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Evaluation of Antifungal and Phytochemical Properties of Violet Tree (*Securidaca Longepedunculata* Fres)

By Junaidu, S., Shehu K., Aliero, A. A., Bawa, J. A. & Suleiman, I.
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Abstract- This study was undertaken to investigate the antifungal activities of aqueous and ethanol extracts of Securideca longepedunculata leaves and root bark against two Aspergillus species (Aspergillus niger and Aspergillus flavus). Agar incorporation method was used for antifungal testing. The results of phytochemical screening demonstrated the presence of flavonoid, saponin, alkaloids, cardiac glycoside and saponins glycosides. Highest growth inhibition (1.16+1.15mm) at 300 mg/ml, (1.67+2.88mm) and higher increase (3.16+0.57mm) at 100 mg/ml were observed. The results showed significant effect ($p<0.05$) of antifungal activities. In the same study, the results however, applied that the phytochemical constituents of S. longepedunculata leaves and root bark extracts can be used as potential antimicrobial agents in the management of microbial diseases caused by pathogenic Aspergillus species which can become an alternative to chemical antibiotics.

Keywords: *antifungal, violet tree, phytochemical, aqueous, ethanol extracts, aspergillus.*

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I. Introduction

Violet tree (*Securidaca longepedunculata*) commonly called Krinkhout in Africa. It is a slender tree with beautiful flowers, belonging to the family polygalaceae. The tree is highly regarded for its medicinal purpose, especially by the vhaVenda people of the Limpopo Province where it occurs (Ndou, 2006). A cooperative approach by ethnobotanists, ethnopharmacologists, physicians and phytochemists is thereby essential to spur the progress of medicinal plants research (Gilani and Rahman, 2005). Medicinal plants have traditionally occupied an important position in the socio-cultural, spiritual and medicinal arena of rural and tribal lives in Sudan (Nelson-Harrison *et al.,* 2002). Through its long history, the Sudan has witnessed the fusion of many cultures, Pharonic, Islamic and Christianity along with the local indigenous cultures. With this unique history and vast variety of climate and flora, traditional medicine together with use of medicinal plants became an important part of the cultural heritage of the Sudan (Elkalifa *et al*., 1999). The abundance of information on traditional medicinal uses of plants in Africa is in danger of disappearing since the knowledge of how to use medicinal plants is mostly passed down orally and even to date is poorly documented (Gurib-Fakim, 2006), although written information has been produced for some specific regions. Moreover, the most serious threat to local medicinal plant knowledge, however, appears to be cultural change, particularly the influence of modernization and the western world view (Voeks and Leony, 2004) which has contributed to under mining traditional values among the young (Giday *et al*., 2003).

Plants are very good sources of medicinal compounds that have continued to play a dominant role in the maintenance of human health since Ancient times (Moriita *et al.,* 2011). Plant extracts or their active constituents are used as folk medicine in traditional therapies of about 80% of the world's population and Over 50% of all modern clinical drugs are of natural product origin (Baker *et al*., 1995: Kumar and Chandrashekar, 2011). The effect of plant extracts on microorganisms have been studied by a very large number of researchers in different parts of the world (Kumar *et al*., 2006; Mathabe *et al*., 2006) and the use of a variety of plant extracts and phytochemicals, both with known antimicrobial properties can be of great significance in therapeutic treatments. Many plants have been used because of their antimicrobial properties, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, such as, the phenolic compounds which are part of the essential oils, as well as in tannin (Nascimento *et al.,* 2000).

This study was design to investigate and determine the phytochemical properties and antifungal activities of violet tree *Securidaca longepedunculata* on *Aspergillus* species.

II. Materials and Method

a) Description of Study Area

Katsina State, covering an area 23,938 sq. km., is located between latitudes 11Â°08'N and 13Â°22'N and longitudes 6Â°52'E and 9Â°20'E. The state is bounded by Niger Republic to the north, by Jigawa and Kano States to the east, by Kaduna State to the South and by

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Zamfara State to the West. A cool dry (harmattan) season from December to February; a hot dry season from March to May; a warm wet season from June to September; a less marked season after rains during the months of October to November, characterized by decreasing rainfall and a gradual lowering of temperature.

b) Collection, Identification and Processing of Plant Material

Fresh roots and leaves of *Securidaca longepedunculata* were collected during the month of May, 2013 at 5:30pm-6:05pm from Kudewa, Kurfi Local Government Area, Katsina State, Nigeria.

The plant was preserved, identified and authenticated at the Herbarium Section, in the Botany Unit of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. Samples deposited at the Herbarium have a Voucher No. D-01SL-7.

The plant materials were properly washed under tap water, rinsed with distilled water, dried under shade and pulverized with a pestle and mortar and kept in a transparent sterile polyethene bag at room temperature for use.

i. *Preparation of Extract*

Two hundred grams (200g), each of dried plant material was extracted by soaking in 1000 ml of ethanol and water (solvent) in 1000 ml of conical flask, and covered with aluminum foil and allowed for 24 hours.

The extracts were filtered and the solvents removed by warming in oven at 40^{0}C for 3 days. The evaporated extract was stored for 48-hours in sterile universal bottles at room temperature, this methods is adopted by Okogun (2000) and Shariff (2001).

c) Qualitative Phytochemical Tests

The plant extracts was screen for the presence of secondary metabolites using standard method (Odebiyi and Sofowora, 1978; Trease and Evans, 1989).

i. *Source and Maintenance of Microbial Test Strains*

Stocked isolate of fungal strains *Aspergillus* species (*A. niger, A. flavus*) was obtained from Mycology Laboratory of Botany Unit Usmanu Danfodiyo University Sokoto, Nigeria. The isolate was maintained on Potato Dextrose Agar.

ii. *Sterilization of Glassware*

The glassware were adequately washed with liquid soap and sufficiently rinsed with tap water and distilled water respectively, air dried and sterilized in hot air oven at 160^{o}C for 1hour, while the conical flask was autoclaved

d) Preparation of Media

i. *Preparation of Sarboroud Dextrose Agar (SDA*)

The sarbouraud dextrose agar (SDA) was prepared according to manufacturer's instructions, SDA (65g) was dissolved in 1000 ml distilled water and 0.5g streptomycin solution was added to inhibit bacterial growth. The conical flask was plugged with cotton and capped with aluminum foil, sterilizing using lender autoclave at 121^{0}C for 15 minutes, cooled to 45^{0}C before been poured into sterilized plates and kept at 30^{0}C (Cheesebrough, 1985).

ii. *Preparation of Potato Dextrose Agar (PDA)*

Potato dextrose agar (PDA) was prepared according to manufacturer's instructions, 39g PDA was dissolved in 1000 ml of distilled water, the suspension was mixed until completely homogenized and 0.5g of streptomycin was added to inhibit the growth of bacteria. The conical flask containing the media were plugged with cotton wool and capped with aluminum foil, sterilized using lender autoclave at 121^{0}C for 15 minutes, cooled for 45^{0}C and pouring in to sterile plates. The plates were kept at 30^{0}C (Cheesebrough, 1985).

e) Antifungal Testing

Antifungal testing of the aqueous and ethanolic extracts of leaves and root bark were determined by Agar incorporation method as described by Brantner (1994) for antifungal testing.

i. *Activity of S. longepedunculata*

Food poison technique was used to determine the antifungal effects of different concentrations of the extracts. Into 100 ml conical flask, 15 ml of media were added. The flasks were plugged with cotton wool, capped with aluminum foil and allowed to stand for 24 hours. The four flasks of 100mg, 200mg, and 300mg of the extract were added. The fourth flask contained only the media.

Five (5ml) each of varying concentration of the leaves and root bark extracts were incorporated in to each flask containing 15 ml of the media, this was then poured in to pre-sterilized petri-dishes and kept at room temperature of 27^{0}C to 30^{0}C, the growing cultures was punch with sterile inoculating needle and then deposited in the centre of the petri dishes containing varying concentration. The control plate was sterilized and containing 20 ml of the media were place at the centre of treated plates.

The results was measured in millimeters (mm) by measuring the fungal growth from two lines vertical and horizontal, the mean were recorded (Singh and Tripatti, 1999). For each treatment 3 replicate were maintained. Mean of three 3 replicates served as the result of each of the varying concentration.

Results obtained were subjected to statistical analysis using one-way analysis of variance ANOVA, with SPSS 16.0 Version. $p<0.05$ considered as significant followed by Duncan's Multiple Range Test to detect significant differences among the means as well as the interactions between the variable.

III. Results

a) Antifungal activities of the different solvent extracts of S. longepedunculata

The growth inhibitions of *Aspergillus* species due to the application of *S. longepedunculata* are presented which revealed that leaves and root bark extracts with different solvents inhibited the growth of all the fungal species Table 1. It was also indicated in the same Table 1, that the phytochemical properties of the extracts appeared more unless where they are not present in the composition.

Table 1 : Phytochemical Composition of S. *longepedunculata* Leaves and Root bark Extracts

Phytochemical	Leaves	Root
Flavonoid	+	+
Tannins	+	-
Saponin	+	+
Glycosides	+	-
Alkaloids	+	+
Cardiac glycosides	-	+
Steroids	+	+
Saponin Glycosides	+	+
Balsams	+	-
Anthraquinones	-	-
Volatile oil	+	+

Keys: -Not present, + Present

Antifungal activities of S. *longepedunculata* was exhibited in root bark extracts on *A. niger* appeared high (at 300 mg/ml 8.83+2.46) and 13.00+00 root bark extract. In *A. niger,* the highest growth inhibition was found in the ethanol leaf extract at 300mg/ml of 3.33 + 0.57mm, as seen in Table 2.

