The Annals of the University Dunarea de Jos of Galati
Fascicle VI – Food Technology (2013), 37(2), 35-46

ORIGINAL RESEARCH PAPER

TESTING OF THE NEW STREPTOMYCES STRAINS FOR PRODUCTION OF PHENOLOXIDASES

CLAUDIA POPA (UNGUREANU)[1], LIDIA FAVIER[2], GABRIELA BAHRIM[1]

[1] *"Dunarea de Jos" University of Galati, Faculty of Food Science and Engineering, 111 Domneasca Street, 47, 800201, Galati, Romania*

[2] *Ecole Nationale Supérieure de Chimie de Rennes, CNRS, UMR 6226, Université Européenne de Bretagne, General*

[*]Corresponding author: claudia.popa@ugal.ro

Received on 4th September 2013
Revised on 3rd October 2013

Thirty wild new strains of filamentous bacteria belonging to the genus *Streptomyces* isolated from different Romanian soil samples and ten strains from Collection of microorganisms of Bioaliment Research Platform (acronym MIUG) were tested and screened for their ability to produce extracellular tyrosinase and laccase. Based on preliminary qualitative screening assays for the extracellular phenoloxidases production carried out by stationary cultivation on Gause agar medium (GMA) supplemented with 1% (w/w) L-tyrosine, nineteen strains were selected as active producers. Furthermore, a quantitative selection based on active strains ability to produce tyrosinase and laccase by submerged cultivation in liquid Gause salts basal medium supplemented with 1 g L^{-1} L-tyrosine and 0.001 g L^{-1} $CuSO_4$, during 168 hours was performed. Results showed that 70% of the *Streptomyces* strains have a good potential for producing tyrosinase and 30% of strains were remarked for their ability to produce laccase. Moreover, it was observed that, *Streptomyces* strains coded MIUG 4.89 and MIUG 4.88 from MIUG Collection have the ability to simultaneously produce both enzymes. The effect of temperature and pH on enzymes activity was also investigated. The optimum temperature for both activity (tyrosinase and laccase) was found to be 30°C. Laccase was found active over a pH range of 4.0 to 6.0 with maximum activity at pH 5.0. The optimum pH for tyrosinases activity was observed to be around 7.0. The obtained results are important for future applications of *Streptomyces* phenoloxidases in different areas.

Keywords: *Streptomyces*, isolation, screening, extracellular phenoloxidases, tyrosinase, laccase.

Introduction

The genus *Streptomyces* is represented in nature by the largest number of species and varieties among the *Actinomycetaceae* family. They are chemoorganotrophic, and most

are aerobic. Most species are saprotrophic, but a few are pathogenic in plants or animals (Bahrim *et al.,* 2011).

The possibility of *Streptomyce*s spp. strains to produce enzymes is continuously investigated because these filamentous bacteria have a large enzyme production potential, in simple biotechnological conditions, with shorter generation time, due to the ease of bulk production and the ease of genetic and environmental manipulation (Syed *et al.*, 2009).

Phenoloxidases are oxidoreductases that catalyze oxidation of phenolic compounds (Duran and Esposito, 1997). They include tyrosinase and laccase, and both enzymes catalyze oxidation of substrate using molecular oxygen as a terminal electron acceptor with concomitant reduction of oxygen to water, no cofactors are needed (Jolivet *et al.*, 1998; Chevalier *et al.*, 1999) .

Tyrosinases are found in prokaryotic and eukaryotic microbes, in mammals, invertebrates and plants. *Streptomyces* spp. tyrosinases are the most thoroughly characterized enzymes of bacterial origin (Della-Cioppa *et al.*, 1998a and 1998b; Matoba *et al.*, 2006). The first bacterial tyrosinases have been purified from cell extracts of *Streptomyces nigrifaciens* (Nambudiri and Bhat, 1972) and *Streptomyces glaucescens* (Lerch and Ettlinger, 1972).

Among the oxidases, laccases are the most extensively studied group of enzymes. They are widely found in plants and fungi as well as in some bacteria and insects (Mayer and Staples, 2002). Laccases have been isolated and characterized from *Streptomyces cyaneus* (Aria *et al.*, 2003), *Streptomyces griseus* (Endo *et al.*, 2003), *Streptomyces lavendulae* (Suzuki *et al.*, 2003) and *Streptomyces coelicolor* (Machczynski *et al.*, 2004).

