation are to test the compatibility between the two biological agents *Pseudomonas* spp. and the *Bacillus* spp. and to determine

the effect of rice seed coating with biological agents (*P. diminuta* A6 and *B. subtilis* 5/B) on seed quality.

MATERIALS AND METHODS

The present investigation was conducted at the Laboratory of Biochemistry, the Indonesian Research and Development Center for Agricultural Biotechnology and Genetic Resources, Bogor, West Java, Indonesia; Laboratory of Seed Science and Technology at Institut Pertanian Bogor, Bogor and Laboratory of Seed Technology at PT. East West Seed Indonesia (EWSI), Purwakarta, West Java, Indonesia. The research was divided into two experiments. The 1st experiment was carried out to test the ability of an antagonism of the biological agents to the growth of *Xoo* incubated on PSA media on the plates. This method used was to observe the inhibition zone using filter paper (the factors tested were the isolate of *Xoo* with four biological agents; the isolate of *Xoo* versus *P. diminuta* A6, the isolate of *Xoo* versus *P. aeruginosa* A54, the isolate of *Xoo* versus *B. subtilis* 5/B, and the isolate of *Xoo* versus *B. subtilis* 11/C), and to test the compatibility among the biological agents used the method of the stretch on the PSA media on the plates (the factors tested were the isolate of *P. aeruginosa* A54 versus *B. subtilis* 11/C, the isolate of *P. aeruginosa* A54 versus *B. subtilis* 5/B, the isolate of *P. diminuta* A6 versus *B. subtilis* 11/C, and the isolate of *P. diminuta* A6 versus *B. subtilis* 5/B).

The experiments 2 st to determine the effect of rice seeds coated with biological agents to improve seed quality and reduce levels of *Xoo* infection, used a completely randomized design with a single factor (seed coating) consisting of seven levels, namely: T_0 = negative control, healthy seed; T_1 = positive control, the seeds contaminated with *Xoo*; T_2 = *P. diminuta* A6 and *B. subtilis* 5/B; T_3 = alginate 3% + 1% peat + *P. diminuta* A6 and *B. subtilis* 5/B; T_4 = arabic gum 3% + 1% gypsum + *P. diminuta* A6 and *B. subtilis* 5/B; T_5 = CMC 1.5% + 1% talc + *P. diminuta* A6 and *B. subtilis* 5/B, and T_6 = bactericide streptomycin sulfate 20%.

Isolates of *Xoo* prototype IV, *B. subtilis* 11/C and *B. subtilis* 5/B, used come from Big Hall of Research of Rice Crop, Sukamandi. Isolates *P. diminuta* A6 and *P. aeruginosa* A54 represent resulted of insulation from healthy rice crop root among rice planting attacked by BLB (Agustiansyah 2011). Isolate *Xoo* bred at gel $CaCO_3$ dextrose yeast medium (YDCA), isolate *P. diminuta* A6 bred at King'S B medium, while isolate *B. subtilis* 11/C bred at gel nutrient medium (NA), each during 48 hours. The rice seed of Ciherang variety used is extension seed. Coating formulas which consist of alginate 3% + 1% peat; gum arabic 3% + 1% gypsum; and CMC 1.5% + 1% talc.

Procedures

Seed coating with biological agents

Making of rice seeds coated use suspense of *Xoo, P. diminuta* A6, and *B. subtilis* 5/B with a density of 15 x 10^8 per cfu mL^{-1} Mcfarland scale 5. Working procedure making of rice seeds coated which have contamination of *Xoo* is same as which have been conducted by Palupi et al. (2012). The water content of seed coating after draining ranging from 10.9-11.5%.

Examination of compatibility and antagonism

Isolate of *Xoo* with a density of 4.5 x 10^8 cfu mL^{-1} Mcfarland scale 4 (Kiraly et al. 1994) counted 100 µl, disseminated at PSA media. Cutting of paper filter (diameter 1 cm) which have been soaked in a suspension of a biological agent with a density of 4.5 x 10^8 cfu mL^{-1} old age 48 hours put down in the middle of petry dish. Then incubated at room temperature for six days, is it every day perceived. Observation of growth inhibition zone conducted by measuring inhibition zone diameter on filter paper. Compatibility evaluation using harmony examination among biological agents that not interfere with one other.

