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Original paper

Halophilic bacteria as a potential management for autism

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Abstract

Autism spectrum disorders (ASD) are serious neurodevelopmental disorders with poorly understood etiology. Autism has more than one clear cause, which connect to each other leading by multiple connected steps to autism development. Because of the increased incidence of autism pathogenesis in recent years, an increased need to effective treatment has been noticed. The fact that autism has been reported as being a result of oxidative stress and gut microbiome imbalances has motivated researchers to treat autism by treating whether one or the other of these two main causes. Nevertheless, the treatment of just one of these factors has had a lot of limitations. Therefore, we propose in this review a natural treatment by using Halophilic bacterial biomolecules with both antagonistic and antioxidant effects, which could target both gut microbiome and oxidative stress disturbances, increasing then the chance of treating autism pathogenesis at an earlier state.

Keywords Autism spectrum disorders, Oxidative stress, Gut microbiota, Halophilic microorganisms, Natural biomolecules.

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Introduction

Autism spectrum disorder (ASD) is a neuro-developmental disorder, which is implicated in cognitive impairments, sociability impairments, language and communication skills impairments, and presence of repetitive or stereotyped behaviors (FM BERCUM & al [1]; J BAIO [2]). It is considered as the second most prevalent neurodevelopmental disorder. ASD has originally described by Kanner in 1943 (L KANNER [3]) and in less than 73 since the recognition of this disorder, its prevalence has dramatically exploded from 1 in 5000 (0.02%) to 1 in 68 (1.47%). This prevalence has been found to be four times more increased in males than in females (M WINGATE & al [4]). Even ifGeier DA and his collaborators (DA GEIER & al [5]) have mentioned in their study that increased levels of testosterone could be the reason why ASD is more prevalent in males, the obvious reasons still unclear.

The real causes leading to ASD still doubtful. Nevertheless, it has thought to be caused by a combination of genetic and environmental factors. Several studies have reported that ASD is linked to gastrointestinal (GI) symptoms and dietary factors, gut microbiome alterations and metabolic dysfunctions (L KANNER [3]; JR WEISSMAN & al [6]). Genetic andenvironmental risk factors are known to induce Reactive Oxygen Species (ROS) production with very high levels that are beyond the antioxidant system capacity. This state of disequilibrium between pro-oxidant processes and antioxidant defense system is named "oxidative stress" and it is considered as being the major cause of neuropsychiatric disorders including autism.

In front of the dramatic increase in incidence of autism in the last decades, emerging studies have focused in finding new treatments which could be effective to this disease. Many studies, have reported the use of: Antibiotics namely Vancomycin (RH SANDLER & al [7]). The use of probiotics and fecal microbiota transplantation (FMT) has been also reported (BMD TIMOTHY [8]). Nevertheless, the success of any of these treatments in affecting the intestinal microbiome still limited and unachieved because of several factors like the antagonistic effect between the transplanted microbes and those existing in the gut, and/or the resistance to antibiotics by the microbiome. Therefore, the finding of other effective new treatments is of interest need. In this work we will try to highlight the potential role that could play a new type of bacteria named "Halophilic Bacteria" in treating autism spectrum disorders.

Halophilic bacteria are a group of extremophile microorganisms that require environments with very high concentrations of salts. This ability of living in such extreme biotopes has made this bacteria more investigated by a lot of researchers. Many studies have reported the diversity, characteristics and assets of halophilic bacteria (A VENTOSA & al [9]; DK MAHESHWARI & S MEENU [10]). They have been reported as a natural source of various biomolecules such as: Enzymes, antimicrobial compounds, polyphenols and flavonoïds, antioxidants, as well as biomolecules with anticancer and anti-diabetic activities… (DPRIYA & al [11]; S SIKKANDAR & al [12]). Taking in consideration all these assets mentioned above, we will try to further discuss the possibility of using the biomolecules produced by halophilic bacteria in treating autism spectrum disorders.

