Journal of Toxicology and Environmental Health Sciences Vol. 3(11), pp. 311-316, 5 October, 2011
Available online at http://www.academicjournals.org/JTEHS
ISSN 2006-9820 ©2011 Academic Journals

Full Length Research Paper

Lipase production by strains of *Aspergillus* species isolated from contaminated body creams

Brooks A. A.[1]* and Asamudo N. U.[2]

[1]Department of Microbiology, University of Calabar, Nigeria.
[2]Department of Microbiology, University of Uyo, Nigeria.

Accepted 12 August, 2011

Aspergillus **species isolated from contaminated body creams were screened for their ability to produce extracellular lipase. Of the 6 strains tested two, identified as *Aspergillus niger* AC-5 and AC-7 and one strain of *Aspergillus fumigatus*. AF-3 were capable of producing extracellular lipase in appreciable amounts. *A. niger* AC-5 was the highest lipase producer, followed by *A. fumigatus* AF-3, while *Aspergillus flavus* showed the least potential for lipase production. The maximal lipase production (14.4 µ/ml) was produced by strain AC-5 in 6 days culture supplied with 3% ($^{v}/_{v}$) olive oil as the carbon source. The optimum pH and temperature for the crude lipase activity were 6.5 and 40°C respectively, for strains tested. The enzyme was stable over a wide range of pH and temperature. The enzymes retained certain levels of their original activities when assayed at 60°C for 1 h. Body creams contaminated with these lipolytic moulds produced off-odours in addition to other physico-chemical changes such as discolouration, formation of tints and scum, emulsion breakage and changes in pH. This is a source of concern and calls for urgent amendment in the existing formulation protocols and strict adoption of good manufacturing practice to ensure microbiologically stable product.**

Key words: Lipase, body cream, lipolytic molds, physico-chemical changes.

INTRODUCTION

The demand for microbial lipases is on the increase owing to their applications in a wide variety of industrial processes. Lipases are very useful in such industries as food, dairy, pharmaceutical and cosmetic, owing to their hydrolytic reactions. Hence they have gained prominence in the area of organic synthesis. The enantioselective and regioselective properties of lipases have necessitated their utilization for fat modifications and for synthesis of cocoa butter substituents, emulsifiers, moisturizing agents, body care products and flavor enhancers. Because of these biocatalytic potentials of lipases, many cosmetic industries and other industrial concerns have shifted their choice of catalysts to utilizing this enzyme for various catalytic reactions.

However, owing to scarce foreign exchange and the depleting sources of suitable substrate for lipase production, the cost of importing this enzyme into the country is very high and this has contributed to the high cost of production and of the final products. Therefore there is need to explore other locally available sources for isolating microorganisms used for the production of this important enzyme.

Lipases or triacylglycerol hydrolase, Ec 3.1.1.3 are enzymes that catalyze the hydrolysis of glycerol ester bond at fat - water interface (Hosseinpour et al., 2011). In anhydrous water immiscible organic solvents they are also able to catalyze the reverse reactions of synthesis and group exchange of esters and the resolution of racemic mixture into optically active alcohols or acids (Gupta et al., 2004). These properties determine the microbiological importance of lipases.

Body creams are esterified products of triglycerides and phospholipids from various vegetable oils, animal and natural fats (Behravan et al., 2005). They also contain a wide variety of other ingredients such as humectants, colouring agents, emollients and fragrances in complex physico-chemical state, which help to make the product efficacious and sufficiently elegant to be acceptable to users. Some of these ingredients may serve as good sources of nutrient, carbons and energy for the growth and proliferation of molds in the product. Such growth may result in contamination and spoilage of

*Corresponding author. E-mail: brooks.akan28@yahoo.com.

the body creams (Brooks and Antai, 2006). Economically, contaminated body creams result in wastage either as immediate loss of the products or as an increasing cost of litigation should the manufacturers are sued for damages suffered by the users. However, rather than discarding the contaminated body creams, as is commonly done in manufacturing industries and cosmetic shops, these contaminated products may be utilized as good sources of isolating molds which can be employed for the production of industrially important lipase.

The purpose of this study therefore is to utilize the contaminated body creams as source for isolation of various strains of *Aspergillus* spp. for the production of extracellular lipase.

