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Original article

Isolation and screening of *Streptomyces* sp. Al-Dhabi-49 from the environment of Saudi Arabia with concomitant production of lipase and protease in submerged fermentation

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ABSTRACT

In this study, *Streptomyces* sp. Al-Dhabi-49 was isolated from the soil sample of Saudi Arabian environment for the simultaneous production of lipase and protease in submerged fermentation. The process parameters were optimized to enhance enzymes production. The production of protease and lipase was found to be maximum after 5 days of incubation (139.2 ± 2.1 U/ml, 253 ± 4.4 U/ml). Proteolytic enzyme increases with the increase in pH up to 9.0 (147.2 ± 3.6 U/ml) and enzyme production depleted significantly at higher pH values. In the case of lipase, production was maximum in the culture medium containing pH 8.0 (166 ± 1.3 U/ml). The maximum production of protease was observed at 40 °C (174 ± 12.1 U/ml) by *Streptomyces* sp. Lipase activity was found to be optimum at the range of temperatures (30–50 °C) and maximum production was achieved at 35 °C (168 ± 7.8 U/ml). Among the evaluated carbon sources, maltose significantly influenced on protease production (218 ± 12.8 U/ml). Lipase production was maximum when *Streptomyces* sp. was cultured in the presence of glucose (162 ± 10.8 U/ml). Among various concentrations of peptone, 1.0% (w/v) significantly enhanced protease production. The lipase production was very high in the culture medium containing malt extract as nitrogen source (86 ± 10.2 U/ml). Protease production was maximum in the presence of Ca^{2+} as ionic source (212 ± 3.8 U/ml) and lipase production was enhanced by the addition of Mg^{2+} with the fermentation medium (163.7 ± 6.2 U/ml).

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1. Introduction

Actinomycetes are Gram-positive filamentous bacteria which produce various commercial enzymes (Al-Dhabi et al., 2016; Al-Dhabi et al., 2019c). Among actinomycetes, *Streptomyces* sp. is the very important industrially useful organisms because of their ability of producing various enzymes (Al-Dhabi et al., 2019a). Actinomycetes are known for their ability to produce many enzymes such as protease, amylase, lipase, pectinase, cellulase, xylanase, glutaminase and asparaginase (Desai et al., 2016). *Streptomyces* sp. that produce various proteases include *S. griseus*, *S. clavuligerus*,

S. thermoviolaceus, *S. rimovuses* and *S. thermovulgaris* utilize both simple and complex molecules as nutrient sources (Al-Dhabi et al., 2018b) Also, *Streptomyces* species liberate various extra cellular enzymes (Gupta et al., 1995; Al-Dhabi et al., 2019d; Arasu et al., 2017). These *Streptomyces* produce types of extra cellular proteases that have been very much related to sporulation and aerial mycelium (Kim and Lee, 1995).

Proteases and lipases have lot of potential and are frequently exploited for various industrial processes. These two enzymes have been widely used in leather, detergents, baking, dairy and pharmaceutical industries. Industrial processes like leather and bating and cleaning of various slaughter house equipment generally require a mixture of lipase and proteases. Hence concomitant production of lipase and protease has industrial significance. Lipases and proteases widely occur in various microorganisms such as, bacteria, fungi, actinomycetes, animals and plants (Arokiyaraj et al., 2015; Balachandran et al., 2015; Al-Dhabi et al., 2019d; Arasu et al., 2019b). These proteolytic enzymes showed tolerance to many abiotic stresses such as high temperature, pH and salinity (Rathan and Ambili, 2011).

