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Production of Dextran from Locally Lactobacillus Spp. Isolates

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Abstract

Background: Dextran is a commercially available bacterial exopolysaccharide (EPS) with several industrial applications in the food industry and in the biomedical industry as an adjuvant, emulsifier, carrier, and stabilizer. The production of dextran at the industrial level occurs through the fermentation of a sucrose-rich medium. Research to optimize dextran production has found that the yield of dextran varies depending the specific conditions for production. The aim of this study was to produce dextran and establish the optimal conditions for dextran biosynthesis from different *Lactobacillus* species isolated from healthy vaginal and infant stool samples.

Methods: *Lactobacillus* spp. were isolated and identified from vaginal and infant stool samples via the VITEK 2 system. The presence of dextran biosynthesis from the different *Lactobacillus* spp. isolates was determined by a screening test for mucoid colonies and confirmed via the ethanol precipitation method. To optimize for the maximum yield of dextran, the effects of various parameters such as temperature, incubation time, pH, inoculum size, aeration, and sucrose concentration were examined.

Results: All *Lactobacillus* spp. isolates were able to produce dextran. The optimal conditions for dextran production was at 24 hours of incubation at 30 °C with 15% sucrose, 4% inoculation size at pH 7.0 in aerobic conditions. This yielded a dextran dry weight of 580 mg/100 mL.

Conclusions: Dextran production from Lactobacillus species isolates vagina and infant stool had the ability to produce dextran.

Keywords: Dextran, *Lactobacillus* spp, Optimum Conditions, Precipitation.

Introduction

Lactobacillus species are a broadly defined group of bacteria that produce lactic acid as their main end-product of carbohydrate metabolism. *Lactobacillus* are a genus of Gram-positive, facultative anaerobic or microaerophilic, rod-shaped, non-spore-forming bacteria (1). *Lactobacillus* populations are found ubiquitously throughout the environment colonizing plants, animals, and humans. In humans, lactobacilli are commonly found inhabiting the oral cavity, gastrointestinal tract, and the female vaginal tract (2). The process of lactic acid fermentation results in the production of several different products including: hydrogen peroxide, organic acids, diacetyl, hydroxyl fatty acids, proteinaceous compounds, and bacteriocins (3). Additionally, *Lactobacillus* spp. can synthesize a diverse group of polymers secreted into the surrounding external environment called, exopolysaccharides (EPS), which are divided into homopolysaccharides and heteropolysaccharides (4).

Dextran is a bacterial homopolysaccharide mainly composed of consecutive α-(1 → 6) linked glucose units (5, 6, 7). Dextran biosynthesis occurs outside of the bacterial cell, requiring the activity of the dextransucrase enzyme (8). At the industrial level, dextransucrase is the most widely (9) used enzyme for dextran biosynthesis (10). Dextran is a widely used and commercially available EPS with a variety of applications in the food and drug industry (11). Dextran has been previously used as a nano based drug delivery system in a variety of

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ways, including in the delivery of anti-cancer agents (12), or as nano carriers for the targeted delivery of doxorubicin to breast cancer cells (13). The use of dextran is also a promising candidate for the oral delivery of insulin (13). Dextran is extremely water soluble and shows no post drug delivery cellular toxicity. Additionally, dextran can be fully metabolized by the body which can minimize the chance of renal failure, making it a highly attractive nanomedicine, nanodrug carrier, and cell imaging system or nanobiosensor (11, 14, 15). The aim of this study was to synthesize dextran from different *Lactobacillus* spp. isolates and establish the optimal conditions for dextran production.

Materials and methods

Microorganisms

Identifying Lactobacillus spp. isolates

Eight different strains of *Lactobacillus* spp. were isolated from vaginal swabs of healthy women and infant stool samples. Each *Lactobacillus* spp. isolated was identified via bacterial culture and observed on the surface of MRS Lactobacillus agar plates. Addition to microscopical examination and biochemical tests, then the final identification of isolates depends on the results of the Vitek 2 system ANC ID card, this card is designed for rapid identification of Lactobacillus spp. **(**16).

