Sys Rev Pharm 2020; 11(2): 273 – 277
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453

Production Technique of Bifido bacterium's Exo-metabolites with High Antimicrobial Activity towards *Staphylococcus aureus*

Markov A.A[1, 3], Timokhina T.H[1], Perunova N.B[2], Malyugina O.A[4]
[1] Tyumen State Medical University, Tyumen, Russian Federation
[2] Institute of cellular and intracellular symbiosis, Orenburg, Russian Federation
[3] Tyumen Industrial University, Tyumen, Russian Federation
[4] State Autonomous Healthcare Institution of the Tyumen Region "City Polyclinic No. 8", Tyumen, Russian Federation

Article History: *Submitted: 28.10.2019* *Revised: 18.12.2019* *Accepted: 31.01.2020*

ABSTRACT

Results of conducted development of the way for obtaining Bifidobacterium bifidum's (B. bifidum) exo-metabolites with high antimicrobial activity are shown in this article. The suggested method of obtaining of B. bifidum exo-metabolites with high antimicrobial effect includes the preparation of nutritious environment, refrigeration to the temperature of cultivation, deep introduction of bifido bacterium's cultures, and cultivation with further excretion of B. bifidum metabolites. Reference culture of Staphylococcus aureus ATCC 25923 was grown on Muller Hinton agar during 24 hours under 37^0 C. The source suspension of Staphylococcus aureus ATCC 25923 was prepared in sterile isotonic solution (0,5 MF - 1,5x10^8 cfu/ml). During the determination of bactericidal activity of Bifidobacterium bifidum daily culture's exo-metabolites towards test strain of Staphylococcus aureus ATCC 25923, it was proved that two-day exo-metabolites of Bifido bacterium bifidum showed full absence of growth of against to Staphylococcus aureus 25923 ATCC by the way of the most bactericidal activity. This method provides the obtaining of Bifidobacterium bifidum's exometabolites with high antimicrobial activity and the research of possibilities of wide appliance in medicine.

Keywords: Bifidobacterium bifidum, exometabolites, antimicrobial activity, Staphylococcus aureus.

Correspondance:
Markov A.A
Tyumen State Medical University, Tyumen, Russian Federation,
Tyumen Industrial University, Tyumen, Russian Federation
Email id : alexdoktor@inbox.ru
DOI: 10.5530/srp.2020.2.42

INTRODUCTION

Nowadays during the surgical treatment of traumatological patient's high frequency of development of such complications as periprosthetic infection and ostiomyelitis was shown [2, 7, 8, 13, 21].

There are often opportunistic microorganisms with resistance to antibacterial drugs found in medical practice, and that, in its turn, pushes scientists to the search for new medication with antimicrobial features.

Bifidobacterium bifidum is important representative of normal microbiota of men Bacteria's of Bifidobacterium genus take part in their host's metabolism, supplying some physiological functions of macro partner that don't code in his genome (utilization of indigestible carbohydrates, exchange of complex proteins, synthesis of vitamins, production of energy for the preservation of homeostasis for intestines' epithelium), and also providing him with nutritious substances [10, 11, 12, 14, 15, 16, 20].

Besides, bifido bacterium modulates synthesis and activity of humoral and cellular factors of local immune. Nowadays new data are accumulated about immunotropic activity of bifido flora [17, 18, 19].

When considering the meaning of bifido flora from the position of associative symbiosis, it can be pointed that Bifidobacterium spp. appears to be the controller of physiological functions by vectors – "dominant-the host", "dominant – associate". Besides, befido bacterium can regulate interactions of "the host – associate" vector, since they can recognize "native" and "alien" strains of mictrosimbiotes, outpacing "immunological signaling" of men, and rendering immunoregulatory action on the system of adaptive immunity (through cytokines), reducing aggression of the host against "local" associates [3].

Integration of bifido bacterium in the human's organism and its important "helper's" role in accomplishing of some of homeostatic functions by macroorganism causes its actuality for applied aspect of using these microorganisms in biotechnology and medicine. Nowadays microorganisms of Bifidobacterium genus are widely used for production of probiotics and different products of functional nutrition [8, 9]. Efficiency of bifido bacterium's strains has special meaning, namely their antagonistic activity, acid-forming ability, spectrum of fermented carbohydrates, synthesis of vitamins and polysaccharides, adhesive and immunomodulatory features. The appliance of probiotics has big perspectives as "physiological" and safe way of regulation of protective reserves of organism and anti-persistent action towards and pathogenic microorganisms, that is one of the way of exploitation of prophylactic and healing effect of drugs containing bifido bacterium [4].