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Proteolytic enzymes from *Streptomyces* spp. are widely used in processing of various agro industrial wastes such as, nails, feather, plant wastes and hair (Bentley et al., 2002; Al-Dhabi et al., 2019b). Proteases from *Nocardiopsis* spp. are well known as very important industrial enzymes and have extensive application in baking, leather, detergent, textile cheese, brewery and dehairing industry (Gohel and Singh, 2012a). It was previously reported that various actinomycetes isolates have ability to hydrolyze fats and oils. Esterases and lipases form a diverse group of enzymes that hydrolyze lipids like triglycerides (Aly et al., 2012; Gurusamy et al., 2019). Actinomycetes such as, *Nocardiopsis alba* and *Streptomyces exfoliates* produce lipases that hydrolyse triglycerides to glycerol and fatty acids (Gandhimathi et al., 2009; Al-Dhabi et al., 2018a). Lipases have lot of applications including, oil processing, fat, diagnosis, cosmetics and detergents (Ninawe et al., 2006). Also lipases have been purified and characterized from *Streptomyces* sp. The extracellular lipase of *Streptomyces exfoliates* have been purified and characterized in molecular level (Wei et al., 1998) and lipase-encoding gene was sequenced (Sommer et al., 1997). Lipase is an inducible enzyme. The enzyme production is frequently stimulated by inducers such as, surfactants, triacylglycerols and vegetable oils, hydrolysis products of oil industry wastes or oil industry wastes. Also, various natural inducers have been used to induce lipase production (Li et al., 2004). Lipases can be widely used as biocatalyst for synthesis of various esters and for trans-esterification process of the oil for biodiesel production (Gulati et al., 2005). Microbial lipases have very broad substrate specificity and high stability toward various organic solvents (Elibol and Ozer, 2000). Microbial synthesis of enzymes has influenced by various factors such as, pH, temperature carbon source, nitrogen source, incubation time and type of fermentation (Palaniyappan et al., 2009; Arasu et al., 2019c). Among these factors, nitrogen, carbon sources and their concentrations have been of potent interest to industries for the use of low cost media. It is well known that about 40% of the production cost of enzymes is estimated to be the cost of the production medium. Hence, it is very important to optimize the bioprocess conditions for the production of low cost enzymes to meet the industrial demand. Moreover, studies on the influence of the carbon and nitrogen sources showed that not all nitrogen and carbon sources would stimulate simultaneous production of enzyme in a single fermentation system. Also, all nutrient supplements should enhance the production of multienzyme in a single bioreactor (Negi and Banerjee, 2010; Valsalam et al., 2019).

2. Materials and methods

2.1. Isolation, screening and identification of lipase and protease producing *Streptomyces* sp. Al-Dhabi-49

In the present study, about 100 g soil sample was collected from Dammam marine region, Jazan marine region and Al-Aselah hot spring region using a spatula. It was immediately transported to the laboratory in ice and used as the sample source for the isolation of actinomycetes (Arasu et al., 2013). Actinomycetes were isolated using actinomycetes isolation medium by standard method. Morphological different strains were purified by repeated streaking and subjected to lipase and protease. For the screening of lipase, tributyrin (1.0%, v/v) was incorporated with minimal medium. Lipase producers showed a clear zone on tributyrin plates (1%) (Sangiliyandi and Gunasekeran, 1996). Further, lipase producing actinomycetes were screened for the production of proteases. Skimmed milk agar medium was used for the production of proteases. 1% skimmed milk was supplemented with minimal

medium and the actinomycetes isolates were streaked on the culture medium and incubated for 5 days at 28 °C. A clear zone appears around the actinomycete isolates if the strain is protease positive (Mittra and Chakrabarty, 2005).

2.2. Identification of actinomycetes from the soil sample for simultaneous production of lipase and protease

In this study, morphological characters such as, colony morphology, spore morphology was analyzed. The physiological parameters such as, temperature, pH, NaCl concentration, utilization of carbon in the growth media were observed. Biochemical tests such as nitrate reduction test, IMViC test, starch hydrolysis, casein hydrolysis, gelatin hydrolysis, urease test, hydrogen sulphide production test and catalase test were carried out.

2.3. Lipase and protease production

The selected actinomycete strain Al-Dhabi-49 was grown on basal medium containing, olive oil (1%) and 1% casein. After sterilization, 1% inoculum was inoculated in 100 ml minimal medium and the culture was incubated for a week 28 °C. Casein was used as the substrate for protease assay and olive oil was used as the substrate for lipase assay.

