

Screening of Glycolytic Enzyme Inhibitory Activity of *Streptomyces* Isolates from Brine Spring and Marine Sediments of India

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ABSTRACT

The objective of the study was to investigate the percentage inhibition of glycolytic enzymes by compounds produced by two strains of *Streptomyces* sp. isolated from different niches of India. The strain *Streptomyces* sp. VITPK9 was isolated from brine spring located in Thoubal district, Manipur, India and the strain *Streptomyces* sp. VITSTK7 was isolated from marine sediments collected from Puducherry coast, Tamil Nadu, India. Both the strains were characterized using molecular taxonomy and identified as *Streptomyces* sp. VITPK9 (JN689333) and *Streptomyces* sp. VITSTK7 (GQ499369). The 16S rDNA analysis showed 97% similarity of the strain VITPK9 with *Streptomyces pseudogriseolus* (X80827) and 86% similarity of the strain VITSTK7 with *Streptomyces longisporoflavus* (DQ442520). The strains were fermented for seven days using Kuster's broth with pH 7-7.2 at room temperature in shake flask incubator at 150 rpm. The ethyl acetate (EA) extract obtained from isolates were screened for α -amylase and α -glucosidase inhibitory activity. The EA extract (500 μ g/ml) of VITPK9 showed significant (69.1%) inhibitory activity against α -amylase and compared to VITSTK7 activity. The EA extract (500 μ g/ml) of VITSTK7 inhibited (64.3%) α -glucosidase and compared to VITPK9. From this study we are concluding that both the extracts showed concentration dependent inhibitory activity against tested glycolytic enzymes. The bioactive compounds need to be extracted and identified from these potential isolates to study its mechanism of action.

Keywords: α -amylase, α -glucosidase, diabetes mellitus, inhibitors, secondary metabolites, *streptomyces*.

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INTRODUCTION

Glucose plays a very important role and served as the primary source of energy for all living organisms except few. At the same time, excessive glucose is also not good. Glucose metabolism related disorders have become a common disease worldwide. A persistent high level of glucose may lead to hyperglycemia which is the main factor for causing Diabetes Mellitus (DM) and is considered as one of the life-threatening disorder worldwide. India, being a diabetic capital and alarming increase in percentage of diabetic patients force to look for suitable measure to control DM [1]. DM is of three types which include Type I, Type II and gestational diabetes which occurs in pregnant women. Of the three, Type II is the

severe one. Type II diabetes is mediated by defects in either insulin secretion or insulin action leading to high level of glucose in the body [2]. To control the hyperglycemia, the preventive measure is to reduce or slowdown the break-down of carbohydrates in the body and is very critical as it may lead to chronic complications if the glucose level is not maintained in normal level. The breaking down of carbohydrates is mediated through the glycolytic enzymes mainly α -amylase and α -glucosidase. Reports suggest that inhibition of α -amylase and α -glucosidase activity proved to be an effective way for controlling DM type II [3].

It's always known that natural resources are the best way to cure/prevent the

diseases/disorders. However, very few attempts have been made to utilize the natural resources. Most of the antidiabetic medicines available in the market are chemically synthesized and few are derived from plant sources. Most of the chemical drugs have been proved to be causing numerous side-effects [4,5]. Exploiting natural resources for effective antidiabetic drugs are of great importance and current focus. The microbial sources are also often explored for effective antidiabetic drugs and could be utilized as an alternative source for synthetic drugs. Among the microbial sources, actinomycetes can be used as a source of antidiabetic compounds. An α -amylase inhibitor, Hoe 467A was isolated from *Streptomyces tendae* 4158. Antidiabetic drugs acarbose, voglibose, valienamine, adiposin-1 etc. were derived from actinomycetes [6]. So far only very few inhibitors were extracted from *Streptomyces* and there may be a possibility of extracting more antidiabetic drugs from novel marine actinomycetes from unexplored habitats. The Thoubal district of Manipur, India is considered as unexplored or less explored habitat for isolation of novel *Streptomyces* and Puducherry coast is under-explored area for isolation of actinomycetes. As India accounts for 90% of type II diabetic cases [7], it dictates an intensive search for novel antidiabetic compounds from microbial sources.

