OPTIMIZATION OF GROWTH CONDITIONS AND CHARACTERIZATION OF ENZYMATIC ACTIVITY OF SELECTED NOVEL *STREPTOMYCES* SPECIES FROM KENYAN SOILS

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Abstract
This study was aimed at unveiling and assessing protease, esterase, amylase and lipase enzymes from selected novel *Streptomyces* species with biotechnological interest. Four *Streptomyces* isolates from Jomo Kenyatta University of Agriculture and Technology farm soil were studied. Physiochemical and biochemical characterization of the isolates was carried out. The isolates grew well at pH 6, 7, 9 and temperatures of 27.5 ^{o}C, 30 ^{o}C, and 32.5 ^{o}C. They preferentially required sodium chloride (0 g/l – 17.5 g/l) for growth. All the isolates produced amylase, lipase, protease and esterase enzymes apart from one isolate that did not produce esterase enzyme.

Key words: *Streptomyces,* enzymatic index (EI), amylase, lipase, protease, esterase

1.0 Introduction

Streptomyces spp. are generally soil-dwelling organisms that exist as semi-dormant spores (Mayfield *et al.,* 1972). They are the most important group of the Actinobacteria with high G+C content of the DNA. Morphological characteristic of Streptomyces include growth by vegetative hyphae measuring between 0.5 – 2.0 µm in diameter and production of an extensive mycelia that really fragments. Members of the group produce aerial hyphae bearing chains of conidiospores on their tips when growing on agar resulting to dull, powdery or velvety appearance of the colonies that are difficult to pick from the surface of the agar plates (Kieser *et al.,* 2000).

In 2001, Streptomyces genome was fully sequenced and it was found to have a linear chromosome of 8,667,507 base pairs long and predicted to contain 7,825 genes, about twice as many as typical free-living bacteria, making it the largest bacterial genome yet sequenced. The numbers of antimicrobial compounds that have been isolated and reported from these species have increased exponentially in the two decades as indicated from reports (Watve *et al.,* 2001). Around 23,000 bioactive secondary metabolites produced by microorganisms have been reported and over 10,000 of these compounds are produced by Actinomycetes, representing 45% of all bioactive microbial metabolites discovered (Berdy, 2005).

1.1 Enzymes from Actinobacteria

1.1.1 Proteases

Proteases are the most important class of industrial enzymes as they account for up to 25% of all commercial enzymes used in the world. It is estimated that two thirds of the industrially produced proteases are from a microbial source (Gerhartz, 1990; Moon and Parulekar, 1991). The majority of these proteases are used in food, pharmaceutical and detergent industries. For example alkaline proteases are used in detergent powders, and in food processing, e.g. in production of protein hydrolysate (Phadatare *et al.,* 1993). Acid proteases are used extensively in meat tenderization and in the production of fermented foods by moulds from soybean, rice and others cereals (Nout and Rombouts, 1990). They are also used in the baking industry for the modification of wheat proteins whereas in the dairy industry they are used in the manufacture of cheese (Boing, 1982).

Porto *et al.* (1996) studied *Streptomyces clavuligerus* cultures for protease production. They reported that the amount of enzyme produced varies greatly with the culture media used. An important advantage of these proteases produced from *Streptomyces* is they are secreted into the media and hence can easily be extracted and purified by filtration (Phadatare *et al.,* 1993).

1.1.2 Lipases and Esterases

Microbial carboxylesterases that include lipases (EC 3.1.1.3) and esterases (EC 3.1.1.1), catalyze the hydrolysis of a broad range of natural and unnatural esters, often showing high enantio-selectivity and region-selectivity. To date there are over 70 commercially available lipases that are extracellularly produced by various micro-organisms (Bornscheuer and Kazlauskas, 1999).

Lipids are commonly used as carbon sources in *Streptomyces* fermentation but studies on their esterase and lipolytic activities are largely indescribed (Tesch *et al.,* 1996; Sommer *et al.,* 1997; Abramic *et al.,* 1999). For example cell-bound lipases have been described in only five well-known Streptomycetes such as *Streptomyces clavuligerus*, *Streptomyces lividans*, *Streptomyces coelicolor, and Streptomyces rimosus* and in the related *Saccharopolyspora erythraea* (Large *et al.,* 1999).

1.1.3 Amylases

Starch hydrolyzing enzymes are widely distributed in *Streptomyces* species (Goldberg and Edwards, 1990), and some of them can attack and hydrolyze raw starch granules (Fairbarn *et al.*, 1986), with the release of maltose as the predominant product. Such enzymes are useful for the industrial conversion of raw starch into sugars for fermentation (Norman, 1978). α-Amylase from *Streptomyces praecox* NA-273 (Takaya *et al,* 1979) has been purified and shown to contain three isoenzymes that convert starch to maltose, without formation of glucose (Suganuma *et al.,* 1980). On the other hand, the purified amylase from a chlorotetracycline-producing strain of *Streptomyces aureofaciens* cleaves starch through an endo-mechanism, producing glucose, maltose and maltotriose in α-configuration as main products (Hostinova and Zelinka, 1978).

