Screening Of Phytase - Producing Bacteria As Probiotic Candidates Of Poultry

by Muh. Aidil Fitriyan Fadjar Suryadi Suryadi

Submission date: 07-Jun-2023 09:02PM (UTC-0500)
Submission ID: 2111421195
File name: Screening_Of_Phytase.pdf (806.4K)
Word count: 3216
Character count: 16969

Proceedings International Conference on Science and Technology (ICST)

e-ISSN: 2722-7375 **pp: 1-8, Vol. 1, Juni 2020**

Screening Of Phytase - Producing Bacteria As Probiotic Candidates Of Poultry

Muh. Aidil Fitriyan Fadjar Suryadi, Anwar Rosyidi, and Muhamad Ali*

Laboratory of Microbiology and Biotechnology, Faculty of Animal Sciences, University ofMataram Jl. Majapahit No. 62 Mataram, Lombok, Indonesia, m_ali@unam.ac.id

Abstract. This research aimed at screening of phytase-producing bacteria previously isolated from the gastrointestinal tract of poultry as probiotic candidates. Two screening indicators were used; (1) the capacity to produce phytase, and (2) the ability to grow in sugar media. This research was conducted at laboratory of Microbiology and Biotechnology, Faculty of Animal Science, University of Mataram for about 4 months since January to April 2019. Initially, bacteria were subculture in LB broth and on *agar* through serial dilutions to get a pure colony. Thereafter, the pure isolates were grown on sodium phytate-containing *agar*. The results shows that 4 bacteria had a capacity to produce phytase indicated by the formation of clearance zone on the sodium phytate-containing *agar*; three of them appeared to be member of lactic acid bacteria (LAB) indicated by their ability to ferment glucose. Since LAB members are generally regarded as safe (GRAS) microorganism, the results may suggest that the three bacterial isolates are potential probiont for poultry, although in vivo study using live poultry should be further investigated to get more comprehensive results.

Keywords: Bacteria; probiotic; poultry; phytase

1. Introduction

Rice bran is recidu of milling rice or grain into rice which has a by-product in the form of rice bran and husk. Rice bran is the results obtained between rice and the skin. Rice bran is a by-product of rice milling that cannot be stored for long periods. Rice bran has a protein content of around 12-14%, fat 7-9%, ash 9-12% and crude fiber 6-27% containing raw material for animal feed. In addition, rice bran has around 1600-1900 kcal/kg [1-2].

Rice bran has a relatively high nutrient content, and is used as a source of energy in animal feed. besides having a relatively high nutrient content, it also has abundant production and does not compete with humans, which will be able to streamline the use of imported raw materials of excessive feed [4]. Rice bran has the main disadvantage of having high enough crude fiber. In addition, bran contains low amino acids and contains phytic acid which binds minerals and proteins proteins to rice bran which can be difficult to digest by poultry digestive enzymes [5].

Phytic acid is a compound secondary which is used as a primary storage place for phosphate found in plant grains [5]. Besides containing phosphate, phytic acid also binds various types of minerals such as calcium, magnesium, and copper which can cause digestive mineral disruption. In addition to being able to bind minerals, phytic acid can bind to proteins so as to reduce the digestibility

of feed protein [6].

The existence of phytic acid is one of the obstacles for using rice bran in large quantities in the preparation of rations for monogastric animals, because there are phytic acid compounds. So, it is necessary to find a solution to break the bond of phytic acid, so that the nutrient and digestibility of rice bran increases.

One of the methods used to hydrolyze phytic acid content in rice bran is using phytase enzymes (indigenus or exogenus) by fermentation. Fermentation technology using microorganisms, one of the right ways to break down phytic acid content in rice bran. The basic principle of this technology is the breakdown of phytic acid by phytase enzymes produced by microorganisms that have been tested for their abilities [7].

The use of these technologies can reduce and even negate the impact of phytic acid without reducing livestock productivity and feeding efficiency [8]. Kies et al., [7], states that the feed added by phytase-producing enzymes is able to increase the absorption of 2 g / kg of phosphorus content.

The group of microorganisms that will be used and developed for this purpose is probiotics [9]. Probiotic is a product that contains non-pathogenic living microbes given to livestock with the aim of increasing the rate of livestock growth, efficiency of feed conversion and maintaining livestock health [10].