One of problems that occur during usage of probiotic drugs containing bifido bacterium is gradual decline of the level of their antagonistic activity, which, in the end, influences healing-prophylactic efficiency of probiotic. Thereby it is necessary to conduct researches to find not only new strains of bifido bacterium, but also ways for further work on the selection of effective variants for the development of probiotic drugs and also their metabolites (metabolitic probiotics) with antagonistic effect towards different kinds of pathogenic and opportunistic microorganisms [1].

The method is known that allows to obtain the biological stimulator with probiotic and immunomodulatory effect that provides the preparation of bifido bacterium's bacterial suspension, its ultrafiltration with further stabilization of the filtrate. In this case ultrafiltration is performed over bacterial suspension of the microorganism with obtaining of filtrate in 50-75%'s quantity of the original suspension' volume and stabilization of it by 80-110^{0}C thermal treatment for 15-30 minutes [5]. However in this method cultivation of microorganisms is performed with the application of

regulated casein-yeast environment and addition of solutions of ammonia and glucose, which doesn't fully provide bifido bacterium with necessary factors of growth. Besides, thermal treatment keeps bio-stimulating features of the product without excluding of antimicrobial features of bifido bacterium's metabolites. Also there was described in literature the method of preparation of bacterial drug on the basis of live bifido bacterium and lacto bacterium by the way of simultaneous integration of ferment of bifido bacterium and lacto bacterium into the sterile milk, mixing and cultivation [6].

Known methods provide the growth of bifido bacterium, that has biological, antimicrobial included, activity, but with that exo-metabolites of bifido bacterium (supernatant cultural liquid), that contain antimicrobial substances, are divided from cells and are not used further.

GOAL

To develop the method of obtaining of Bifido bacterium bifidum's exo-metabolites, that has high antimicrobial activity for wide application in medicine.

MATERIALS AND METHODS

The suggested method of obtaining of Bifidobacterium bifidum (B. bifidum)'s exo-metabolites with high antimicrobial effect includes the preparation of nutritious environment, refrigeration to the temperature of cultivation, deep introduction of bifidobacterium's cultures, cultivation with further excretion of B. bifidum's metabolites. For cultivation the nutritious Schaedler Broth was used (HIMEDIA, India); as clean cultures dilutions of industrial strain of B. bidium 791 (ZAO "Exopolis", Kovrov, Russia) on the basis of Schaedler Broth were added; obtained metabolites of B. bifidum were filtered through membranous bacterial filters "Millipore" (USA).

The method of obtaining of exo-metabolites was conducted as follows: Schaedler Broth was prepared by the instruction attached to commercial nutritious environment. The nutritious environment (Schaedler Broth) was sterilized by the autoclaving under 121^0C (1,1 athm) during 15 minutes, frozen to the temperature of cultivation, spilled into sterile test tubes by 9 ml. (10 tubes) and 8 ml. (10 tubes). Further workable solution of bifido bacterium was prepared. For this there was taken 1 flask of dry lyophilized "Bifidobacterium" with strain B. bifidum 791, containing 5 doses of drug (with 1×10^7 of microbial cells in one dose), respectively the concentration of microbial cells was 5×10^7, which were diluted in 10 ml. of Schaedler Broth.

Test tubes were incubated in thermostat under 37^0 C during 24, 48, 72 and 96 hours. Further obtained cultures of B. bifidum were centrifuged under 3000 turns / minute during 30 minutes. Supernatant cultural liquid with exo-metabolites was taken by glass pipette and sterilized, filtering through membranous bacterial filters "Millipore" (USA).

Reference culture of Staphylococcus aureus ATCC 25923 was grown on Muller Hinton agar during 24 hours under 37^0 C. The source suspension of Staphylococcus aureus ATCC 25923 was prepared in sterile isotonic solution (0,5 MF - $1,5x10^8$ cfu/ml).