Screening of Lactobacillus spp. for dextran production

Screening test for mucoidy and ropiness

Dextran production was determined via screening test for mucoid colonies and ropiness. The screening test was carried out on customized sterile dextran medium (15% sucrose concentration, 5.0 g bacto–peptone, 5.0 g yeast extract, 15 g K2HPO4, 0.01 g $MnCl_2H2O$, 0.01 g NaCl, 0.05 g $CaCl_2$, 50 g agar, pH 7.0). For each *Lactobacillus* spp., 2 µl of 24 h old culture was added to the screening medium and incubated at 37 °C for 24-48 h under anaerobic conditions. Following incubation, the mucoidy of colonies was determined by examining the visual appearance, and the ropiness was determined by touching the colonies with a sterile inoculation loop (17). The isolates that produced mucoid colonies and had a ropy phenotype were recorded as dextran producing.

Ethanol precipitation method

Each *Lactobacillus* isolate was inoculated in dextran screening broth at 2% of *Lactobacillus* suspension at a concentration of 9 x 10^8 cfu/ml. Following 24-48 h incubation at 37 ˚C, the culture medium was precipitated with an equal volume of chilled ethanol, shaken vigorously, centrifuged at 10,000 g for 15 min. The supernatant was then decanted. The *Lactobacillus* isolates that had EPS precipitate were recorded as dextran producing **(**18).

Production and precipitation of dextran

Dextran production and precipitation was performed according to the procedure described previously (18). Briefly, 250 ml flasks containing 100 ml of autoclaved dextran production medium (sucrose 150 g. peptone 5.0 g, K_2HPO_4 15.0 g, $MnCl_2.4H_2O$ 0.01 g, yeast extract 5.0 g, NaCl 0.01 g, $CaCl_2$ 0.05 g, added to 1 liter of distilled water, pH 7.0) was inoculated with 2% of the *Lactobacillus* spp. suspension at a concentration of 9 x 10^8 cfu/ml, incubated at 37 ˚C for 24 h. The culture medium was precipitated using an equal volume of chilled ethanol, shaken vigorously, centrifuged at 10,000 g for 15 min. The supernatant was then decanted. To purify the dextran, the precipitate was washed with distilled water. The dextran-water mixture was then re-precipitated with an equal volume of chilled ethanol. The precipitate and washing steps were repeated twice. The precipitated dextran was then dried at 40 °C for 45 min (19).

Determining optimal conditions for dextran production

For all experiments, the *Lactobacillus (L.) gasseri* (LV1) isolate was used for examining optimal dextran production conditions. After the optimal amount was determined for each factor (temperature, incubation time, etc.), the subsequent experiments to test optimal dextran production used these optimized conditions. Following each experiment, dextran was isolated via ethanol precipitation method and the dextran dry weight was calculated for each condition after dried at 40 C for 45 min, so the final dextran powder was weighted, as final purified dextran (19, 20).

Optimum conditions
The temperature used to use to test dextran production was 37 ˚C for 24 h and PH 7 with inoculum size 2% and sucrose Concentration 15% under unaerobic conditions.

Effect of temperature
The temperature of the incubation was tested at various ranges, 27, 30, 37, 40 and 43 ˚C for 24 h to determine the best temperature that give high production of dextran

Effect of Incubation Time
The incubation times was tested at 24, 48, or 72 h to determine the best incubation time that give high production of dextran.

Effect of Initial pH
The pH of the cultivation media was varied at 3, 5, 7, 9, or 11, the media was incubated for the optimal incubation time, at the optimal temperature.

Effect of Inoculum Size
The effect of inoculum size on dextran production was studied by inoculating at 1, 2, 4, or 6% of inoculum (9 x 10^8 CFU/ml). The media was incubated at the optimal temperature, for the optimal length, at the optimal pH.

Effect of Sucrose Concentration
The culture medium was inoculated at various sucrose concentrations of 10, 15, and 20%. All other conditions were set to the predetermined optimal levels to determine the best sucrose concentrations that give high production of dextran.

Effect of aeration
To determine the optimal level of aeration for dextran synthesis, the inoculated culture medium was incubated under anaerobic or aerobic conditions with shaking at 50 g. The medium was adjusted and incubated for the previously determined optimal dextran producing conditions.