Various types of microorganisms used as probiotics are isolated from the intestinal contents of digestion, mouth, and animal manure. At present, microorganisms that are widely used as probiotics are strains of Lactobacillus, Bifidobacterium, Bacillus spp, Streptococcus, and Saccharomyces cereviceae. These microorganisms must be non-pathogenic, gram- positive, specific strains, anti-E. coli, resistant to bile, live, attached to the intestinal mucosa, and contain at least 30 × 109 CFU/g [11-12].

The ability of bacteria to produce phytase enzymes can increase the efficiency of the use of rice bran which contains phytic acid. Therefore researchers conducted research aimed at getting the bacteria that produce phytase enzymes to be used as probiotics in poultry.

2. Material and Metods

Preparation of Media LB (*Lysogeny Broth*) Agar

One-hundred millilitres LB Agar consisted of 1% *Trytone Powder*, 1% NaCl, 0.5 % *Yeast Extract*, 1.5 % Agar and 100 ml aquades. Then, mixture using *Hot Plat Stirrer* until homogen. The medium was homogen adjusted to be 7.2 with 0.1 M Sodium hydroxide (NaOH) or Hydrochloric acids (HCl). Then the mixture was autoclaved and ready to used.

Preparation Media LB (*Lysogeny Broth*)

One-hundred millilitres LB Agar consisted of 1% *Trytone Powder*, 1% NaCl, 0,5% *Yeast Extract*, and 100 ml aquades. Then, mixture using *Hot Plat Stirrer* until homogen. The medium was homogen adjusted pH to be 7.2 with 0.1 M Sodium hydroxide (NaOH) or Hydrochloric acids (HCl). Then the mixture was autoclaved for sterilisation and ready to used it.

Isolation of Bacteries Phytase

The scrinning of bacteries done by first step was isolated bacteries from gastro intestinum of poultries. Then, test phytase, caracteritation, and biochemic. The isolation done with serial dulation (Figure 1) of bacteries was got from gastro intestinum. The solution of bacteries took 100 μl and dulated on aquades 900 μl with 3 dulation (10^{-1}, 10^{-2}, and 10^{-3}). Every dulation took 100 μl which was growthed on LB Agar during 24 – 48 hours at incubator shaker using *spread plate* method.

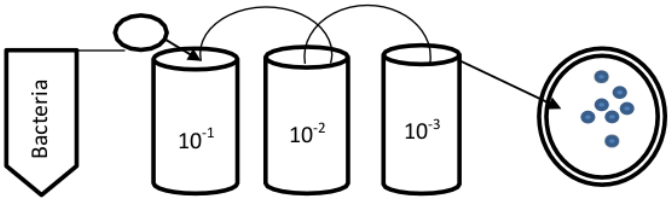


Figure 1. The Process Isolation of Bacteries

Bacteries has grown on media LB, then characterized to know morphology and difference of every colonies bactery. Bacteri has morphology colony different spreated and cultured in media liquid LB.

Biochemic Test (Phytase, Gram, Catalase, and Media Sugar Water) Phytase Test

The single colony of bactery tested phytase to know ability of bactery to produce enzyme phytase. Phytase test using media selection consisted of 1 g glucosa, 0,25 g NH_4NO_3, 0,025 g KCl, 0,025 g $MgSO_4.7H_2O$, 0,1 g $CaCl_2H_2O$, 0,2 g Sodium phytat, 0,75 g Agar and 50 ml aquades (pH 5,5). The all ingredients of media heated up using *magnetic stirrer*. And then sterilation in autoclaved during 15 minutes at 121 ℃ with 15 lbs. Media was sterilitation pour 15-20 ml in the petridisk, and then took 0,005 ml every isolate and *B. amyloliquefaciens* (control positive) with OD_{600} 0,2 and drop it in medium phytat, incubated in uncubator during 24 - 48 hours at ℃. After that, characteritation which bactery can cut content of phytat in media and measuring size of clearing zone surrounded growth of colony bactery.

Gram Test (Coloration)

Coloration used Hucker method for bactery cell purpose to know kind of cell bactery using cristal violet, lugol, foxin and foxin.

Catalase Test

Catalase Test using 3 % hydrogen perocsyda (H_2O_2). The marker of bacteri positive catalase has bubble gas surround of colony bacteri if there was not bubble gas in surround of colony bacteri that is negative catalase.