Oxidative Stress in Autism

Oxidative stress is a disequilibrium state that occurs between prooxidants production and antioxidants functions, and leads to a dysfunctional state of living cells (H SIES & al [13]). Thereby, increased oxidative stress could lead to several serious abnormalities such as: membrane lipid abnormalities, mitochondrial dysfunction and immune dysregulation, as in most of the current disorders and their complications (D TIMOFTE & al [14]; AM VLASCEANU & al [15]; AC NICOLAE & al [16]; AP STOIAN & al [17]) and with increased relevance to the current review, in the majority of the neuropsychiatric disorders (IM BALMUS & al [18]; A CIOBICA & al [19]; A CIOBICA & al [20]; M PADURARIU & al [21]; A CHAUHAN & V CHAUHAN [22]), as well as it could lead to severe macromolecules damage namely deoxyribonucleic acid (DNA), proteins and lipids, which all might contribute to critical psychiatric disorders including autism, since, as mentioned above, oxidative stress is an important player in the neuropsychiatric pathology.

Within normal physiological conditions, living cells could maintain the dynamic pro-/antioxidant balance (E GRANOT & R KOHEN [23]). Nevertheless, during oxidation and reduction processes in most life activities, cells produce increased levels of ROS and Reactive nitrogen species (RNS) which results in pro-/antioxidants imbalance leading to oxidative stress (A MONICZEWSKI & al [24]). In fact, because of the high oxygen demand in addition to the high content of lipids, nerve tissue is exceptionally sensitive to oxidative damage implying ROS/RNS (A MONICZEWSKI & al [24]).Under normal conditions, the first ROS produced during respiration is superoxide ($O_2^{\cdot-}$). This superoxide from the reduction of molecular oxygen is a principal source of deleterious free radicals and hydroperoxide (H_2O_2) which is a key radical anion that has been suggested to be the precursor of other ROS (I FRIDOVICH [25]; M REPETTO & al [26]). Once H_2O_2 is formed, it reacts via Fenton reaction with transition metal ions (Fe^{2+}, Cu^{+}) to generate an extremely reactive hydroxyl radical ($^{\cdot}OH$)(JM MCCORD & ED DAY [27]). This radical is responsible for the most toxic effects on

cells and it is suggested to initiate lipid peroxidation directly due to its very high chemical reactivity (JM MCCORD & ED DAY [27]).

In addition to ROS, RNS are produced in a well-regulated way to help maintain homeostasis at the cellular level in the normal healthy tissues. Actually, they are supposed to cause oxidative damage, destroy cellular macromolecules, and to promote neuronal damage (M VALKO & al [28]). Nitric oxide (NO) is an important physiological messenger implicated in principal functions in the Central Nervous System (CNS) such as the regulation of cerebral blood flow and memory (AP HERNANSANZ & al [29]). Moreover, it even affects the development and function of the CNS. NO is produced in mitochondria from L-arginine by a reaction catalyzed by NO synthase (AP HERNANSANZ & al [29]).Due to its very short half life, it is converted to other RNS for instance, nitrosonium cation (NO^+), nitroxyl anion (NO^-), nitrate (NO_3^-), or peroxynitrite anion ($ONOO^-$) which is a potent oxidant resulting from the reaction between NO and $O_2^{\cdot-}$, during mitochondrial respiration (A NAVARRO [30]). It is very important to mention that NO is the only free radical that doesn't promote lipid peroxidation chain reaction because of its quick reactivity with peroxyl radicals leading to chain termination (A OHARA & M SAYURI [31]). Lipid peroxidation has been described as the main mechanism involved in the damage of cell structures and functions which lead to cell death. Its process is a generator of hydrocarbon ($R\cdot$), lipid ($L\cdot$), peroxyl ($ROO\cdot$), and lipid peroxide ($LOO\cdot$) radicals in which the majority is capable to provoke a singlet state of the oxygen molecule ($^1O_2$). This form of oxygen has been demonstrated to be able to penetrate the phospholipid bilayer easily so it might initiate other peroxidation chains (RM CORDEIRO [32]).