Study of new sources for phenoloxidase production are of practical importance because presently there is an increasing interest in using tyrosinase in industrial applications: in the environmental technology, for the detoxification of phenol-containing waste waters and contaminated soils, biosensors for the monitoring of phenols and in cosmetic and food industries. Moreover, tyrosinase is suggested to be a potential tool in the treatment of melanoma. Laccase possesses great biotechnological potential because of its wide reaction capabilities as well as broad substrate specificity. Promising applications include biosensors for drug analysis and phenols in tea, polymer synthesis, textile-dye bleaching, bioremediation, clarification of juices and wines.

The aim of this paper was to study the potential of *Streptomyces* spp. strains newly isolated from soil samples, compared to MIUG Collection strains to produce extracellular tyrosinase and laccase. The optimal range of temperature and pH on the activity of these enzymes were determined, in order to use them in various practical applications.

Materials and methods

Microorganisms

The *Streptomyces* strains used in this study were newly isolated from various soil sources and also provided from the Microbial Cultures Collection (acronym MIUG) of the Bioaliment Research Center, Faculty of Food Science and Engineering of "Dunărea de Jos University", Galati, Romania.

Soil and reagents

Soil samples used for streptomycetes isolation were aseptically collected, from different places located near Galati (Romania), using sterile test tubes and kept at 5°C.

L-tyrosine, catechol and ingredients for culture media formulation of analytical grade were purchased from Sigma-Aldrich.

Isolation of streptomycetes from soil

The soil samples were first pretreated with 1% (w/w) Ca CO_3 and then were incubated for 7 days at 28^0C. Approximately five grams of treated soil samples were suspended in 45 mL sterile water and then stirred vigorously for an efficient homogenization. These samples were then serially diluted (up to 10^{-5}) with sterile water and spread plated on the Gause medium No.1 (GMA) containing (g/L): potato flakes 20; K_2HPO_4 0.5; $MgSO_4$ •$7H_2O$ 0.5; KNO_3 1; NaCl 0.5; $FeSO_4$•$7H_2O$ 0.01; agar, 25. The pH was adjusted to 7.2 prior to sterilization. The plates were incubated for 7 days at 25°C. *Streptomycetes* spp. colonies were examined and taxonomically recognized on the basis of morphological characteristics according to the Bergey's Manual description (Lechevalier, 1989). Pure cultures of *Streptomycetes* isolates were maintained on Gauze slant agar medium at 4°C.

Screening of isolates for phenoloxidases production

The selection on qualitative basis of phenoloxidases producers was realized by spot inoculation and stationary cultivation of pure cultures on GMA medium supplemented with 1g L^{-1} L-tyrosine at 25°C for seven days. Their capacity to produce extracellular melanin pigments around the colonies was investigated. The colonies which formed a brown intense extracellular pigmentation characteristic of phenolic compound transformation based on extracellular phenoloxidase (tyrosinase or laccase) production were selected and then used for the quantitative screening step.

The quantitative selection was performed based on the *Streptomyces* strains ability to produce extracellular phenoloxidases by submerged cultivation. Five ml of inoculum (cells suspension) were inoculated into 250 mL Erlenmeyer flasks containing 100 mL of liquid Gause medium (GLM) containing in (g/L) : potato flakes 20; K_2HPO_4 0.5; $MgSO_4$ •$7H_2O$ 0.5; KNO_3 1; NaCl 0.5; $FeSO_4$•$7H_2O$ 0.01, pH=7. The culture medium was supplemented with 1 g L^{-1} L-tyrosine as inducer and 0.001g L^{-1} $CuSO_4$.

Cultures were incubated on an orbital shaker (Jeio Tech, Korea) at 150 rpm, at temperature of 25ºC for 7 days. Samples were taken at different time intervals to analyze the culture growth dynamics and to measure the extracelullar tyrosinase and laccase activity. Control cultivation in GLM without L-tyrosine and $CuSO_4$ was also done under the same culture conditions. All experiments were carried out in duplicate.

Determination of culture growth and biomass concentration

The dynamics of culture growth was established by measuring the optical density of the cultures medium at 600 nm using JASCO UV/VIS spectrophotometer (Japan). Biomass concentration was monitored as dry weight. It was determined by collecting wet biomass by centrifugation at 12000g, for 10 min. The pellet was washed twice with distilled water. The washed cells suspension was then placed in a pre-weighed aluminum pan and dried at 105°C until constant weight.