Table 2 : Antifungal activities of S. longepedunculata Leaves and Root bark Extracts on A. niger

Extract	Conc. (mg/ml)	Leaf extracts M ±SD(mm)	Root bark extracts M ±SD(mm)
Aqueous	0	21.3^{fg} + 2.46	21.33^{fg} + 2.46
	100	10.00^{abcd}+1.00	14.00^{d}+3.50
	200	9.17^{abcd}+3.25	13.00^{ef} + 0.50
	300	8.83^{abc}+ 2.46	13.00^{fg}+00
Ethanol	0	21.33^{e} + 2.47	21.33^{de} + 2.46
	100	9.00^{bc}+ 3.12	4.33^{b}+1.52
	200	6.00^{ab} + 2.59	4.33^{b} + 1.75
	300	4.83^{ab}+1.15	3.33^{ab} + 0.57

^{a,b,c} Means in a column with different superscripts are significantly different ($p<0.05$) Values are means + standard error of three replications

The antifungal activity of S. *longepedunculata* in both the root bark and leaf extracts increase as a result of increase in the concentrations in mg/ml, Table 1. It could be deduced that, he aqueous and ethanolic extracts of the root bark and leaf significantly different ($P<0.05$) in increase of the antifungal activities in *A. niger* and *A. flavus* respectively.

In *A. flavus* highest growth inhibition was observed in the aqueous root extract at (300mg/ml of 3.00 +0.50mm), least growth inhibition (13.83+2.51mm) was observed in aqueous leaves extract at 100mg/ml, to the more higher concentrations (at 300mg/ml) of leaf (6.33+1.44) and root bark (3.16+0.57) as seen in Table 3.

Table 3 : Antifungal activities of S. *longepedunculata* Leaves and Root bark Extracts on *A. flavus*

Extract	Conc. (mg/ml)	Leaf extract M ±SD(mm)	Root extract M ±SD(mm)
Aqueous	0	24.66^{g} + 2.02	24.67^{f} + 2.02
	100	13.83^{cde}+2.51	8.50^{bc}+ 3.50
	200	8.17^{ab}+4.80	7.00^{ab}+ 4.92
	300	4.67^{a}+2.75	3.00^{a}+0.50
Ethanol	0	6.67^{ab} + 4.31	6.16^{b}+2.56
	100	6.67^{ab} + 4.31	6.16^{b}+2.56
	200	6.33^{ab} + 5.39	4.50^{b} + 0.00
	300	6.33^{ab} + 1.44	3.16^{ab} + 0.57

^{a,b,c} Means in a column with different superscripts are significantly different ($p<0.05$)

Values are means + standard error of three replications

IV. Discussion

The present study revealed the phytochemical and antifungal screening of *Securidaca longepedunculata* samples, which co-opt the rich sources of bioactive compounds in potential use of diseases management. It was reported in this study, that the presence of various secondary metabolites like tannins, saponins, alkaloids, flavonoids and others in qualitative analysis extracted from *S. longepedunculata* might be responsible for great medicinal importance. These findings are in conformity with those reported by (Donald *et al.*, 2011; Auwal *et al.*, 2012) on phytochemical composition and acute toxicity of root bark extracts of *S. longepedunculata*. The present of bioactive compounds is an indication that *S. longepedunculata* has medicinal potential; this is due to the fact that each of the compounds identified has one or more therapeutic usage. Absent of anthraquinone worth nothing medically as earlier observed by (Ajiboye *et al.*, 2010).

Results of the antifungal activities of ethanol and aqueous extracts of *S. longepedunculata* root bark and leaves ware tested against the organisms *A. niger and A. flavus* at three different concentrations, the extracts indicate significant effects ($P<0.05$) inhibitory activities of aqueous and ethanol extracts. This might be due to the fact that the extracts can exhibit remarkable activity. Antifungal activities of the ethanol extracts appeared to be more effective then aqueous extracts, since ethanol could extracts a wide variety of active component as compared to aqueous. Flavonoid together with the other secondary metabolites identified in the presence study have been severally reported to show curative activity against diverse pathogens, used traditionally analgesic antimicrobial, anti tumor headache, venereal diseases, constipation and coughs. This report is in line with findings of Abubakar *et al.* (2011) who investigated the growth inhibition and broad spectrum activity (14 to 27 mm) of *Vernonia* spp., from the crude ethanol extracts and chloroform fractions against some clinical bacterial strains and found the activity of chloroform fraction to be higher on *Corynbacterium ulcerans* and *Klebsiella pneumoniae* (27 mm), while the chloroform fractions of *V. oocephala* and *V. ambigua* were more active on *Proteus mirabilis* (27 mm) and *Salmonella typhi* (22 mm), respectively. They added that the minimum inhibitory concentration (MIC) values ranged from 1.25-2.5 mg/mL for all the organisms tested.

However, the phytochemical screening and antifungal activities of the concentrations at 100, 200 and 300mg/ml of the extracts used in this research revealed the presence of active compounds like tannins, saponins, alkaloids, flavonoid, steroids/terpenes, tannins and glycosides. The antifungal activity exhibited against the organisms *A. niger* and *A. flavus* and the susceptibility of these organisms may be a pointer to their potentials as a component or drug against the organisms tested in this study.

V. Conclusion

The results of this study confirm the potential use of Violet tree, *Securidaca longepedunculata* as antifungal agents against infections caused by *Aspergillus niger* and *Aspergillus flavus*. The presence of these importance substances suggests that *S. longepedunculata* may possess myriads of therapeutic tendencies and ability to manage numerous malaises caused by *Aspergillus* species. The overall result concludes that the extracts used in this research are of potent antifungal activity. Thus, should be explored further for pharmaceutical uses as this is important in combating the recent observed emergence of drug resistance organisms.

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Study on Enzyme Activity in the Production and Optimization of High Temperature Alkaline α-Amylase Enzyme by Bacillus Lichenoformis using Low Cost Medium Derived from Agricultural byproducts

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Keywords: ***α****-amylase, bacillus lichenoformis, low cost medium, agricultural by products, fermentation, alkaline enzyme.*

GJSFR-C Classification : *FOR Code: 860399*

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Strictly as per the compliance and regulations of :

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M. Sathiyamoorthy [α] & Dr. S. Theneshkumar [σ]

Abstract- Production of α-amylase enzyme by Bacillus Lichenoformis using stirred tank fermentor (BIOSTAT – E) was carried out. The strain was obtained from National Chemical Laboratory, Pune, India. Corn starch is used as the substrate. The enzyme production was studied by changing the various parameters like temperature, pH, rpm and substrate concentration. The enzyme activity shows maximum at a temperature of 35^0C – 37^0C, pH 8 and 300 rpm. The maximum enzyme production was achieved, for 1% concentration of cornstarch at 35.6^0C and pH 9 using the fermentation medium contains yeast extract and peptone and the enzyme activity was found to be 55.93 DUN/ml. Since the cost of yeast extract and peptone is very high, so the further work was done using some low cost carbon and nitrogen sources like defatted cotton seed, defatted soya flour and mustard seed which are extracted from agricultural byproducts. The enzyme activity for using the low cost medium was found to be nearly triple such as 121.49 DUN/ml. The enzyme production reaches the steady phase at 24 hours. So it is highly recommended that using the low cost medium for the α-amylase enzyme gives better biomass cell concentration and enzyme activity as well.

Keywords: *α-amylase, bacillus lichenoformis, low cost medium, agricultural by products, fermentation, alkaline enzyme.*

I. Introduction

Enzymes are proteins which catalyze variety of reactions in the biological system. When enzymes were first intensively studied in the last two centuries this chemical nature was obscure and even the reactions catalyzed were frequently ill defined. It was natural and therefore, that individual enzymes were given names by their discoverers. Most enzymes are studied and need to be named before any significant information about their structures exists. Whenever the 'same' enzyme from different organism is studied, it is found that Proteins different in detailed structure (and some times in gross structure) can have essentially the same catalytic properties. In the recommendations of the "International Union of Biochemistry Nomenclature Committee (1984), therefore, an enzyme name does not specify a structure but instead defines the Principal reaction catalyzed.

Enzymes are classified in to six classes. Enzymes in the first three classes all catalyze transfer reactions, with stoichiometry A+B → P+Q, but differ in other respects. Oxidoreductases catalyze reaction in which one or more electronics (usually two) are transferred from a donor (reducing agent) to an acceptor (Oxidizing agent). In many oxidoreductases the oxidized substrate can be regarded as a hydrogen donor, and for these enzymes the term dehydrogenase is preferred. Hydrolases catalyze hydrolytic reaction, i.e. reactions in which water is the acceptor of the transferred group. The transferases thus comprise all enzymes catalyzing transfer reaction that are not oxide reductases or hydrolases. Lyases catalyze elimination reaction, where the bond is broken without oxidoreduction or hydrolysis and in most cases have stoichiometry. A → P+Q.

The six classes are further sub divided in to subclasses, to specify the type of reaction more fully and to indicate the reactants. All the enzymes have a property of either intra cellular or extra cellular in nature. But most of them are extra cellular in nature.

a) Intracellular Enzymes

Enzymes occur in all living cells, where they catalyze and regulate reactions of Biochemical pathways essential to the existence of the living system. In general substrates for these enzymes are small molecular weight molecules, e.g. Sugars, amino acids, carboxylic acids, which are able to permeate the membrane. Their catalytic properties are regulates by conformational changes in their three dimensional structure accomplished by allosteric cofactor molecules.

