International Journal of Molecular and Clinical Microbiology 1 (2013) 248-254

International Journal of
Molecular and Clinical Microbiology

A comparative investigation on zearalenone toxin production patterns during mycoprotein production by *Fusarium solani* using carbon and nitrogen resources

Fereshteh Jafarian[1] (Ms.c), Arash Chaichi Nosrati[1] (Ph.D), Leila Modiri[1] (Ph.D), Mohammad Faezi Ghasemi[1](Ph.D)

1Division Microbiology, Department of Molecular and Cell Biology, Faculty of Basic Sciences, Lahijan branch, Islamic Azad University (IAU), Lahijan, Gilan, I.R Iran,

ARTICLE INFO

Article history:
Received 21 February 2013
Accepted 7 April 2013
Available online 1 June 2013

Keywords:
Zearalenone toxin,
Mycoprotein,
Fusarium solani

ABSTRACT

Fusarium is a fungus that is commonly found as saprophytes with parasitic life on some living organisms. Mycoprotein or fungus proteins with physical and chemical features are known as food in recent years. When they are involved in temperature and wet stress, release materials such as mycotoxins or fungus toxins remained in food. Zearalenone and dependent compounds have distinct role in many fungus toxicity and beings especially despite acute toxicity. Also the global allowed rate of zearalenone in foods is 30-1000 nano gr/ppb. The submerged culture environment to produce fungus biomass was Vogel basic medium.Then, the produced fungus biomasses were harvested at 4500rpm for about 20 minutes by centrifugation, washed and rinsed twice and desiccated overnight in room temperature. The dry weight biomass was measured and used in order to measure protein rate extracts for toxin estimating based on producer instructions. Then, 50ml substrate was added and after 5 minutes stopper enzyme was added. Finally, the zearalenone toxin amount was measured by ELISA reader system. The results show that after nitrogen resource, the carbon resource play the second role in production and increase of toxin rate in mycoprotein biomass extracts. Verifying the optimum submerged environment in which all optimal conditions of each steps were applied (the first priority of optimal environment include 0.25% of Starch originated carbon resource and 0.25% of Urea originated nitrogen resource) showed the rate of protein production was 0.642% that is have more increased about 0.207% in relation to basic submerged environment. In this environment the amount of zearalenone toxin was 0.99 ppb/gr. In the other way, when we use 2.64% of Rice bran as replacement carbon resource, using 0.75% of Urea in compare with 0.50% of Soy bean peptone as nitrogen resources we could observe an increase of toxin rate in biomass about 0.66 %. While the amount of Urea reduced to 33% and even we use 0.25% of Starch instead of Rice bran, the toxin in biomass reduced into 71% and reached to 0.99 ppb/gr. When we use 2.30% of Meat peptone, applying 0.50% of Urea in compares with 0.50% of Soy bean peptone increase up to 66% of zearalenone toxin in biomass and reached to 0.77 ppb/gr.

1. Introduction

Fungi are important because of high vigor and strengthen enzymes system, while they can be harmful as well. The fungus genes are used in researches to understand cell's basic biological process and medicine (Deacon, 2006). Fusarium is a fungus that is commonly found as saprophytes with parasitic life on some living organisms (Banerjee et

*Corresponding author. Fereshteh Jafarian
Tel: 09126053863
E-mail address: fe.j863@yahoo.com

al., 1995; Brock, 1989). This fungus is active biologically and some types of this fungus are toxin causing, also damage animals, and humans while scientists are looking for cheap resources of protein for people. One of these resources is mycoprotein (Hosseini et al., 2010). This protein is applied in livestock and poultry rations as microbial proteins so wide studies is performed on microorganisms as a fair replace for protein resources. Protein production of mycoprotein is one of the most important steps (Carlile and Watkinson, 1997). SCP production is preceded by microorganisms such as yeast and bacteria. Mycoprotein or fungus proteins with physical and chemical features are known as food in recent years (Ohno, 1991). However the fungi have a long history in food security and food production. The used strategy of these organisms' especially filamentous fungi is performed to produce microbial protein for human too (Anupama, 2000; Collins and Rinald, 1977; Hashemieh, 2009; Moore-landecker, 1996). When they are involved in temperature and wet stress, release materials such as mycotoxins or fungus toxins remained in food. Mycotoxins are known as secondary materials products thus it means that, however these materials haven't main role in natural metabolism and growth but are not motivated monopoly by growing fungus to produce toxin (Hussein and Brasel, 2001). Zearalenone is a non stroide estrogenic mycotoxin produced by many types of Fuzarium Spp. This fungus grows on grains specially Barley, Oats, Rice and Sorghum and usually are scattered not only in temperate but also in some moderate regions even some are adapted with equatorial tropic and subtropical weather condition (Verma et al., 2000). Zearalenone and dependent compounds have distinct role in many fungus toxicity and beings especially despite acute toxicity. In these animals, infertility and sexual dysfunction is stipulated as a difficult in livestock industry (Ghazi Khansari and Hadiani, 2003; Hashemieh, 2009).