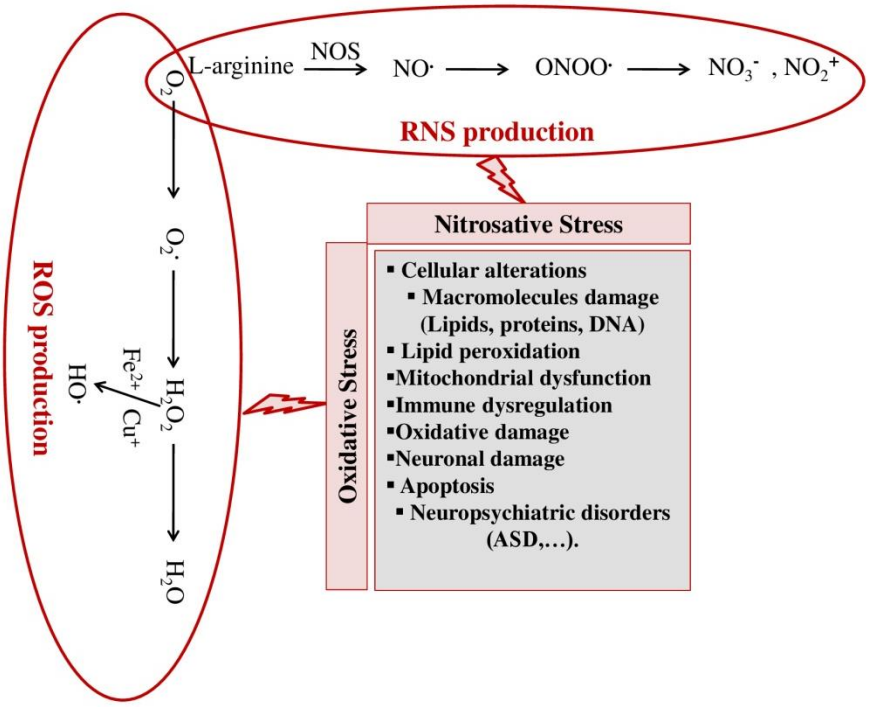

Figure 1. Mechanisms of reactive oxidative and nitrosative species (ROS and RNS) production and their contribution to oxidative stress and autism.

The main question here is: How cells maintain the equilibrium and neutralize the excessive accumulation of ROS/RNS? Normally, Cells use an antioxidant system constituted by a certain number of enzymes which cooperate together in order to neutralize the overproduction of ROS/RNS. The main enzymes (primary enzymes) involved in the direct elimination of these free radicals are: Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Besides, other endogenous enzymes named "secondary antioxidant enzymes" such as; glutathione reductase and glucose-6-phosphate dehydrogenase; are acting by maintaining a steady concentration of glutathione and nicotinamide adenine dinucleotide phosphate (NADPH) which are necessary for optimizing the primary antioxidants functions (JMC GUTTERIDGE [33]; B CHANCE [34]; G VENDEMIALE & al [35]). On the other hand, other antioxidant compounds are used by living cells to remove ROS/RNS, for instance ascorbic acid, Vitamin A, Vitamin E, amino acids (arginine, citrulline, creatine, taurine), metals (selenium, zinc), and

tocopherols (A MONICZEWSKI & al [24]). Many studies have reported the use of these antioxidant enzymes and compounds levels as biomarkers that reveal the implication of oxidative stress in autism disorder. For example, compared to controls, autistic patients have shown decreased activity of SOD in plasma (S SOGUT & al [36]) as well as in erythrocytes (O YORBIK & al [37]; NA MEGUID & al [38]). Nevertheless, other studies have shown increased activity of SOD in plasma (A LASZLO & al [39]) and in erythrocytes (L VERGANI & al [40]; SS ZOROGLU & al [41]), or even unchanged plasma SOD activity (SP PASCA & al [42])in patients with autism. A recent study made in 2002 (O YORBIK & al [37]), has found that autistic patients have shown decreased activity of GPx in plasma and erythrocytes, and decreased catalase activity in erythrocytes. Other researchers have confirmed the reduction of catalase activity in erythrocytes (L VERGANI & al [40]; SS ZOROGLU & al [41]).