The importance of the microorganisms in enzyme production is due to high production capability, low cost and susceptibility to genetic manipulation. Actually, the enzymes of microbial origin have high biotechnological interest such as in the processing of foods, manufacturing of detergents, textiles, pharmaceutical products, medical therapy and in molecular biology (Pilnik and Rombouts, 1985; Falch, 1991; Rao *et al.,* 1998). Knowledge of the spatial and temporal variation of enzymes in such ecosystems, the organisms producing the different enzymes and factors affecting enzyme activity are important to understand. Besides this, industrially useful enzymes with novel applications, or which improve upon the activities of ones being currently used, are frequently being sought (Marrs *et al*., 1999). The aim of this study was to unveil and assess amylase, lipase, protease and esterase enzymes from the isolates that could be of commercial importance hence improving industrial and pharmaceutical applications as well as other sectors where they may be of use.

2.0 Materials and Methods

2.1 Growth of Actinobacteria isolates

Five *Streptomyces* species isolates from International Centre for Insect Physiology and Ecology (ICIPE) microbial bank with biotechnological interest were used. Four of the *Streptomyces* isolates were from Chyulu National Park (Chy 4-10, Chy 15-10, Chy 15-5 and Chy 2-3) and one from Ruma National Park (Ruj 7-1).

The isolates were fermented in a differential broth media in a shaker incubator (Gallen Kamp, Germany) (200 rpm, 28ºC) for 96 h. The original stocks of the isolates from which the working stocks were prepared were kept in a freezer (Sanyo MDF-594 AT, Japan) at - 80°C.

2.2 Physiochemical characterization of the Actinobacteria Isolates

In order to carryout screening of enzymes from the isolates, optimization of growth conditions and media composition was done to achieve good results.

2.2.1 Effect of pH on Growth of the Actinobacteria Isolates

An optimum pH requirement for the isolates was determined. International Streptomyces Project (ISP_2) broth media adjusted to varying pH ranges of 3, 6, 7 and 9, using 1N sodium hydroxide and 1N hydrochloric acid was used. The cultures were incubated in a shaker incubator (Gallen Kamp, Germany) (30ºC for 48 h at 100rpm) and optical density readings were read at 600nm using a UV spectral photometer (Shimadzu UV 240, Japan).

2.2.2 Effect of Temperature on Growth of the Actinobacteria Isolates

Growth of the five isolates was monitored by spectrophotometric measurement of the optical density at 600 nm. Experiments were performed at 15, 20, 25, 27.5, 30, 32.5, 35, 36, 37, and 38°C. Prior to the experiments, bacteria were acclimatized to the temperature conditions in the growth experiments. All cultures were grown in liquid International Streptomyces Project (ISP_2) media on a rotary shaker incubator (Gallen Kamp, Germany) (15, 20, 25, 27.5, 30, 32.5, 35, 36, 37, and 38°C for 12 h at 100 rpm) in the dark. Precultures of acclimatized strains that were used for setting up growth experiments were grown overnight. Experiments were performed in 100-ml Erlenmeyer flasks in triplicate for each isolate. The medium used for the experiments was preincubated in a flat bed incubator for 6 h under the same temperature conditions as the temperature conditions in the experiment. Measurement of the optical density was started 72 h after inoculation. The optimal growth temperature was determined graphically.

2.2.3 NaCl tolerance Test

For this test, NaCl broth was used. 50 ml of the medium was autoclaved at 121°C for 15 min in clean 100 ml conical flasks. 100µl of test strain inoculums were inoculated into the medium contained in conical flasks and incubated on rotary shaker (Gallen Kamp, Germany) (30°C for 96 h at 100 rpm). Growth of the five isolates was monitored spectrophotometrically by measuring the optical density at 600 nm.

2.3 Screening of the Isolates for Enzymes

2.3.1 Determination of Amylolytic Activity

The methodology used was described by Hankin and Anagnostakis (1975). Isolates were inoculated in modified nutrient agar with 0.2% of soluble starch (Sigma Aldrich, Germany). After incubation in a flat bed incubator (Carbolite 301 Controller, Jencons, United Kingdom) (at optimum temperatures for each isolate for 96 h), the cultures were treated under iodine vapours, which allowed the visualization of clear halos around the colonies.