According presented standards by Iran standard, the allowed rate of zearolenone in wheat is under 200 mg/ppm. Also the global allowed rate of zearalenone in foods is 30-1000 nano gr/ppb (Edwards et al., 1987; Hedayati, 2002; Kazemi et al., 2009).

2. Material and Methods

2.1. Vogel culture environment

The submerged culture environment to produce fungus biomass was Vogel basic medium. Also it was used in order to provide inoculum from spores' suspension. So that to compose Conidies on slope surface of SDA, was created by Normal saline solution include tween80 0.1% or suspension fluid environment and after counting by Neobar ,5ml transferred to Erlen meyer containing 100ml of Saboraud's broth culture environment plus Chloramphenicol and were incubated in 200rpm during 48 hours in temperature of 30°C.

2.2. Carbon resources

To verify the impact of different carbon resources in amount of 1% of carbonic resources include Saccharose, Starch, Maltose, Wheat bran, Rice bran, Whey and Potato wastes added to 50ml of based cultivated environment and after adding 5ml of inoculum to each Erlen, was placed about 48 hours in temperature of 30C in Incubator in 200rpm.

2.3. ELISA reader system

The produced fungus biomasses harvested 4500rpm for about 20 minutes by centrifugation, washed and rinsed twice and by desiccated overnight in dry temperature by 50°C to be dried completely. Also, the dry weight biomass was measured for 2gr and after powdering dried Mycelium, it was used of it in order to measure protein rate extracts for toxin estimating based on producer instructions.

Then, 50ml Substrate was added and after 5 minutes stopper enzyme was added. Finally, the zearalenone toxin amount was measured by ELISA reader system.

In order to verify the impact of different Starch rate, the presences of 0.25, 0.5, 0.75, 1, 1.25 and 1.5 of the material added to cultivating environment, adding 5ml of inoculum to each balloon were incubated based on recent order. All separation stages of biomass from cultivated environment and toxin measurement was mentioned by following the recent order and preceded equally.

2.4. Nitrogen resources

In order to verify the impact of different rate of Starch and Urea in this stage, the percent's of 0.25, 0.5, 0.75, 1,1.25 and 1.5 from Starch and percent's of 0.25,0.5 and 0.75 from Urea added

to cultivating environment, adding 5ml of inoculum to each balloon were incubated based on recent order. All separation stages of biomass from cultivated environment and toxin measurement in biomass was mentioned by following the recent order and preceded equally.

To measure the impacts of applied natural resources on the rate of zearalenone toxin, we used the cultivating environment with a variety of cheap resources include Potato wastes and Rice bran. In order to provide extract, after heating wastes about 30 minutes in 100°C and filtration with filter paper and separation of impure materials and sterilizing, calculating the rate of dry matter (DM) availabled in 10ml, the needed amount in applying in cultivated environment was calculated and added to cultivated environment in sterilized condition.

In order to verify optimal conditions in production of mycoprotein in method of one factor at a time, all optimal condition achieved by each of verifications was provided in group for fungus in submerged culture environment so that after preparation of 100ml from the above environment and inoculation with 18 ml spore suspension, incubation was proceeded during 48 hours in temperature of 30°C and finally the rate of protein was measured.

In this step, it is used of second and third priorities of carbonic resource include Potato waste and Rice bran in order to verify the protein production by using of a cheap resource. Also, it is used of second priority of nitrogen resource means, Meat peptone to provide the optimal condition achieved by verification in each group for fungus in submerged culture environment for these resources. The primary Carbonic and Nitrogen resource was Starch and Urea.