This study was carried out to investigate the percentage inhibition of glycolytic enzymes by compounds extracted from two different isolates of *Streptomyces* isolated from different niches of India and to compare the results. The isolate *Streptomyces* sp.VITPK9 was isolated from brine spring located in Thoubal district, Manipur, India and the isolate *Streptomyces* sp. VITSTK7 was isolated from marine sediments collected from Puducherry coast of Bay of Bengal, Tamil Nadu, India.

MATERIALS AND METHODS

Sampling and isolation:

The isolate *Streptomyces* sp. VITPK9 was isolated from brine spring located in Thoubal district, Manipur, India [Latitude (N) 24°45', Longitude (E) 94°15'] and the isolate *Streptomyces* sp.VITSTK7 was isolated from marine sediments collected

from Puducherry coast, Tamil Nadu [Latitude (N) 11°56', Longitude (E) 79°53']. The soil samples were dried in laminar air-flow for 8-12 hrs and 1 gm of the dried soil sample were serially diluted and plated on Starch Casein Agar (SCA) (Himedia, India). The plates were incubated for seven days at room temperature.

Molecular taxonomic characterization of the isolate:

The isolates were subjected to morphological, physiological and biochemical characterization. For the morphological study, International Streptomyces Project (ISP) method were followed [8-10]. The spore arrangements were observed by Scanning Electron microscopy (F E I Quanta FEG 200). Physiological characterization was carried out using different range of pH, NaCl concentration and various range of temperature. 16S rDNA sequencing was carried out as reported earlier [10]. The 16S rDNA nucleotide sequence was submitted to GenBank, NCBI, USA. The related strains were selected from NCBI data base for alignment using a software tool CLUSTAL W 1.83 version of DDBJ program and the tree was constructed using Tree view software (Win 32) 1.6.6.

Fermentation and extraction of the secondary metabolites:

The isolates VITPK9 and VITSTK7 were inoculated in Kuster's broth with pH 7.2 and incubated at 28°C in rotary shaker at 150 rpm for seven days. After seven days of incubation, the broth was centrifuged at 4000 rpm for 15 minutes and the supernatant was collected. The supernatant was mixed with equal volume of ethyl acetate (1:1 v/v ratio) and kept for overnight incubation in a rotary shaker. The ethyl acetate (EA) extract was concentrated using rotary evaporator. The EA extract obtained were used for further studies [10].

Assay of α -amylase Inhibitory activity:

The EA extract obtained from both the *Streptomyces* isolates were screened for α -amylase inhibition [11]. EA extract (500 μ l) was mixed with the 500 μ l of the 0.02 M sodium phosphate buffer (pH 6.9) containing α -amylase (SRL, India) solution of 0.5 mg/ml and incubated for 10 min at 25°C. 500 μ l of 1 % starch solution in 0.02 M

sodium phosphate buffer (pH 6.9) was added to each tube at the interval of 5 sec. The mixture was incubated for 10 min at 25°C. The reaction was stopped by adding 1 ml of the dinitrosalicylic acid (Himedia, India). The test tubes were then incubated in boiling water bath for 5 min and cooled to room temperature. The mixtures were diluted using distilled water and the absorbance was measured at 540 nm. The percentage of inhibition was calculated using the below formula:

$$\% \text{ Inhibition} = \left[\frac{\text{Absorbance of control at 540} - \text{Absorbance of extract at 540}}{\text{Absorbance of control at 540}} \right] \times 100$$

Assay α -glucosidase inhibitory activity:

The EA extract (50 μ l) was mixed with 100 μ l of the 0.1M phosphate buffer (pH 6.9) containing the α -glucosidase solution (SRL, India) of 1 U/ml and incubated at 25°C for 10 min [11]. Add 50 μ l of 5 mM p-

nitrophenyl-D-glucopyranoside solution (Himedia, India) to 0.1 M phosphate buffer in each tube and incubated for 5 min at 25°C. The absorbance of the mixture was read at 540 nm. The percentage of inhibition is expressed as:

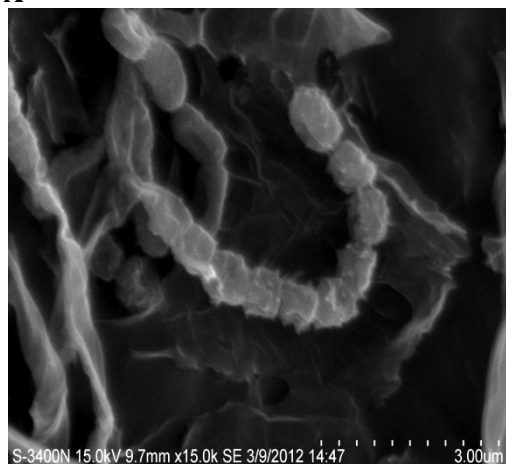
$$\% \text{ Inhibition} = \left[\frac{\text{Absorbance of control at 540nm} - \text{Absorbance of extract at 540nm}}{\text{Absorbance of control at 540nm}} \right] \times 100$$

RESULTS

Taxonomy:

Morphological, physiological and biochemical characterization showed that isolates VITPK9 and VITSTK7 were *Streptomyces* species. Both isolates VITPK9 and VITSTK7 showed white aerial mycelium. The optimized growth media is ISP1 and Kuster's agar respectively and the surface of the spore was found to be smooth (Fig. 1)

A



B

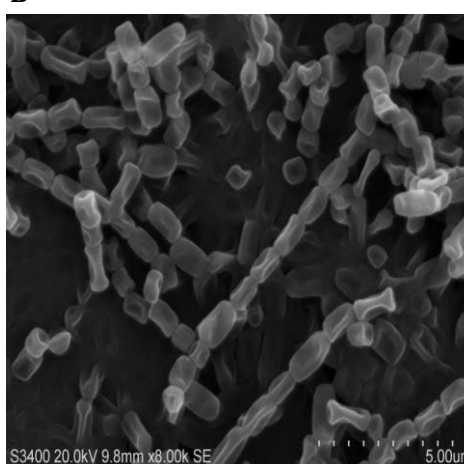


Fig. 1: Scanning Electron Microscopic Image of Spore Arrangement A) *Streptomyces* Sp.VITPK9 B). *Streptomyces* Sp.Vitstk7.

The culturing conditions were characterized for both the isolates; optimum pH was found to be 7.0, temperature was 27°C and 25°C respectively, NaCl concentration was 5 % and 2 % respectively for VITPK9 and VITSTK7. The biochemical characterization revealed that utilization of starch, urea, oxidase, casein, and gelatin by both the isolates and negative for hypoxanthine and voges proskauer (Table 1). The molecular taxonomic characterization using 16S rDNA

nucleotide sequence revealed that the isolates belonged to the genus *Streptomyces* and designated as *Streptomyces* sp. VITPK9 (JN689333) in (Fig. 2) and *Streptomyces* sp. VITSTK7 (GQ499369) in (Fig. 3). The Blast search of 16S rDNA sequence of VITPK9 showed 97% similarity with *Streptomyces pseudogriseolus* (X80827) and VITSTK7 showed 86% similarity with *Streptomyces longisporoflavus* (DQ442520).

Table 1: Characteristics of *Streptomyces* Isolates

Characteristics	VITPK9	VITSTK7
Morphological characterization		
Growth media	ISP1 media	Kuster's agar
Aerial mycelium	White	white
Substrate mycelium	Cream white	Yellowish white
Reverse side pigment	Nil	Red
Spore surface	Smooth	Smooth
Physiological characterization		
Optimum pH	7	7
Optimum temperature	27 °C	25 °C
Optimum NaCl concentration	5%	2%
Biochemical characterization		
Hypoxanthine	-	-
Starch	+	+
Urea	+	+
Oxidase	+	+
Methyl red	+	-
Casein	+	+
Gelatin	+	+
Catalase	+	-
Indole	-	+
Voges proskauer	-	-

