enzymes, when it comes to the enzyme's thermal stability aspect. A lot of work has been carried out on their industrial production and still it is on-going process for unraveling their newer sources. The present study is aimed at isolation, screening and characterization of bacteria capable of producing amylase and cellulase from soil sample of dumpsite area of Patna, Bihar.

Materials and Methods :

Collection of Samples : Soil samples were collected from two different dumpsite areasin Boring road (Site A) and Chhajjubagh (Site B), Patna, Bihar. The soil samples were collected aseptically in sterile vial with the help of sterile spatula at the depth of about 10 cm and transported to the laboratory aseptically for further processing.

Isolation and purification of bacteria : The collected soil samples were subjected for isolation of bacteria through serial dilution technique. About 1 g of the soil sample was weighed and added into 9 ml of the normal saline (0.85% NaCl) and serially diluted from 10^{-1} upto 10^{-5}. From each dilution, 0.1 ml of the aliquot was spread on nutrient agar plates (peptone-5g/l, beef extract-3g/l, NaCl-5g/l and agar-15g/l) (Aneja, 2003).Then, the plates were incubated at 37°C for 3 days.After incubation, the plates were observed regularly and morphologically different colonies were purified by sub-culturing through streaking and serial dilution. Each pure isolates were maintained as stock culture in slants at 4°C for further use.

Screening of amylase and cellulase producing bacteria

For amylase:- Screening of amylase producing bacterial isolates were carried out by streaking each pure isolate onstarch nutrient agar (SNA) media containing soluble starch (1%), peptone (5g/l), beef extract (3g/l), NaCl (5g/l), agar (15g/l) of pH (7.0±0.2) (Aneja, 2003) and were incubated at 37°C for 24 h. After 24 h of incubation, plates were flooded with Lugol's iodine solutions. Formation of clear zones around the bacterial colony indicates positive result, while no halo zone as negative test (Arotupin, 2007).

For cellulase:- Screening of cellulase producing bacterial isolates were carried out by streaking each pure isolate an carboxymehyl cellulose agar (CMCA) media containing carboxymethyl cellulose (1%), $NH_4H_2PO_4$ (1g/l), KCl (0.2g/l), $MgSO_4.7H_2O$ (1g/l), yeast extract (1g/l), agar (15g/l)of pH (7.2±0.2) and were incubated at 37°C for 24 h. After incubation, plates were flooded with aqueous solution of 1% congo red for 15 min at room temperature and washed with 1M NaCl. The presence of clear halo zone around the bacterial colony indicates the positive result,while no halo zone as negative test (Arotupin, 2007).

Characterization of amylase and cellulase positive isolates : Identification of amylase and cellulase positive bacterial isolates were done on the basis of morphological and biochemical characteristics.

Morphological Characteristics : The positive isolates were characterized on the basis of the colony characteristics such as color, margin, texture and elevation, Gram's staining etc.(Aneja, 2003). For Gram's staining, the heat fixed smears of amylase and cellulase positive isolates were prepared individually on glass slide, then flooded with crystal violet and led to stand for 30 sec and rinsed with distilled water for 5 sec. Then, the slides were covered with gram's iodine mordant and allowed to stand for 60 sec. After, that the slides were decolorized with ethanol (dropwise) and then, the slides were again rinsed with distilled water and counter stained with safranin for about 30 sec. Finally, the slides were rinsed with distilled water for 5 sec and dried and examined under a compound microscope under 40X magnification.

Biochemical Characteristics : It was done by performing some biochemical test such as gelatin

hydrolysis test, urease test and nitrate reduction following Aneja (2003).

Gelatin hydrolysis test : This test is used to determine the ability of an organism to produce proteolytic enzymes (gelatinases) that liquefy gelatin. The medium used was nutrient broth to which 12% gelatin was added, converting it into a semisolid medium. For this the test isolates were inoculated individually in aseptic condition to a sterile tube of gelatin medium and incubated at 37°C for 48 h. After incubation, tubes were placed into a refrigerator for few minutes, where solidification of media was regarded as positive test, while no solidification as negative test.

Urease test : This test is used to determine the ability of an organism to produce the enzyme urease that hydrolyzes urea. It was done by growing bacteria in urea broth for 48 h at 37°C. After incubation, the broth cultures were examined for color change. The increase in pH causes the indicator to change from orange red to deep pink and was a positive test for urea hydrolysis.

Nitrate reduction test : The nitrate reduction test was performed by growing bacteria in a culture tube with a nitrate broth medium. The test isolates were inoculated individually in aseptic condition to a sterile tube of containing nitrate medium and incubated at 37°C for 24 h, the culture was examined for the presence of gas and nitrate ions in the medium. For that, the medium will react with these reagents to produce a red color.

Results and Discussion :

Isolation and purification of bacteria : In this study, isolation of amylase and cellulase producing bacteria were isolated from dumpsite area in Patna district of state Bihar.Based on the colony morphology, 20 different bacterial colonies were isolated from the soil samples. The number of isolates were high (12) in the soil sample collected from sampling site A (Chhajjubagh) and the least (08) bacterial isolates was found in site B (Boring road) sample. Among 20 isolates, 10 dominant colonies (designated as I, II, III, IV, V, VI, VII, VIII, IX, X)were randomly selected and purified for screening purpose (Table 1).