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b) Extracellular enzymes

Extra cellular enzymes were originally defined as enzymes which are external to the cell wall and in contact with surrounding medium. At present we consider transport the membrane as the primary secretion event. Thus for the purpose of this review the term & erection is used to refer to the transmembrane passage of protein and the term extra cellular to those proteins that have undergone this process. The biological function of this kind of enzymes may be seen in the hydrolysis of macro molecules which are too large to be transported in to the cell.

c) Animal tissue Enzyme

Enzymes used in Industry are isolated from animal and plant tissues, as well as from Micro organisms. One of these three sources may be favored for a given enzyme. For example, some proteolytic enzymes isolated from animals may be advantageous in special fields of application. The enzyme chymosin, also known as rennet, is an acid protease used in the milk-clotting step of cheese production. A mixture of chymosin and its zymogen prochymosin, which may be converted chymosin by low pH treatment, are currently obtained from the abo-masum of an unweaned calf. Animal glands, e.g. the pancreas, are sources for hydrolyzing enzymes used as a digestive acids. The pancreas is a very rich sources of enzymes. It contains about 23% of trypsinogen and 10 -14% of chymotrypsinogen. So called pancreatin, a digestive aid, contains several enzymes such as amylase, lipase and protease.

d) Plant tissue enzymes

Plant protease isolated from pineapple (bromelain) and the papaya plant (papain) have been used for meat tenderizing and chill proofing beer. Useful amylolytic enzymes occur in plant tissues such as barely, wheat, rye, Potatoes, sweet potatoes, beans, soy beans, α - amylase, β - amylase, which starts at the non-reducing ends of the outer chains of the starch and proceeds by gradual removal of maltose units and de branching enzyme which hydrolyzes the α -1 - 6 linkages of starch, were detected in these plants.

e) Microbial enzymes

Microorganisms have become increasingly important as producers of industrial enzymes and in fact most enzymes used in industry today are of Microbial origin. Attempts are now being made to replace enzymes which traditionally have been isolated from animal tissue and plant tissues with enzymes from Microorganisms. Examples for partial replacement of plant and animal enzymes in dudes. Amylases and endo - β - glucanases of malted Barley and wheat by enzymes from Bacillus and Aspergillus in the beer, distillery, baking and textile industries. Plant and animal proteases by Aspergillus and Thermoactinomyces protease for meat tenderization and for chill proofing beer.

f) Uses of α – amylase

The enzyme α–amylase is used as a biocatalyst in many small scale and large scale industries some of the uses are.

- The Bacterial α–amylase used in starch hydrolysis industries, Brewing industries, Detergents industries and textile industries.
- The fungal α–amylase used in starch industries and baking industries.
- The α–amylase from Malt used as a digestive aid and supplement to bread.
- The α–amylase from Aspergillus Orygaze is used to produce starch liquefying syrups.
- The α–amylase from Bacillus Subtillis used in Desizing textile industries, Alcohol fermentation industries and glucose producing industries.
- The α–amylase produced from Aspergillus Niger is highly acid resistant is used as a digestive acid at pH-5.
- The α–amylase from Bacillus lichenoformis is used in all starch industries and detergent industries and to produce starch sizing pastes for use in paper coatings.

II. Objective of the Study

Enzymes are Proteins which catalyze variety of reaction in the Biological systems. There are many methods used to produce the enzymes among that the biological methods are widely used. In this type of biological method of production, solid state fermentation is applied for the production. In all the types of fermentation processes, the cultures has been prepared using yeast extract and peptone etc. These are added to the culture in terms of nutrients as a carbon and nitrogen sources for the microorganism. The cost of these chemicals are much expensive. So the alternative method has been proposed for the preparation of culture medium using some low cost agricultural byproducts such as defatted cotton seed, defatted soybean, mustard seed etc. The fermentation has to carryout using these type of low cost medium to check the productivity and enzyme activity.

III. Experimental Setup

a) Biostat E fermentor

The fermentation was carried out in a B. BRAUN CO, Biostat E fermentor. It is a compact and comprehensive fermentation system on a laboratory scale, which can be used in microbiological and biotechnological research and development. Biostat E fermentors are designed for use in discontinuous fermentation (Batch operations) as well as in continuous

process. The measurement and control system used in compatible with computers. The Biostat E is protected against unauthorized use with a main key. All modules of the measurement and control section are separately switched on. Therefore they can be installed or removed independently from the control in spite of the central mains switch. Additional modules can be inserted without interruption or disturbance of operations.

The lower front panel of the basic device is provided with installation ports for at least 4 dosing pumps of the four, three are peristaltic pumps for the supply of acid, alkali and antifoam agent, the fourth is prepared to install precision dosing pumps.

The arrangements of the various technical appliances in the basic devices are:

- Thermostat system which containing heating and cooling water circuit for tempering as well as for sterilization.
- Gas supply system including exhaust equipment.
- Motor and drive system for the stirrer shaft drive.

The recorder, of 6 channels dot printer records the following measurement values in the basic devices.

- Temperature
- Speed
- pH Value & Antifoam consumption

The culture vessel is mounted on the console laterally fixed at the fermentor where there are the corresponding borings for the feet of the culture. Simultaneously the connection to the stirrer drive is guaranteed. For starting operating the device the filling state of the fermentor thermostat is to be checked. The set point temperature is adjusted at the corresponding digital switch of the module. A good mixing of the culture vessel is a prerequisite. For that a stirrer system is provided which is driven by a controlled DC motor. The stirrer speech can be directly adjusted by the digital switch of the speed controlled module. The adjustable speed range is 50 – 1500 minutes^{-1}.

The pH – value in the culture medium can be electro chemically determined via a combined – glass electrode. The pH set point desired can be adjusted with the digital switch of the pH controller.

b) Dimensions of the fermentor

Total volume of the fermentor	:	6 lit.
Working volume	:	5 lit.
Max working temperature	:	138^{o} C
Max working pressure	:	124^{o} C
Diameter of the fermentor	:	17.5 cm
Height of the fermentor	:	40 cm
Agitator type	:	6 Blade, Paddle type Agitator

IV. Materials and Methods

a) Microbial strain

Bacillus Lichenoformis, NCIM 2051 Received from National Chemical Laboratory, Pune, India.

b) Chemicals

Beef extract
Peptone
NaCl
$MgSO_4$
KH_2PO_4
$CaCl_2$
Yeast extract
Agar
Corn Starch
Defatted Cotton Seed
Defatted Soya flour
Mustard Seed

c) Medium

i. *Universal medium for bacteria*

Beef extract	:	1.0 %
Sodium Chloride	:	0.5 %
Peptone	:	1.0 %
pH	:	7.0 - 7.2

Sterilize the medium, and adjust the pH at 7.2. Add 2% Agar for making slants.

ii. *Corn starch medium: (Basal Medium)*

Corn starch	:	1 %
Yeast extract	:	0.2 %
Peptone	:	0.5 %
$MgSO_4$	:	0.05 %
KH_2PO_4	:	0.05 %
NaCl	:	0.15 %
$CaCl_2$	:	0.015 %

iii. *Low cost medium*

Corn starch	:	1 %
$MgSO_4$	:	0.05 %
KH_2PO_4	:	0.05 %
NaCl	:	0.15 %
$CaCl_2$	:	0.015 %
Soya bean	:	0.5 %
Mustard Seed	:	2 %
Cotton seed	:	3 %

d) Procedure

Shake flask cultures were operated at constant temperature of 37^{0}C and fixed rpm with 100 ml of medium in a 500 ml Erlenmeyer flask and inoculated with the culture. Fermentation studies were carried out in above described B. Braun Biostat E fermentor with the cultural conditions of 37^{0}C, pH 7, and 300 rpm. Since it is an aerobic fermentation, the aerobic rate was maintained at 1 vvm. For every six hours the sample were collected from the sampling point provided in the top of the culture vessel, and analyzed.

i. *Stock Culture*

Bacillus Lichenoformis NCIM 2051 was maintained in an Agar slant at 4^{0}C.

ii. *Sub Culture Maintenance*

Subculture was prepared using a universal Bacteria medium and it was maintained in an incubator at 37°C.

iii. *Pre inoculum*

Take 100 ml of the Universal medium inoculate this with a stock agar culture in a 500 ml Erlenmeyer flask and kept in a shaker at 300 rpm and 37^{0}C. It is also called as seeding of culture.

iv. *Enzyme activity*

One unit of enzyme activity (DUN) is defined as the quantity of enzyme that causes 1 % reduction of blue color intensity of starch-iodine solution in 1 min. The optical density was first measured at 660 nm using an UV spectrophotometer.

V. Results and Discussion

a) Enzyme Activity determination

Different techniques have been used to measure enzyme activities. There is no general method equally applicable to all enzymes. The enzyme activity may be depends on the time, enzyme concentration, substrate concentration.

Extra cellular amylase activity was determined by measuring the decrease in iodine color reaction showing dextrinization of starch. The reaction contained 1 ml of enzyme (cell free supernatant) and 10ml of 1% starch solution incubated at 40^{0}C for 10 min. The reaction was stopped by adding 10ml of 0.1N HCl. 1 ml of this acidified solution was added to 10ml 0.1N HCl. From this 1ml was added to iodine solution (0.05% iodine in 0.5% KI). The optical density of the blue colored solution was determined of 660 nm one unit of enzyme activity (DUN) is defined as the quantity of enzyme that causes 1% reduction of blue color intensity of starch iodine solution at 40^{0}C in 1 min.