Results

Screening of Lactobacillus spp. for Dextran production
Screening test for mucoidy and ropiness
All *Lactobacillus* isolates were examined for dextran production by screening for the presence of mucoid colonies and ropiness on the surface of the dextran screening medium. Isolates with mucoid colonies and ropiness were recorded as dextran producing. Our findings show that all *Lactobacillus* spp. were observed to have mucoid colonies and some degree of ropiness and were therefore positive for dextran production (Table 1). Of the 8 isolates, 2 produced mucoid colonies with a mild degree of ropiness, 5 isolates produced mucoid colonies with moderate ropiness, and 1 isolate produced mucoid colonies with a large degree of ropiness. Figure 1 shows the *L. gasseri* isolate with the highest degree of ropiness in the mucoid colonies (Fig. 1).

Table 1. Screening for dextran production by Lactobacillus spp. isolates determined by mucoidy and ropiness.

Bacterial isolates	Mucoid formation
L. gasseri (LV1)	+++
L. gasseri (LV2)	+
L. acidophilus (LV3)	++
L. acidophilus (LV4)	++
L. acidophilus (LV5)	++
L. gasseri (LS1)	++
L. fermentum (LS2)	++
L. plantarum (LS3)	+

+++: high production of dextran
++: moderate production of dextran
+: weak production of dextran
LV: isolated from vagina, LS: isolated from infant stool

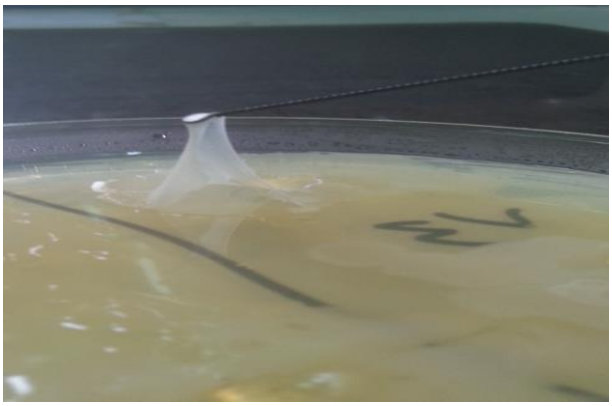

Fig. 1. Ropiness of mucoid colonies of *L. gasseri* (LV1) on dextran screening medium.

Screening for mucoid colonies is a commonly used method for detecting EPS production from lactic acid producing bacteria (21). Previous experiments have successfully screened for EPS producing bacteria by identifying mucoid colonies and ropiness. This method has been used to identify EPS production from *Lactobacillus* and *Streptococcus* spp. isolated from fermented milk products (22).

Ethanol precipitation method

The ethanol precipitation method was used to confirm dextran production from the Lactobacillus isolates. The results from the ethanol precipitation method are slightly different from that of the mucoid screening test where showed more easy in collection and separation of dextran. One of the isolates, *L. gasseri* (LV1), was observed to produce high levels of dextran when using the ethanol precipitation method. The other isolates varied in their ability to produce dextran (Table 2).

Table 2. Screening for dextran production by *Lactobacillus* spp. isolates using ethanol precipitation method

Bacterial isolates	Ethanol precipitation
L. gasseri (LV1)	+++
L. gasseri (LV2)	+
L. acidophilus (LV3)	++
L. acidophilus (LV4)	++
L. acidophilus (LV5)	++
L. gasseri (LS1)	++
L. fermentum (LS2)	++
L. plantarum (LS3)	+

+++: high production of dextran;
++: moderate production of dextran
+: weak production of dextran; LV: isolated from vagina; LS: isolated from infant stool

Dextran Production

The *L. gasseri* (LV1) isolate, with the highest dextran production of 260 mg/100 ml, was used to screen for optimal dextran producing conditions. After optimizing for all conditions, the maximal yield of dextran from *L. gasseri* was determined to be 580 mg/100 ml. Previous studies optimizing for dextran yield have found *L. rhamnosus* to produce 210.28 mg/100 ml EPS under optimal conditions (23), while *L. salivarius* yielded 450 mg/100 ml of EPS (24).