Quantitative screening of amylase and cellulase producing bacterial isolates : Out of 10 selected isolates, only 02 produced clear halo zone around the colony on SNA plates and of which isolate IV showed maximum halo zone formation of diameter 4mm (Fig. 1). Similarly for cellulase, 02 isolates showed clear halo zone around the CMCA medium, which confirms them to be cellulase producer (Verma et. al., 2012). Isolate VII showed maximum zone formation of diameter 5mm on CMCA media (Fig.2). However, other bacterial isolates showed low amylase and cellulase activity with the zone formation in SNA media and CMCA media, respectively (Table 1). On the basis of halo zone isolate IV and VII were selected as best amylase and cellulase producer and subjected to phenotypic characterization.

Table 1. Isolation and screening of bacterial isolates for amylolytic and cellulolytic activity

Sampling site	Total no. of isolates	Isolates	Amylase positive isolates	Halo zone	Cellulase positive isolates	Halo zone
Site A	12	I	-ve	-	-ve	-
		II	-ve	-	+ve	3 mm
		III	-ve	-	-ve	-
		IV	+ve	4 mm	-ve	-
		V	-ve	-	-ve	-
		VI	-ve	-	-ve	-
Site B	08	VII	+ve	2 mm	+ve	5 mm
		VIII	-ve	-	-ve	-
		IX	-ve	-	-ve	-
		X	-ve	-	-ve	-
Total	20	10	2 +ve		2 +ve	

Fig. 1. Clear halo zone on SNA plate by isolate IV

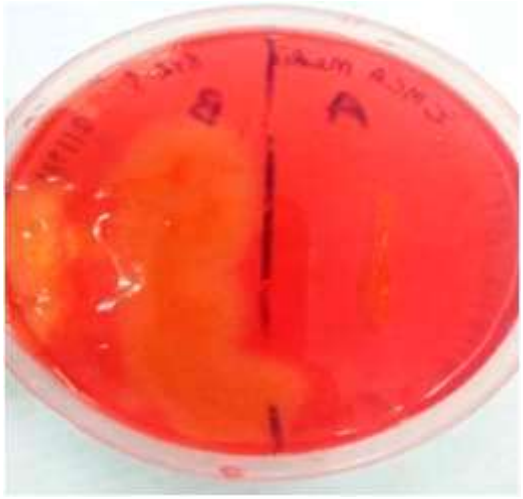

Fig. 2. Clear halo zone on CMCA plate by isolate VII

Phenotypic characterization : Phenotypic characterization of best isolates was done by studying morphological and biochemical features.Colony morphology was studied of all the 20 bacterial isolates at the purification time. Table 2 represents the morphological characteristics of isolates. Morphologically they were quite different where, 08 isolates were bacilli and only 02 were coccus. Baclli were Gram positive as well as gram negative, while both coccus were Gram negative. Biochemical test were done of best isolates i.e, isolates IV and VII selected after screening process (Table 3).

Table 2.Morphological characteristics of bacterial isolates

Isolates	Morphological Characteristics				Gram stain	Shape
	Colour	Texture	Margin	Elevation		
I	White	Smooth	Entire	Raised	+ve	Rod
II	White	Smooth	Entire	Raised	+ve	Rod
III	Cream	Mucoid	Iregular	Flat	-ve	Rod
IV	Cream	Mucoid	Iregular	Flat	+ve	Rod
V	Yellow	Smooth	Entire	Raised	-ve	Coccus
VI	White	Smooth	Entire	Raised	+ve	Rod
VII	White	Smooth	Entire	Raised	+ve	Rod
VIII	White	Smooth	Entire	Raised	+ve	Rod
IX	Cream	Mucoid	Iregular	Flat	-ve	Coccus
X	Yellow	Smooth	Entire	Raised	+ve	Rod

Table 3. Biochemical Characteristics of best isolate

Isolates	Tests	Isolates	
		Isolate IV	Isolate VII
1.	Nitrate reduction	+ve	-ve
2.	Urease production	+ve	+ve
3.	Gelatin hydrolysis	+ve	-ve

The microbial population in our environment is large and complex and their number varies depending on the nature of the environment.In the present study, isolation of bacterial capable of producing amylase and cellulase was carried out from soil sample collected from dumpsite of household and other organic wastes. The reasoning behind the same was that areas rich in organic wastes could be probable source of potent bacteria capable of producing hydrolytic enzymes. High productivity of metabolites, here enzymes is one of the important criteria of producer strain. For this screening of potent isolate is primary step. In the present investigation among randomly selected 10 bacterial isolates, 02 isolates were screened to be amylase positive and 02 isolates were cellulase

positive of which isolate IV and VII gave prominent halo zone diameter. Similar to the result of the present study, the use of starch nutrient agar and iodine for detecting amylase producing microorganisms have been reported by (Fogarty and Kelly, 1979; Iverson and Millis, 1974), who started that starch hydrolysis can be detected on plates as a clear zone surrounding a colony, whereas the use of CMCA and Congo red plus 1M NaCl for detecting cellulase producing microorganisms have been reported by (Kasana el.al. 2008), who stated that cellulase hydrolysis can be detected on plates as a clear zone surrounding a colony. Both Isolate IV and VII were Gram +ve rods. Both gave positive test for urease hydrolysis, while isolate IV gave positive test for nitrate reduction and gelatin hydrolysis and isolate VII were negative for same. For proper identification of isolate further biochemical and genotypic characterization should be carried out for generic and species level identification.

Conclusion :

The results obtained in the present study confirm that bacteria inhabiting soil of dumpsite area are rich source of hydrolytic enzymes i.e, amylase and cellulase. Isolate IV and VII are primarily screened as potent isolates for amylolytic and cellulolytic activity, respectively. They may be further studied for quantification of enzyme production and proper identification.

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