+ positive; - negative

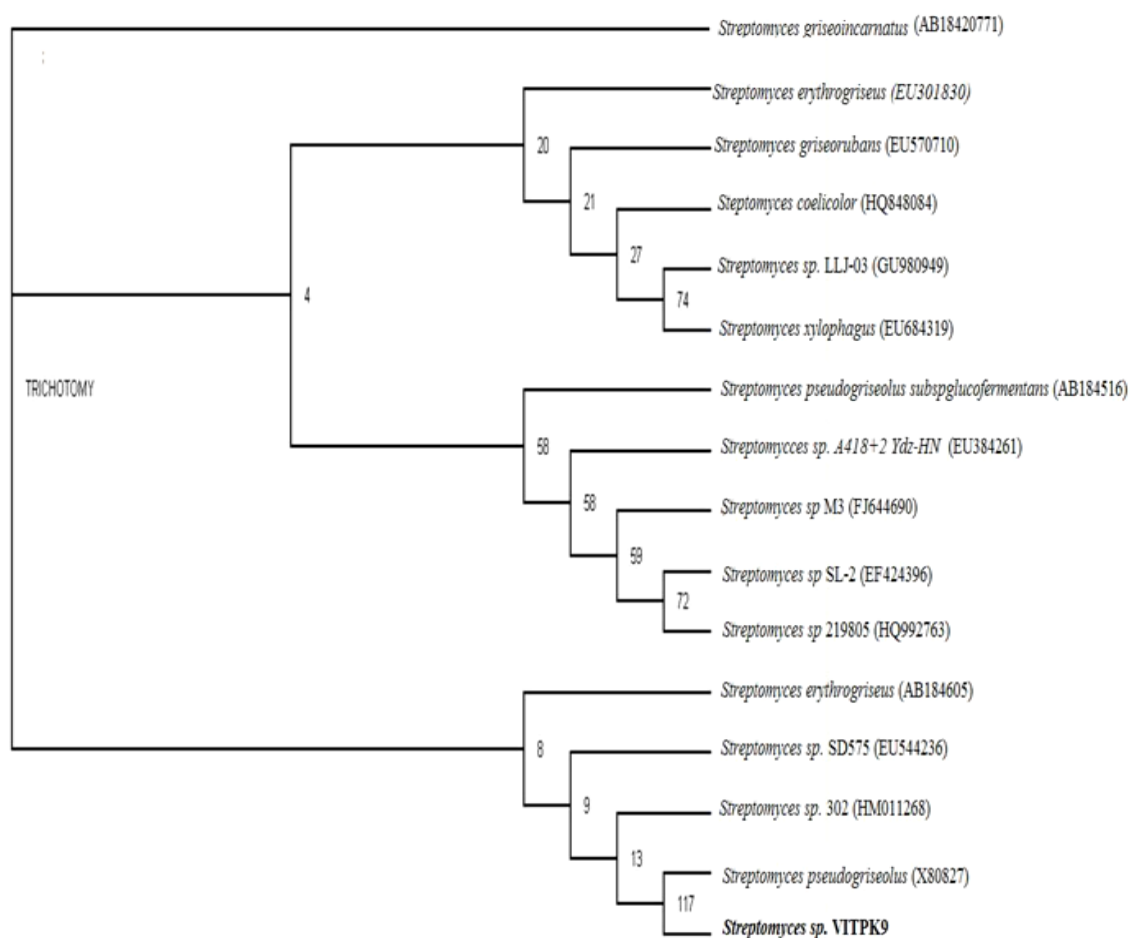


Fig. 2: Phylogenetic Analysis of *Streptomyces* Sp.VITPK9 Shown Using Neighbour-Joining Method Based on Tree View Version Software. Bootstrap Values are Represented at the Nodes of the Tree.