RESULTS AND DISCUSSION

For the control Schaedler Broth was spilled on sterile tubes by 1,8 mln (pH 7,6). Tubes were signed (#1 to #6). The source suspension of Staphylococcus aureus (S. aureus) was thoroughly mixed and moved by 0,2 ml. in pipette into the tube #1. After thorough mixing it was transferred by new sterile measured 0,2 ml. pipette from this tube to the next et cetera up to 6 tube. At once after titration the seeding by 0,1 ml. from every was made over the yolk salt agar (YSA) for the determination of common microbial number (CMN).

YSA was used as an elective differential environment for the cultivation of staphylococcus, that helped to determine the feature of pathogenicity – the presence of lecitovitelasse. After the incubation of tubes during 24 hours under 37^0C the similar seeding was made over YSA for the determination of CMN.

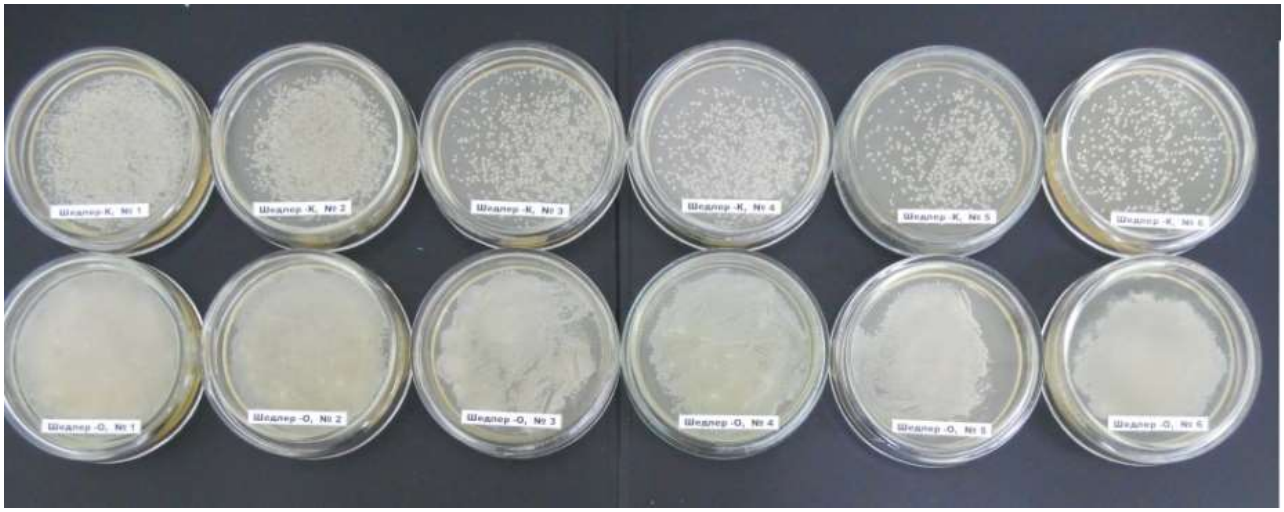

Figure 1. Culture growth control against to Staphylococcus aureus 25923 ATCC
(Without the influence of exo-metabolites)
The initial concentration of the test strain 15 000 000 cfu/ml.
($\mathbf{1,5x10^7}$; $\mathbf{1,5x10^6}$; $\mathbf{1,5x10^5}$, $\mathbf{1,5x10^4}$; $\mathbf{1,5x10^3}$; $\mathbf{1,5x10^2}$ cfu/ml)

During the determination of bactericidal activity of exo-metabolites of daily B. bifidum's culture towards test-strain of Staphylococcus aureus ATCC 25923, pH of daily solution of B. bifidum's exo-metabolites was measured and the decrease of this indicator was registered to 6,0 in comparison with the source 7,6 of Schaedler Broth's pH. Exo-metabolites of B. bifidum were spilled by 1,8 ml. in serile tubes, which were signed from #1 to #6. The source suspension of S. aureus was thoroughly mixed and moved by 0,2 ml. in pipette into the tube #1.

After thorough mixing it was transferred by new sterile measured 0,2 ml. pipette from this tube to the next et cetera up to 6 tube. For each new dilution a new (sterile) micropipette is used. At once after titration the seeding by 0,1 ml. from every was made over the YSA for the determination of common microbial number CMN. After the incubation of tubes during 24 hours under 37^{0}C the similar seeding was made the determination of CMN with the purpose of bactericidal effect of exo-metabolites. Result of the seeding after titration: the quantity of grown colonies corresponded to the calculated concentration of microorganisms in 1 ml.