Phenoloxidases assay

The culture broth was centrifuged at 1000 rpm for 10 minutes at 4°C and the cell-free supernatant was used as crude enzyme extract. Tyrosinase activity was evaluated based on the principle of tyrosine oxidation to dihydroxyphenylalanine (DOPA), which is further oxidised to o-quinone in the presence of phenoloxydase. The reaction mixture contained 1mL phosphate buffer (0.1 M, pH 6.5), 0.5 mL enzyme extract and 0.5 mL L-tyrosine (0.001mM). The rate of absorbance change is directly proportional to the enzyme concentration and varies linearly during 5-10 minutes, after a lag period. One unit of tyrosinase activity represents the quantity of enzyme that determines change of the absorbance at wavelength (λ) of 280 nm with a rate of 0.001 per minute, at 25°C.

Laccase activity was measured spectrophotometrically using catechol as substrate. The laccase reaction mixture contained 1 mL phosphate buffer (0.1 M, pH 6.5), 0.5 mL catechol (0.001 mM) and 0.5 mL crude enzyme extract. Oxidation of catechol was monitored by determining the increase in absorbance at 410 nm, after 5 min and 25ºC (Maria *et al.*, 1981). One unit of laccase activity was defined as the amount of enzyme oxidizing 1μmoL of catechol per minute in the reaction conditions.

Effect of pH and temperature on enzymes activity

The effect of pH on enzymes activity was studied in phosphate buffer (0.001 mM) over a wide pH range (3.0-8.0). The optimum temperature activity was investigated at optimum pH value, in the range of temperature of 10…60^{0}C for 20 minutes.

Results and discussion

Streptomyces spp. strain isolation and morphological characterization

Thirty indigenous new strains of filamentous bacteria belonging to the streptomycetes group were isolated from different habitats. Macroscopic and microscopic characterization of pure cultures was done by following the directions given in the Bergey's Manual of Systematic Bacteriology. This analysis indicated that the isolated strains belong to the genus *Streptomyces* (Lechevalier, 1989). The morphological characteristics of the newly isolated strains were compared to those of three strains from MIUG Culture Microorganisms Collection, coded MIUG 4.38, MIUG 4.88 and MIUG 4.89. Microscopic observation revealed that all isolates have typical actinomycetes characteristics such as vegetative mycelium and in some cases aerial mycelium and typical spore formation. Some strains formed also a secondary growth zone around the primary colony. The most common aerial mycelium colors were

white, grey and pink. Details of the main morpho-cultural and physiological characteristics of the 30 *Streptomyces* isolates are given in Table 1. Results showed that sixteen of these isolates exhibited brown and light brown substrate mycelium while the others are red, green and yellow. They produced spiral chain spore or rectiflexible (with 2 curves) chain proper; generally with a smooth surface.

Table 1. Morphological characteristics of the colonies of the thirty selected streptomycetes (stationary cultivation on Gause's medium No.1, for 7 days at 25°C). aP (+) sign means positive melanin reaction of the screened isolates text

***Streptomyces* spp. code**	**Isolation site**	**Spore morphology**		**Colour of**		**Extracellular pigmentation[a]**
		Shape	**Surface**	**Aerial mycelium**	**Substrate mycelium**	
MIUG 4.38	Clay soil	Spiral chain	Smooth	Grey	Red	+
MIUG 4.88	Pasture soil	Spiral chain	Smooth	Grey	Brown	+
MIUG 4.89	Pasture soil	Spiral chain	Smooth	White-grey	Brown	+
S1	Garden soil	Spiral chain	Smooth	White	Green	+
S2	Sandy soil	Spiral chain	Rugose	Cream-white	Light brown	+
S3	Garden soil	Rectiflexibles chain	Smooth	White	Light yellow	+
S4	Garden soil	Rectiflexibles chain	Warty	Pink	Light brown	+
S5	Garden soil	Spiral chain	Smooth	White	Light brown	-
S6	Garden soil	Spiral chain	Smooth	White-grey	Light yellow	-
S8	Forest soil	Spiral chain	Smooth	White	Brown	-
S9	Forest soil	Spiral chain	Smooth	Grey	Light brown	-
C1	Garden soil	Rectiflexibles chain	Warty	White	Red	-
C2	Garden soil	Spiral chain	Smooth	White	Green	-
C3	Garden soil	Spiral chain	Smooth	Pink	Light brown	+
C4	Forest soil	Spiral chain	Smooth	Cream-white	Brown	+
V2D	Garden soil	Spiral chain	Smooth	Grey	Brown	-
V3C	Rotten wood	Rectiflexibles chain	Smooth	Grey	Brown	+
V3D	Forest soil	Spiral chain	Rugose	White	Light yellow	+
SB	Sandy soil	Spiral chain	Smooth	Cream-white	Red	+
SC	Pasture soil	Spiral chain	Warty	White	Green	+
SD	Garden soil	Rectiflexibles chain	Spiny	White	Light yellow	+
SE	Pasture soil	Rectiflexibles chain	Rugose	White	Green	-
L1	Sawdust	Spiral chain	Rugose	Grey	Brown	
LP1	Sawdust	Spiral chain	Smooth	Cream-white	Brown	+