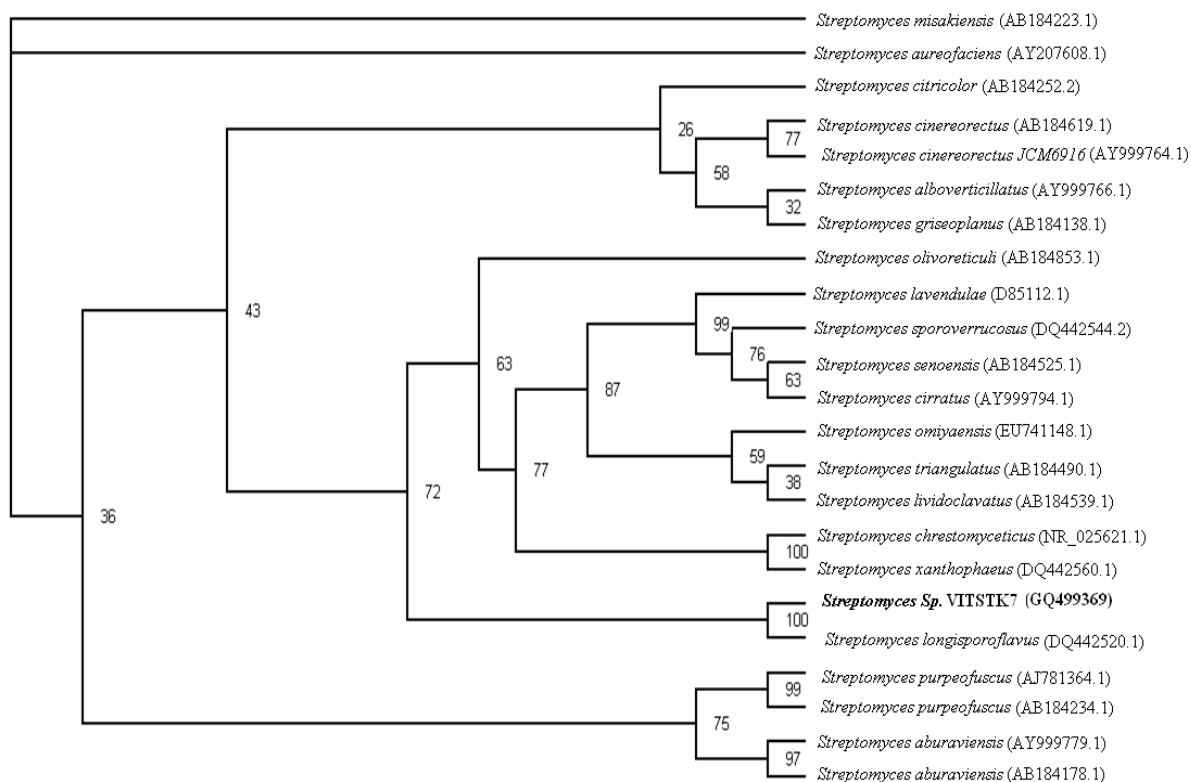


Fig. 3: Phylogenetic Analysis of *Streptomyces* Sp.VITSTK7 Shown Using Neighbour-Joining Method Based on Tree View Version Software. Bootstrap Values Are Represented at the Nodes of the Tree.

α -amylase and α -glucosidase Inhibitory Activity:

The EA extract from both the isolates proved to be a significant inhibitor of both α -amylase (**Fig. 4**) and α -glucosidase (**Fig. 5**). Both the extracts showed concentration dependent (100-500 μ g/ml) inhibitory activity against tested glycolytic enzymes. The highest concentration of the extract (500 μ g/ml) of VITPK9 showed significant (69.1%) inhibitory activity against α -amylase and compared to VITSTK7 activity which showed 51.53 %. The lowest concentration (100 μ g/ml) of the extract of VITPK9 and VITSTK7 showed 26.96% and 10.8% respectively. Interestingly, the highest concentration of extract (500 μ g/ml) of VITSTK7 inhibited (64.3 %) α -glucosidase and compared to VITPK9 activity which showed 57.89%.

DISCUSSION

Streptomyces are ubiquitous in nature and have always been well-known for its

excellent production of secondary metabolites ranging from enzymes to antibiotics. The present study was carried out to screen the inhibitory activity of both the isolates on glycolytic enzymes. *Streptomyces* sp.VITPK9 was isolated from brine spring located in Thoubal district, Manipur, India Manipur, being a Indo-Burma hotspot diversity and considered as virgin area for isolation of *Streptomyces* [12]. *Streptomyces* sp.VITSTK7 was isolated from marine sediments collected from Puducherry, Tamil Nadu. The marine realm covers 70% of the earth's surface and thus provides an excellent platform for the exploration of novel actinomycetes [13,14]. India being the diabetic capital, it is very important to take appropriate measures for better control and management of DM. The rise in glucose level in blood can only be controlled either by insulin or inhibition of glycolytic enzymes like α -amylase and α -glucosidase in the body. The mechanism of

enzyme inhibition is that they delayed the breaking down of carbohydrates into

glucose and thus slowdown the increase in blood glucose concentration.