2.4. Optimization of lipase and protease production

The isolate, Al-Dhabi-49 having hyper production of lipase and protease was selected for optimization of lipase and protease production. The effect of fermentation period on enzyme production was studied in the production medium in submerged fermentation and incubated for 7 days. To the fermentation medium 1% inoculum was inoculated and incubated. The sample was withdrawn and enzyme assay was carried out. The effect of pH on lipase and protease production was performed by varying pH of the culture medium from 6 to 11, whereas the other process parameters were constant. The effect of culture medium temperature for lipase and protease production was carried out by varying temperatures, whereas the other culture parameters were constant. The carbon sources such as, glucose, lactose, fructose, maltose, arabinose, starch, xylose and trehalose (1%, w/v) were added with the medium in 250 ml Erlenmeyer flask. Effect of nitrogen source on the enzyme production was performed with various nitrogen sources (beef extract, yeast extract, peptone, glycine, ammonium sulphate, ammonium nitrate and urea (1%, w/v) at 1% level. Effect of ionic sources (Ca^{2+} , Mg^{2+} , Mn^{2+} , Cu^{2+} and Hg^{2+}) on the enzyme production was performed with various ions at 0.1% level.

2.5. Industrial applications of protease and lipases

2.5.1. Gelatin hydrolysis and release of silver from X-ray film

In this study gelatine hydrolysis was analyzed as described previously by. A piece of used X-ray film (2 × 2 cm) was cut and incubated with alkaline protease at 35 °C (pH 8.0) in an orbital shaker at 150 rpm.

2.5.2. Organic solvent stability of *Streptomyces* sp. Al-Dhabi-49 lipase for industrial processes

The effects of organic solvents such as, ethanol, acetone, methanol, isopropanol, dimethyl sulphoxide, ethyl acetate and chloroform on lipase activity were studied. The enzyme was incubated with organic solvents (50% v/v) at 150 rpm for 30 min. To the control sample organic solvent was not added under the same experimental protocols. Finally, the residual enzyme activity was measured as described previously.

3. Results

3.1. Isolation and screening of actinomycetes for lipase and protease production

Actinomycetes were isolated from the soil sample for the production of industrial enzymes. Samples were collected from three different locations of Saudi Arabia. The subjected soil sample showed maximum actinomycetes population in Jazan marine region, followed by Dammam marine region and Al-Aselah hot spring region. In this study, actinomycetes showed small variations in numbers in three selected stations from 32 to 41×10^6 CFU g^{-1} dry soil (Table 1). In this study, total actinomycetes counts were measured on starch casein agar plates and protease, and lipase producing actinomycetes were measured on skimmed milk agar medium and tributyrin agar medium, respectively. The ability of soil actinomycetes for lipase, and protease production was screened and the number of positive colonies which has the enzymatic activity was analyzed. Initial screening revealed the ability of producing proteases, and lipases by the isolated actinomycetes species on culture plates. Among the actinomycetes isolate, strain *Streptomyces* sp. Al-Dhabi-49 which was isolated from station 2 showed good activity on skimmed milk agar plates and tributyrin agar plates. Based on lipase and protease activity produced by an individual actinobacterial isolate, *Streptomyces* sp. Al-Dhabi-49 was selected.

3.2. Identification of *Streptomyces* sp. Al-Dhabi-49

The selected actinomycete is filamentous type, Gram-positive, branched and non-acid fast. Biochemical analyses revealed that this organism showed positive to catalase experiment, positive to nitrate reduction, hydrolyzed starch, Indole negative and Voges-Proskauer negative. Based on biochemical and morphological experiments this strain was characterized as *Streptomyces* sp. 16S rDNA analysis (accession number KC292820) was also carried out and this strain was identified as *Streptomyces* sp. Al-Dhabi-49. The selected isolate showed 21 mm zone on tributyrin agar plates and 27 mm zone on casein agar plates.

3.3. Effect of fermentation period on lipase and protease

The production of protease was found to be maximum after 5 days of incubation (139.2 ± 2.1 U/ml) and depleted after 6 days (97.2 ± 1.1 U/ml). Lipase activity was not detected upto 24 h of incubation. It was found to be maximum after 5 days of incubation (253 ± 4.4 U/ml) and reduced marginally after 6 days (172 ± 2.1 U/ml) (Table 2).

3.4. Effect of various pHs on lipase and protease production

This experiment was performed for 5 days at various pH values (6.0–11.0). In this study the production of proteolytic enzyme increases with the increase in pH up to 9.0 (147.2 ± 3.6 U/ml)

Table 2

Effect of fermentation period on lipase and protease production by *Streptomyces* sp. Al-Dhabi-49.