LP2	Rotten wood	Rectiflexibles chain	Spiny	White-Grey	Green	+
LP4	Sawdust	Spiral chain	Smooth	Cream-white	Light brown	-
RA	Clay soil	Rectiflexibles chain	Smooth	Pink	Brown	+
RB	Garden soil	Spiral chain	Warty	Red-grey	Brown	+
RC	Pasture soil	Rectiflexibles chain	Smooth	Light-grey	Green	+
RD	Rotten wood	Spiral chain	Warty	Yellow	Light brown	+
RE	Sawdust	Rectiflexibles chain	Rugose	Grey	Green	+
RF	Sawdust	Rectiflexibles chain	Smooth	Red-grey	Brown	-
SNA	Sawdust	Spiral chain	Smooth	White	Brown	+

Screening of phenoloxidases producers

The isolated strains from soil samples and the ones from the collection of microorganisms (MIUG) were then tested and screened for their ability to produce tyrosinase and laccase. Preliminary qualitative screening assays for the enzyme production were carried out in Petri dishes, by observing the extracellular melanin pigments formation on the reverse side of the GMA, during 7 days of stationary cultivation. Only 19 of the studied strains have produced extracellular melanoid pigments on the reverse side of solid medium.

In addition, the preselected strains were investigated for the extracellular phenoloxidase production by cultivation in GML broth supplemented with 1 g L^{-1} L–tyrosine, as inducer and 0.001 g L^{-1} $CuSO_4$. Among all the tested bacterial strains, only 13 strains of *Streptomyces* showed a good potential for tyrosinase production (figure 1) and 8 strains were evidenced for their ability to produce laccase (figure 2).

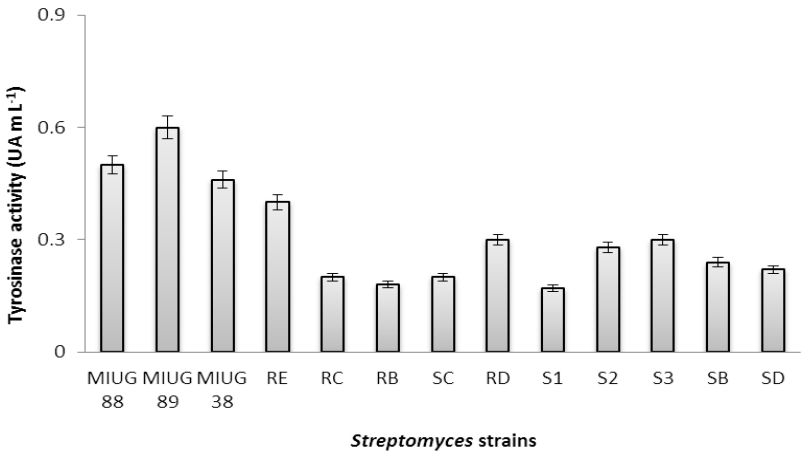

Figure 1. The potential of the selected *Streptomyces* strains to produce tyrosinase

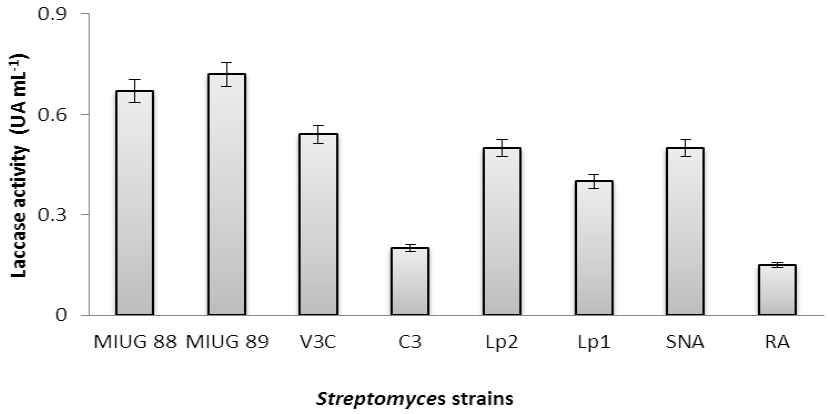

Figure 2. The potential of the selected *Streptomyces* strains to produce laccase

The strains coded MIUG 4.88, MIUG 4.89, MIUG 4.38, RE, SNA, V3C, LP2 showed high yield of enzymes production and were selected for further studies in order to characterize the biotechnological conditions. The *Streptomyces* strains coded MIUG 4.89 and MIUG 4.88 from the MIUG Collection, simultaneously produced both enzymes.