Lately, a recent study have reported the change in levels of two principal antioxidant proteins namely; ceruloplasmin (a copper-transporting protein) and transferrin (an iron-transporting protein); which are synthesized in brain and other tissues in autistic children. Their levels have been reduced in the serum of children with autism in comparison to their sibilings**(**A CHAUHAN & al [43]). Pascaand his group (SP PASCA & al [42]), were interested in studying the change of amino acids levels in autism. They have found higher total homocysteine levels in autistic children plasma compared to controls. All these studies highlight the major potential role of oxidative damage in autism.

Although, in addition to oxidative stress, gut microbiome abnormalities have been reported in numerous studies as major cause of autism spectrum disorders (ASDs) (HE VUONG & EY HSAIO [44]; BMD TIMOTHY [8]; CA HEBERLING & al [45]). In fact, the human gut microbiome is a very complicated ecosystem that contains several types of microbes, especially, bacteria. Besides the primary role played by the microbiome in digestion and synthesis of vitamins and cofactors, it plays an important role in regulating the host physiology, metabolism, nutrition and brain function (HE VUONG & EY HSAIO [44]). As mentioned in many studies, the balance of gut bacteria composition and growth are key factors that influence the development of ASD. When analyzing fecal samples from ASD patients for microbial composition determination, Finegold and his group (SM FINEGOLD & al [46]) have noticed the existence of abnormal *Clostridia* taxa, and overgrowth of *Clostridia* together with decreased numbers of *Bifidobacteria*. In a more recent study (S SIKKANDAR & al [12]), he found that *Desulfovibrio* was of special note. Despite its very small amount over total bacterial population (0.3%), its proportion was 10-fold elevated in autistic individuals compared to healthy ones. Actually, it has been reviewed that *Desulfovibrio* and *Clostridia* are the main microbes that contribute to ASDs (CA HEBERLING & al [45]). On one hand, *Desulfovibrio* are known to produce hydrogen sulfide (H_2S) as main sulfur metabolism product. Thus, the sulfur metabolic deficiencies found in autistic individuals could be the result of increased need of ASDs patients for *Desulfovibrio*. Moreover, a study performed by Newton. DF and his group in 1998 (DF NEWTON & al [47]), has suggested that *Desulfovibrio* might play an important role in changing the gut bacterial ecosystem, which could lead to autism. On the other hand, Clostridial toxins have been known as a cause of gut permeability by changing the gut epithelial cells morphology, which create a substantial increase in the paracellular space (K AKTORIES & I. JUST [48]). This increased gut permeability induces the leak of molecules outside the gut toward the cerebrospinal fluid. In the same context, a recent study (CA HEBERLING & al [45]), has tried to gather all this parameters in order to establish a pathogenesis model of ASD. The proposed model contains circular relationships that could be broken down to three major routes: Oxidative stress and resulted sulfur metabolic deficiencies, intestinal bacterial abnormality, and intestinal permeability.

First off, increased oxidative stress induces sulfur metabolic deficiencies that result in transmethylation and transulfuration pathways deficiencies leading to serious changes in genetic expression, impaired removal of toxic heavy metals and reduced sulfating detoxification of xenobiotic compounds. Moreover, because of the rate limiting step of cystein in glutathione production, the metabolic deficiency could aggravate the oxidative stress state and lead to further harmful implications. In addition, gut microbiome composition abnormalities cause the production of toxins, hydrogen and sulfide. All these together lead to GI inflammation that causes in turn increased gut permeability. This increased intestinal permeability gives rise to increased blood circulation of molecules contained into the gut (Lipopolysaccharids (LPS), cytokines, short chain fatty acids (SCFAs), …) and ensuing rupture of the blood-brain-barrier causing thereby autism spectrum disorder pathology.