The result of the second seeding (after incubation for 24 h.): in plates with concentration of test-strain S. aurerus ($1,5x10^7$; $1,5x10^6$; $1,5x10^5$ cfu/ml.) absolute growth of microorganisms was registered, what indicted to the absence of bactericidal activity towards high concentration of test-strain. In plates with low concentration of test-strain of S. aureus ($1,5x10^4$; $1,5x10^3$; $1,5x10^2$ cfu/ml.) the decrease of quantity of colonies in comparison with control seedings was marked. This fact points to the bacteriostatic and possibly bactericidal activity of daily exo-metabolites towards low concentrations.

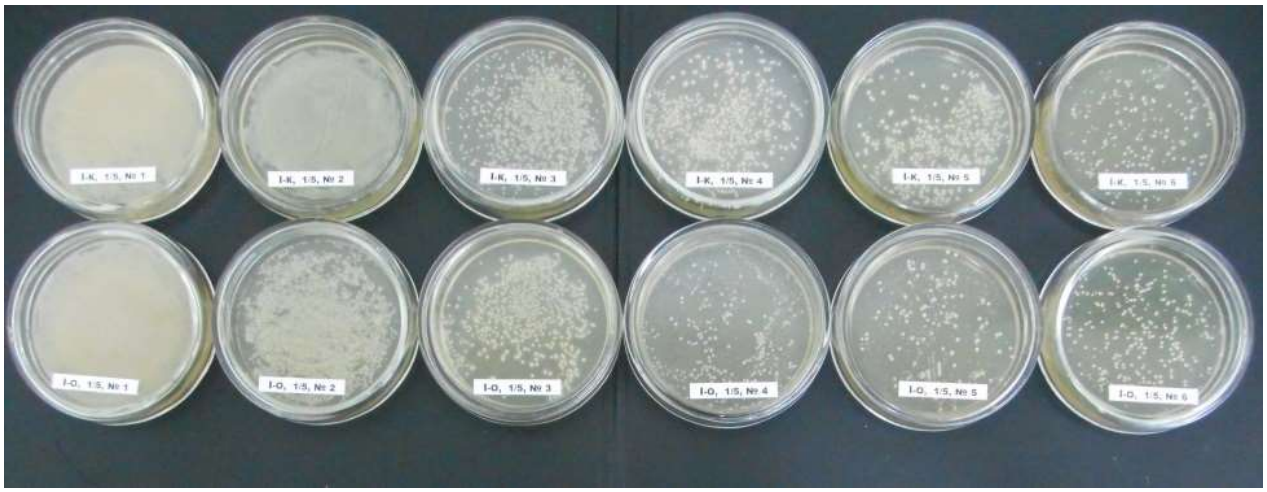

Figure 2. The study of the antimicrobial properties of exo-metabolites of Bifido bacterium bifidum obtained within 24 hours, against to Staphylococcus aureus 25923 ATCC. Bacteriostatic effect.
($1,5\mathbf{x10^7}$; $\mathbf{1,5x10^6}$; $\mathbf{1,5x10^5}$, $\mathbf{1,5x10^4}$; $\mathbf{1,5x10^3}$; $\mathbf{1,5x10^2}$ cfu/ml)

The decrease of pH was registered among two-day exo-metabolites of B. bifidum to 4,71; thre-dat – 4,67; four-day – 4,61 in comparison with the source 7,6 of Schaedler Broth's pH.

Two-day metabolites of B. bifidum showed 100% bactericidal activity towards different concentrations of test-strain of S. aureus (from $1,5x10^7$ to $1,5x10^2$ cfu/ml), which was confirmed by the absence of growth of microorganism's colonies in all plates; two-day B. bifidum's exo-metabolites showed 100% bactericidal activity towards test-strain of S. aureus, from the titer of $1,5x10^6$ to $1,5x10^2$ cfu/ml; during the titer of $1,5x10^7$ cfu/ml non-significant quantity of colonies (10-20) was registered, and that also confirmed high bactericidal activity of two-day exo-metabolites of B. bifidum