Evaluation of seed quality

Examination of seed physiological quality conducted with the UKDdp method in germinator of APB IPB 72-1 type. Seed physiological quality tested i.e. vigor index (%), and growth rate (%/etmal). Calculate the vigor index by the formula: (Sadjad et al. 1999).

$$\text{Vigor Index} = \frac{\text{Total of normal shoot at the five days germination}}{\text{Total investigated seeds}} \times 100\%$$

Calculate the growth rate (%/etmal) by the formula: total addition normal shoot at every day (interval 24 hours) up to observation of normal shoot to last days (7 days after seedling) (Sadjad et al. 1999).

In addition is also perceived by the quality of seed pathologist, covering degradation mount *Xoo* infection and existence of agent involve in seed after coating.

Data analysis

The data obtained from each experiment is analyzed. If between treatment there are a marked difference, hence analysis continued with Analysis of Variance and Duncan's Multiple Range Test (Gomez and Gomez 1995) using Statistical Analysis System (SAS) version 9.1.

RESULTS AND DISCUSSION

Antagonism testing between *Xoo* with biological agents and also compatibility testing to one another

The result of examination of a biological agent by in vitro to the growth of *Xoo* colony indicated that isolate *B. subtilis* 5/B; *B. subtilis* 11/C; *P. diminuta* A6; and *P. aeruginosa* A54 tested have ability inhibition growth of *Xoo* which different each other. This matter can be seen from formed by transparent zone around isolate (Figure 1).

The diameter of inhibition zone widest of got at isolate *P. diminuta* A6 (3.7 cm), and differ from biological agent otherly. Resistivity is smallest got by at isolate *B. subtilis* 11/C (1.6 cm), and do not differ from isolate *P. aeruginosa* A54 (Table 1).

Table 1. The result of inhibition test at four biological agents to growth of *Xoo*

Isolate codes	Biological Agents	Diameter of inhibition zone (cm)
A6	*Pseudomonas diminuta*	3.7 a
A54	*Pseudomonas aeruginosa*	1.9 c
5/B	*Bacillus subtilis*	2.8 b
11/C	*Bacillus subtilis*	1.6 c

Note: Same letters in column are not significantly different at p = 0.05 according to DMRT

Result of compatibility test between is fourth of biological agent to indicate that combination between isolates *P. diminuta* A6 and *B. subtilis* 5/B had a good growth compatibility with no antagonism, whereas combination between isolate *P. diminuta* A6 and *B. subtilis* 11/C, or between isolates *P. aeruginosa* A54 and *B. subtilis* 11/C, and also between isolates *P. aeruginosa* A54 whit *B. subtilis* 5/B show resistance between one other (Figure 2).

Effect of seed coating with biological agent on seed quality in rice

Results of the experiment showed that seed coating with a biological agent to have an effect on reality to the quality of seed, (vigor index and growth rate of the shoot), and also can decrease to *Xoo* infection level at seed compared to positive control. The vigor index and growth rate highest founded at treatment *P. diminuta* A6 + *B. subtilis* 5/B (96,50% and 30,86%/etmal) (Table 2).

The decrease of *Xoo* infection level highest founded by *P. diminuta* A6 + *B. subtilis* 5/B (from 0.74 x 10^6 becoming 0.28 x 10^6 cfu mL^{-1}), whereas lowerst decrease by coating treatment of CMC 1.5 + talc 1% + *P. diminuta* A6 and *B. subtilis* 5/B (from 0.74 x 10^6 becoming 0.68 x 10^6 cfu mL^{-1}). Population of *P. diminuta* A6 highest founded by coating treatment of CMC 1.5 + talc 1% + *P. diminuta* A6 and *B. subtilis* 5/B (2.08 x 10^6 cfu mL^{-1}), whereas the highest of population *B. subtilis* 5/B by treatment of gum arabic 3% + gypsum 1% + *P. diminuta* A6 and *B. subtilis* 5/B, that is 0.43 x 10^6 cfu mL^{-1} (Table 3).