For amylase activity determination requires the standard chart for starch iodine solution. Take six test tubes in that add 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of 1% starch solution respectively and add 1, 0.8, 0.6, 0.4, 0.2 and 0 ml of water reply. Add 10ml of iodine solution (0.05% I_2 and 0.5% KI) in all the six text tubes. The inference is the light blue color formation. The optical density of the blue colored solution was measured at 660 nm in the UV spectrophotometer. The standard graph was drawn by plotting starch concentration Vs absorbance. From the standard graph the enzyme activity was calculated.

b) Production of enzyme

The growth pattern of Bacillus Lichenoformis NCIM 2051 and α–amylase production was observed for three days in basal medium with 1% cornstarch as a carbon source. The formation of α–amylase started from 4 hours. The maximum enzyme production was achieved at 24 hours. The pH of the broth increased from 7 at the beginning to 8.9 at the end of fermentation. The maximum yield was achieved at 35^{0}C.

c) Effect of corn starch concentration

The effect of corn starch concentration was further studied. The α–amylase production was studied, by changing the Corn starch concentration at 0.5%, 1% and 2.5%. It was found that with an increase of starch concentration in the medium beyond 1%, enzyme production did not increase. At higher starch concentration, enzyme production was comparatively lower and the time required to reach the maximum enzyme level was longer.

d) Effect of pH

The bacterium was found to grow at pH 3-11, with growth resulting in an increase of the patient's media's pH. Enzyme production started at 5.0 and ceased at pH 10.0. Maximum enzyme production occurred at pH 6-9. Very little enzyme production in the medium at initial pH of 3 - 4. At higher pH values (10-11), growth was quite high, but the amount of enzyme production was very low.

e) Effect of temperature

The strain was found to grow and produce enzyme at temperatures from 25 to 50^{0}C. Maximum enzyme production was observed at 35^{0}C. Growth and enzyme production both started decreasing drastically above 40^{0}C.

f) α–amylase production in low cost medium

The α–amylase production was further studied by using the low cost medium which containing the carbon and nitrogen sources like corn flour, mustard seeds. Since the cost of yeast extract and peptone in the Basal medium is very high, we can replace the yeast extract and peptone with the above mentioned things. The low cost medium produced 2 times more enzyme than the high cost synthetic medium (yeast extract and peptone). The medium containing 0.5% defatted, 2% mustard seed in the place of yeast extract and peptone, was found to yield high enzyme activity of α–amylase. The experiments were conducted for 6 different batches

with various concentrations, which are given in the below table and graph.

Table 1 : Enzyme production for 1% corn starch concentration

Time hours	pH	Temp ^0C	$\%PO_2$	rpm	Enzyme Activity DUN/ml
0	7.0	36.9	104.7	300	0.003
3	7.1	36.8	101.8	300	2.01
6	7.2	37.0	100.2	300	3.37
12	5.8	37.1	22.6	300	4.51
18	7.3	35.7	89.3	300	29.31
24	8.2	35.6	86.8	300	55.84
48	9.0	35.6	96.4	300	55.93
72	8.9	36.2	98.7	300	56.11

Table 2 : Enzyme production for 2.5% corn starch concentration

Time hours	pH	Temp ^0C	$\%PO_2$	rpm	Enzyme Activity DUN/ml
0	6.3	37.0	100.8	300	0.0007
3	5.8	36.4	100.1	300	1.37
6	6.1	35.9	92.7	300	2.56
12	6.7	35.7	95.6	300	8.48
18	6.9	35.4	97.9	300	33.49
24	7.1	35.3	98.3	300	40.83
48	7.9	35.1	88.5	300	40.71
72	8.5	34.8	84.3	300	40.74

Table 3 : Enzyme production for 0.5% corn starch concentration

Time hours	pH	Temp ^0C	$\%PO_2$	rpm	Enzyme Activity DUN/ml
0	6.1	37.0	120.3	300	0.0007
3	6.1	36.7	110.8	300	1.53
6	6.9	36.6	102.6	300	4.92
12	7.5	35.9	100.9	300	12.19
18	7.8	35.8	98.7	300	39.43
24	7.9	35.6	98.5	300	43.38
48	8.3	35.7	98.1	300	42.31
72	8.8	35.4	83.6	300	42.34

Table 4 : Enzyme production using Basal medium + 0.5% defatted soya flour

Time hours	pH	Temp ^0C	$\%PO_2$	rpm	Enzyme Activity DUN/ml
0	7.0	37.0	120.3	300	0.98
3	7.1	38.3	110.4	300	11.91
6	7.3	37.1	93.6	300	27.56
12	8.1	36.3	83.9	300	48.29
18	8.7	36.1	70.8	300	61.49
24	8.9	35.9	64.7	300	81.24
48	8.7	36.3	53.9	300	81.31
72	8.9	37.8	48.7	300	80.9

Table 5 : Enzyme production using Basal medium + 3% defatted cotton seed

Time hours	pH	Temp ^0C	$\%PO_2$	rpm	Enzyme Activity DUN/ml
0	7.0	37.0	120.1	300	1.90
3	7.3	38.1	117.3	300	18.53
6	7.9	37.5	93.5	300	46.93
12	8.5	36.3	83.8	300	64.71
18	9.1	35.3	77.9	300	84.18
24	9.7	35.5	64.2	300	91.5
48	9.9	36.1	56.9	300	92.3
72	10.3	38.5	28.5	300	91.9

Table 6 : Enzyme production using Basal medium + 2% mustard seed

Time hours	pH	Temp ^0C	$\%PO_2$	rpm	Enzyme Activity DUN/ml
0	7.0	37.0	120.1	300	2.41
3	7.3	38.1	117.3	300	12.49
6	7.9	37.5	93.5	300	32.33
12	8.5	36.3	83.8	300	65.91
18	9.1	35.3	77.9	300	95.41
24	9.7	35.5	64.2	300	121.49
48	9.9	36.1	56.9	300	120.83
72	10.3	38.5	28.5	300	121.10

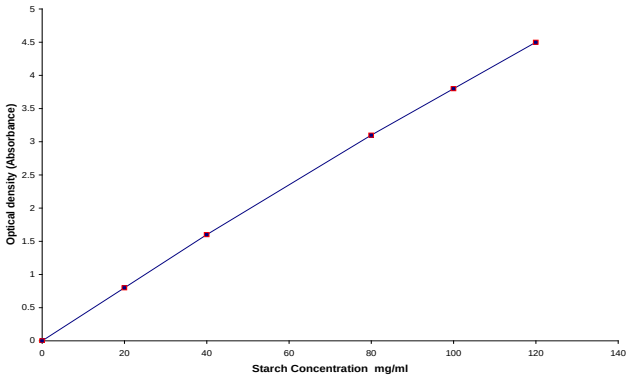

Figure 1 : Standardization graph of starch iodine solution for α–amylase activity