MATERIALS AND METHODS

Source of body creams

A total of 42 samples of 8 types of body creams which are produced in Nigeria, were purchased from cosmetic shops in Akwa Ibom State, Nigeria. All the creams were unexpired with properly fitted covers and flaps.

Preparation of samples for the isolation of *Aspergillus* species

The sample containers were properly disinfected with aqueous mixture of 70% ethanol ($^{v}/_{v}$) and 1% HCl ($^{v}/_{v}$) before they were opened to remove the contents. The total contents were then mixed by shaking manually for 5 min. Only representative portions of the contents were used for the microbial analysis.

One gram of the respective samples was aseptically weighed into different sterile screw capped tubes containing 3.0 ml of sterile Tween 80 plus six 5 mm glass beads. The total volume was finally made up to 10.0 ml with sterile Ringer solution ($^{1}/_{4}$ strength).

To isolate *Aspergillus* spp., serial dilutions up to 10^{-6} was carried out using Ringer solution and 0.1 ml of the last two dilutions were transferred to pre-labeled duplicate plates and mixed with pre-cooled molten Sabouraud dextrose agar (SDA) containing 40 mg of Chlortetracycline. The plates were gently swirled to mix properly and then allowed to set. Thereafter the plates were inverted and incubated at 28°C for up to 7 days.

At the end of the incubation period, the mold cultures were purified by repeated sub-culturing. Pure cultures were preserved on SDA slants and identified using cultural and morphological characteristics described in Barnett and Hunter (1972) manual for fungi taxonomy. Colonies identified as *Aspergillus* spp. were selected for further studies.

Screening of *Aspergillus* spp. for lipase production

Screening was carried out using mineral medium described by Macedo et al. (1997) with olive oil emulsion as the carbon source. Strains that showed hydrolytic halos on this medium were selected for further lipase determination.

Extraction of crude lipase

The production of crude lipase was studied in 250 ml Erlenmeyer flask containing 50.0 ml of mineral medium with olive oil emulsion as carbon source. The medium composed of KNO_3, 3.0 g; KH_2PHO_4. 1.0 g $MgSO_4$ $7H_2O$, 1.0 g; sterile olive oil, 2% ($^{v}/_{v}$). The growth medium was inoculated with the mycelia of the test organisms and incubated at 28°C on a rotary shaker at 120 rpm for up to 7 days. At the end of incubation period, the mycelia mats were aseptically skimmed off and further separation of spores and mycelia fragments was achieved by suction filtration through Whatman No. 1 filter paper contained in a Buchner funnel using Compton vacuum pump model D/35/VM. The resulting clear culture filtrates were assayed for lipase activity.

Assay of lipase activity

Lipase activity was assayed using the method of Yadav et al. (1993). This involved incubating a reaction mixture containing 5.0 ml of olive oil emulsion, 20 ml of 0.1 M phosphate buffer (pH 6.5) and 1.0 ml of the culture filtrate (lipase crude extract) at 40°C for 30 min with shaking at 130 rpm. After incubation, the reaction was stopped by adding 15.0 ml of acetone-ethanol (1:1) mixture to liberate free fatty acid. The mixture (that is, free fatty acid solution) was titrated with 0.05 N NaOH in the presence of phenolphthalein as indicator. All the tests were conducted in triplicates and mean activities determined. One unit of lipase activity was defined as the amount of crude enzyme which liberated 1 µ/ml of fatty acid per minute.

Effect of pH on lipase activity and stability

The optimum pH for lipase activity was determined by assaying the enzyme at various pH 5.0 to 9.0 on phosphate buffer. Lipase stability was studied by incubating the enzyme at the above stated pH regimes for 1 h at room temperature.

Effect of temperature on lipase activity and stability

The effect of reaction temperature on the activity and stability of lipase were determined at temperatures ranging from 30 to 70°C. Thermal stability was investigated by incubating the crude enzymes at 30, 40, 50, 60 and 70°C for 1 h. Immediately after incubation at each temperature, the enzyme was immersed in an ice bath for 30 min and then the activity was tested under standard conditions.

Effect of substrate concentration on lipase activity

The Effect of substrate concentration on lipase activity was determined using different concentrations of olive oil ranging from 1 to 4 %, according to the method of Hosseinpour et al. (2011).