Screening tests applied to 13 strains of *Streptomyces* spp., isolated from different samples of soils withdrawn from East Antarctica, showed that 73 % of the strains have a good potential for producing tyrosinase (Bahrim *et al*., 2004). After 72 h of submerged cultivation in liquid Gauze medium containing 1g L^{-1} L-tyrosine, two strains coded MIUG 4.109 and MIUG 4.113 had a good potential for production of extracellular tyrosinase, 2.0 and 1.2 times higher than the control strain *Streptomyces* MIUG 4.88 (Bahrim and Negoita, 2007).

Effect of pH and temperature on the activity of laccase and tyrosinase

Figure 3 and figure 4 present the effect of pH on the activity of the phenoloxidases produced by the *Streptomyces* strains. The optimum pH values of the laccase were found to be 5.0 and for tyrosinase 7.0, respectively. Increasing or decreasing of pH beyond this value resulted in a decline of the enzyme activity. The information on the effect of pH and temperature on laccase production by *Streptomyces* strains is scarce. Some studies indicated that an initial pH between 4.5 and 6.0 is suitable for enzyme production (Suzuki *et al*., 2003; Arias *et al*., 2003).

Other studies reported an optimal pH value for the purified laccase from *Streptomyces psammoticus* of 8.5. Similar results were obtained by Niladelvi and Prema (2007) for laccase isolated from *Streptomyces psammoticus*. It is important to note that these results were of high importance since the optimum pH of the majority of *Streptomyces* laccases is known to be in the acidic range (Arias *et al.*, 2003; Suzuki *et al.*, 2003).

Cordi *et al.* (2007) observed that the optimum pH for strain L1 (isozyme of laccase) was 4.0 whereas the optimum pH for strain L2 was 5.0. Han *et al.* (2005) extracted laccase from *Trametes versicolor* which showed high enzyme activity at a broad range of pH and temperature, with an optimum activity at pH 3.0. Laccase extracted from *Stereum ostrea* showed highest activity at pH 6.0 (Valeriano *et al.*, 2009).

Streptomyces tyrosinases have their pH optima in the neutral and slightly acidic conditions. Lerch *et al.* (1972) showed that the optimum pH for tyrosinase from a *Streptomyces glaucescens* strain was 6.8. Thomas *et al.* (1990) also found that tyrosinase from *Streptomyces Michiganensis DSM 40015* was active over a pH range of 4 to 7 with a maximum activity at pH 7.0. Similar results were obtained by Raval *et al.* (2012) for tyrosinase from *Penicillium jensenii*.