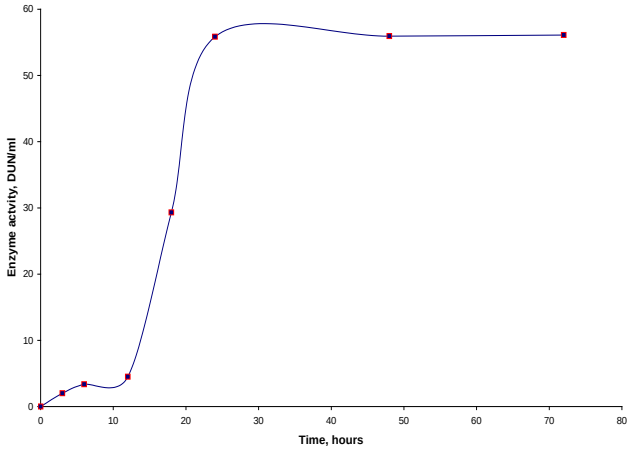

Figure 2 : Enzyme activity for 1% corn starch concentartion

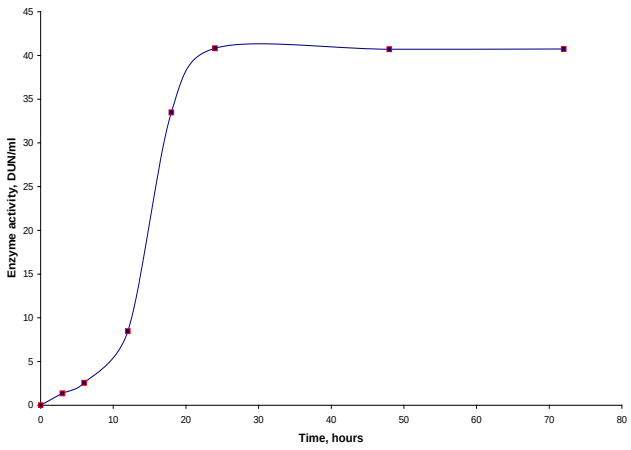

Figure 3 : Enzyme activity for 2.5% corn starch concentartion

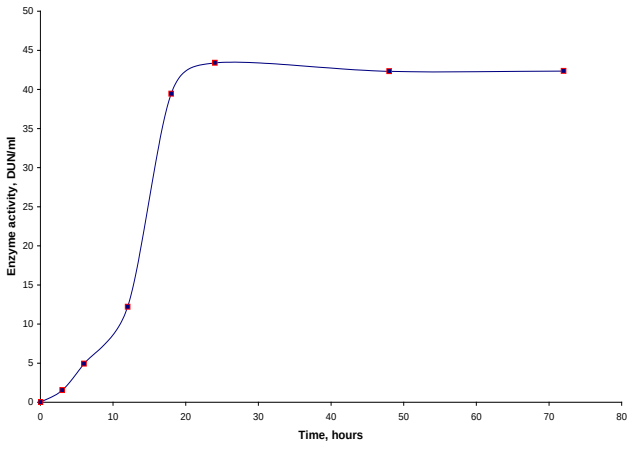

Figure 4 : Enzyme activity for 0.5% corn starch concentartion

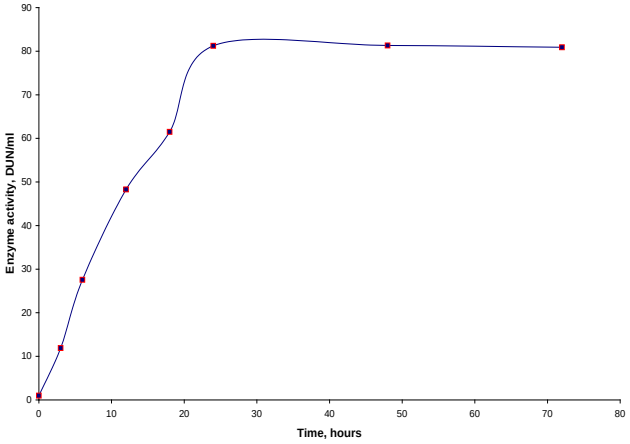

Figure 5 : Enzyme activity for Basal medium with 0.5% defatted Soya flour

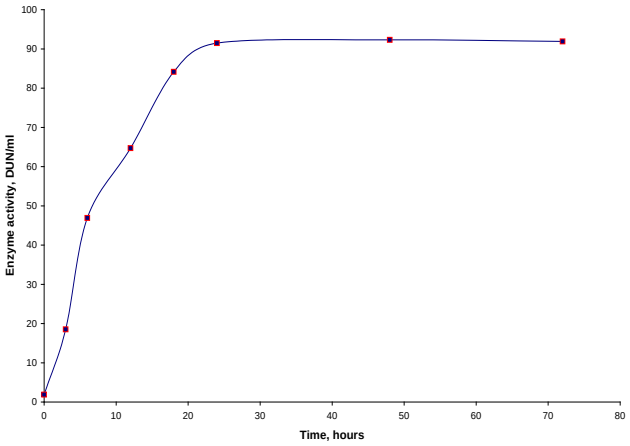

Figure 6 : Enzyme activity for Basal medium with 3% defatted Cotton seed

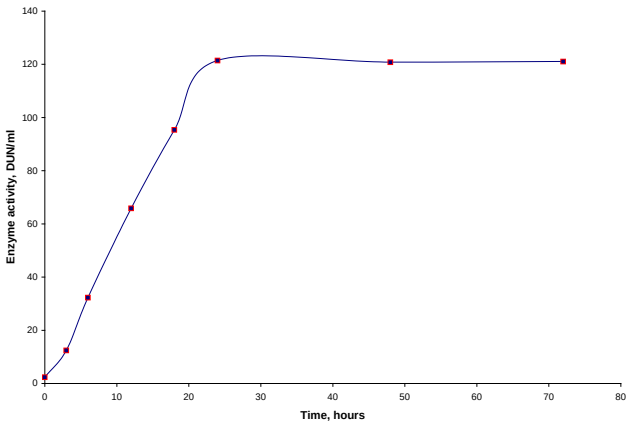

Figure 7 : Enzyme activity for Basal medium with 2% Mustard seed

VI. Conclusion

The Bacterial strain, *Bacillus lichenoformis* NCIM 2051 was obtained from National Chemical Laboratory, Pune, which produced high temperature alkaline α–amylase enzyme. The optimum cultural conditions are found to be 35°C, pH 7 and 300 rpm. The α–amylase produced from this Bacterial strain, *Bacillus lichenoformis* was quite active even at 100°C, however it showed optimum activity at 90°C, and also it exhibited optimum activity in the broad pH range 5.5 – 10, thus α-amylase *of Bacillus lichenoformis* seems to have a very broad pH range. A low cost synthetic medium producing large quantities of α–amylase has been developed from *bacillus lichenoformis* was used for α–amylase production. The α–amylase of this strain showed excellent stability at high temperatures and over a wide pH range. The enzyme activity were determined and optimized. The low cost medium which contains, Defatted soya flour, Defatted cottonseed, and Mustard seed, produces around three times more enzyme than the high cost synthetic medium using yeast extract and peptone in the B. Braun Biostat E fermentor. So it is further suggested to change the cheapest different nitrogen sources components in this low cost medium like corn steep liquor etc.

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Influence of Colchicine Treatments on Character Expression and Yield Traits in Cowpea (*Vigna Unguiculata* L. Walp)

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Abstract- Mutagenesis has been exploited to enhance genetic variability in cowpea (Vigna unquiculata L. Walp.); an important legume in the tropical and subtropical regions of the world. Mutation is regarded to be a shortcut breeding technique, which has produced new and high yielding varieties through heritable changes in genetic constitution of characters in some leguminous crops. Effects of 0.1% aqueous solution of colchicine for different periods of time, viz; - 0, 2, 4 and 6 hours were tested on the quantitative and yield characters of a cowpea variety popularly known as 'Oloyin' in M1 generation. Lethal dose value (LD40) of 59% was observed at 2 hours treatment. Treatment was significant (P = 0.05) for seedling emergence percentage (67 – 13%), plant height (21.52 – 15.63 cm), number of leaves (11.08 – 4.98), number of nodes on main stem (6.26 - 4.5), survival percentage (63.50 – 12.50%) and number of days to first flowering (55.52 – 47.30) while treatment was not significant for all other characters studied.

Keywords: mutagenesis, genetic variability, induced variation.

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Influence of Colchicine Treatments on Character Expression and Yield Traits in Cowpea (*Vigna Unguiculata* L. Walp)

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Abstract- Mutagenesis has been exploited to enhance genetic variability in cowpea (Vigna unquiculata L. Walp.); an important legume in the tropical and subtropical regions of the world. Mutation is regarded to be a shortcut breeding technique, which has produced new and high yielding varieties through heritable changes in genetic constitution of characters in some leguminous crops. Effects of 0.1% aqueous solution of colchicine for different periods of time, viz; - 0, 2, 4 and 6 hours were tested on the quantitative and yield characters of a cowpea variety popularly known as 'Oloyin' in M1 generation. Lethal dose value (LD40) of 59% was observed at 2 hours treatment. Treatment was significant (P = 0.05) for seedling emergence percentage (67 – 13%), plant height (21.52 – 15.63 cm), number of leaves (11.08 – 4.98), number of nodes on main stem (6.26 - 4.5), survival percentage (63.50 – 12.50%) and number of days to first flowering (55.52 – 47.30) while treatment was not significant for all other characters studied. The results revealed that colchicine can be used to induce variations in cowpea which may be of agronomic importance in the production of this crop.

Keywords: mutagenesis, genetic variability, induced variation.

I. Introduction

Cowpea (*Vigna unguiculata* L. Walp) is one of six major cultivated crop species of the family *Leguminosae* distributed throughout the tropics (Padulosi and Ng, 1997; Pasquet, 2001). It is the second most important grain legume crop after groundnut (Blade *et al*., 1997). Cowpea has been reported as an important food crop throughout Sub –Saharan Africa (SSA) (Kitch *et al*., 1998) and one of the major sources of plant protein in the developing countries including Nigeria (Adekola and Oluleye, 2007). Its grain and leaves have high quality protein and vitamins which serves as an excellent food supplement in developing countries (Kitch *et al*., 1998). Millions of relatively poor people in low income countries in the tropics rely on it for their livelihood and as protein supplement (Ajayi, 2014). Hence, it is a key staple food crop for ever-increasing population both in the rural and urban areas. Cowpea has a great ability to fix atmospheric nitrogen in the soil, thereby improving soil nutrients (Adetiloye *et al*., 2013). Ajayi and Adesoye (2013) reported that cowpea production has been consistently hindered by low grain yields and quality, and lack of improved cultivars. Dhanavel *et al* (2012) reported induced mutation as a valuable supplement to conventional breeding in crop improvement programs, but has been least applied in grain legumes like cowpea. Induced mutations have been used successfully to improve yield and yield components of many crops like *Oryza sativa, Hordeum vulgare, Triticum durum, Vicia faba, Cicer arietinum, Cajanus cajan,* in the world (Khan and Wani, 2006). Improvement of legumes such as cowpea through induced mutation could make it possible to identify new genes and thus broaden the spectrum of heritable changes and expand cowpea germplasms.

To enhance the limited genetic variability in cowpea, mutagenesis has been exploited and efforts made at identifying the proper mutagens in cowpea breeding which can produce mutants for future breeding programs (Achaya *et al*., 2007). Acceleration of frequency of mutation in cowpea has been accomplished by exposure of seeds to mutagenic agents like ionizing radiation and / or chemical mutagens (Natarajan, 2005). Colchicine treatment was reported as one of the best tools of inducing and enhancing genetic variability in some food crops within a very short period of time (Gnanamurthy *et al*., 2013). Hence, this study focused on the influence of colchicine treatments on characters expression and yield of cowpea.

II. Materials and Methods

This research was conducted at the Research Laboratory of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. Modified techniques of Khan and Wani (2006); Kumar and Verma (2011); Ajayi (2014) were employed for this study.

A cowpea variety popularly known as "Oloyin" was obtained from a local farmer in Akungba-Akoko, Ondo State, Nigeria. A total of 800 healthy seeds of uniform size were used for this study. Two hundred seeds per treatment were soaked in distilled water for 12 hours, after which the water was drained, and seeds spread and air dried on a filter paper before being treated with colchicine.

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Two hundred seeds per treatment were soaked in 0.1% (w/v) aqueous colchicine solution for 2, 4 and 6 hours at room temperature while the control was left untreated. Seeds were later transferred into a sterile cloth, tied and rinsed in running water for 20 min to terminate the residual effect of colchicine. The treated seeds were later air dried for 15 hours before sowing.

Treated seeds along with the control were sown in the field to generate M1 generation in a randomized complete block design with five replications, during the rainy season of 2013 at the Research Field of Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Nigeria. Two hundred seeds from each treatment and control were planted in the field adopting an intra-row spacing of 50cm and inter- row 30cm.

a) Data collection

Data on 15 quantitative traits were collected from 10 randomly selected plants per replicate for control and from all surviving plants per replicate for the treated plants. The quantitative traits included;- seedling emergence percentage (7- 20 days after sowing); plant height (cm) taken at 4weeks and 8 weeks after sowing; number of leaves; terminal leaflet length (cm); terminal leaflet width (cm); number of main branches; number of nodes on main stem taken at 7 weeks after sowing; survival percentage; days to first flowering; number of peduncles per plant; peduncle length (cm); number of pods per plant; pod length (cm); number of seeds per pod and 100-seed weight (g) were taken at maturity.