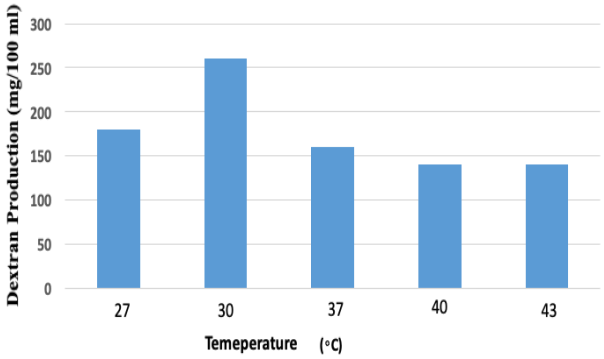

Fig. 2. Effect of temperature on dextran production from *L. gasseri* (LV1).

Effect of Temperature

Dextran production by *L. gasseri* (LV1) was examined at different temperatures, (27, 30, 37, 40, and 43 ˚C). Results showed that the optimal temperature for dextran production was 30 ˚C which produced a dextran dry weight of 260 mg/100 ml. At 27 ˚C the dry weight for dextran was 180 mg/100 ml, at 37 ˚C dry weight was 160 mg/100 ml, at 40 °C and 43 ˚C the dextran dry weight was 140 mg/100 ml (Fig. 2). Previous studies have also found 30 °C to produce the maximal yield of dextran. Research by Abedin *et al*. (7) examining the production of dextran at different temperatures, ranging from 15 °C to 45 °C found the maximum dextran production and the greatest enzyme activity of dextransucrase by L. acidophilus was at 30 °C. A separate study by Abedin *et al*. (7) used 30 ˚C for dextran production from *L. mesenteroides*. In a separate study by Salman and Salim (18), the optimal temperature for dextran production from *L. mesenteriodes* isolates from fish intestine was 30 °C.

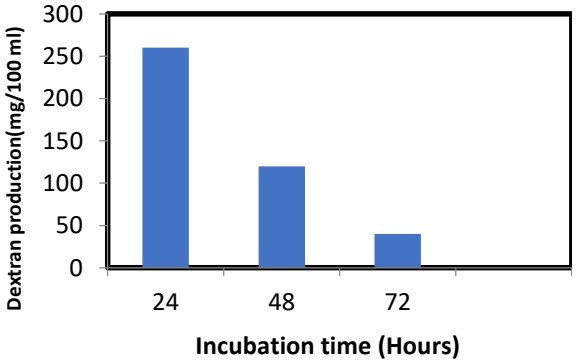

Fig. 3. Effect of Incubation time on dextran production from *L. gasseri* (LV1).

Effect of incubation time

The effect of incubation time of dextran production by *L. gasseri* (LV1) was examined by studying different incubation times (24, 48, or 72 h). Results showed that the optimal incubation time for dextran production was at 24 h incubation, producing a dextran dry weight of 260 mg/100 ml. Incubation for 48 h resulted in a dextran dry weight of 120 mg/100 ml and at 72 h the dry weight was 40 mg/100 ml (Fig. 3). Previous work by Salman and Salim (18) studied the effect of incubation time on dextran production from *L. mesenteriodes* and observed the optimum incubation time for dextran production to be at 24 h. A separate study by Mohanasrinivasan et al (25) optimizing for L. mesenteriodes dextran production mentioned that the optimum incubation time EPS production from lactic acid bacteria was also at 24 h. A separate study by Abedin et al. (7) studied dextran production from L. acidophilus and found that it was increased at (20-48) h, with the maximum production of dextran at 20 h after which the yield gradually decreased with increased incubation time (19).

Effect of Initial pH

After determining the optimal temperature and incubation times for dextran yield, *L. gasseri* (LV1) was incubated at varying pH levels (3, 5, 7, 9, 11). The optimum pH for dextran production was determined to be at pH 7, which produced a dextran dry weight of 260 mg/100 ml. At pH 3 the dextran dry weight was 20 mg/100 ml, at pH 5 dextran dry weight was 80 mg/100 ml, at pH 9 was 60 mg/100 ml and at pH 11 the dextran dry weight was 20 mg/100 ml (Fig. 4). The optimal pH for dextransucrase production by lactic acid bacteria has been previously determined to be between a pH of 6.5 to 7.0 (26). Previous work by Joshi and Koijam (27) studied the effect of different pH levels on dextran production and found that the production progressively increased until reaching maximum production at pH 6.5. Additionally, in a separate study the optimal pH for dextran production from *L. mesenteriodes* was also previously determined to be at a pH 7.