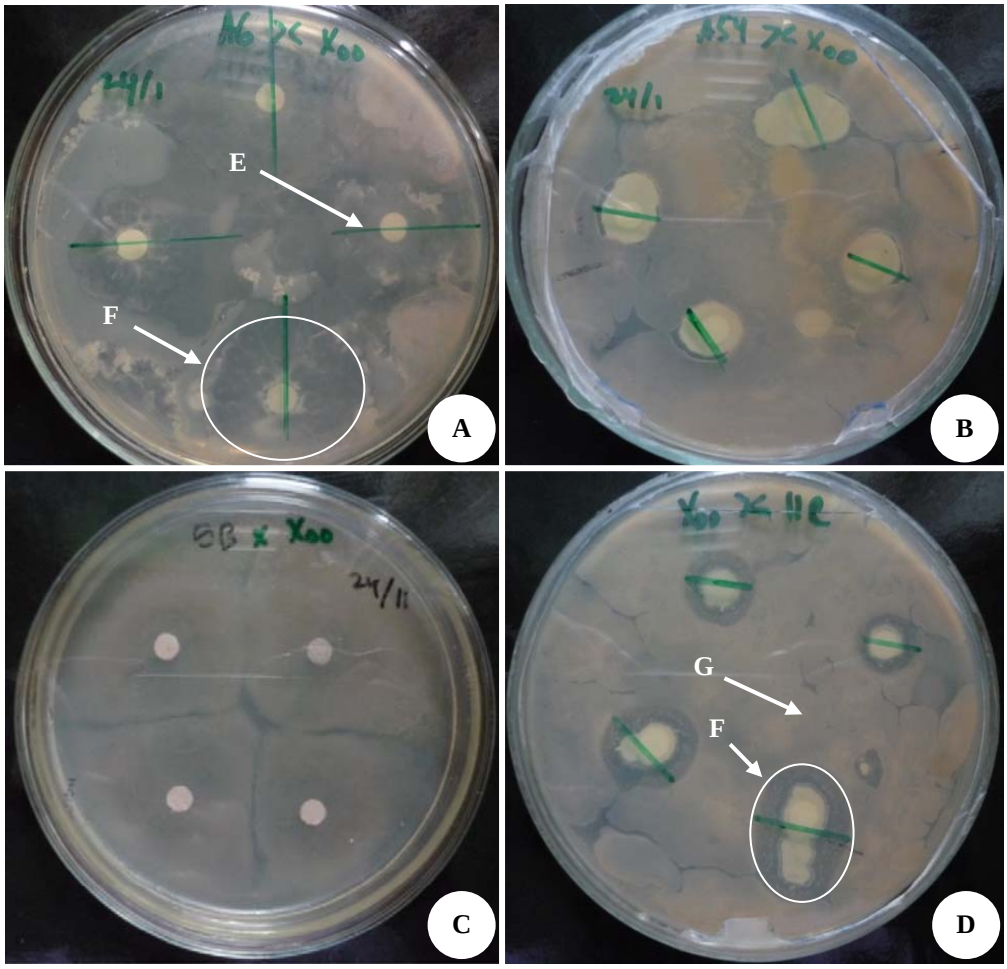

Figure 1. The result of inhibition ability biological agents to the growth of *Xoo*. A.. *P. diminuta* A6 versus *Xoo*; B. *P. aeruginosa* A54 versus *Xoo*; C. *B. subtilis* 5/B versus *Xoo*; D. *B. subtilis* 11/C versus *Xoo*; E. filter paper of biological agent carrier; F. inhibition ability biological agents; G. colony of *Xoo* bacteria