RESULTS

A total of six strains of *Aspergillus* spp. were isolated from contaminated body creams based on hydrolytic halos on lipid medium which indicated the potential for the production of lipase. However, only two strains *Aspergillus niger* (AC-5 and AC-7) and one strain of *Aspergillus fumigatus* (AF-3) were found to exhibit high lipase production potential on the lipid medium. *A. niger* AC-5 was the highest lipase producer while *A. flavus* showed the least potential for lipase production.

Figure 1 shows the time course of lipase production in relation to incubation period. The highest lipase

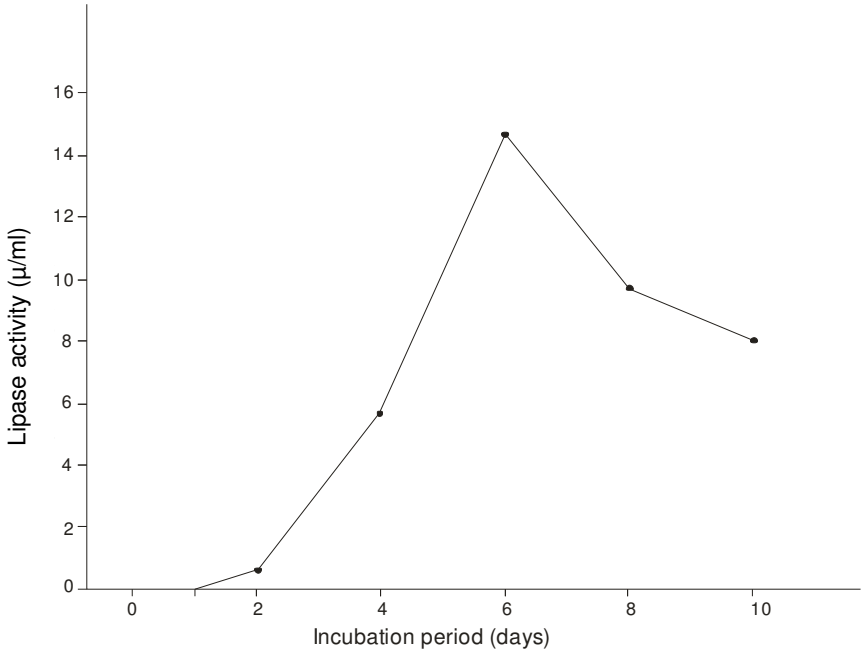

Figure 1. Time course of lipase activity of *A. niger* in relation to incubation period.

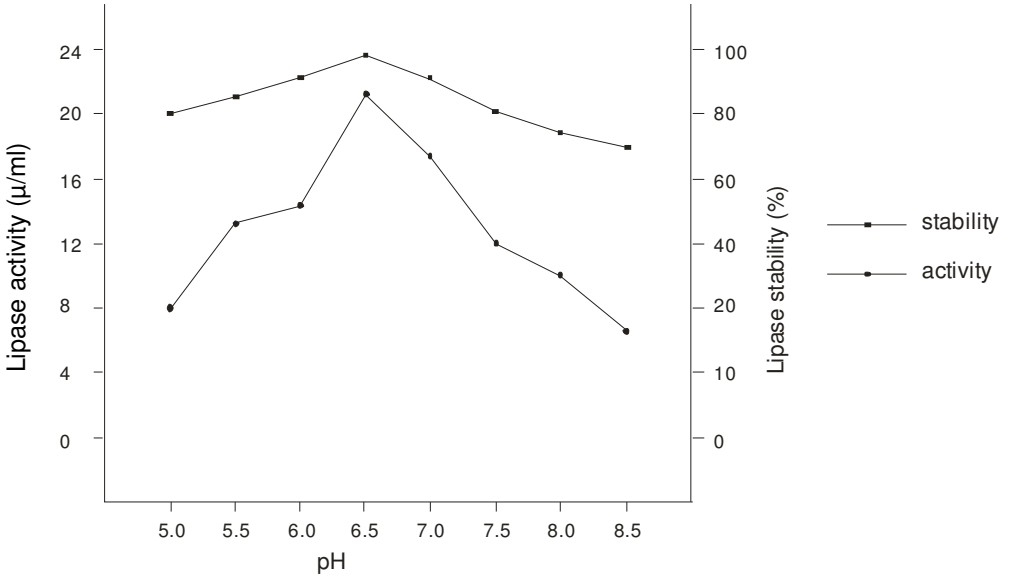

Figure 2. Effect of pH on activity and stability of lipase from *A. niger*.

production was reached on the 6th day of incubation. During this time, the lipase production by *A. niger* AC-5, AC-7 and *A. fumigatus* AF-3 were 14.4, 10.2 and 8.6 µ/ml respectively.