2.3.2 Determination of the Esterasic Activity

The media used was as described by Sierra (1975), containing (g/l): peptone 10.0, NaCl, $CaCl_2$ $2H_2O$ 0.1, agar 18.0. To the sterilized culture media, previously sterilized Tween 80 was added in a final concentration of 1% (v/v). The medium was inoculated with the isolate and incubated (at optimum temperatures for each isolate for 96 h). An opaque zone of crystals was recorded as positive reaction for hydrolysis of Tween 80 (Sands, 1990).

2.3.3 Determination of the Lipolytic Activity

The media used was as described by Sierra (1975), containing (g/l): peptone 10.0, NaCl, $CaCl_2$ $2H_2O$ 0.1, agar 18.0. To the sterilized culture media, previously sterilized Tween 20 was added in a final concentration of 1% (v/v). The medium was inoculated with the isolates and the presence of hydrolytic halos observed after four days of incubation.

2.3.4 Determination of the Proteolytic Activity

To determine protease activity, a media containing nutrient broth 8 g/l, sugars 1 g/l and agar 18 g/l was used according to (Vieira, 1999). After autoclaving, 15 ml of skimmed milk separately autoclaved was added before cooling and mixed well. 20 ml of media was poured in Petri-dishes and the isolates inoculated. After incubation in a flat bed incubator (Carbolite 301 Controller, Jencons, United Kingdom) (at optimum temperatures for each isolate for 48 h), casein hydrolysis was indicated by the presence of clear halos around the colonies.

2.4 Determination of the Enzymatic Index

The isolates were grown in modified differential broth media for 24 h. After that, aliquots of 100µl for each of the isolates broth were inoculated on the specific culture media for each enzyme to be investigated. The cultures were incubated in a flat bed incubator (Carbolite 301 Controller, Jencons, United Kingdom) (at optimum temperatures for each isolate for 96 h). The enzymatic index (EI) was expressed by the relationship between the average diameters of the degradation halo over time (24, 48, 72 and 96 h) according to (Hankin *et al.,* 1971).

2.5 Degradation Assay of the Crude Extra-Cellular Proteins on Substrates

2.5.1 Starch

Actinobacteria isolates were cultivated in modified differential broth medium containing 0.3% soluble starch. The cultures (100mL in 500ml flasks) were incubated in an orbital shaker (at isolate optimum temperatures for 144 h at 100rpm). Crude extra-cellular proteins were extracted from the supernatant after centrifugation (Refrigerated centrifuge, H-2000, Japan) of the culture broth (10,000 × g, 20 min, 4 °C). 40 g of soluble starch was added into 500ml culture supernatant and the suspension stirred gently at 4 °C.

After 30 min, the suspensions were centrifuged (Refrigerated centrifuge, H-2000, Japan) (10,000 × *g* for 10 min), and the precipitated starch washed twice with 500ml of 20mM acetic acid-potassium acetate buffer at pH 5.5. The adsorbed enzymes were eluted from starch by shaking in 250 ml of 20 mm sodium borate buffer (pH 6.8) (40 °C for 2 h) and the released enzyme solutions was transferred in to a dialysis tubing and dialyzed overnight. The dialyzed enzyme solutions were used as the crude extra-cellular proteins for amylase activities. Paper disc (Whatman® qualitative filter paper, Grade 1, Aldrich chemical co. ltd., United States of America) were prepared

using a paper punch and impregnated with the prepared enzyme solutions. This was achieved through soaking of the paper discs in the enzyme solution and thereafter draining the excess solution for 10 s. This was followed by placing the impregnated paper discs in Petri-dishes containing nutrient agar supplemented with 0.2% soluble starch (g l^{-1}). After incubation in a flat bed incubator (Carbolite 301 Controller, Jencons, United Kingdom) (27.5°C, 30°C and 32.5°C for 72 h), Petri-dishes were flooded with an iodine solution which allowed visualization of clear halos around the colonies (Hankin and Anagnostakis, 1975). Halo diameters were measured and data recorded.

2.5.2 Tween 20

Aliquots of Actinobacteria (100µl) were inoculated into 100ml portions of sterile 10% reconstituted skim milk (Kumura *et al*., 1991) and incubated in a flat bed incubator (Carbolite 301 Controller, Jencons, United Kingdom) (27.5°C, 30°C and 32.5°C for 72 h), followed by another incubation (6°C for 72 h). These portions were centrifuged (Refrigerated centrifuge, H-2000, Japan) (20,000 x *g* at 4°C for 30 min). The supernatants were filtered using 0.45-µm cellulose acetate filter units (Toyo Roshi Kaisha, Ltd., Japan). The filtrates were used as the crude extra-cellular proteins for lipase activity experiment. Paper discs were prepared, impregnated with the filtrates and placed in Petri-dishes containing media described by Sierra (1975), supplemented with 1% (v/v) Tween 20. Zones of crystals around the discs were observed, measured and data recorded after incubation (27.5°C, 30°C and 32.5°C for 72h).