Fermentation period (Days)	Enzyme activity (U/ml)	
	Protease	Lipase
1	2.5 ± 0.2	0 ± 0
2	37 ± 2.2	12 ± 1.1
3	68 ± 1.5	30 ± 4.2
4	84 ± 6.1	112 ± 3.8
5	139.2 ± 2.1	253 ± 4.4
6	97.2 ± 1.1	172 ± 2.1

and enzyme production depleted significantly at higher pH value (59.5 ± 2.8 U/ml). In the case of lipase, production was maximum in the culture medium containing pH 8.0 (166 ± 1.3 U/ml). At pH 10.0, lipase production was registered as 49.8 ± 2.1 U/ml and enzyme production was completely depleted at pH 11.0 (Table 3).

3.5. Effect of temperature on lipase and protease production by *Streptomyces* sp. Al-Dhabi-49

The culture was incubated up to 5 days at specific temperature and lipase and protease production was evaluated. The production of protease increased with the increase in fermentation temperature up to 40 °C. The maximum production of protease was observed at 40 °C (174 ± 12.1 U/ml) by *Streptomyces* sp. Al-Dhabi-49. Lipase activity was found to be optimum at the range of temperatures (30–50 °C) and maximum production was achieved at 35 °C (168 ± 7.8 U/ml) (Table 4).

3.6. Effect of various carbon sources on lipase and protease production from *Streptomyces* sp. Al-Dhabi-49

The result on the ability of *Streptomyces* sp. on production of commercial enzymes by utilizing various carbon sources are given in Table 5. Among the evaluated carbon sources, maltose significantly influenced on protease production (218 ± 12.8 U/ml). When compared with other carbon sources, *Streptomyces* species produces less protease (162 ± 10.1 U/ml) by utilizing glucose. Lipase production was maximum when *Streptomyces* sp. was cultured in the presence of fructose (162 ± 10.8 U/ml). When compared with other carbon sources, *Streptomyces* sp. produce less lipase (121 ± 4.3 U/ml) by utilizing glucose as carbon sources (Table 5).

3.7. Effect of different nitrogen sources on enzymes production from *Streptomyces* sp. Al-Dhabi-49

The optimum amount of protease (263 ± 4.7 U/ml) produced by *Streptomyces* sp. Al-Dhabi-49 was observed in the production medium containing peptone. Among various concentrations of peptone, 1.0% (w/v) significantly enhanced protease production. The lipase production was very high in the culture medium containing malt extract as nitrogen source (86 ± 10.2 U/ml) and production

Table 1

Total viable counts of actinomycetes in the soil sample from three different locations of Saudi Arabia.

Stations	Colony forming unit (1×10^6 CFU g^{-1}) (10^{-5} dilution)		
	Protease producing organism	Lipase producing organism	Total
1	18	14	32
2	20	16	36
3	30	11	41

1: Al-Aselah hot spring region; 2: Dammam marine region; 3: Jazan marine region.

Table 3

Effect of pH on enzymes production by *Streptomyces* sp. Al-Dhabi-49.

pH	Enzyme activity (U/ml)	
	Protease	Lipase
6	12.1 ± 2.1	43.6 ± 10.1
7	22.8 ± 1.8	88.4 ± 10.1
8	102.1 ± 2.1	166 ± 1.3
9	147.1 ± 2.6	112 ± 19.7
10	119.2 ± 2.2	49.8 ± 2.1
11	59.5 ± 2.8	10 ± 0.2

Table 4
Effect of temperature on enzymes production by *Streptomyces* sp. Al-Dhabi-49.

Temperature (°C)	Enzyme activity (U/ml)	
	Protease	Lipase
20	18.2 ± 1.2	7.1 ± 2.1
25	58 ± 2.7	18.8 ± 1.1
30	79 ± 1.1	159 ± 2.7
35	106 ± 2.1	168 ± 7.8
40	174 ± 12.1	150 ± 4.7
45	164 ± 5.8	148 ± 1.2
50	108 ± 4.7	142 ± 2.2

Table 5
Effect of carbon sources on enzymes production by *Streptomyces* sp. Al-Dhabi-49.