The results of the effects of pH on lipase activity and stability are shown in Figure 2. Crude lipase from *A. niger* AC-5, AC-7 and *A. fumigatus* AF-3 were most active at pH 6.5. The enzyme from *A. niger* AC-5 was stable at pH range 5.0 to 8.0; AC-7 and AF-3 lipases were stable at pH range 6.0 to 9.0 and 5.0 to 10.0 respectively, at room temperature for 1 h. The effect of reaction temperatures on the enzyme activity and stability are depicted in Figure 3. The optimum temperature for lipase activity was 40°C for AC-5, 45°C for AC-7 and 35°C for AF-3. Extracellular

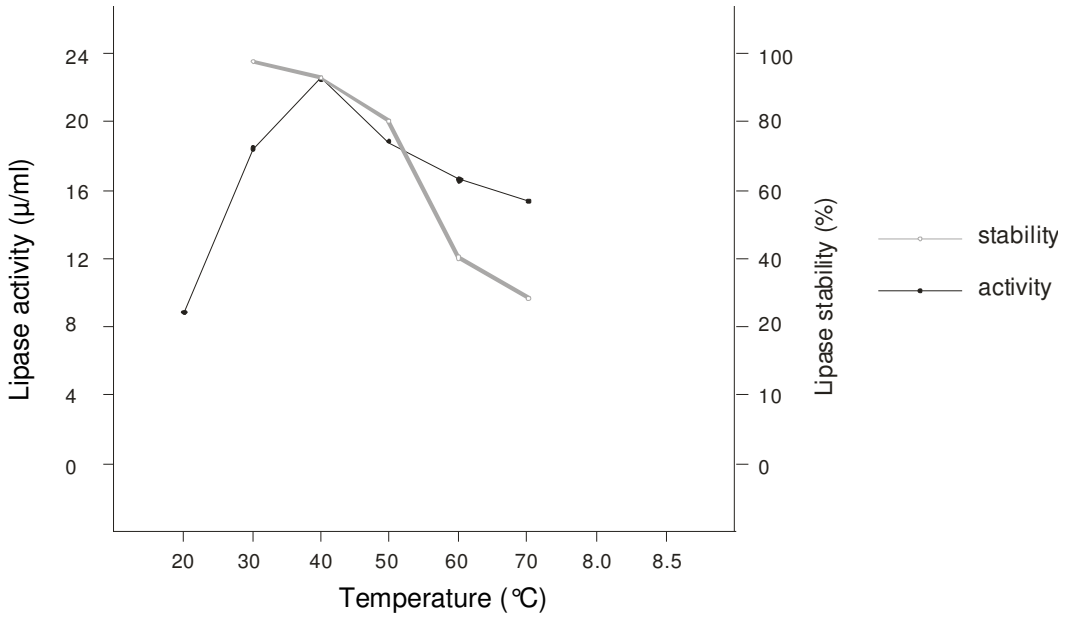

Figure 3. Effect of reaction temperature on activity and stability of lipase from *A. niger*.