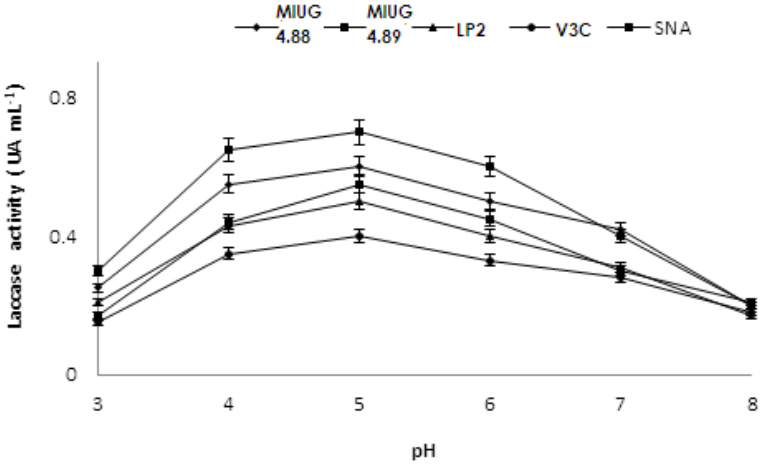

Figure 3. Effect of pH on laccase activity by *Streptomyces* strains

The influence of temperature on the phenoloxidases activity produced by *Streptomyces* spp. was studied by incubating the reaction mixture at different temperatures, ranging from 10 to 60° C and pH 5.0 for laccase and pH 7.0 for tyrosinase, for 20 minutes. Maximum activity values for phenoloxidases production were obtained at 30°C, as showed in figure 5 and figure 6. Previous studies reported that the optimum temperature range for laccase production is between 25°C and 30°C (Pointing *et al.*, 2000). For example, the maximum laccases activity of *Streptomyces Griseus MTCC 4734* was recorded at 30°C (Sampoorna *et al.*, 2009). Kizhokkedalte *et al.* (2007) showed that for *Streptomyces psammoticus* the optimum temperature for laccases production was 45°C.

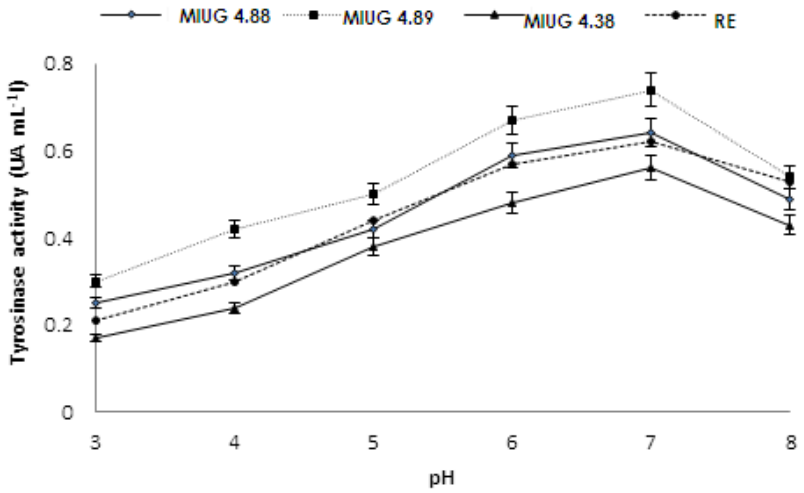

Figure 4. Effect of pH on tyrosinase activity by *Streptomyces* strains

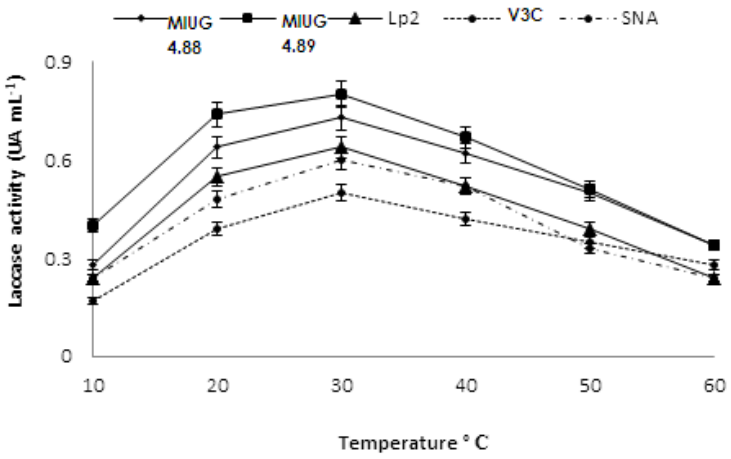

Figure 5. Effect of temperature on laccase activity by *Streptomyces* strains

Suzuki *et al.* (2003) reported that laccase from *Streptomyces lavendulae* has been thermostable, being stable at 70 °C. Farnet *et al.* (2000) found that pre-incubation of enzymes at 40°C and 50°C greatly increased laccase activity. The *P. ostreatus* laccase is almost fully active in the temperature range of 40-60°C, with maximum activity at 50°C. This activity remains unaltered after prolonged incubation (more than 4 h) at 40°C (Palmieri *et al.*, 1993). Nyanhongo *et al.* (2002) showed that laccase produced by *T. modesta* was fully active at 50°C and was very stable at 40°C but half-life decreased to 120 min at higher temperature (60°C).