Carbon source (1%)	Enzyme activity (U/ml)	
	Protease	Lipase
Glucose	162 ± 10.8	121 ± 4.3
Lactose	182 ± 11.4	159 ± 4.9
Fructose	202 ± 11.4	169 ± 10.3
Maltose	218 ± 12.8	142 ± 10.1
Starch	186 ± 5.4	131 ± 8.2
Trehalose	197 ± 1.6	129 ± 4.2
Control	168 ± 11.4	130 ± 2.2

was very low in the presence of ammonium sulphate (86 ± 10.2U/ml) (Table 6). Lipase production was increased in the presence of 1.5% malt extract in the culture medium.

3.8. Effect of ions on lipase and protease production from *Streptomyces* sp. Al-Dhabi-49

Protease production was maximum in the presence of Ca²⁺ as ionic source (212 ± 3.8 U/ml) and enzyme production was significantly affected by Hg²⁺ (30.5 ± 8.7 U/ml). However, lipase production was enhanced by the addition of Mg²⁺ with the fermentation medium (163.7 ± 6.2 U/ml) and Hg²⁺ severely inhibited lipase production (63.1 ± 5.6 U/ml) (Table 7).

3.9. Effect of *Streptomyces* sp. Al-Dhabi-49 lipase on the stability of organic solvent

The lipase was very much stable in the presence of various organic solvents. In this study, it was very clear that this lipase was highly stable in acetonitrile (103.4%), toluene (104.3%), chloroform (102.1%), acetone (107.9%), ethyl acetate (106.4%) and n-hexane (104.2%) and enhanced enzyme stability.

3.10. Hydrolysis of gelatin layer of used X-ray film from *Streptomyces* sp. Al-Dhabi-49

Enzymatic treatment of used X-ray film with protease enzyme resulted in the removal of gelatin layer from the X-ray film. After

Table 6
Effect of nitrogen sources on enzyme production by *Streptomyces* sp. Al-Dhabi-49.

Nitrogen source (1%)	Enzyme activity (U/ml)	
	Protease	Lipase
Beef extract	172 ± 10.1	167 ± 4.2
Malt extract	252 ± 10.1	173 ± 10.8
Peptone	263 ± 4.7	157 ± 17.9
Glycine	179 ± 5.9	98 ± 16.9
Ammonium sulphate	177 ± 11.9	86 ± 10.2
Ammonium nitrate	159 ± 9.1	148 ± 18.2
Urea	159 ± 4.8	149 ± 12.4
Control	168 ± 11.8	143 ± 10.6

Table 7
Effect of ions on enzymes production by *Streptomyces* sp. Al-Dhabi-49.

Ionic sources (0.1%)	Enzyme activity (U/ml)	
	Protease	Lipase
Ca ²⁺	212 ± 3.8	130 ± 2.1
Cu ²⁺	43 ± 2.9	131 ± 11.2
Mg ²⁺	170 ± 3.4	169.7 ± 4.1
Mn ²⁺	159 ± 3.3	111 ± 6.9
Hg ²⁺	30.5 ± 8.7	63.1 ± 5.6
Control	135 ± 5.6	130 ± 2.2

enzymatic treatment, the X-ray film lost about 7.5% based on initial film weight. The silver ions in the form of silver chloride stripped out from the used X-ray film may be useful in preparing photographic paper, in photochromic lenses and also used in preparing wound healing products.