3. Results

According to graph 1, A (Starch) B (Rice bran) C (Urea), E (Meat peptone) and F (Soy bean peptone) are the materials that we use for construct the culture medium. The best resources of carbon were A, B, C and the best resources of nitrogen were F, E and D that were used in construct of environments. The amount of zearalenone toxin was measured in different cares in which, the measured amount of toxin directly, sc is the actual amount of error-free and Mean, is the average of Elisa measures. The carbon materials include Starch (st), Rice bran (br) and Potato waste (pme) are considered as the carbonic resources and Meat peptone (p), Soy bean peptone (psb) and Urea (u) are considered as nitrogen resources.

The verifications showed that when we use of Starch 0.25% and Urea 0.25%, the amount of zearalenone toxin in biomass is 0.99 ppb/gr. (graph 2).

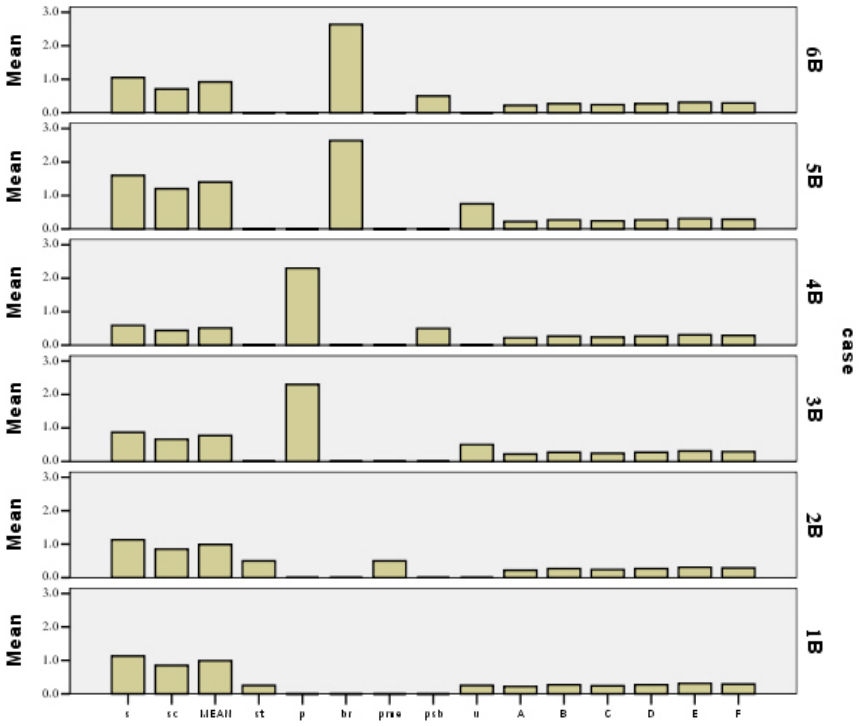
The amount of measured toxin in biomass in environment of Rice bran at a range of 264% and 0.75 % of Urea is equal to 1.40 ppb/gr. (graph3)

When we use Starch of 0.25% associated with Urea 0.25% in cultivation process, the amount of zearalenone toxin in biomass is equal to 0.99ppb/gr while if we use Meat peptone of 0.50% instead of Urea and so when we increase the amount of Starch, the toxin amount tend to 0.99 ppb/gr. (graph4)

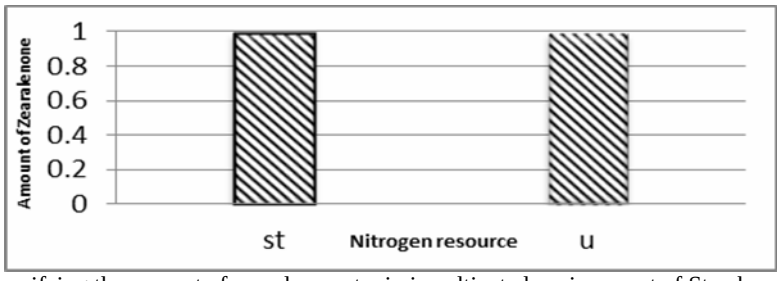
In the cases of the 2.30% of Meat peptone and 0.50% of Urea, the toxin amount is 0.77 ppb/grWhile, if we use 0.50% of Soy bean peptone instead of the Urea, the toxin amount is reduced to a range of 0.51 ppb/gr. (graph5)