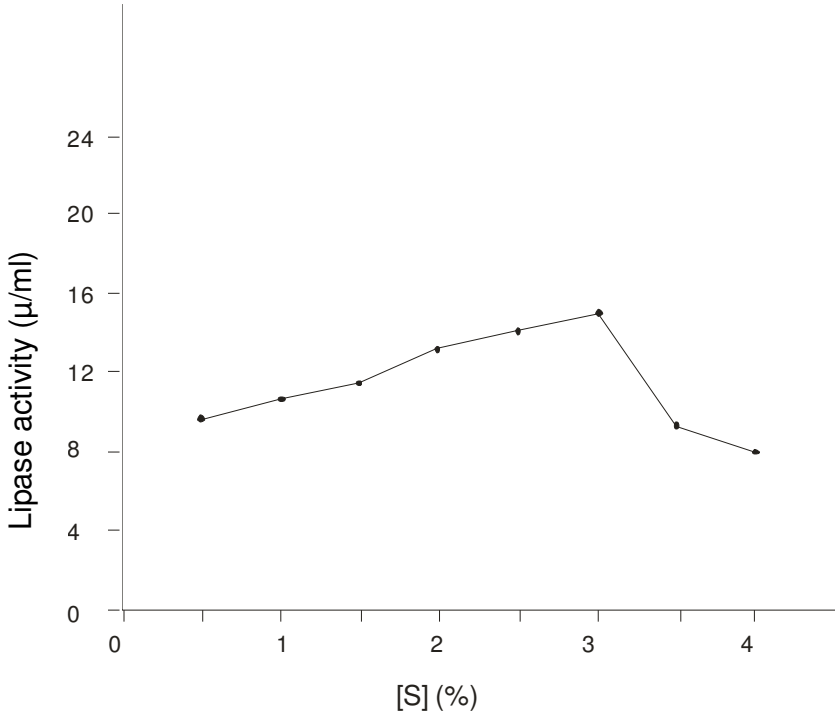

Figure 4. Effect of substrate concentration on activity and stability of lipase from *A. niger*.

lipase from AC-5 retained 65 and 58% of the original activity after 60 min at 45, 50 and 60°C respectively. Similar trend of reduction in activity was observed in AC-7 and AF-3 at higher temperatures. Figure 4 shows the influence of substrate concentration [S] on the enzyme activity. All the lipases from the three strains exhibited maximal activities at the [S] of 3% olive oil. At [S] above this value, the activities began to decline.

DISCUSSION

The ability of different strains of *Aspergillus* spp. which were isolated as contaminants in body creams, to excrete lipolytic enzyme has been demonstrated in this investigation. The elaboration of extracellular lipase by *Aspergillus* spp. isolated from contaminated body creams is particularly interesting because it indicates the possibility of utilizing contaminated/spoiled body creams as sources for isolating lipase - producing microorganisms, instead of discarding the products as being practiced in many manufacturing companies and cosmetic shops. This is a good step toward employing these contaminated products as substrates for large scale cultivation of molds for industrial production of lipase.

Some of the properties which enable lipase from various mold sources to be commercially exploited include thermal stability, pH stability, substrate specificity and activity in organic solvents (Costa and Peralta, 1999). In this study, lipase from the three strains of *Aspergillus* spp. were stable at 40, 45 and 50°C. A similar observation was made by Ghosh et al. (1996), who reported that lipase produced by *A. niger* and some bacteria were stable in solution at 50°C. Lipase from *A. niger* AC-5 retained 65, 58 and 54% of original activity at 45, 50 and 60°C respectively. Although Costa and Peralta (1999), reported that majority of lipases from plant and animal sources lost completely their activity at temperature above 40°C, microbial lipases have temperature optima in the range of 30 to 60°C (Lithauer et al., 2002). Reports exist on microbial lipases with optima in both lower and higher ranges (Sunna et al., 2002; Lee et al., 1999). A few microbial lipases have been reported which are stable at 100°C or even beyond to 150°C with a half – life of a few seconds (Rathi et al., 2001). A highly thermotolerant lipase has been reported from *Bacillus stearothermophilus,* with a half life of 15 – 25 min. at 100°C (Bradoo et al., 1999). From the above assertions therefore, it could be inferred that microbial lipases generally are more thermostable than similar enzyme from plant and animal sources.

In this study the highest level of lipase activity (14.4 µ/ml) was obtained at 3% olive oil. The effect of different concentrations of olive oil on lipase activity has been investigated by several workers. Hosseinpour et al. (2011) investigated the effect of several concentrations of olive oil on lipase activity by *A. niger* NCTM 584. They obtained the best lipase activity (4455.88 µ/L) at the concentration of 12 g/L. Shah et al. (2007) reported the optimum concentration of olive oil for lipase production by *Bacillus* sp. as 1.0%. An optimum lipase activity from *A. niger* NNRL3 was recorded by Adham and Ahmed (2009) at 2.0% olive oil concentration, while Contesini et al. *(*2009) reported that the best lipase activity from *A. niger* AC-54 in a solid state fermentation was obtain with 1.6%w/v. From the above reports including that from this study it can be deduced that the presence of olive oil as carbon source is essential for the growth of lipolytic molds and as inducer for the production of lipase. The differences in the concentrations of olive oil required for optimal activities depends on the microbial species employed.