Sugar Water Test

Sugar water test purpouse to know ability of bacteries growth in simple media using 20 g sugar on 100 ml aquades. The media sugar water test sterilized using autoclave at 121 ℃ on 15 lbs. after that, 5 μl isolate bacteries put in media sugar and incubated for 24 hours. The marker of bacteri that can growth on media sugar water was change clear media to be murky.

3. RESULTS AND DISCUSSION

The content of phytat acid in feed of poultry such as soybean and rice bran that was problem to maximal absorption protein and mineral posphor fot poultry, one of solution to solving the problem using phytase enzyme that can cutting phytat held phospor [13]. For resulting phytase enzyme done using probiotic which has ability made phytase enzyme.

Isolation Of Producing-Phytase Bacteries

This study found 4 types of bacteria that can be used as phytase enzymes. The morphological forms of the four types of colonies are (1) bacteria colony A has a medium size, convex colony growth, there is a core point in the middle, jagged edge and white, (2) bacteria colony B has a large size, rounded, intact white and clear, (3) C bacteria colonies are round, small, convex, clear, white

color, and (4) bacteria D has oval, small, and clear colony. Figure 3 and Table 1 showed the results and bacterial growth in dilutions 10^{-3}.

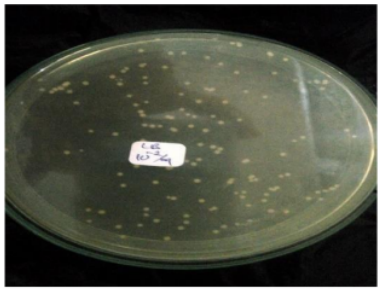


Figure 3. Some Colonies of Bacteria

The four types of bacteri colonies were purified through serial dilution and then grown on a liquid LB media that was incubated for 24 - 48 hours in a incubator shaker. Figure 4 show the results culture one of the bacteria that was successfully isolated. The Figure 4 resulted of the purification process of one of the bacterial colonies to obtain a single colony. Bacterial growth was marked by turbidity of liquid LB media which initially looks clear like the figure 4. Bacteria that grew in liquid LB media were then grown on solid LB media for 24 hours at 37 ° C in incubator shaker. Purification of the bacteria was more repeated until getting a single uniform colony as shown in Figure 5 and Table 1.

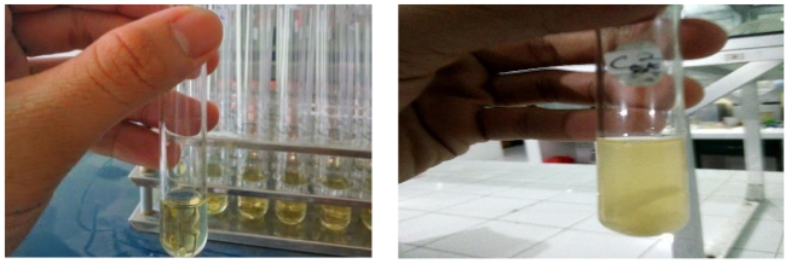


Figure 4. Culture one of bacteria isolate

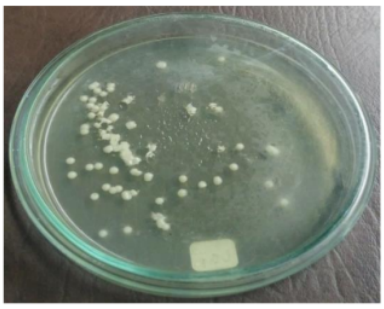


Figure 5. The Single Colony One of Bacteria Biochemical Test

Scrinning Of Producing-Phytase Bacteries

This phytase-producing bacterial test was tested on solid LB media containing sodium phytate with a concentration 0.4% and as a control positive bacterium Bacillus amyloliquefaciens subsp. *Plantarum* (K). After the growth of several bacterial isolates in the agar media containing sodium phytate obtained the results of bacteria A, B, C, and D have the activity of breaking down phytic acid as indicated by the presence of a clearing zone on the culture media containing sodium phytat. Figure 6 and Table 1 showed the ability of bacteria to break down sodium phytate characterized by a clearing zone around the growing of colony.