When we use 2.64% of Rice bran as carbon resource and 0.75 % of Urea as nitrogen resource, the measured toxin is equal to 1.40 ppb/gr whileif we use 0.50% of Soy bean peptone instead of Urea, the toxin amount is reduced to 0.92 ppb/gr. (graph6)

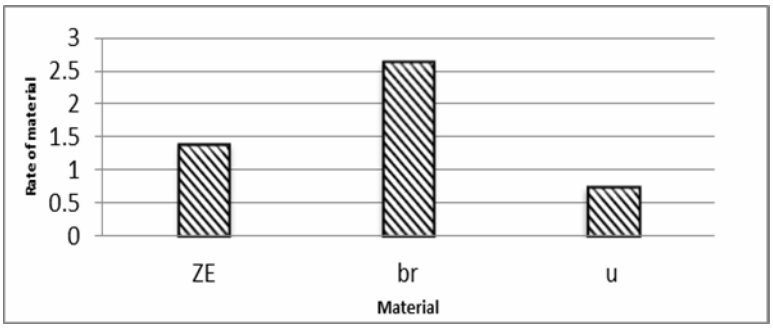
When the Urea amount is changed from 0.25% to 0.75% and when we use Rice bran instead of Starch, the toxin amount in biomasses increase from 0.99 ppb/gr to 1.40 ppb/gr. (graph7)



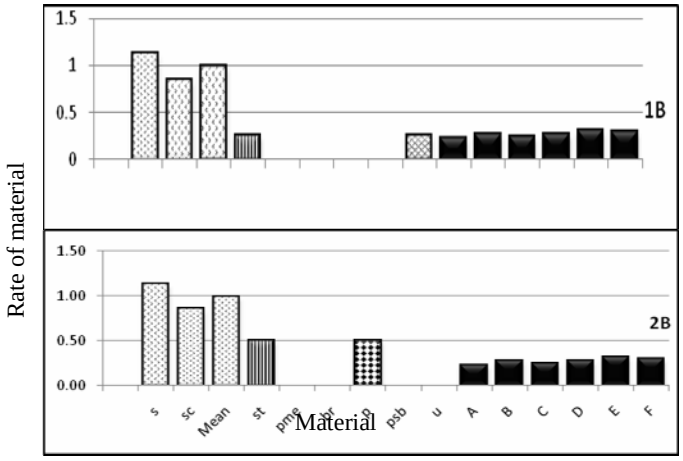
Graph 1: the average of materials and zearalenone toxin in different cares.



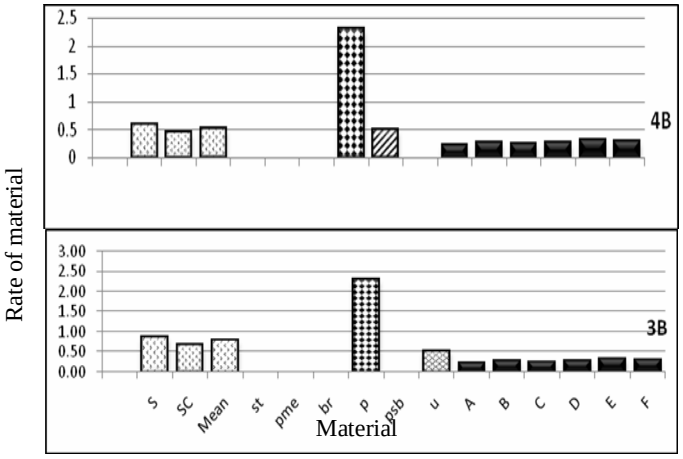
Graph 2: verifying the amount of zearalenone toxin in cultivated environment of Starch and Urea 0.25%.



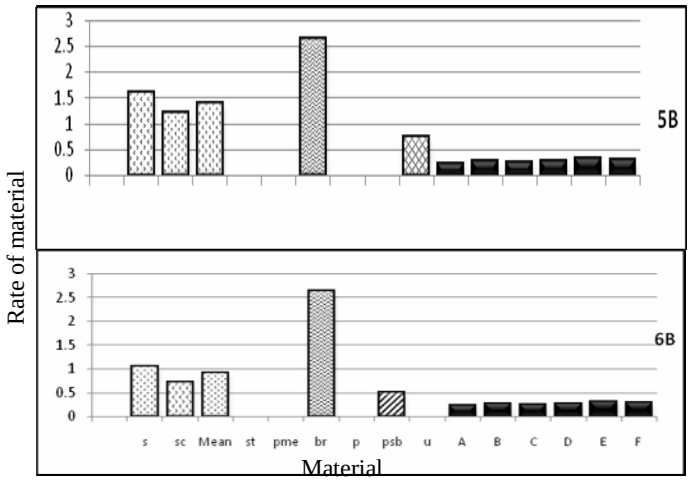
Graph 3: verifying the amount of zearalenone toxin in cultivated environment of Rice bran and Ure