According to all these studies discussed above, it seems that oxidative stress is a principal cause of ASD. In fact, oxidative stress and gut microbiome sound to be together the base of the entire pathways whether leading or aggravating autism disorders. Therefore, admitting that the cyclic relationships giving above is true, then we should come up with treatments that could deal with oxidative stress and gut microbiome problems at one in order to ensure better treatment efficiency.

Halophilic Bacteria as Potential Treatment of Autism

Nowadays, because of the increasing problem of drug resistance (antimicrobial resistance, antineoplastic resistance …) we notice a dire need to research, isolate, identify and use novel biomolecules with biotechnological importance. Thus, newer ecosystems are yet being sought in order to find and identify new potential producers of natural bioactive compounds. Under the same objective, microbes that inhabit extreme environments in which they withstand harsh conditions of salinity, temperature, potential of hydrogen (pH), pressure, and severe solar radiation, have attracted considerable attention in the last decades (SH VASAVADA & al [49]). In this study, we are interested in halophilic bacterial biomolecules which have earned tremendous importance in recent years due to their potential to be "molecules of the future" which benefit mankind (KK TONIMA & K. SAVITA [50]).

Halophiles are highly diverse salt-loving micro-organisms, which are adapted to high osmolarity and generally require salts for their survival in hypersaline environments (I AARZOO & al [51]; A VENTOSA & al [9]). According to their salts requirement, halophilic microorganisms may be categorized as: Slight halophiles grow optimally at 0.3 *M* -0.8 *M* (1.8-4.7%) of salts, moderate halophiles grow optimally at 0.8 *M* - 3.4 *M* (4.7-20%), extreme halophiles requiring 3.4 *M* - 5.1 *M* (20-30%) of salts, or halotolerants which could live in the presence as well as in the absence of salts. Nevertheless, nonhalophiles grow optimally at less than 0.3 *M* (1.8%) (A VENTOSA & al [9]; A VENTOSA [52]).

Despite the fact that salts are extremely needed for all life types, halophiles are distinguished by their exigency of hypersaline conditions for growth and proliferation. Actually, it's well known that high osmolarity in such harsh conditions could be deleterious to cells because of the loss of water to the external medium till the achievement of the osmotic equilibrium (S DASSARMA & P ARORA [53]). Thereby, the main question is: How could halophiles support these conditions? Further, how could they require them to proliferate?

In fact, halophilic bacteria are able to live in such environments due to their cellular machinery which is able to prevent loss of cellular water within these circumstances. To make it clear, in order to counterbalance the external osmotic pressure and prevent loss of water molecules, halophiles accumulate high compatible solute concentrations either by synthesizing them or by possessing the transporters which take them up in the cytoplasm. These solutes allow cells to maintain their water molecules until an isoosmotic balance with the medium is achieved and cells volume is maintained (EA GALINSKI [54]; S SIKKANDAR & al [12]). Many osmoregulatory solutes have been reported. For instance, halophiles usually accumulate amino acids (glycine, proline, betaine, ectoine), and polyols (sucrose, trehalose, and glycerol). As well, they may accumulate inorganic ions such as potassium, chloride, and sodium as the case of some extreme halophiles, which accumulate potassium chloride(KCl) at an equal concentration to the external sodium chloride (NaCl) one (S SIKKANDAR & al [12]). It's important to mention that these solutes don't disrupt metabolic processes and haven't net charge at physiological pH. Otherwise, they simplify the adaptation of proteins so they could function at elevated salt concentrations. For example, negatively charged amino acid residues cover the surface of halophilic proteins for increasing their solubility in saturated sodium conditions (F VEILLIEUX & al [55]). Generally, secondary metabolites are produced by micro-organisms as a response to the effect of biotic and abiotic factors of their environment. From this viewpoint, the interesting capacity of halophilic bacteria to survive and produce into hypersaline conditions in which the growth of the majority of organisms is limited in addition to their simple nutritional requirements (VF RODRIGUEZ [56]), their feasible genetic manipulation (several genetic tools developed for nonhalophilic bacteria could be applied to halophiles) (A VENTOSA & al [9]), their increased stability in adverse biotopes, and the high stability of their products (MBS DONIO & al [57]); point out the high possibility of these bacteria to serve sources of significant newer secondary metabolites. All together show that halophiles have a promising biotechnological application, especially in medical biotechnology.