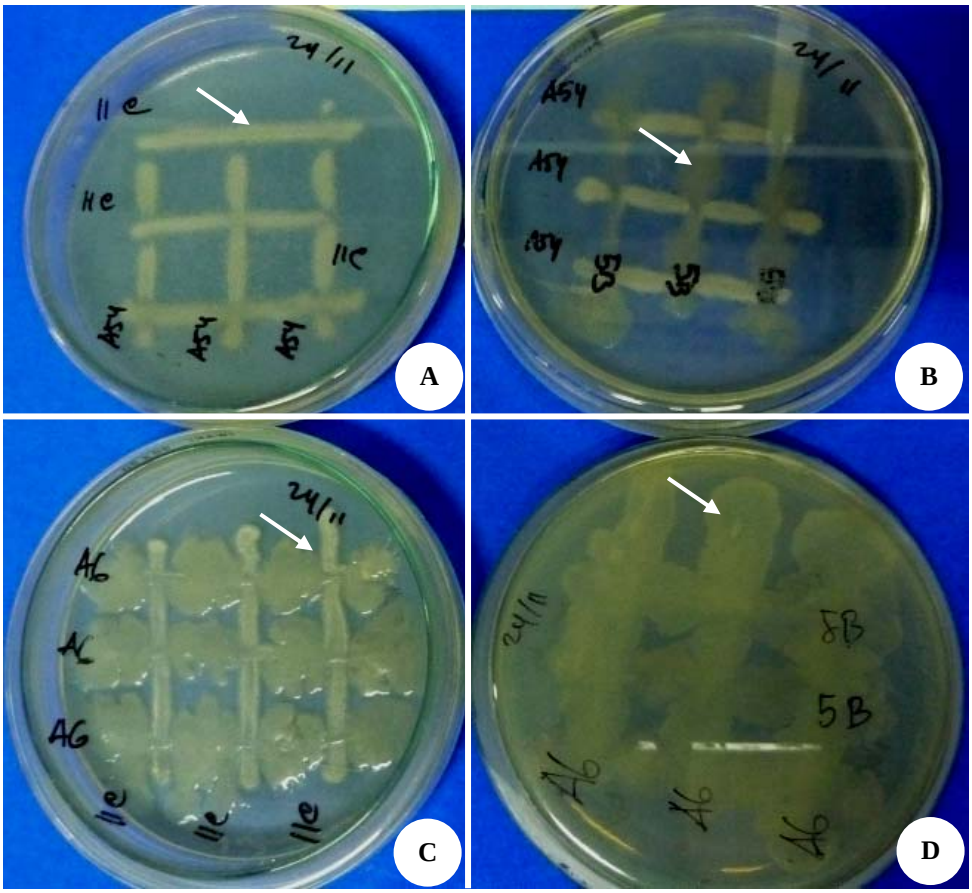

Figure 2. The result of compatibility test between many biological agents. Isolates of: A.. *P. aeruginosa* A54 versus *B. subtilis* 11/C are not compatible (inhibition); B. *P. aeruginosa* A54 versus *B. subtilis* 5/B are not compatible; C. *P. diminuta* A6 versus *B. subtilis* 11/C are not compatible; D. *P. diminuta* A6 versus *B. subtilis* 5/B are compatible

Table 2. The effect of coating formulas with biological agents to seed vigor

Treatments	Vigor index (%)	Growth rate of shoot (%/etmal)
Negative control, healthy seed	91.50 ab	20.83 d
Positive control, the seeds contaminated with *Xoo*	89.50 b	23.01 c
P. diminuta A6 + *B. subtilis* 5/B	96.50 a	30.86 a
Alginate 3% + 1% peat + A6+5/B	89.50 b	20.80 d
Arabic 3% + gipsum 1% + A6+5/B	89.00 b	20.63 d
CMC 1.5% + 1% talc + A6+5/B	91.50 ab	20.83 d
Bactericide streptomycin sulfate 20%	93.00 ab	28.49 b

Note: Same letters in column are not significantly different at p = 0.05 according to DMRT

Table 3. The effect of coating formulas to decreasing level infection of *Xoo* and survival of biological agents on the seed

Treatments	Population of (10^4 cfu mL^{-1})		
	Xoo	*P. diminuta* A6	*B. subtilis* 5/B
Negative control, healthy seed	55.75 c	0.00 d	0.00 e
Positive control, the seeds contaminated with *Xoo*	73.75 a	0.00 d	0.00 e
P. diminuta A6 + *B. subtilis* 5/B	28.25 e	23.25 c	12.75 d
Alginate 3% + 1% peat + A6+5/B	35.50 d	28.25 bc	20.75 c
Arabic 3% + gipsum 1% + A6+5/B	35.00 d	29.25 b	43.00 a
CMC 1.5% + 1% talc + A6+5/B	68.00 b	208.00 a	33.00 b
Bactericide streptomycin sulfat 20%	1.50 f	0.00 d	0.00 e