2.5.3 Tween 80

Preparation of crude extra-cellular proteins was done similarly to the one of lipases as stated above. However, impregnated paper discs were placed in Petri-dishes containing media described by Sierra (1975), supplemented with 1% (v/v) Tween 80. Zones of crystals around the discs were observed, measured and data recorded after incubation at (27.5°C, 30°C and 32.5°C for 72 h).

2.5.4 Skimmed Milk

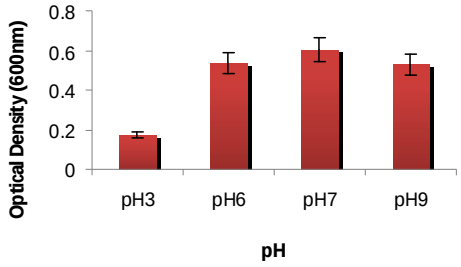
Actinobacteria isolates were grown in 100 ml of differential broth medium supplemented with 1.5 ml skimmed milk in 250 ml shake flasks in an orbital shaker (Gallen Kamp, Germany) (200 rpm at 27.5 °C, 30 °C and 32.5 °C). Cells were harvested through centrifugation after 9 days of growth (2500 ×***g*** for 30 min). The supernatant fluid was passed over a 0·22 mm filter (Millipore), concentrated (against polyethylene glycol 4000), and dialyzed overnight at 4 °C against 0·01 mmol l^{-1} Tris-HCl, pH 7·5, containing 5 mmol l^{-1} $CaCl_2$ (Tris-$CaCl_2$ buffer). Dialyzed supernatants were used as the extra-cellular proteins. Paper discs were impregnated with the dialyzed supernatants and placed in Petri-dishes containing media described by Vieira (1999), supplemented with skimmed milk. After incubation (27.5 °C, 30 °C and 32.5 °C for 48 h), clear zones of hydrolysis were observed, measured using a ruler and data recorded.

3.0 Results

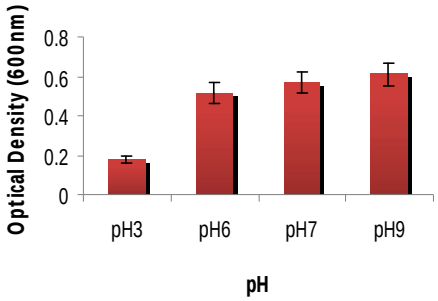
3.1 Physiochemical Characterization of Isolates

3.1.1 pH Tolerance by the Isolates

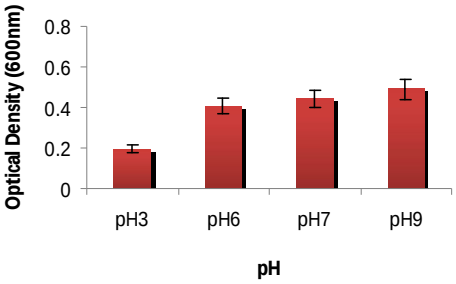
All the isolates were able to grow at acidic, neutral and alkaline pH conditions. However, the different pH conditions yielded different growth levels for the various isolates. pH 7 recorded highest growth of isolate Chy 4-10 with an optical density (OD600 = 0.604nm). Lowest growth of the isolate was recorded at pH 3 (OD600 = 0.173nm) (Figure 1a). Increased growth of isolate Chy 15-10 was recorded at pH 9 (OD600= 0.610nm) whereas minimal growth was at pH 3 (OD600 = 0.178nm) (Figure 1b). For isolate Chy 15-5, pH 9 (OD600 = 0.591nm) recorded highest growth of the isolate followed by pH 7 (OD600 = 0.549nm). Minimal growth of the isolate was recorded at pH 3 (OD600 = 0.205nm) (Figure 1c). For isolate Chy 2-3, increased growth of the isolate was recorded at pH 9 (OD600 = 0.489nm) whereas pH 3 had the lowest growth with an optical density (OD600 = 0.193nm) (Figure 1d). Lastly, highest growth of isolate Ruj 7-1was recorded at pH 6 (OD600 = 0.619nm) whereas minimal growth was at pH 3 (OD600 = 0.215nm) (Figure 1e).