Data were analyzed using Statistical Package for Social Science (SPSS) version 20 (SPSS, Inc., Chicago IL). Analysis of variance (ANOVA) was performed, followed by least significant difference (LSD; P = 0.05% level of significance) computation for mean separation.

III. Results and Discussions

Chemical mutagenesis has been a beneficial technique in the improvement of yield characters in crop breeding. Variability of quantitative traits influencing yield have been greater in mutagenic progenies than in control. Ability of mutagens to enter the cell of the living organisms to interact with DNA produces the general toxic effects associated with their mutagenic properties (Mensah *et al*., 2007). It has been widely proved that chemical mutagens induce physiological damages (injury), gene mutations and chromosomal aberration in M1 individuals which can be detected and measured from seed germination or emergence of seedlings, survival reduction (lethality), plant height reduction (injury) and fertility reduction or sterility (reduction in pod and seed formation) (Kumar *et al*., 2009) which might not be restricted to M1 generation (Mak *et al*., 1986). Analysis of variance revealed that treatment effect was significant ($P \leq 0.05$) for emergence percentage, plant height, number of leaves, number of nodes on main stem, survival percentage, and number of days to flowering in the M1 generation whereas treatment effect was not significant for all other traits (Table 1). Means for emergence percentage from 7th day after sowing to 20th day after sowing established that colchicine treatment reduced emergence of seedlings with direct correlation with the duration of exposure. At 20days after sowing, emergence of seedlings ranged from 67% (control) to 25% (6 hours). Colchicine significantly decreased the emergence of seedlings compared with the control. About 40% reduction of emergence was found at 2 hours of exposure; therefore, LD_{40} value (59%) was fixed at 2 hours duration of treatment (Table 2). Reduction in emergence of seedlings and survival as a result of colchicine treatments agrees with the findings of many workers on cowpea (Kumar *et al*., 2009; Girija and Dhanavel, 2009; Gnanamurthy *et al*., 2013) and in many other crops like black gram (Deepalaskshmi, 2000; ThangaHamavathy, 2002). The damage to the biological materials according to these findings might be considered as an indication of the mutagenic effects.

Survival percentage at maturity ranged from 63.50% (control) to 12.5% (6 hours). Survival was significantly reduced with increased duration of colchicine treatment (Table 4). The linear relationship of treatment duration on survival has been observed by many workers. In most cases, the mortality may be due to poor seedling vigor resulting from inability to overcome the toxic effect of colchicine (Zlesak *et al*., 2005).

Plant height was significantly reduced by all treatments generally compared with the control, with the highest duration of time producing the shortest heights, 7.46cm (4 weeks) and 15.63cm (8 weeks); whereas the control had the highest heights of 15.57cm at week 4 and 21.52cm at week 8 (Table 3). The reduction of growth observed with increase in duration of treatment is very common in M1 generation of many mutated crops, which is actually as a result of reduction in the rate of cell division among the treated plants and also linked to chromosomal abnormality, reduction of auxin levels, inhibition of auxin synthesis and failure of assimilation mechanisms (Riley, 1954).

Number of leaves was more in the control than other treatments: the number of leaves being 11.08 (control), 8.86 (2 hours), 4.98 (4 hours) and 6.79 (6 hours). There was a significant difference in treatment effects for number of leaves. The treated plants were found to possess longer leaflets and wider leaflets compared to control as also confirmed by Ajayi *et al*. (2010). This may actually be a useful trait for breeding for its potential to increase the net photosynthetic area which may lead to increase in photosynthetic assimilates going into grains as a positive effect of seed yield (Priya, 2006). It has also been proved that

successful colchicine treatment has been found to result in plants that possess characters such as thicker-wider leaves with bigger and fewer stomata number (Uhlik, 1981).

Number of nodes on main stem showed a significant difference among the treatments, with the 2 hours having the highest number of nodes (6.26), followed by the control (6.22), and while the lowest number of leaves was for the 4hour treatment (4.50). Control flowered earlier (47.30 days) than all other treatments. Increase in duration of exposure delayed flowering. The mean number of days to flowering among the treated plants was 47.50 (2 hours), 54.97 (4 hours) and 55.12 (6 hours). The difference between the control and other treatments was significant. This delay in flowering with direct correlation with increased exposure period is not novel as it has been observed by many workers especially in soybean (Maheshwari *et al.*, 2003; Pavadai and Dhanavel, 2005), in mungbean (Khan and Wan, 2005).

The decrease in peduncle length, number of pods per plant, pod length and number of seeds per pod all contradict the results of Odeigah (1998) on M1 generation of cowpea but consistent with Kumar *et al.* (2009), who reported that reduction in pod number may be as a result of inhibiting action of enzymes, changes in enzymatic activities and toxicity of the mutagen on these traits, while reduced seed yield can be attributed to high seed sterility and reduced pod number, also as consequences of physiological and biochemical disturbances in the development of plants (Prabhakar, 1985) resulting from mutagenic treatment (Ajayi *et al.*, 2014). Seed weight was however enhanced by 2hour duration of exposure but was further reduced by treatment at higher exposure of time. Some of the characters studied decreased linearly as the duration of treatment increased why most of them showed irregular pattern of behaviour with increase in duration of treatment.

IV. Conclusion

From the results obtained, seed treatment has proven to be a viable method for inducing variability in cowpea through mutagenesis. The level of dissimilarity among the plants as a result of treatment was high and this indicates a possible improvement through this approach. Although colchicine reduced most of the morphological characters at M1, this is always expected as it is a usual phenomenon in M1 generation.

Table 1 : Mean square values of all traits for colchicine treatment

CHARACTER	DF	REPLICATION	TREATMENT	ERROR
EP (20 DAS)	3	452.66*	3753.75*	47.24
PH(8 WAS)	3	50.04*	36.04*	5.99
NL	3	2.45	34.64*	3.86
NN	3	1.34*	5.66*	0.78
TLL (cm)	3	1.21	1.78	2.31
TLW (cm)	3	0.84	0.56	0.82
NMB	3	0.63	0.83	1.19
SUP	3	358.91*	3176.98*	32.45
PPP	3	39.36	94.48	53.51
PEL (cm)	3	55.85*	50.19	14.42
NDF	3	45.07	97.51*	19.94
NPP	3	5.62	2.58	8.22
PL (cm)	3	2.88	1.48	1.35
SP	3	0.86	3.52	3.67
SW (g)	3	0.04	0.81	0.89

*: Significant

EP: Emergence percentage; PH: Plant height; NL: Number of leaves per plant; NN: Number of nodes on main stem; TLL: Terminal leaflet length; TLW: Terminal leaflet width; NMB: Number of main branches; SUP: Survival percentage; PPP: Peduncle per plant; PEL: Peduncle length; NDF: Number of days to flowering; NPP: Number of pods per plant; PL: Pod length; SP: Seeds per pod; SW: Seed weight; DAS: Days after sowing; WAS: Weeks after sowing

Table 2 : Emergence percentage of colchicine treated cowpea and control from 7 DAS to 20 DAS (Mean ±Standarderror)

Treatment	7DAS	8DAS	9DAS	13DAS	18DAS	20DAS
Control	55.00±7.70[a]	62.00±5.55[a]	63.50±5.45[a]	65.00±5.53[a]	67.00±5.26[a]	67.00±5.26[a]
2Hrs	28.50±4.07[b]	38.50±7.85[b]	44.00±7.05[b]	55.00±8.60[b]	55.50±8.11[b]	59.00±7.81[b]
4Hrs	11.00±2.44[c]	11.50±2.44[c]	12.00±3.00[c]	15.00±4.10[c]	16.80±4.58[c]	18.50±5.35[c]
6Hrs	11.50±2.31[c]	12.00±2.12[c]	12.50±2.50[c]	14.00±2.80[c]	13.00±2.42[c]	13.00±2.42[c]
LSD	12.27	13.48	11.46	11.76	9.66	9.47
CV (%)	33	32	25	23	18	18

Mean values followed by same letters within a column are not significantly different

DAS: Days after sowing; LSD: Least significant difference; CV: Coefficient of variation

Table 3 : Mean and Standard error values of plant height of colchicine treated and untreated cowpea

Week	Control	2Hrs	4Hrs	6Hrs	LSD	CV (%)
4WAS	15.57±0.6[a]	10.93±0.7[b]	6.69±0.50[c]	7.46±0.78[c]	1.93	13.9
8WAS	21.52±1.8[a]	17.53±1.4[b]	16.05±2.03[b]	15.63±2.04[b]	3.37	13.84

Mean values followed by same letters within the same row are not significantly different

LSD: Least significant difference; CV: Coefficient of variation; WAS: Weeks after sowing

Table 4 : Quantitative traits (Mean ± Standard error) of control and colchicine treated cowpea

Treatment	NL	NN	TLL	TLW	NMB	SUP	PPP	PEL	NDF	NPP	PL	NSP	SW
Control	11.08±1.29a	6.22±0.43b	11.73±0.07a	7.57±0.16a	7.30±0.41a	63.50±4.23a	27.56±3.29a	24.90±1.59a	47.30±1.43c	9.00±0.89a	14.65±0.54a	10.78±0.75a	12.92±0.51a
2hrs	8.06±0.42b	6.26±0.26a	15.04±0.70a	8.38±0.56a	7.84±0.42a	52.00±6.49b	28.52±4.27a	25.32±0.86a	47.50±0.83c	8.76±1.09a	14.62±0.36a	9.37±0.68a	12.50±0.32a
4hrs	4.98±0.35c	4.26±0.22c	14.36±0.81a	7.84±0.41a	6.40±0.60a	17.50±5.06	18.89±2.69a	24.85±2.36a	54.97±1.53b	8.58±1.65a	13.84±0.68a	11.06±0.73a	13.30±0.81a
6hrs	6.79±0.90c	4.50±0.36c	14.91±0.55a	7.96±0.36a	6.94±0.34a	12.50±2.37	24.25±1.91a	18.70±3.32a	55.52±3.99a	7.38±1.16a	13.58±0.71a	11.19±0.90a	12.44±0.38a
LSD	2.7	0.84	NS	NS	NS	7.85	NS	NS	6.15	NS	NS	NS	NS
CV (%)	24.78	11.52	10.43	11.41	15.34	15.66	29.49	16.19	8.72	34	8.21	18.06	7.37

Mean values followed by same letters within the column are not significantly different

NL: Number of leaves per plant; NN: Number of nodes on main stem; TLL: Terminal leaflet length; TLW: Terminal leaflet width; NMB: Number of main branches; SUP: branches; SUP: Survival percentage; PPP: Peduncle per plant; PEL: Peduncle length; NDF: Number of days to flowering; NPP: Number of pods per plant; PL: Pod length; SP: Seeds per pod; SW: Seed weight; LSD: Least significant difference; CV: Coefficient of variation.