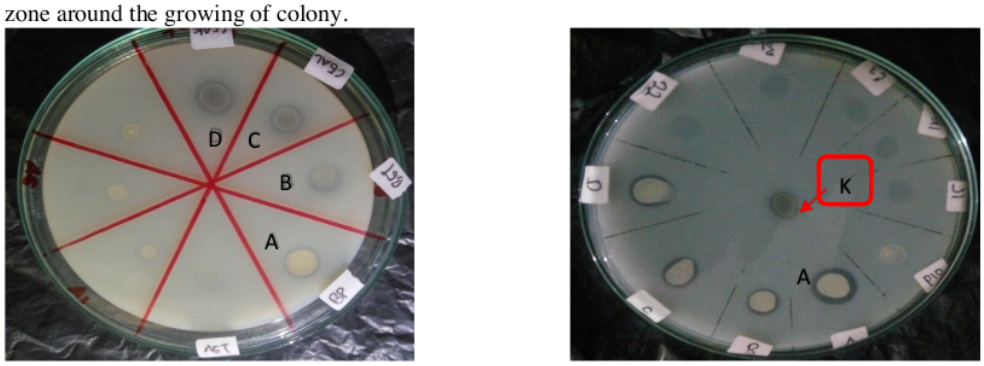


Figure 6. Result of phytase test bacteries (A, B, C, and D) in media LB sodium phytat

This study accordance with what has been done by Kusdianto [14], who managed to isolate phytase-producing bacterial enzymes from the soil tested on selective media using sodium phytate. The presence of a clearing zone around the growth of a growing bacterial colony proves the bacteria as able to produce phytase.

This research has also been carried out by Amin and Liliyanti [15], who have succeeded in isolating and characterizing phytase-producing bacterial enzymes from fish intestines. The some of bacteries are tested on selective media and produce clearing zones around the growth of bacterial colonies and good isolates characterized by the highest clearing zone.

The ability of bacteria A (Bp), B (AGT), C (BGT), and D (Ct) to form a clearing zone indicates that these bacteria produce phytase enzymes that are able to hydrolyze the content of sodium phytate on solid LB medium containing sodium phytate. The bacteria D have the best ability to break down sodium which is characterized by the formation of the highest clearing zone based on the growth of the colony as accordance with the opinions of Amin and Liliyanti [14]. There is a clearing zone around of the growth of colonies caused by bacteria furthermore phytic acid hydrolysis into phosphorus (P) by extracellular phytase produced by bacteria A, B, C, and D which diffuses into the medium test.

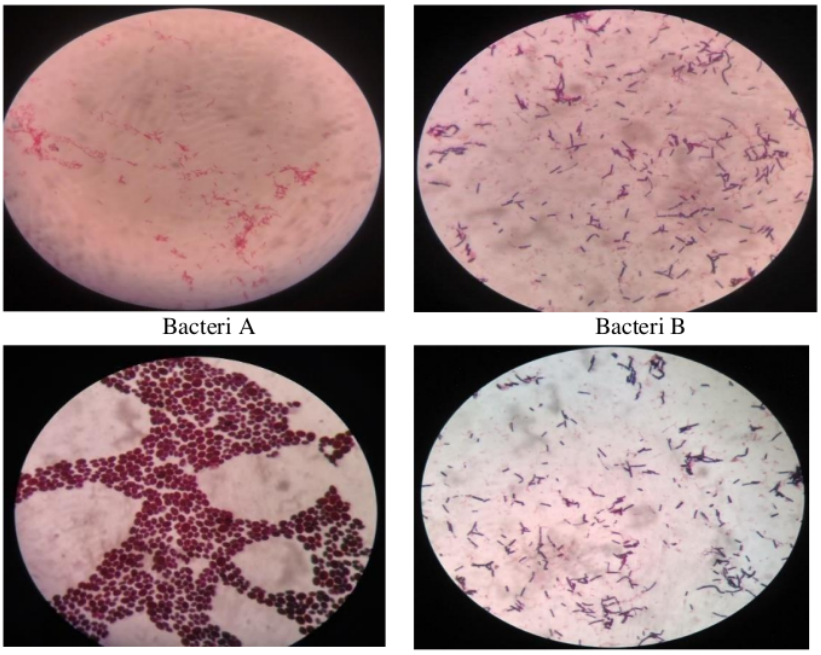
The microorganism can result extracelluar phytase which can hydrolyze content of phytat acid on feed being P that cause clearing zone around of growth colonies [16]. Probiotics produced are used as feed additives in foods to increase the availability of phosphate, by means of bacteria containing probiotics that have the ability to produce phytase enzymes will hydrolyze phytate reserves contained in feed ingredients such as rice bran.