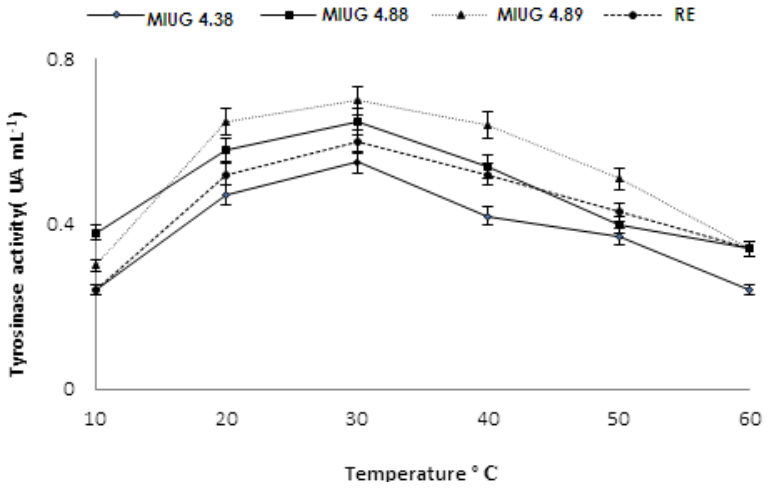

Figure 6. Effect of temperature on tyrosinase activity by *Streptomyces* strains

Tyrosinases are in general reported not to be very thermostable enzymes. The optimum temperature value for laccase production is 30°C. A similar result was obtained by Thomas *et al.* (1990) and Lerch *et al.* (1972) for tyrosinases production by *Streptomyces Michiganensis DSM 40015* and *Streptomyces glaucescens*. Raval *et al.*, (2012) reported an optimal temperature for tyrosinase activity from *Penicillium jensenii* at 30°C.

Conclusion

Sixteen strains belonging to the genus *Streptomyces* newly isolated from soil and three strains belonging to the Microbial Culture Collection (MIUG) were screened based on their ability to produce phenoloxidases.

The effect of temperature and pH was found to be important parameters that influenced phenoloxidases activity. The optimum temperature for tyrosinases and laccase produced by studied *Streptomyces* spp. was found to be 30°C. Laccase and tyrosinase were found to be active over a pH range of 4.0 to 6.0 with maximum activity at pH 5.0 and pH 7.0, respectively.

Acknowledgments

This work has benefited from financial support through the Project SOP HRD-TOP ACADEMIC 107/1.5/S-ID 76822.

References

Arai, T., Mikami, Y. 1972. Choromogenecity of *Streptomyces*. *Applied Microbiology*, **23**, 402-406.

Arias, M.E., Arenas, M., Rodriquez, J., Soliveri, J., Ball, A.S., Hernandez, M. 2003. Kraft pulp biobleaching and mediated oxidation of a non-phenolic substrate. Substrate by laccase from *Streptomyces cyaneus* CECT3335. *Applied Environmental Microbiology*, **69**, 1953-1958.

Bahrim, G., Negoita, T.G, Minghong, C. 2004. Preliminary screening to put into evidence the potential to produce tyrosinase of some *Streptomyces* sp. strains isolated from east Antarctic soils. XXVIII SCAR Open Science Conference, Bremen Germany, Abstract www.scar28.org/ SCAR/SCARmeeting/ Wednesday/PDF/ S_01_poster.pdf.

Bahrim, G., Negoita, T. 2007. *Streptomyces* strains from east antarctic soils as tyrosinase producers. VI Argentine and III Latin-american symposium on antarctic research. http://www.dna.gov.ar/CIENCIA/SANTAR07/CD/PDF/CVRE409.PDF.

Bahrim, G., Coman G., Cotarlet M., Popa C. 2011. Enzy-*Streptomycete*s As Valuable Bioingredients And Biopreservatives, pp. 52-89. In: Anima Sharma, Harhik Pathak (eds.), *Microbial Technology "The Emerging Era*", Lap Lambert Academic Publishing, Germany.

Bento, I., Arménia Carrondo, M., Lindley, P.F. 2006. Reduction of dioxygen by enzymes containing copper. *Journal of Biological Inorganic Chemistry*, **11**, 539-547.

Chevalier, T., Rigal, D., Mbeguie-A-Mbeguie, D., Gauillard, F., Richard-Forget, F., Fils-Lycaon, B.R .1999. Molecular cloning and characterization of apricot fruit polyphenol oxidase. *Plant Physiology* **119**, 1261-1269.

Cordi, L., Minussi, R.C., Freire, R.S., Duran, N. 2007. Fungal laccase: copper induction, semi-purification, immobilization, phenolic effluent treatment and electrochemical measurement. *African Journal of Biotechnology*, **6**, 1255-1259.