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Isolation and Phenotypic Characterization of Lactobacillus Sakei and Pediococcusspp. Antagonists from Algerian Meat

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Abstract- The aim of the present work was to isolate antagonist cultures in order to use them in biopreservation. LAB were isolated from Algerian meat and characterized at the genus level based on phenotypic characteristics. That following these spot agar test was achieved to assess their potential antagonistic towards pathogens: Bacillus cereus, Bacillus subtilis ATCC6633, Escherichia coli ATCC8739, Salmonella Typhimurium ATCC14028, Staphylococcus aureus ATCC6538, and Pseudomonas aeruginosa. Biochemical tests had ended this study to characterize the potent isolates at the spice level. As a results, thirty LAB had been differentiated to: 53% belong to Lactobacillus or Lactobacillus-like; 23% to Pediococcus; 20% to Lactococcus or Vagococcus; and 4% to Streptococcus. The antagonist test had observed activity of five isolates against only St. aureus with inhibition zone ranging from 0.58 to 5.16 mm.

Keywords: exploratory test, lab, crude bacteriocins, spoilage, pathogens.

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M. Naimi [α] & M. B. Khaled [σ]

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Keywords: exploratory test, lab, crude bacteriocins, spoilage, pathogens.

I. Introduction

Meat is rich in nutrients, so provided a desired environment for growth for different groups of micro-organisms (Guiraud et al. 1980; Stiles 1994; Bibek et al. 2008). Lactic acid bacteria are part of the initial microbiota, typically mesophilic which can grow easily at 5-45°C, under aerobic, anaerobic or microaerobic terms. These bacteria form a group of diverse genera with *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus* that form the core of the group. However, from a practical food-technology point of view, the following genera are considered the principal: *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus,* and *Weissella*. Lactic acid bacteria; may be characterized as Gram-positive sphere or rod shaped, non-spore-forming, oxidase and catalase negative, do notreduce nitrates to nitrite and sulfate to sulphide, able to produce lactic acid either by Homofermenter or Heterofermenter way; and are associated not only with meat but also with beverages, vegetables and dairy as well normal micobiota of mouth, intestinal and vaginal microbiota of mammals (Carr et al. 2002; Axelsson 2004; Doyle et al. 2006). Since do not pose any health risk to human there are designated as GRAS « Generally Recognized As Safe » organisms (Klaenhammer et al. 2005; Castellano et al. 2008; Dortu et al. 2009; Jeevaratnam et al. 2005). At this time, lactic acid bacteria are exploited as one of three cultures: probiotic, protective or starter (Carr et al. 2002; Castellano et al. 2008; Lücke 2000; Holzapfel 1995; Gálvez et al.2007). Also using theirs antimicrobial end products such bacteriocins as dual anti- and probiotics is well-known. Lactic acid bacteria have been isolated and characterized from meat and meat products (Schillinger, and Lücke 1987; Morishita, and Shiromizu 1986; Samelis et al. 1994; Najjari et al. 2008; Bromberg et al. 2004; Jones et al. 2008; De-Martinis, and Freitas 2003; Al-Allaf et al. 2009; Chaiyana 2007; Castellano et al. 2004). As no universal selective medium exists for the cultivation of all genera, elective media appear for more than one genus and selective media assigned for well-defined genera wile changing pH, addition of inhibitory agents, or used with other temperature-time terms (Reuter 1985), as well as some color indicators (Najjari et al. 2008; Dallbelo et al.). Moreover, the choice of the medium is related to the biotope, and for example, for meat and meat products MRS medium are often used. Therefore, this medium has been recommended for the isolation of "LLPW" group (*Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Weissella*) other secondary genera as *Lactoccucs* and *Streptococcus* can grow over (Reuter 1985; Schillinger and Holzapfel 2003; Carr et al. 2002). Self-evidently, characterization is largely based on morphology, mode of glucose fermentation, lactic acid produce, ability to grow at different temperatures, at high salt concentrations, and acid or alkaline tolerance. These characteristics are a basic and still very important to identify lactic acid bacteria (Axelsson 2004; Doyle et al. 2006). The objective of this study was to isolate and characterize, through phenotypic characteristics, *Lactobacillus* and *Pediococcus* from Algerian meat, in

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order to initiate an isolates collection and to probable use as bioprotective agents for meat products.

II. Material and Methods

a) Sample Meat Collection

Six samples, each one in three units, every unit about one hundred gram measuring five centimeters cub of fresh lamb meat, liver, and small intestine were cut out according to destructive technique using sterile instruments (scalpel, clamp) under aseptic condition (Larpent 1997), from retail stores and butchers in Saida region, (Algeria). Samples were introduced in label sterile bags, immediately transferred in isotherm box at 4°C to the laboratory, being analyzed on arrival.

b) LABMeatIsolation

Samples have been prepared for analysis according to ISO 6887-2, for each sample 25 g of meat cutting into small cubes was aseptically transferred to a sterile stomacher bag homogenized with 225 ml of saline-peptone water (NaCl 8.5 g/l; bactopeptone 1g/l) for 1 min using stomacher (LAB BLINDER © 400) to obtain a 1:10 dilution. Serial dilutions 10^{-1} -10^{-6} were then made directly or after enrichment for 1 day at room temperature, and 100 µl aliquots were spread onto duplicate plates of MRS-BG agar (bromocresol green: 0.0025% (w/v)). Plates were incubated microaerobically at 30°C for 2 days (Najjari et al. 2008; Dallbelo et al.) Bacterial count was performed according to ISO 4833. Colonies were selected from plates on the basis of theirs colors and size. Such colonies were sub cultured differentially on MRS-BG agar and pure isolates were maintained on MRS-BG as slants agar at temperature of 4°C for short-term use. Stock cultures were maintained frozen at -18°C on 20% glycerol (De valdez 2001). Each isolate was propagated twice on MRS broth before use. Overnight culture was employed in the tests. All isolates were initially subjected to macroscopic exams and orientation tests; Gram stain (Chaskes 2009), catalase (Hart and Shears 1997) and endospore (Guiraud et al. 1980).

c) Characterization and Differentiation of LAB Meat Isolates tothe Genus Level

A preliminary identification in order to differentiate isolates at the genus level (Axelsson 2004; Doyle et al. 2006) was carried out using the following tests: CO_2 from glucose, growth at different temperatures, salt tolerance at 6.5-10 % (w/v) and pH tolerance at pH 3.9, 4.4, 9.6. Incubations were made at: 30°C for 3 days, 7-10°C for 7 to 10 days, 15-45°C for 3 to 5 days, 30°C for 2 to 3 days, in the same order (Schillinger and Lücke 1987). Lactic acid production was determined according to NF V.04.206.

d) Antagonism Test

To select antagonists among lactic acid bacteria isolates an antagonism test was achieved based on the agar spot test, according to (Schillinger and Lücke 1989) originally described by Fleming et al. (1975), on TSA-YE medium (Tryptic Soy Agar supplemented with 0.6% Yeast Extract) towards the following pathogens: *B. cereus, B. subtilis ATCC 6633, E. coli ATCC 8739, S. typhimurium ATCC 14028, St. aureus ATCC 6538,* and *P. aeruginosa.* Incubation was carried out at 30°C for 2 days under anaerobic conditions means to reduce lactic acid and hydrogen peroxide effect. Isolates were selected on the basis of positive results showed the presence of clear zone around spots.

e) Characterization of the Selected LAB Meat Isolates to Species Level

A secondly identification at the species level was carried out by both assimilation and production tests: Arginine (Schillinger and Lücke 1987), Nitrate, Urea, and Hydrogen sulfide (Guiraud et al. 1980; Larpent and Gourgaud 1990; Forouhandeh et al. 2010). Incubations were made at: 30°C for 2 days, 30°C for 3 days, and 30°C for 2 weeks, in the same order.Carbohydrate fermentation profile was determined on MRS-BCP (bromocresol purple: 0.017% (w/v)). Sterile solutions of the sugars at 10 % (w/v) were added at final sugar concentration of 2 % (w/v). All strains were tested for fermentation of the following sugars: L-Arabinose, D (+) Glucose, Starch, D (+) Maltose, D (+) Galactose, Saccharose, D Mannitol, L Rhamnose, D (+) Lactose, Esculine, Arabinose, D Fructose, Raffinose, D Xylose, Sorbitol, D Cellubiose, Ducitol. 100 µl aliquots of sterile liquid paraffin were added to ensure anaerobic conditions. Incubations were made at 37°C for 2 days.