These phytase producing enzymes can be used as feed addictive for monogastric animals which are less able to produce phytase enzymes in their digestive tract. so, the adding up phytase enzymes by microorganisms that can produce phytase enzymes important to increase growth of poultry.

The Characteritation of Bacteri Phytase Gram Test (Staining)

Gram staining was done to facilitate the process of identifying and knowing the morphology of

bacterial cells and to distinguish gram-positive and gram-negative bacteria (Rostinawati, 2008). Based on the results of gram staining that has been carried out microscopically with a magnification of 100 x obtained results of gram staining from bacteria A, B, C, and D as showed in Figure 7 and Table 1.



Bacteri A Bacteri B

Bacteri C Bacteri D

Figure 7. The result of staining gram

. Tabel 1. Morphology and Biochemic Analysis bacteries of digestivus poultry

No.	Code of Bacteries	Types coloni and size of bacteri	Color of colony	cells	Gram	Biochemic Test			Size of clearing zone	
						Fitase	Katalase	Sugar	Koloni (mm)	Zona bening (mm)
1	A (Bp)	Convex Middle	White	Bacilloc occuss	+	+	-	+	5	2
2	B (AGT)	Round Big	Clear	Bacill	+	+	-	-	5	1
3	C (BGT)	A little convex and round Small	Clear	Bacill	-	+	-	+	4	1
4	D (Ct)	Round and oval Small	Clear	Bacill	+	+	-	+	6	4

The figure 7 showed the cell of bacteria A is gram-positive *basillococcus*. The bacteria C is gram-negative *bacill*, while B and D bacteria are gram-positive *bacill*. Bacterial cells A, B, and D are purple indicating that they can bind to the color of violet crystals, whereas red or gram negative bacterial cells indicate that the bacteria cannot bind to the color of violet crystal and only colored by a solution of fuchsin (counter dye). Based on the results of morphological and biochemical analysis consisting of gram test, phytase test, catalase test and sugar media test on phytase-producing bacteria at Table 1.

The result of catalase test from bacteria A, B, C and D was got negative catalase results in Table 1, because they cannot produce air bubbles. The result of positive catalase test characterized by the formation of oxygen bubbles which showing results of the evolution produced by enzymes that convert hydrogen peroxide into air and oxygen [17].

Testing on Sugar Water Media

The ability of bacteria to grow in simple media such as sugar water will make it easier to make probiotics by using sugar water as a prebiotic. Special characteristics of lactic acid bacteria are able to grow at high levels of sugar, alcohol and salt, and also able to fermented monosaccharides and disaccharides [18].

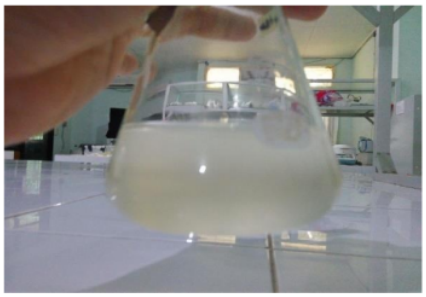


Figure 8. The result of sugar water media

Based on the results of test of the ability bacteria A, B, C, and D to grow in pure sugar water getting results (Figure 8), only bacteria B is not able to grow in sugar water media.

CONCLUSION

The isolated bacteria (A, B, C and D) are able to produce phytase enzymes as evidenced by the presence of clearing zones around bacterial colonies that were grown on selective media. The bacteria B has the best ability of the four bacteries marked by the formation of the highest clearing zone around the growth of the colony. The existence of the clearing zone was caused by the phytic acid hydrolysis process by phytase enzymes, resulting in diffusion. Besides being able to produce phytase enzymes, it is also able to grow in sugar water media which will facilitate the making of probiotics.

Acknowledgement

The authors grateful to Muhamad Ali,Ph.D lecture of faculty animal science, university of mataram has supported this research until getting bacteries and resulting product probiotics for poultry.