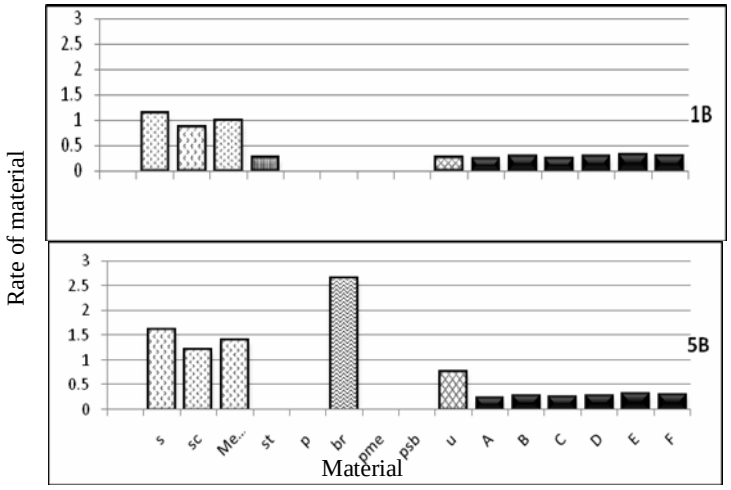
Graph 4: compare verification of zearalenone toxin in biomass in one factor at a time of first and second cares



Graph 5: compare verification of zearalenone toxin in biomass in one factor at a time of fourth and third cares



Graph 6: compare verification of zearalenone toxin in biomass in one factor at a time of fifth and sixth cares



Graph 7: compare verification of zearalenone toxin in biomass in one factor at a time of first and fifth cares

4. Discussion

The results show that, when the Urea amount is 0.25% and Starch originated carbon resource is 0.25%, the amount of toxin will be 0.99ppb/gr and when Rice bran originated carbon resource is 2.64% and the Urea originated nitrogen resource amount is 0.75%, the toxin in biomass reached to 1.40 ppb/gr that show the increase of toxin and stated that after nitrogen resource, the carbon resource play the second role in production and increase of toxin rate in mycoprotein biomass extracts, that we are matched to Nahvi and Shafiei, Bo Jim et al, Ahangy et al and Verma et al (Ahangi et al., 2005; Hosseini et al., 2010; Nahvi and Shafiei, 1984; Verma et al., 2000).

Verifying the optimum submerged environment in which all optimal conditions of each steps were applied (the first priority of optimal environment include 0.25% of Starch originated carbon resource and 0.25% of Urea originated nitrogen resource) showed the rate of protein production was 0.642% that is have more increased about 0.207% in relation to basic submerged environment. In this environment the amount of zearalenone toxin was 0.99 ppb/gr.

In the other way, when we use 2.64% of Rice bran as replacement carbon resource, using 0.75% of Urea in compare with 0.50% of Soy bean peptone as nitrogen resources we could observe an increase of toxin rate in biomass about 0.66 %. While the amount of Urea reduced to 33% and even we use 0.25% of Starch instead of Rice bran, the toxin in biomass reduced into 71% and reached to 0.99 ppb/gr.

When we use 2.30% of Meat peptone, applying 0.50% of Urea in compares with 0.50% of Soy bean peptone increase up to 66% of zearalenone toxin in biomass and reached to 0.77 ppb/gr.

Hadiany, Hedayaty and in Mazandaran tested samples of Corn and Wheat from stores, and for measuring the amount of toxic of Zearalenone used TLC-Densitometry and competitive enzyme immunoassay method with microtitre plate. 75% and 64/4% of samples had too much higher than the level of allowable pollution (100-212 ng/g) and their toxic reported 80/5%. The minimum amount of its pollution to this toxic was 29µg/kg and the results of all of the stores to the pollution to the Zearalenone were positive (Hashemieh, 2009; Hedayati, 2002).