In the last decades, halophiles have been given increased interest. They become more investigated since a lot of researches have focused on reporting their new secondary metabolites production, in addition to their potential biotechnological uses. In this review, we will focus on discussing the possible uses of halophilic bio-molecules in medical and pharmaceutical biotechnologies, notably the potential use of these attractive biocompounds to treat autism spectrum disorders.

Since decades, several interesting halophilic bio-molecules have been reported for several applications. At first, halophiles have been known as rich sources of stable hydrolytic enzymes capable to function under harsh conditions which generally lead to most proteins denaturation or precipitation. The most famous halophilic enzymes are: Amylases, lipases, proteases, cellulases, pectinases, and nucleases. These enzymes are intensively demanded in multiple industrial sectors (H ONISHI [58]; H ONISHI & al [59]; A VENTOSA & al [9]; S DASSARMA & P ARORA [53]). Moreover, according

to data, halophilic microorganisms are well-known by their large antagonistic activities against a wide range of microbes. In 1982, seven screened strains of Haloarchea have shown an antagonistic activity by producing proteinaceous antibiotic named "Halocins" (reported by LA GIDDINGs & DJ NEWMAN [60]). These Halocins act by controlling Haloarcheal populations and seem to have similar functions with bacteriocins produced by eubacteria to control eubacterial populations (MA RILEY [61]). Recently, Donio and coworkers have identified an halophilic bacterial strain *Bacillus sp.BS3* able to produce important pharmacologically biosurfactants with antibacterial, antifungal, and antiviral activities against: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aerugenosa*, and *Salmonella typhi*; *Trichophytonrubrum*, *Aspergillus niger*, *Aspergillus flavus*, *fusarium sp*.; and "White Spot Syndrome Virus (WSSV)", respectively (MBS DONIO & al [57]). It is extremely important to mention that the genus *Bacillus* is a part of lactic acid bacteria, which constitute a part of the gut microbiome. *Bacillus* doesn't have side effects on human health and possesses a lot of health benefits. In addition to hydrolytic and antimicrobial activities, halophiles have been found to produce further interesting biomolecules with antioxidant and cytotoxic activities. In 2006, Iizuka and his group (T IIZUKA & al [62]) have identified two antibiotic depsipeptides produced by a slightly halophilic myxobacterial strain SMH-27-4 isolated from Kanagawa, Japan. Both biocompounds were found able to inhibit nicotinamide adenine dinucleotide (NADH) oxidase enzyme which is considered one of the major sources of superoxide anion in human as well as bacterial cells. Besides, two novel fungal metabolites have been screened from a halotolerant fungus (*Aspergillus variecolor B-17*) by Wang and his group (W WANG & al [63]). These metabolites were capable to exhibit moderate cytotoxic activity against murine leukemia P-388 cells, human promyelocytic leukemia HL-60, hepatocellular carcinoma BEL-7402, and lung adenocarcinoma A549 cell lines. Furthermore, one of the same biomolecules has shown a radical scavenging activity against1,1-diphenyl-2-picrylhydrazyl(DPPH) with half maximal inhibitory concentration(IC_{50}) value similar to that of the ascorbic acid. More studies have focused on searching halophilic secondary metabolites with antioxidant activity. For instance, Lu and coworkers (ZY LU [64]) have reportedin 2008the finding of a new antioxidant biomolecules; from a halotolerant fungal strain *Peniciliumcitrinum B-57*; manifesting a moderate antioxidant activity compared to that of ascorbic acid. A recent study made by SikkandarS and his group in 2013 (S SIKKANDAR & al [12]) has shown the great potential of two halophilic strains: *Halobacterium salinarium* and *Halobacterium volcanii* in producing "carotenoïds" which are strong antioxidant pigments. The extracted carotenoïds have revealed a powerful antioxidant activity in comparison to that of the positive control which is Butylated hydroxytoluene (BHT), in addition to a potent anticancer activity on human liver carcinoma cell lines Hep G2. In the light of the potent halophilic biomolecules identified and their high pharmaceutical potential exploitation, scientifics have become more interested in finding new halophilic pharmaceutical metabolites. For example, recently, 1178 halophilic bacterial isolates were studied for various biological activities in order to determine their biomedical significance. 63 Out of 1178 bacterial extracts were found able to produce significant pharmaceutical metabolites. From the 63 active cultures; 14 isolates have shown antifungal activity, three isolates have exhibited anticancer activity against colon and uterine cancers, two bacteria have revealed antigastric ulcer activity (against *Helicobacter pylori*), and one culture have shown antioxidant activity. Furthermore, 13 isolates were able to produce amylases while 4 produce proteases. The 63 active cultures were also tested in order to evaluate their effects on some neuropsychiatric disorders. The obtained results have shown that 32% have revealed anti Parkinson's activity, 22% have shown antidepressant activity, 11% have exhibited anti dementia activity, whereas 3% have revealed anti anxiety activity (KK TONIMA & K SAVITA [50]). For our best knowledge, this study is the first that was interested in confirming the possible halophilic microbial activities on memory enhancement and neurological disorders management. Taking in consideration all these studies cites above, especially basing on the reported antioxidant and antimicrobial activities of halophilic bacteria in addition to the significant antioxidant and antimicrobial results obtained for our halophilic bacteria isolated from Dead Sea it seems that halophilic metabolites could offer a good treatment of neuropsychiatric disorders, notably autism.