References

[1] Murni, R, Suparjo, Akmal, dan B. L. Ginting. 2008. Textbook on Waste Utilization for Feed

Technology. Animal Food Laboratory. Faculty of Animal Science. University of Jambi. Jambi.
[2] Kurniati. 2016. The content of crude fat, organic matter, and extract ingredients without nitrogen silage complete feed made primarily of banana stems (usa paradisiaca) with different incubation times. *Thesis*. Faculty of Animal Science. University of Hasanuddin. Makasar.
[3] Scott, M. L, M. C. Neiseim, and R. J. Young. 1982. Nutrition of Chicken. 3rd Edition, Published M, L. Scott and Associates: Itacha, New York
[4] Rasyaf, M. 2004. Regarding Chicken Food. Edition 8, Publisher Kanisius, Yogyakarta. Rostinawati, T. 2008. Screening and Identification of Chitinase-Producing Enzymes Bacteria From Sea Water in Pondok Bali Coastal Waters. Independent Research. faculty of Pharmacy Universitas Padjadjaran Jatinangor.
[5] Wu P, Tian JC, Walker CE, Wang FC. 2009. Determination of phytic acid incereals - A brief review. Int J Food Sci Technol. 44:1671-1676.
[6] Kies, A. K., K. H. F. Van Hemert and A. C. Sauer. 2001. Effect of fitase on protein and amino acid digestibility an energy utilitation. World's Poultry Science Jurnal. 57:109- 1026.
[7] Gupta, R. K., Gangoliya, S. S., & Singh, N. K. 2015. Reduction of phytic acid and enhancement of bioavailable micronutrients in food grains. Int J Food Sci Technol, *52*(2), 676-684.
[8] Zurmiati, M.E. Mahata, M. H. Abbas, dan Wizna. 2014. Probiotic Application For Duck. Indonesian Animal Husbandry Journal. 16(2):19
[9] Kompiang IP. 2009. The use of microorganisms as probiotics to increase poultry production in Indonesia. Agricultural Innovation Development 2:177-191.
[10] Syahrurachman, A.,1994, Mikrobiology medicen, Revised Edition, Publisher Bina Rupa Aksara, Jakarta.
[11] Pal, A., L. Ray and P. Chattophadhyay. 2006. Purification and immobilization of an *Aspergillus terreus* xylanase: Use of continuous fluidized column reactor. Ind. J. Biotechnol. 5: 163 – 168.
[12] Salminen S, Wright AV, and Ouwenhand A. 2004. Lactic Acid Bacteria Microbiology and Fungtional aspects. Marcel Dekker, New York. Pp 211- 254.
[13] Greiner R, Konietzny U. 2006. Phytase for food application. Food Technol Biotechnol. 44:125-14.
[14] Kusdianto, H. 2004. Isolation of phytase-producing bacteria and its activity testing. *Tesis*.Magister. Institut of Pertanian Bogor. Bogor.
[15] Amin, M., dan Liliyanti, M. 2018. Isolation And Caracteritation Of Producing-Phytase Bacteri Local Strain As Candidat Probiotic For Fish. Final Report of Beginner Lecturer Research. University 45 Mataram. Mataram.
[16] Hadioetomo RS. 1993. Procedur and tecnich practice Microbiology at laboratory.Gramedia, Jakarta
[17] Sajidan, A. M. P. Nuhriawangsa dan A. Ratriyanto, 2004. Application of Phytase-Producing (non-recombinant) Bacteria in Wheat Pollard Mixed Feed on Performance of Broilers.Bull. Animals UGM. 28(3): 114-121.
[18] Sangadji, I. 2004. Phytase Enzymes and Their Role in Breaking Fitat Acid Bonds in Feed Materials. Personal Paper. Introduction to the Philosophy of Science (PPS702). Magister and Doctor. Institut of Pertanian Bogor.

Screening Of Phytase - Producing Bacteria As Probiotic Candidates Of Poultry

ORIGINALITY REPORT

3%	2%	1%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.atlantis-press.com Internet Source	1%
2	iwaponline.com Internet Source	1%
3	www.wellingtoncollege.org.uk Internet Source	<1%
4	d-nb.info Internet Source	<1%
5	repository.unipa.ac.id Internet Source	<1%
6	Applied Environmental Biotechnology Present Scenario and Future Trends, 2015. Publication	<1%
7	Melissa Fox Young, Usha Ramakrishnan. "Chapter 10 Iron", Springer Science and Business Media LLC, 2017 Publication	<1%

8	U Suryadi, A F Prasetyo. "Probiotics based on Local Microorganism as a subtitute of Antibiotic Growth Promotor (AGP) on Broiler productivity", IOP Conference Series: Earth and Environmental Science, 2018 Publication	<1%

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		