The problem of pollution of grains and food to ziralenone is a world problem assigned by Zakharova, Furlong et al, Zamany Zade and Khorsandy, Hadiany, Hedayaty, Ezekiel et al and observing necessary issues result in reduction of this mycotoxin, such that researches in Egypt in a 1990 decade, the countries of east Europe and central Europe in 1990 – 2006, the south of brazil in 1995, Germany in 2006- 2005 and in Iran in 2002 indicated the compounding of agriculture system of these countries in

deletion or effective reduction of ziralenone in produce of wheat and the other grains or beans (Hashemieh, 2009; Hedayati, 2002; Hosseini et al., 2010; Kazemi et al., 2009).

References

Ahangi, Z., Sho Jaosadati, S. A., Nikoopur, H. 2005. Study of mycoprotein production using Fusarium oxysporum PTML 5115 and reduction of its RNA content. Pakistan Journal of Nutrition. 7(2):240-243.

Anupama, p., Ravindra., 2000. Value-added Food: Single cell protein: Biotechnology advance. 18:459-479.

Banerjee, U. C., Chisti, Y., MOO-Young, M. 1995. Effect of substrate particle size and alkaline pretreatment on protein enrichment by Neurospora sitophila, Resources, conservation and recycling. 1:139-146

Brock, T.D., 1989. A text of industrial microbiology. Sunderland, MA: Sinauer Associates Inc. 76-306.

Carlile, M. J., Watkinson, S.C., 1997. The Fungi, Academic press. 1-482.

Collins, M.S., and Rinald, M.G., 1977. Cutaneous infection in man caused by Fusarium moniliforme. Sabouraudia.; 15: 151-160.

Deacon, J., 2006. Fungal Biology, 4th ed, Black well publishing. 1-371.

Edwards, S., Cantley, T.C., Rottinghaus, G. E., et al. 1987. The effects of zearalenone on reproduction in swine I. The relationship between ingested zearalenone dose and anoestrus in non-pregnant, sexually mature gilts.

Ghazi Khansari, M., Hadiani, M.R., 2003. The effect of estrogenic mycotoxins in fertility disorder. J Reprod Infertility. 4(1):75-82.

Hashemieh, M. 2009. Master's thesis. In vitro production of fungal protein by Fusarium solani PTML5285.

Hedayati, M.T., 2002. A survey on wheat samples for Mycotoxin Zearalenone from Mazandaran province, Journal of Mazandaran University of Medical Sciences.15 (49):89-94.

Horner, W.E., Helbling, A., Salvaggio, J.E., and Lehrer, S.B. 1995. Fungal Allergens. Clinical Microbiology Reviews. 8(2): 161-179.

Hosseini, M., Khosravi-Darani, K., Mohammadifar, M., Nikoopour, H., Hosseini, H., Valaii, N., 2010. Mycoprotein production by Fusarium venenatum in surface culture using a central composite design. Iranian Journal of Nutrition Sciences & Food Technology. 4 (4):45-52.

Hussein, H.S., Brasel, J.M. 2001. Toxicity, metabolism and impact of mycotoxins on human and animals. Toxicol. 167:34-101.

Kazemi, A., Mohtadi Nia, J., and Mahdavi, R., 2009. Survey of storage Wheat contamination to Zearalenone producer Fusarium Sp. in East Azarbaidgjan. ZUMS Journal. 17 (68):53-64.

Moore-landecker, E., 1996. Fundamentals of the Fungi, prentice Hall. 1-574.

Nahvi, I., and Shafiei, R., 1984. Single cell protein production from raw starch in fed_batch culture by coculture of Cryptococcus aerius and Saccharomyces cerevisiae. Pajouhesh & Sazandegi. 75:33-38.

Ohno, N., 1991. Purification and properties of amylases extracellulary produced by an imperfect fungus. Bioscience, Biotechnology and Biochemistry (BBB). 56(3): 456-471.

Opinion of the Scientific Committee of European Commission on food: fusarium toxins: Zearalenone (ZEA). 2000.

Pittet, A., 1998. Natural omlurences of mycotoxins in foods and feeds: an updated rewiew. Rev Med Vet. 149:92-479.

Verma, G., Nigam, P., Singh, D., Chaudhary, K., 2000. Bioconversion of raw starch to ethanol in a single step process by co-culture of amylolytic yeast and Saccharomyces cerevisiae Bioresource Technology. 72, 261-266.