Note: Same letters in column are not significantly different at p = 0.05 according to DMRT

Discussion

The diameter of inhibition zone widest of got at isolate *P. diminuta* A6 (3.7 cm), at isolate *B. subtilis* 5/B (2.8 cm), *B. subtilis* 11/C (1.6 cm), and at isolate *P. aeruginosa* P54 (1.9 cm), (Table 1). The result of this research strengthens result of research of Agustiansyah (2011), which rhizobacteria (biological agent) owning ability which different each other in inhibition growth of *Xoo*.

The ability inhibition growth of this pathogen related to the ability of isolate biological agent in producing cyanide hydrogen (HCN) and siderophore. The isolates biological agents which is used on trial (*P. diminuta* A6*; P. aeruginosa* A54*, B. subtilis* 11/C*, and B. subtilis* 5/B) can produce siderophore and is special of isolate *P. diminuta* A6 also can produce HCN (Agustiansyah 2011). The HCN compound represents one of the secondary metabolite yielded by *Pseudomonas* spp. and have the character of antimicrobe (Fuente et al. 2004). The siderophore is iron (Fe) sticky compound, in a condition lacking Fe which is secreted by a microbe (Dwivedi & Johri 2003). Siderophore production by a biological agent that one of the mechanism and character in the depressing growth of the pathogen. According to Kazempour (2004), mechanism of a biological agent as pathogen antagonist pass competition to Fe nutrient which is also used for the growth of another microorganism.

The increase of growth rate of shoot indicated of increasing a physiological quality of seed coating. Repairing of viability and seed vigor is that expected to be caused by the happening of increasing of hormone synthesis i.e. IAA or gibberellin (GA_3) as the starter of amylase enzyme activity which plays a part in germination (Gholami et al. 2009). Biological agents of *P. diminuta* A6 and *B. subtilis* 5/B which used in this research is known can produce hormone IAA (Agustiansyah 2011). The positive effect from the treatment of biological agent also reported to happened at germination of rice seed (Ashrafuzzaman et al. 2009) and maize (Gholami et al. 2009). Agustiansyah et al. (2010) reported on seed treatment of Ciherang variety rice with matriconditioning + isolate *P. diminuta* A6, immersion in *P. diminuta* A6 or *P. aeruginosa* A54 isolates are the best treatment in improving the viability and seed vigor.

The biological agent used for the coating of can depress growth of *Xoo*. *P. diminuta* A6 and *B. subtilis* 5/B isolates which used in this research can produce siderophore, and cyanide hydrogen (HCN) (Agustiansyah 2011), having the character of antimicrobe. Decreasing of availability of Fe (iron) of necessary for pathogen growth and development effect of chelating by siderophore, inhibiting the growth of pathogen (Siddiqui 2005). Awais et al. (2007) reporting that *Pseudomonas* spp. producting of 2.4 diacetyl phloroglucinol compounds (known to inhibiting the growth of *Xoo*), and *Bacillus* spp. producting of bacitracin compound having the character of antimicrobe. Agustiansyah et al. (2010) reporting that immersion of seed in *Bacillus* spp suspension and also in *Pseudomonas* spp suspense, and treatment of combined matriconditioning with *Bacillus* spp. and also *Pseudomonas* spp. can depress growth of *Xoo* on rice seed of Ciherang variety.

The results of this research showed that isolates *P. diminuta* A6 has the highest antagonistic potential against *Xoo* based on the diameter of inhibition zone on *Xoo*, followed by isolate *B. subtilis* 5/B. Isolate *P. diminuta* A6 and *B. subtilis* 5/B had a good growth compatibility with no antagonism. Therefore, isolates *P. diminuta* A6 and *B. subtilis* 5/B were selected and used as biocontrol agents in further studies. Treatment with *P. diminuta* A6 + *B. subtilis* 5/B gave the best of increase of seed vigor, and the decrease of *Xoo* infection level in the seeds.

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