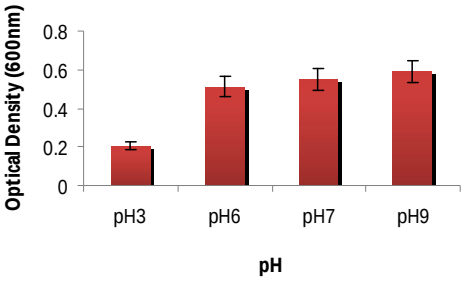
1a: Chy 4-10



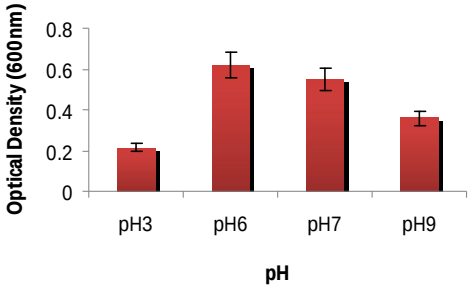
1b: Chy 15-10



1c: Chy 15-5



1d: Chy 2-3

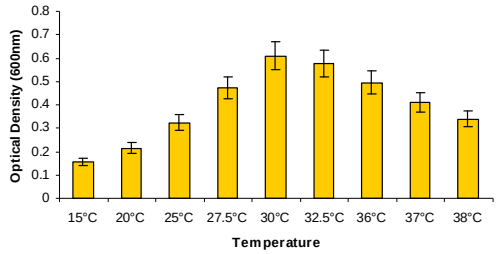


1e: Ruj 7-1

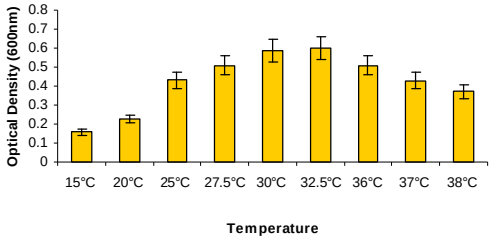
Figure 1a, b, c, d and e: Effect of pH on growth of the isolates

3.1.2 Effect of Temperature on Growth of the Isolates

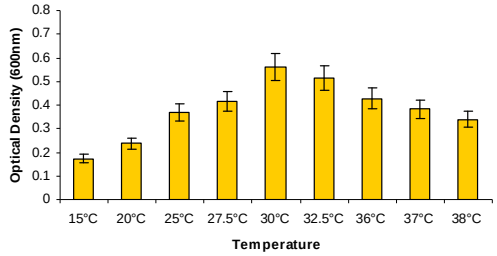
In all the isolates investigated, there was a linear increase of growth at temperature range 15 °C to 32.5 °C. With further increases in temperature, growth of the isolates was either plateau or with a linear decrease. The optimal growth temperature had the highest OD at 600nm. The optimum growth temperature for isolate Chy 4-10 was 30 °C (OD600=0.610nm). Beyond 30 °C, there was a decline in growth of the isolate (Figure 2a). For isolate CHY 15-10, 32.5 °C (OD600=0.602nm) was the optimum temperature for growth of the isolate (Figure 2b) whereas 30 °C (OD600=0.562nm) was the optimum temperature for growth of isolate Chy 15-5 (Figure 2c). For isolate Chy 2-3, the optimum temperature for growth was 32.5 °C (OD600=0.518nm) (Figure 2d). Lastly, 27.5 °C was the optimum temperature for growth of isolate Ruj 7-1 as it gave the highest growth (OD600=0.653nm) when compared with other temperature regimes (Figure 2e).



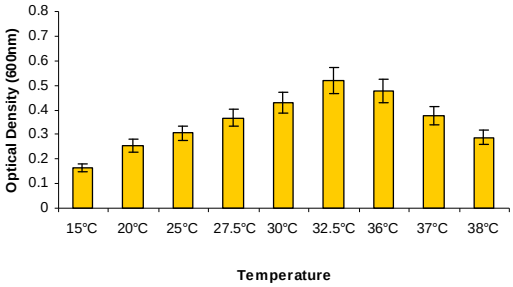
2a: Chy 4-10



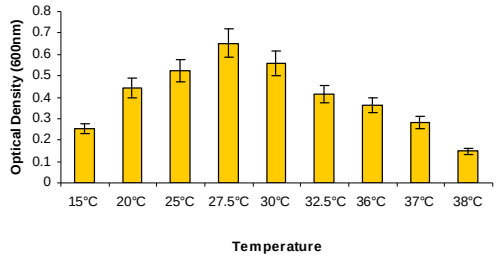
2b: Chy 15-10



2c: Chy 15-5



2d: Chy 2-3

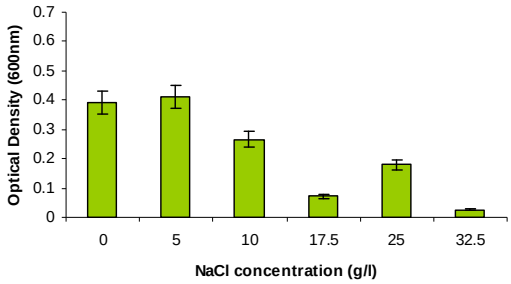


2e: Ruj 7-1

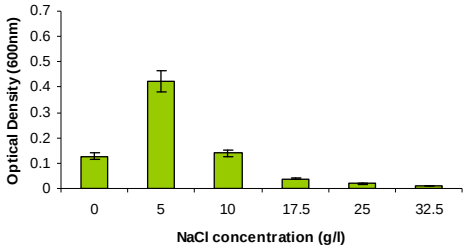
Figure 2a, b, c, d and e: Growth of isolates under different temperature regimes