III. Results

Thirty-three isolates were pricked from dilutions 10^{-4} and 10^{-5}. In the case without enrichment only eight isolates were pricked from dilutions 10^{-1}. So a total of forty-one bacteria were isolated from different parts of fresh lamb meat, including liver and small intestine. Loads of 2.10^2, 2.10^6 and 3.10^6 UFC / g for directly, after enrichment, liver and small intestine, were taken in. Colonies macroscopic exams show five colors (green, light green with green center, white, white with green center and grey), two forms (punctiform and circular), opaque with smooth surface, of sizes from 1 to 3 mm. The colonies were picked from plates with 100 to 150 total colonies. Thirty isolates were non-spore-forming, catalase negative and Gram-positive (bacilli/coccobacillior cocci, some of them form tetrad). These lactic acid bacteria isolates produce various ratios of lactic acid of 0.74 to 1.26% and were farther characterised at the genus level, most (66.66%) seems to be true psychrotrophic growing at 7°C, as: fifteen mesophilichomofermentative bacilli (*atypical Streptobacteria*), eight among them were able to grow at 10-15°C but not at

45°C, failed to stand in the presence of 10-6.5% NaCl, as well to different pH except 9.6, thus, they appears belong to the genera: *Lactobacillus* and or *Lactobacillu-slike.* While the others were able to grow at 10-15°C but not at 45°C, also in the presence of 10% but not at 6.5% of NaCl, unable to bear different pH except 9.6 other than one isolate, they appears belong to the genus *Lactobacillus*; one thermophilichomofermentative bacilli (*Thermobacteria*) the only able to grow at 45°C but not at 15°C, stand in the presence of 6.5% NaCl and to pH 9.6, such description be like the genus *Lactobacillus*; seven mésophilichomofermentativecocci (*Streptococcus*), six of whom were able to grow at 10 15 °C but not at 45°C, do not with 10-6.5% NaCl and on different pH except 9.6, that to say *Lactococcus* or *Vagococcus*,only one isolate are unable to grow at 10°C appears to be *Streptococcus;* and seven mésophilicho-mofermentativecocci (*Tetracoccus*) able to grow at 10°C except for two, grow all at 15°C but not at 45°C, can't do it at 10-6.5% NaCl, as well to different pH except 9.6, characteristics of genus *Pediococcus.* The antagonist test point out five isolates potency bacteriocinogenic, were antagonistic to Gram-positive target strains: *B. cereus, B. subtilis ATCC 6633* and *St.aureus ATCC 6538* with inhibition diameters ranging from 0.5 to 5.16 mm. On the basis of biochemical tests carried towards their characterization at the spice level; one isolate assumed *Lactobacillus* or *Lactobacilluslike* are arginine positive, urea negative, are neither nitrite nor H_2S producer, ferment weakly esculine, mannitol, D-sorbitol, are negative reaction for L-rhamnose, L-arabinose, raffinose and dulcitol, take these specific characters with *Lactobacillussakei*. Another are arginine and urea negative, are neither nitrite nor H_2S producer, ferment all sugars except for L-rhamnose, L-arabinose and sorbitol, weakly reaction for dulcitol; the three other isolates are Nitrate, H2S, arginine and urea negative, ferment all sugars except for L-rhamnose, L-arabinose, raffinose, sorbitol and dulcitol. These four isolates were *Pediococcus. spp*.

IV. Discussion

Isolation been began with an enrichment so as to increase the initial biomass and to give a better chance to detect lactic acid bacteria. Whereon, total counts are 2.10^2 on fresh lamb meat, at attempt without enrichment, those counts may reflect the exact population of the products at the time of sampling. While, after enrichment counts are ranged from 2.10^6 to 3.10^6 UFC/g on fresh meat, liver and small intestine, respectively. Similar densities from fresh sheep-meat around 10^6 CFU/g were found by Najjari et al. (2008). Liver and small intestine showed the highest bacterial population, these are in agreement with results obtained by Olaoye and Onilude (2009). Five different types of colony were observed on plates, where upon colonies were picked on the basis of theirs colors and size that have the same colony morphology noted by Najjari et al. (2008) and Dallbelo et al. (). Such colonies are: green; light green with green center; white; white with green center and grey. As result, forty-one bacteria were isolated; among them thirty isolates were non-spore-forming, catalase negative and Gram-positive. These results are consistent with the group of genera of lactic acid bacteria (Carr et al. 2002; Schillinger and Holzapfel 2003; Axelsson 2004; Doyle et al. 2006). These isolates displayed various forms: bacilli, coccobacillior cocci, some of them with tetrad formation. As to lactic acid, isomer L/D, and CO_2 production from glucose, such parameters were useful for the characterization (Hayward1957; Axelsson 2004). Thereby, all our isolates converted glucose quantitatively to lactic acid suggesting that belonging to the homofermetative following genera *atypical Streptobacteria*, *Thermo-bacteria*, *Streptococcus* and *Tetracoccus* (Stiles et al. 1997; Axelsson 2004; Doyle et al. 2006), So with a wide prevalence of homofermentative. Similar to that observed by Niemand and Holzapfel (1984) having isolated 67 strains, only two were heterofermentative. In addition, homofermentative lactic acid bacteria are potency to be used for biopreservation of meat (Vermeiren et al. 2004). Titratable acidity shows deferent capacity to produce lactic acid from 0.74 to 1.26%, this may have an effect antagonist, on typical spoilage microbiota mainly Gram-negative bacilli, while decreasing pH (Niemand and Holzapfel 1984). Moreover, (Stiles 1994) noted suitable for use lactobacilli with are aciduric, producing low pH in meats. In fact Inhibitory activity of lactic acid lies in the reduction of pH, and in the action of undissociated acid molecules. Further, (L+) lactic acid is inhibitorier than (D-), since the (D-) isomer is not hydrolyzed by human lactate dehydrogenase and may cause health problems, only strains producing mainly (L+) lactic acid should be selected (Ammor and Mayo 2007). All our isolates except one were able to grow at 15°C most among them were psychrotrophic growing at 7°C. An advantage, since the psychrophilic character is noted as a key in the selection of protective cultures (Vermeiren et al. 2004). Mesophilichomofermentative were *Lactobacillus*, *Lacto-bacilluslike* genus *Carnobacterium*called "atypical meat lactics". This genus resemble lactobacilli but they do not grow on acetate media (Stiles et al. 1997, are unable to grow at 0°C and are arginine positive (Carr et al. 2002). Our *Lactobacillus* and *Lactobacilluslike* were: bacilli/co-ccobacilli; able to grow at pH 9.6 but do not at pH 3.9. Are therefore in agreement with description of *atypicalStreptobacteria*found associated with red meat (Samelis et al. 1994; Carr et al.2002). Growing at pH 9.6, this also was found in previous study (Chaiyana 2007). Such *atypicalStreptobacteria* havebeen found inhibit the growth of *St. aureus*and others undesirable bacteria with bacteriocins (Carr et al. 2002). Bacteriocins can be

used with or without released cultures as food-grad (Hugas 1998; Lücke 2000; Vermeiren et al. 2004; Savadogo et al. 2006; Castellano et al. 2008; Carr et al. 2002). Mésophilichomofermentativecocci were: *Lactococcus* or *Vagococcus*, except one unable to grow at 10°C was *Streptococcus*; those with tetrad formation were *Pediococcus* (Schillinger and Lücke 1987). Antagonism test carried out *in vitro* to asses' isolates potential inhibitor has ended to select five isolatespotency bacteriocinogenic against Gram-positive: *B. cereus, B. subtilis ATCC 6633* and *St.aureus ATCC 6538*, with inhibition diameters ranging from 0.5 to 5.16 mm.. This result isn't wonder; as known the inactivity of bacteriocins against the Gram-negative due to the protective barrier provided by the lipopolysaccharides (Abee et al. 1995; De-Martinis and Freitas 2003; Bromberg et al. 2004) and bacteriocinsare only active against Gram-positive (Dortu et al. 2009). Biochemical characteristics allowed to identify one isolate as *L. sakei*(Schillinger and Lücke 1987;Korkeala and Mäkelä 1989; Carr et al. 2002) this isolate shows properties of the subgroup S6/b include this spice described by Morishita and Shiromizu (1986), therewith weak reaction; fermetingD-xylose, mannitol, sorbitol, esculine; and little growing in 7.5% NaCl. The same identity description of group 4 represented only by *L. sakei*reported by Korkeala and Mäkelä (1989) therewith weak reaction; fermeting D-xylose, sorbitol and little growing in 8% NaCl. Also his pattern agrees with *L. sakei*identified by Samelis et al. (1994) there with fermenting D-xylose and growing in 8 % NaCl. Furthermore, this isolate is saccharose positive, another clean character to *L. sakei* (Carr et al. 2002). So it is of great importance to note that various researchers have used different criteria to describe *a typical Streptobacteria* which sometimes makes it difficult to make a point comparison and to take meaning conclusions (Carr et al. 2002). Others four isolates, were distinctive for tetrad formation, identified at genus as *Pediococcus,* three isolates have common fermenting all sugars except L-rhamnose, L-arabinose, raffinose, dulcitol, while the fourth has ability to ferment all sugars except L-rhamnose, L-arabinose, sorbitol, dulcitol. Due too far differences patterns from those described in the literature, themselves ever changing, sometimes even contradictory, this was mainly attributable to high variability observed in the same species of the genus. It seems at that time not possible to make clear statements about *Pediococcus* sp.

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3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

Segment Draft and Final Research Paper: You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else's analysis.

- Do not give permission to anyone else to "PROOFREAD" your manuscript.

- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)
- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

Criterion for Grading a Research Paper (Compilation) by Global Journals Inc. (US)

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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