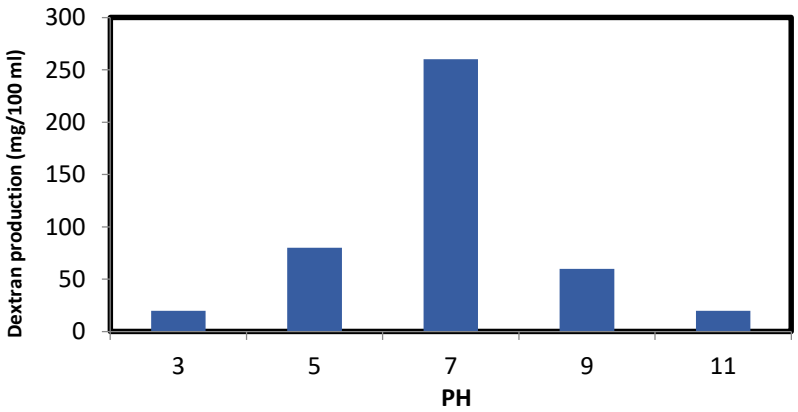

Fig. 4. Effect of pH on dextran production from *L. gasseri* (LV1).

Effect of Inoculum Size

To examine the effect of inoculum size on dextran production, *L. gasseri* (LV1) was incubated at various inoculum sizes (1, 2, 4, 6%) at a concentration of 9×10^8 cell/ml. Our findings showed that the best inoculum size for dextran production was at 4%, yielding a dextran dry weight of 340 mg 100/ml. At 2% inoculum size the dextran dry weight was 260 mg 100/ml, and at 6% the dextran dry weight was determined to be 220 mg/100 ml. The lowest dextran dry weight recorded was 60 mg/100 ml at a 1% inoculum size (Fig. 5).

Previous work has found that the best inoculum size for dextran production from *L. mesenteroides* spp. *mesenteroides* was at 4% (18). Additionally, the best inoculum size for production of the bacterial EPS, levan, from *L. mesenteroides* ssp. *cremoris* was at 4% (28).

Demirci *et al*. (29) has mentioned that an increase in the amount of inoculum possibly had no positive effect on the yield of EPS. Their findings show that the most suitable inoculum size for EPS production from *Xanthomonas axonopodis* was at 5%.

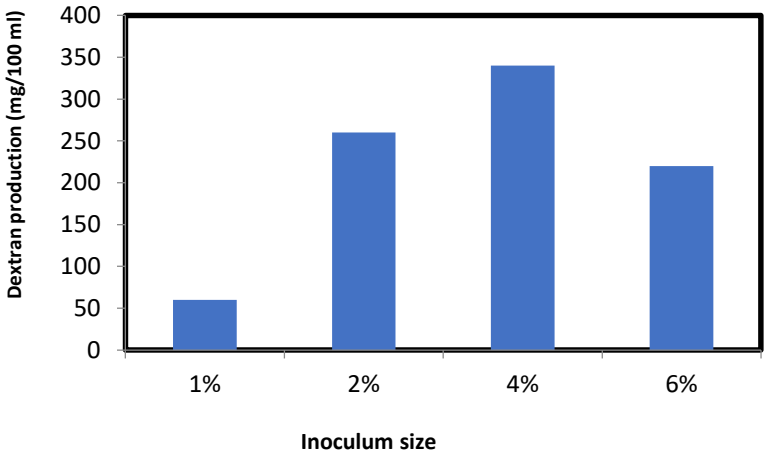

Fig. 5. Effect of inoculum size on dextran production from *L. gasseri* (LV1)

Effect of Sucrose Concentration

To determine the optimal sucrose concentration for dextran production, *L. gasseri* (LV1) was inoculated in production medium with different sucrose concentrations of (10,15, and 20%). The sucrose concentration yielding the best dextran production was recorded at 15%, with a dextran dry weight of 340 mg/100 ml. At a 10% sucrose concentration the dry weight was 100 mg/100 ml, while at a 20% sucrose concentration it was 120 mg/100 ml (Fig. 6). Similar to our findings, previous work has found that the best sucrose concentration for dextran production from *L. mesenteroides* ssp. *mesenteroides* was recorded in medium with 15% sucrose concentration (18).