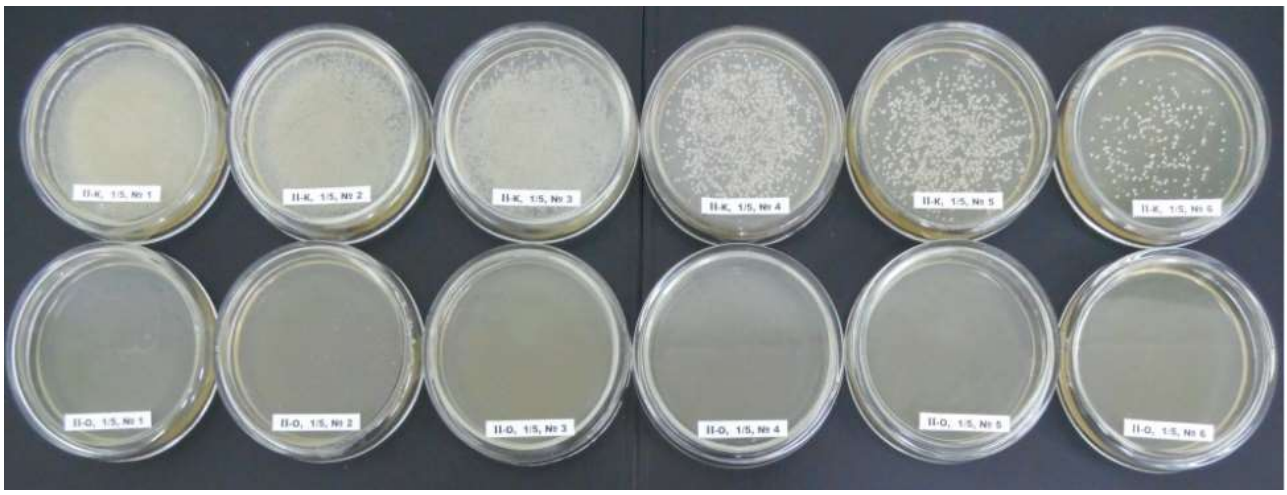

Figure 3. The study of the antimicrobial properties of exo-metabolites of Bifido bacterium bifidum obtained within 48 hours, against to Staphylococcus aureus 25923 ATCC. Bactericidal effect.
(1,5**x10^7; 1,5x10^6; 1,5x10^5, 1,5x10^4; 1,5x10^3; 1,5x10^2** cfu/ml)

Three-day exo-metabolites of B. bifidum showed 100% bactericidal activity towards concentrations of test-strain S. aureus (1,5x10^5, 1,5x10^4; 1,5x10^3; 1,5x10^2 cfu/ml), which was confirmed by the absence of growth of microorganism's colonies. Exo-metabolites showed relative bactericidal activity towards test-strain S. aureus in concentrations of 1,5x10^7, 1,5x10^6 cfu/ml.

Four-day exo-metabolites of B. bifidum showed 100% bactericidal activity towards concentrations of test-strain S. aureus (1,5x10^5, 1,5x10^4; 1,5x10^3; 1,5x10^2 cfu/ml), which was confirmed by the absence of growth of microorganism's colonies.

Exo-metabolites showed relative bactericidal activity towards test-strain S. aureus in concentrations of 1,5x10^6, 1,5x10^{7}cfu/ml (growth of occasional colonies was evidenced, some of them were different due to smaller size, change of pigment and the lack of factor of pathogenicity – lecithinase activity).

CONCLUSION

By this way, it was proved that two-day exo-metabolites of Bifido bacterium bifidum showed full absence of growth of against to Staphylococcus aureus 25923 ATCC by the way of the most bactericidal activity.

This method provides the obtaining of Bifido bacterium bifidum's exo-metabolites with high antimicrobial activity and the research of possibilities of wide appliance in medicine.