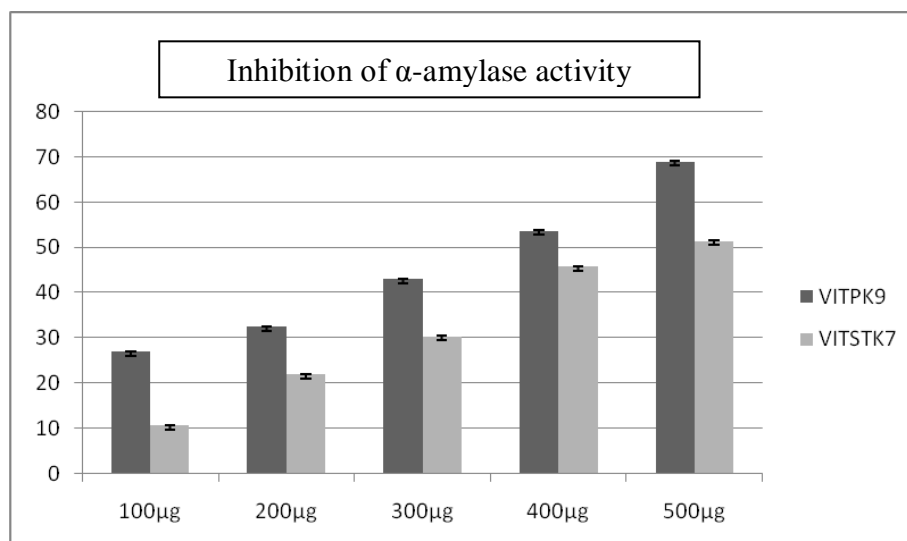


Fig. 4: Inhibition of A-Amylase Activity by Ethyl Acetate Extract of VITPK9 and VITSTK7. The Values are Mean \pm S.D.

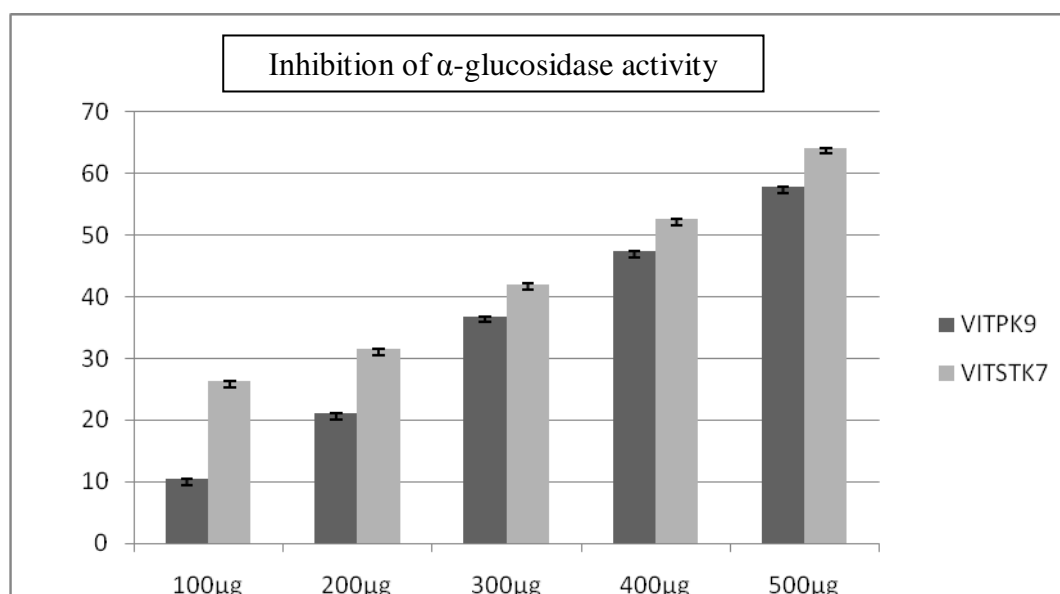


Fig. 5: Inhibition of A-Glucosidase Activity by Ethyl Acetate Extract of VITPK9 and VITSTK7. The Values Are Mean \pm S.D.

CONCLUSION

The results of our study showed that EA extract of both *Streptomyces* sp. VITPK9 and VITSTK7 found to be a potential inhibitor of α -amylase and α -glucosidase activity. The bioactive secondary metabolites need to be extracted and identified from these potential isolates to study its mechanism of action.

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