3.1.3 NaCl Tolerance by the Isolates

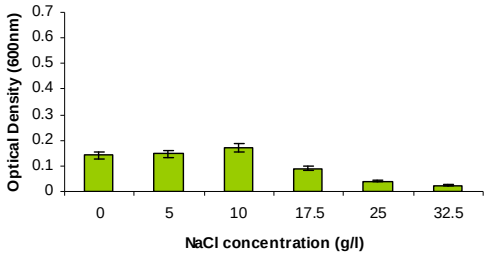
Isolates were subjected to different sodium chloride concentrations to determine the concentration for optimum growth for each isolate. All the isolates were observed to tolerate sodium chloride. Optimum growth for isolate Chy 4-10 was recorded in 5 g/l NaCl (OD600 = 0.411nm) whereas the lowest growth was recorded in 32.5 g/l NaCl (OD600 = 0.026nm) (Figure 3a). 5 g/l NaCl gave the highest growth of isolate Chy 15-10 (OD600=0.423nm). Lowest growth on the other hand was recorded in 32.5 g/l NaCl (OD600=0.010nm) concentration (Figure 3b). For isolate Chy 15-5, 10 g/l NaCl recorded highest growth (OD600=0.169nm) whereas 32.5 g/l NaCl was the concentration with the lowest growth of the isolate (OD600 = 0.023nm) (Figure 3c). For isolate Chy 2-3, optimum growth was recorded in 5 g/l NaCl (OD600 = 0.388nm) whereas lowest growth was at 32.5 g/l NaCl (OD600=0.009nm) concentration (Figure 3d). And lastly, optimum growth of isolate Ruj 7-1 was recorded in 17.5 g/l NaCl (OD600 = 0.584nm) sodium chloride concentration (Figure 3e).



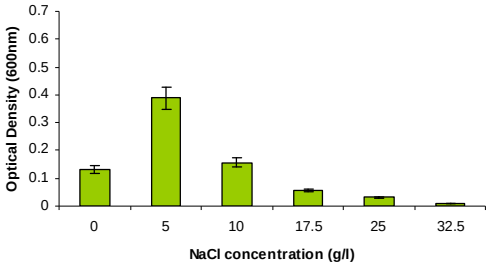
3a: Chy 4-10



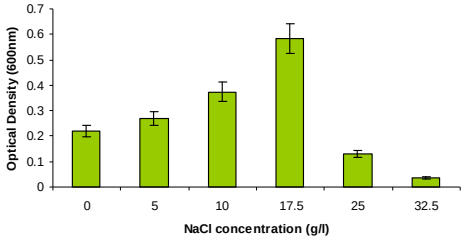
3b: Chy 15-10



3c: Chy 15-5



3d: Chy 2-3



3e: Ruj 7-1

Figure 3a, b, c, d and e: Growth of isolates under different sodium chloride concentrations

3.2 Enzymatic Activity

All the studied isolates hydrolyzed lipids, skim milk and starch apart from isolate CHY4-10 that did not hydrolyze Tween 80 (Table 1).

3.3 Enzymatic Index

On amylase-like activity, isolate Chy 4-10 expressed the highest EI (5.2±0.354) followed by isolate Ruj 7-1 with an EI of (4.4±0.354). Low EI was expressed by isolate Chy 2-3 with an EI of (3.4 ±0.354) (Table 2). EI on esterase-like activity for isolate Ruj 7-1 (5.3±0.652) was the highest followed by isolate Chy 15-5 (3.8±0.652). Isolate Chy 4-10 did not express EI on esterase-like activity (Table 2). EI on lipase-like activity for isolates Ruj 7-1 (4.2±0.548) and Chy 15-5 (4.1±0.548) were higher when compared with other isolates respectively (Table 2). Low EI on lipase-like activity was expressed in isolate Chy 2-3 (3.0±0.548) (Table 2). Isolate Chy 4-10 expressed the highest EI in terms of protease-like activity (7.3±0.707) compared to other isolates (Table 2).

3.4 Activity of the Crude Extra-Cellular Proteins on Starch, Tween 20, Tween 80 and Skimmed Milk

Enzymatic activities of crude extra-cellular proteins from the isolates were determined using various substrates. Amylase-like activity was recorded highest in isolate CHY 4-10 (27.33±0.63) and lowest in CHY 2-3 (11.00±0.63). Isolates RUJ 7-1 (17.33±0.84) and CHY 4-10 (0.00±0.84) presented highest and lowest esterase-like activity respectively whereas isolate RUJ 7-1 (23.00±0.98) recorded the highest lipolytic activity compared to other isolates (Table 3).