4. Discussion

In the present study, *Streptomyces* sp. Al-Dhabi-49 was used for the production of lipase and protease which was isolated from the soil. Previously, lipase and protease production by various microbial consortium have been used. In our study, lipase produced by *Streptomyces* sp. Al-Dhabi-49 was found to be highly resistant against proteolysis (Ilavenil et al., 2015). Earlier, involvement of protease activity in the production of extracellular lipase was studied earlier and *in vitro* analysis on effect of lipase and proteases showed that lipase activity was critically affected by proteases (Lopes et al., 1999). Production of protease was reported from various actinomycetes species, including, *Streptomyces albidoflavus* (Bressollier et al., 1999; Roopan et al., 2019), *Streptomyces caespitosus* (Inouye et al., 2007), *Streptomyces* sp. 594, *Streptomyces gulbargensis* (Das and Prasad, 2010), *Streptomyces clavuligerus* (Thumar and Singh, 2007) and *Streptomyces pulvereus* MTCC 8374 (Jayasree et al., 2010). In a study, Narayana and Vijayalakshmi (2008) reported that *Streptomyces albidoflavus* showed proteolytic activity in the culture medium after 24 hrs of incubation and found to be maximum level after 72 h. In our study the fermentation parameters significantly influenced on lipase and protease production. In *Bacillus subtilis*, dextrose significantly enhanced protease production (Das and Prasad, 2010). In *Streptomyces clavuligerus*, gelatine was found to be the best nitrogen source for the production of protease (Thumar and Singh, 2007). However, in the case of thermophilic *Bacillus* sp. strain SMIA-2, ammonium nitrate enhanced protease production (Nascimento and Martins, 2004). The physio-chemical factors such as temperature and pH also affected lipase and protease production. In *Streptomyces albidoflavus*, protease was highly active between 40 and 70 °C. Also, enzyme was highly stable in the presence of Ca²⁺ and Ba²⁺ ions. However, incubation of enzyme with EDTA and copper significantly affected enzyme activity. The actinomycete such as, *Streptomyces albidoflavus* showed maximum protease production after 72 h incubation. Enzyme production has been found to be optimum at 35 °C and pH 7.0, respectively (Narayana and Vijayalakshmi, 2008). The actinomycete, *Streptomyces clavuligerus* strain MIT-1 showed maximum production of protease at pH 9.0 (Thumar and Singh, 2007). Lipase synthesis has been initiated by Tween 80 and enhanced production was reported (Henriette et al., 1993). It was also reported that supplementation of lipase enhanced lipase production (Plou et al., 1998). Oil contaminated sites are rich sources of oil degrading microbial consortium (Rajan, 2010). Morphological biochemical tests have been previously used for the identification of *Streptomyces* sp. The isolate LP10 has been identified as *Streptomyces* sp. based on Bergey's

manual of systematic bacteriology (Williams et al., 1989) and this has been further confirmed by molecular approach (Tork et al., 2010). Previously, Haba et al. (2000) screened 47 strains of the genera *Rhodococcus*, *Pseudomonas*, *Bacillus*, *Staphylococcus* and *Candida* for the production of lipases. These all isolates showed activity on the waste oil and enzyme production has been found to be maximum in *P. aeruginosa* (1.703 IU/l) and *Pseudomonas* sp. (2.748 IU/ml). Recently, Vishnupriya et al. (2010) isolated a lipase producing *Streptomyces griseus*. In this strain, process parameters were affected by palm oil, olive oil, Sunflower oil, pH, temperature, carbon and nitrogen source, fermentation period and various inducers critically affect lipase production. This kind of result also has been found in *Bacillus* strain H1 (Handelsman and Shoham, 1994). The surfactants such as, Tween 80 enhanced lipase activity in *Bacillus stearothermophilus* (Gowland et al., 1987), *Bacillus* sp. (Sidhu et al., 1998) and *Rhizopus delemar* (Espinosa et al., 1990). Lipase production in the culture has been influenced by the concentration of nitrogen and carbon sources, the culture medium temperature, incubation period and aeration (Elibol and Ozer, 2000). In *Streptomyces* sp., lipase production was found to be maximum at pH 7.0 and at 37 °C (Sirisha et al., 2010).

5. Conclusion

Streptomyces sp. Al-Dhabi-49 was isolated from the environmental soil samples of Saudi Arabia and was evaluated for the concomitant production of lipase and protease. Optimization studies evidenced that proteolytic enzyme increases with the increase in pH up to 9.0 (147.2 ± 3.6 U/ml) and enzyme production depleted significantly at higher pH values. Whereas, the lipase production was maximum in the culture medium containing pH 8.0 (166 ± 1.3 U/ml). The maximum production of protease was observed at 40 °C (174 ± 12.1 U/ml) by *Streptomyces* sp. Lipase activity was found to be optimum at the range of temperatures (30–50 °C) and maximum production was achieved at 35 °C (168 ± 7.8 U/ml). Both these industrial enzymes produced in a single fermentation system and thus can be highly useful for various industrial processes for the bulk level production enzyme associated products with huge commercial values.

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