The exposure of the enzymes to different pH regimes gave stability at pH range of 5.0 to 8.0. This is confirmed by the similarity depicted by the pH curve for the three mold lipases. The stability of these extracellular lipases over a wide range of temperature and pH regimes is very useful for the body creams, because this property will enhance the stability of the products over a wide range of manufacturers' and consumers' storage conditions. Based on the findings made in this work, it can be concluded that contaminated body creams can be exploited as an alternative source of isolating lipolytic molds which can elaborate extracellular lipases with efficient catabolic effect on the fatty acid and phospholipid components of the body creams and varying capabilities of stabilizing the products over a wide range of environmental conditions.

ACKNOWLEDGEMENT

The authors are grateful to the authority of the University of Calabar for providing grant and facilities for this study.

REFERENCES

Adham N, Ahmed E (2009). Extracellular lipase of *Aspergillus niger* NRRLS; production, partial purification and properties. Indian J. Microbiol., 49(1): 77-83.

Barnett HL, Hunter BB (1972). Illustrated Genera of Imperfect Fungi, 3rd Ed. Mineapolis: Burgress Publishing Company, pp. 4-8.

Behravan J, Bazzaz F, Malaekeh P (2005). Survey of bacteriological contamination of cosmetic creams in Iran (2000). Int. J. Dermatol., 44(6): 482-485.

Bradoo S, Saxena RK, Gupta R (1999). Two acidothermotolerant lipases from new variants of *Bacillus* spp. World J .Microbiol. Biotechnol., 15: 87-91.

Brooks AA, Antai SP (2006). Bacteriological status of some cosmetic products in three states of Southern Nigeria. Nig. J. Microbiol,. 20(3): 1345-1354.

Contesini F, Da Silva V, Maciel R, De Lima R, Barros F, De Oliveira CP (2009). Response surface analysis for the production of an enantioselective lipase from *Aspergillus niger* by solid–state fermentation. J. Microbiol., 47(5): 563- 571.

Costa MAF, Peralta RM (1999). Production of lipase by soil fungi and partial characterization of lipase from a selected strain *(Penicillium wortmanii).* J. Bas. Microbiol., 39(1): 11-15.

Ghosh PK, Saxena RK, Gupta R, Yadav RP, Davidson S (1996). Microbial lipases: Production and applications. Sci. Prog., 79: 119-157.

Gupta R, Gupta N, Rathi P (2004). Bacterial lipases: An overview of production, purification and biochemical properties. Appl. Microbiol. Biotechnol. 64: 763-781.

Hosseinpour MN, Najafpour GD, Younesi H, Khorrami M (2011). Submerged culture studies for lipase production by *Aspergillus niger* NCIM 584 on soya flour. Middle East J. Sc. Res., 7(3): 362-366.

Lee OW, Koh YS, Kim BC, Choi HJ, Kim DS, Suhartono MI, Pyun YR (1999). Isolation and characterization of a thermophilic lipase from

Bacillus thermoleovorans ID-1. FEMS Microbiol. Lett. 179: 393-400.
Lithauer D, Ginster A, Skein E (2002). *Pseudomonas luteola lipase*: a new member of the 320 – residue *Pseudomonas* lipase family. Enz. Microbiol. Technol., 30: 209-215.
Macedo GA, Park YK, Pastore GM (1997). Partial purification and characterization of an extracellular lipase from a newly isolated strain of *Geotrichum* sp. Microbiol. Rev., 28: 90-95.
Rathi P, Saxena RK, Gupta R (2001). A novel alkaline lipase from *Burkholderia cepacia.* J. Appl. Microbiol., 93: 930-936.
Shah KK, Patel PM, Bhatt SA (2007). Lipase production by *Bacillus* sp. under different physio-chemical conditions. J. Microbiol., 47(5): 563-571.
Sunna A, Hunter L, Hutton CA, Berquist PL (2002). Biochemical characterization of a recombinant thermoalkalophillic lipase and assessment of its substrate enantioselectivity. Enz. Microbiol. Technol., 31: 472-476.
Yadav RP, Saxena RK, Gupta R, Davidson S (1993). Lipase production by *Aspergillus* and *Penicillium* species. Int. J. Food Microbiol., 19(3): 217-227.