Table 1: Hydrolysis of substrates by the Actinobacteria isolates

	Substrates			
Isolate	Starch	Tween20	Tween80	Skimmed milk
CHY4-10	+ve	+ve	-ve	+ve
CHY15-10	+ve	+ve	+ve	+ve
CHY15-5	+ve	+ve	+ve	+ve
CHY2-3	+ve	+ve	+ve	+ve
RUJ7-1	+ve	+ve	+ve	+ve

Table 2: Enzymatic index of the isolates on the various substrates

Isolate	Enzymatic Index			
	Amylase (starch)	Esterase (T80)	Lipase (T20)	Protease (skim milk)
CHY 4-10	5.2 ± 0.354^{a}	0.0 ± 0.00^{d}	3.5 ± 0.548^{bc}	7.3 ± 0.707^{a}
CHY 15-10	4.0 ± 0.354^{c}	3.1 ± 0.652^{bc}	3.5 ± 0.548^{bc}	4.4 ± 0.707^{b}
CHY 15-5	3.7 ± 0.354^{cd}	3.8 ± 0.652^{b}	4.1 ± 0.548^{ab}	4.8 ± 0.707^{b}
CHY 2-3	3.4 ± 0.354^{d}	2.2 ± 0.652^{c}	3.0 ± 0.548^{c}	2.9 ± 0.707^{c}
RUJ 7-1	4.4 ± 0.354^{b}	5.3 ± 0.652^{a}	4.2 ± 0.548^{a}	4.8 ± 0.707^{b}

*The enzymatic index represents the halo diameter of degradation/diameter of colony in cm.
*Averages followed by the same letter don't differ among themselves (test *t* of Student, $p < 0.05$).

Table 3: Enzymatic activities of crude extra-cellular proteins

	Enzymatic activities (Diameter in mm + SE)			
Isolate	Amylase	Esterase	Lipase	Protease
CHY4-10	27.33 ± 0.63^{a}	0.00 ± 0.84^{d}	17.33 ± 0.98^{b}	21.33 ± 0.837^{a}
CHY15-10	21.67 ± 0.63^{c}	11.33 ± 0.84^{b}	13.67 ± 0.98^{c}	17.67 ± 0.837^{b}
CHY15-5	20.67 ± 0.63^{c}	16.33 ± 0.84^{a}	19.00 ± 0.98^{b}	15.33 ± 0.837^{c}
CHY2-3	11.00 ± 0.63^{d}	8.33 ± 0.84^{c}	10.33 ± 0.98^{d}	14.33 ± 0.837^{d}
RUJ7-1	24.00 ± 0.63^{b}	17.33 ± 0.84^{a}	23.00 ± 0.98^{a}	20.33 ± 0.837^{a}

* Mean values with the same letter are not significantly different at 95% confidence level (t- test).

4.0 Discussion

Physiochemical studies were carried out since most enzymes applied for industrial purposes have limitations, in exhibiting low activity and low stability at wide range of pH and temperature and secondly 30 - 40 % of production cost of industrial enzymes is estimated to be accounting for the cost of growth medium. As the composition of culture medium strongly influences enzyme production (Giarrhizzo *et al*., 2007). The study was therefore to optimize cultural conditions in order to achieve higher enzyme activities. Physiochemical characterization of the isolates Chy 4-10, Chy 15-10, Chy 15-5 and Chy 2-3 on pH showed optimal growth of the isolates at pH range of 6 - 9. These results were in accordance to Gava (1998) who reported that majority of Actinomycetes isolated from

rhizosphere and non-rhizosphere soil grows at a pH range varying from 6.5 to 8.0. In addition, the wide pH range is an advantage when it comes to production of enzymes adapted to alkaline conditions in order to have good enzymatic stability. Isolate Ruj 7-1 yielded good growth, characterized by abundant mycelium, in culture media with pH 6.0 suggesting its tolerance to acidic condition. This isolate would therefore be useful in production of acid tolerance enzymes. The optimum pH level allows for optimal metabolic reactions characterized by enzymes hence the increase in growth of microorganisms (Moreira & Siqueira, 2002). Isolates CHY4-10, CHY15-10, CHY15-5, CHY2-3 and RUJ7-1 showed optimum growth at 30 °C, 32.5 °C, 30 °C, 32.5 °C and 27.5 °C respectively. Minimal growth was recorded below 27.5 °C and above 32.5 °C. Isolates Chy 15-10 and Chy 2-3 had a wider temperature range (15 °C – 32.5 °C) hence they would produce enzymes that are more stable when temperatures exceed 27.5 °C. These results also confirmed that isolates Chy 4-10, Chy 15-10, Chy 15-5 and Chy 2-3 originated from a relatively warmer ecosystem than isolate Ruj 7-1 (27.5 °C). Therefore, for isolation of enzymes from these isolates, temperature during fermentation would be different. According to Goodfellow et al. (1990), bacterial growth rates increase with temperature up to the optimum temperature, at which the growth rate is maximal. Enzymatic processes are thought to limit further increases in growth rates at temperatures above the optimum temperature.