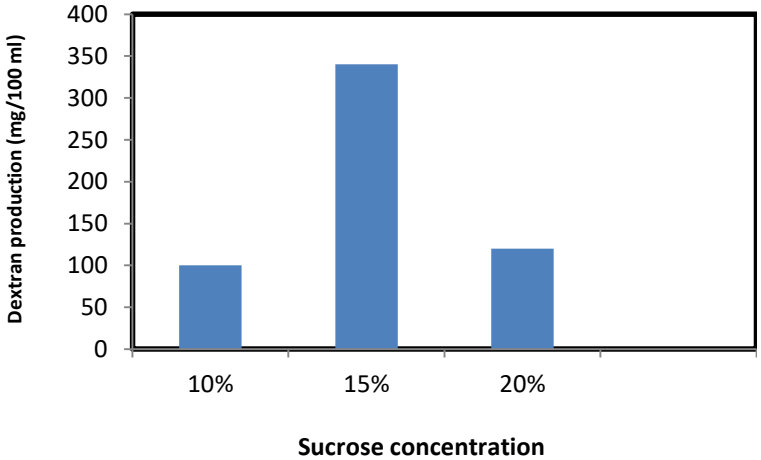

Fig. 6. Effect of sucrose concentration on dextran production from *L. gasseri* (LV1).

Effect of aeration

The production of dextran by *L. gasseri* (LV1) under anaerobic and anaerobic conditions was examined. Our findings showed that the amount of dextran produced under the aerobic condition reached 580 mg/100 ml, while the yield in the anaerobic condition was 340

mg/100 ml (Fig. 7). Work by Srinivas and Padma (8) found the optimal production for dextran by the *Weissella confusa* bacterium was at an aeration ratio of with shaking at 150 g. *Leuconostoc lactis* isolates produced EPS under shaking condition of 100 g (30). The positive effect of aeration on dextran production related with the highest dextranase activity in aeration condition (31).

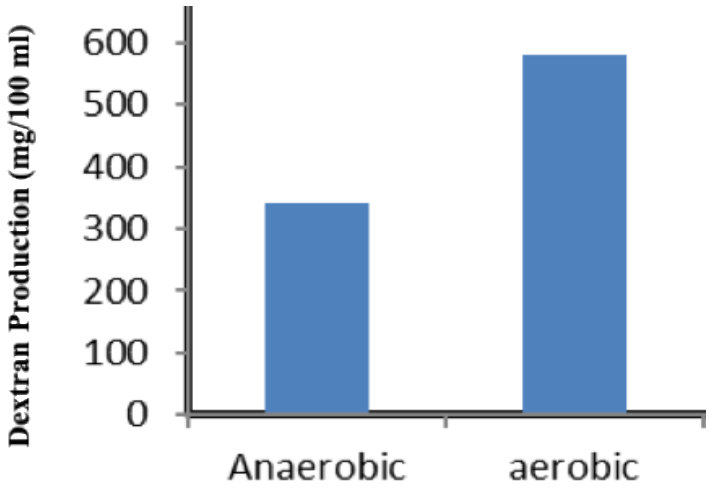

Fig. 7. Effect of aeration on dextran production from *L. gasseri* (LV1).

Discussion

The Lactobacillus spp. were isolated from healthy women's vagina, and infant's stool, all isolates were subjected to the cultural, microscopical and biochemical test as well as to vitek 2 system for identification to species. Eight isolates were identified as 5 isolates from vagina and 3 isolates from infant stool. The isolates from vagina were distributed as (3) *L. acidophilus* and (2) *L. gasseri* while isolates from infant stool were distributed as, one isolate of each of *L. gasseri*, *L. fermentum* and *L. plantarum*, all isolates of Lactobacillus spp. were tested for dextran production using the mucoidy method and the ethanol precipitation method, all isolates had ability to produce dextran. The optimum conditions for the production of dextran were tested such as temperature, incubation time, pH, inoculum size, aeration, sucrose concentration. The optimum condition for dextran production was 30 °C for 24 h at pH 7 in aerobic condition with 4% inoculum size and 15% sucrose concentration. Dextran dry weight was 580 mg/100 ml at optimum conditions.

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