REFERENCES

1. Adlerberth I., Wold A.E. Establishment of the gutmicrobiota in Western infants. Acta. Paediatr., 2009; 98: 229-238.
2. Baimagambetov S.A., Balgazarov A.S., Ramazanov Z.K., Markov A.A., Ponomarev A.A., Turgumbayeva R.K. Abdikarimov M.N. Modern models of endoprostheses and periprosthetic infection. Biomedical Research (India). 2018; 29: Iss.11.
3. Bondarenko V.M. Stabilizing effect of metabolic antibiotic Hilac Forte towards normal microflora of intestines. Farmateka, 2005; 1 (97): 44-49.
4. Bukharin O.V., Perunova N.B. Symbiotic interactions of man and microorganisms. Physiology of Man. 2012; 38 (1): 128-138.
5. Bukharin O.V., Perunova N.B., Ivanova E.V. Bifidoflora in associative symbiosis of man. Instrument of Cellular and Intracellular symbiosis. UrO RAs. Yekaterinburg, 2014.
6. Cani P.D., Delzenne N.M. The role of the gut microbiota in energy metabolism and metabolic disease. Curr. Pharm. Des. 2009; 15: 1546-1558.
7. Markov A. Problems of the surgical treatment of patients with fractures of the proximal femur on the basis of osteoporosis. Sys. Rev. Pharm. 2019; 10(1): 143-145.
8. Martens E.C., Chiang H.C., Gordon JI. Mucosal glycan foraging enhances fitness and transmission of a saccharolytic human gut bacterial symbiont. Cell Host Microbe 2008; 4: 447-457.
9. Martin F.P., Sprenger N., Yap I.K., Wang Y., Bibiloni R. et al. Panorganismal gut microbiome-host metabolic crosstalk. J. Proteome Res. 2009; 8: 2090-2105.
10. Neschislyaev L.P., Chistokhina L.P. The Mergod of obtaining of biological stimulator. Patent of RF 2224018. Publ. 20.02.2014.
11. Paliy O., Kenche H., Abernathy F., Michail S. High-throughput quantitative analysis of the human intestinal microbiota with a phylogenetic microarray. Appl. Environ. Microbiol. 2009; 75: 3572-3579.
12. Resta S.C. Effects of probiotics and commensals on intestinal epithelial physiology: implications for nutrient handling. J. Physiol. 2009; 17: 4169-4174.
13. Rayner C.R., Baddour L.M., Birmingham M.C., et al. Linezolid in the treatment of osteomyelitis: results of compassionate use experience. Infection 2004; 32: 8-14.
14. Roy D. Bifidobacteria: friends in your food. Alimentech., 1992; 5(2): 14-17.
15. Sheil B., Shanahan, L. Mahony B. Probiotic Effects on Inflammatory Bowwel Disease. The Journal of Nutrition. 2007; 137: 819-824.
16. Shenderov B.A. Medical microbial ecology and functional nutrition. Microflora of man and animals and its functions. M.: Grant, 1998; Vol. I: 288.
17. Shenderov B.A. Skiba N.E. Manvelova M.A. Stepanchuk U.B. The Method of preparation of probiotoc in the basis of living bifidobacterium and

lactobacterium. Patent of RF № 2060673 publ. 27.07.1996
18. Tannock G. What immunologists should know about bacterial communities of the human bowel. Seminars in Immunology. 2007; 19(2): 94-105.
19. Wilmes P., Simmons S.L., Denef V.J., Banfield J.F. The dynamic genetic repertoire of microbial communities. FEMS Microbiol. Rev. 2009; 33: 109-132.
20. Yankovskiy D.S., Dyment G.S. Bifidobacterium and lactobacillus as optimal basis for modern probiotics. Current questions of pediatric. 2006. 3(12). 1-10.
21. Wald D.S. Wound healing under pathological conditions. Inf. Medicina propraxi. 2002; 10: 6-1.
22. Pathak, A. and Mathew, K.J., 2017. Need for care to caregivers: Psychological distress and its socio-demographic correlates among the relatives of persons with mental illness. International Journal of Psychosocial Rehabilitation. Vol 21 (2) 3, 12.
23. Srinath, R. and Sendilvelan, S., 2017. Behavioral Problems Perceived by the Alcoholic and His Family A Study among Males in Rural Areas. International Journal of Psychosocial Rehabilitation. Vol 21 (2) 13, 19.
24. Sari, S.P., Dwidiyanti, M., Wijayanti, D.Y. and Sarjana, W., 2017. Prevalence, demographic, clinical features and its association of comorbid depressive symptoms in patients with schizophrenia. International Journal of Psychosocial Rehabilitation, 21(2).
25. Fuadi, L., 2017. Influence of Behavioral Counseling Techniques, Token Economy and Parent's Parenting Class of Behaviour Prosocial X Syamsulhude Tegallinggah. International Journal of Psychosocial Rehabilitation, 21(2).
26. Uhrmann, L.S., Nordli, H., Fekete, O.R. and Bonsaksen, T., 2017. Perceptions of a Norwegian clubhouse among its members: A psychometric evaluation of a user satisfaction tool. International Journal of Psychosocial Rehabilitation, 21(2).