Growth of the isolates in culture medium with varying NaCl levels (0 g/l to 32.5 g/l) confirmed tolerance to saline conditions. All the isolates recorded growth in absence of sodium chloride but isolate Chy 4-10, Chy 15-10 and Chy 2-3 indicated an increase in growth at 5 g/l sodium chloride concentration which was similar to that of *Nocardiopsis kunsanensis* sp. nov., a moderately halophilic actinomycete (Chun *et al.,* 2000). Isolate Chy 15-5 and Ruj 7-1 grew optimally at higher NaCl concentrations (10 g/l and 17.5 g/l) respectively meaning they were more tolerant and also required higher NaCl concentrations for them to grow. In terms of enzyme stability, isolate Ruj 7-1 would likely produce enzymes that are more stable to a wide range of NaCl concentrations compared to the other isolates.

Streptomyces are an important source of enzymes and bioactive products (Bull *et al*., 1992). Most produce secondary metabolites that have antibacterial, anti-fungal, anti-tumor or antiprotozoal activities making them a target for isolation in large-scale screening programs in industries.

Growth of the isolates on solid media containing the various substrates as the only carbon sources demonstrated that these isolates secreted enzymes as per the studied substrate. All the studied isolates hydrolyzed lipids, skim milk and starch. Tween 80 was hydrolyzed by all the other isolates apart from isolate CHY 4-10. Isolate Chy 4-10 recorded highest degradation of starch (27.33 ± 0.63^{a}) and skim milk (21.33 ± 0.837^{a}) whereas Ruj 7-1 had the highest degradation of Tween 20 (23.00 ± 0.98^{a}) and Tween 80 (17.33 ± 0.84^{a}). Hydrolysis of the various substrates was an indication of the ability in the various isolates to produce lipases, proteases, amylases and esterases that are industrially important enzymes. These enzymes also play an important role in promotion of plant growth and biological control of plant diseases (Moreira and Siqueira, 2002). Starch is the most important organic reserve compound of plants and among the good starch decomposers are the Actinomycetes which produce organic acids, CO_2, and dextrin during the decomposition process (Moreira and Siqueira, 2002).

Enzymatic index (EI) was determined by directly correlating the diameters of the halo of degradation and that of colony (Lin *et al.,* 1991). The EI is a practical tool that facilitates and speeds the selection and the comparison of the enzymatic production of different isolates. Fungaro and Maccheroni (2002) suggested that EI larger than 1.0 were indicative of excretion of enzymes. It was observed that all the isolates possessed at least one enzymatic activity tested (Table 3).

The amylolytic activity was observed in all isolates with Chy 4-10 showing the highest EI of (5.2 ± 0.354). Isolate Chy 4-10 had a higher EI when compared with *Actinomyces pyogenes* that had an EI of 1.2cm in a previous study carried out by Aysha *et al*., (2006).

Among the producers of esterases, four isolates showed positive results apart from isolate Chy 4-10. Isolate Ruj 7-1 had the highest EI of (5.3 ± 0.652). This was an indication of the potential this isolate had as producer of these enzymes. In this experiment, all the isolates produced extracellular lipases although EI differed among the isolates due to the differences in levels of lipase production. Isolate Ruj 7-1 gave the highest EI in lipase activity (4.2 ±

0.548) followed by isolate Chy 15-5 (4.1 ± 0.548). This is a positive indication of the potential these isolates have in terms of lipase production.

All the isolates produced proteases though the best proteolytic production was observed in isolate Chy 4-10 (7.3 ± 0.707). These results form a platform for further studies and also revealed alternative sources of amylase, lipase, esterase and protease enzymes with applicable biotechnological potential in different areas such as in the nutrition, detergent, paper, pharmaceutical, textile and leather industries.

5.0 Conclusion and Recommendation

This study showed that all the isolates were Gram positive and they grew optimally between 27.5°C - 32.5°C, pH ≥ 6 and at 5 - 32.5g/l NaCl concentration. The isolates also hydrolyzed Tween 20, Tween 80, skim milk, starch; indicating that they are an important source of lipase, esterase, protease and amylase enzymes. However, further purification procedures of the proteins secreted by these isolates through various chromatographic techniques are necessary in order to characterize them and determine their activity.

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