Della-Cioppa, G., Garger, S.J., Holtz, R.B., McCulloch, M.J., Sverlow, G.G. 1998a. Method for making stable extracellular tyrosinase and synthesis of polyphenolic polymers therefrom. *US Patent* 5801047.

Della-Cioppa, G., Garger, S.J., Sverlow, G.G., Turpen, T.H., Grill, L.K., Chedekal M.R. 1998b. Melanin production by Streptomyces. *US Patent* 5814495.

Duran, N., Esposito, E. 1997. Lignin biodegradation and effluent treatment by ligninolytic fungi, pp. 269. In: De Melo, I.S., Acevedo, J.L. (eds.), *Microbiologia Ambiental*, CNPMA/ EMBRAPA Publishers, S.P., Brazil.

Han, M.J., Choi, H.T., Song, H.G. 2005. Purification and characterization of laccase from the white 469 rot fungus *Trametes versicolor*. *Journal of Microbiology Method*, **43**, 555-560.

Jolivet, S., Arpin, N., Wichers, H.J., Pellon G. 1998, *Agaricus bisporus* browning: a review. *Mycological Research*, **102**, 1459-1483.

Lechevalier, H. 1989. A practical guide to generic identification of actinomycetes, pp. 2344-2347. In: S.T. Williams (eds.) Bergey's manual of systematic bacteriology, Williams and Wilkins, Baltimore.

Lerch, K., Ettlinger, L. 1972. Purification of a tyrosinase from *Streptomyces glaucescens*. European Journal of Biochemistry. **31**, 427–437.

Machczynski, M.C., Vijgenboom, E.,Samyn,B.,Canters, G.W. 2004. Characterization of SLAC: a small laccase from *Streptomyces coelicolor* with unprecedented activity. *Protein Science*, **13**, 2388–2397.

Matoba, Y., Kumagai, T., Yamamoto, A., Yoshitsu, H., Sugiyama, M. 2006. Crystallographic evidence that the dinuclear copper center of tyrosinase is flexible during catalysis. *Journal of Biological Chemistry*, **281**, 8981–8990.

Mayer, A.M., Harel, E. 1978. Polyphenol oxidases in plants. Phytochemistry 18, 193–215.

Mayer, A.M, and Staples, R.C. 2002. Laccase: New functions for an old enzyme. *Phytochemistry*, **60**, 551-565.

Nambudiri, A.M.D., Bhat, J.V. 1972. Conversion of p-cumarate into caffeate by *Streptomyces nigrifaciens*. *Biochemical Journal*, **130**,425-433.

Niladevi, K..N, Prema P. 2005. Mangrove actinomycetes as the source of ligninolytic enzymes. *Actinomycetologica*, **19**, 40–47.

Nyanhongo, G.S., Gomes, J., Gubitz, G., Zvauya, R., Read, J.S., Steiner, W. 2002. Production of laccase by a newly isolated strain of Trametes modesta. *Bioresource Technology*, **84**, 259-263.

Palmieri, G., Giardina, P., Marzullo, L., Desiderio, B., Nitti, G., Cannio, R., Sannia, G.1993. Stability and activity of a phenol oxidase from the ligninolytic fungus *Pleurotus ostreatus*. *Applied Microbiology Biotechnology*, **39**, 632-636.

Suzuki, T., Endo, K., Ito, M., Tsujibo, H.,Miyamoto, K., Inamori, Y.A. 2003. Thermostable laccase from *Streptomyces lavendulae REN-7*: purification, characterization, nucleotide sequence and expression. *Bioscience, Biotechnology and Biochemistry*, **67**, 2167–2175.

Syed, D.G., D., Agasar, A., Pandey. 2009. Production and partial purification of α- amylase from a novel isolate *Streptomyces gulbargensis*. *Journal of Industrial Microbiololgy and Biotechnology*, **36**,189–194.

Thomas, B.R., Yonekura, M., Morgan, T.D., Czapla, T.H., Hopkins, T.L., Kramer, K.J. 1989. A 625 trypsin-solubilized laccase from pharate pupal integument of the tobacco hornworm, *Manduca sexta 626*. *Insect Biochemistry*, **19**, 611-622.

Thurston, C.F. 1994.The structure and function of fungal laccases. *Microbiology*, **140**, 19-26.

Valeriano, V.S. Silva, A.M.F. Santiago, M.F. Bara, M.T.F. Telma, A.G. 2009. Production of laccase by *Pynocorpus sanguineus* using 2, 5- xylidine and ethanol. *Brazilian Journal of Microbiology,* **40**, 790-794.