As we already mentioned, the balance of gut microbial growth and composition in addition to oxidative stress are the base of the whole pathways leading or aggravating autism spectrum disorders. Therefore, halophilic bacteria could be a perfect treatment of autism, because they sound to manifest both antioxidant and antagonistic activities, which maybe a solution for treating oxidative stress and the gut microbiome imbalances at the same time. Accordingly, GI inflammation may be prevented leading to reduced gut permeability. Consequently, the blood circulation of gut molecules (LPS, cytokines, SCFAs, and other bacterial products…) may be reduced, which might stop the ensuing rupture of the blood-brain-barrier preventing thereby the cause of ASD.

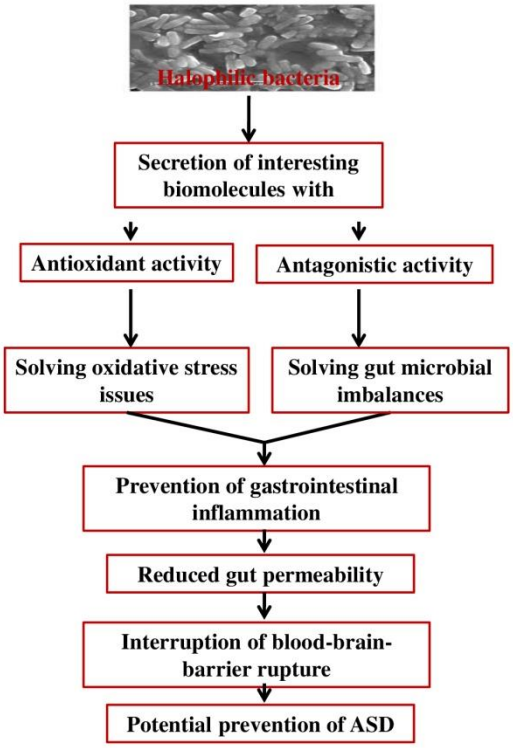

Figure 2. A proposed pathway of autism spectrum disorders treatment using halophilic biomolecules with antioxidant and antagonistic activities.

Potential Halophilic Bacterial Biomolecules Therapy in Autism

Autism spectrum disorder (ASD) is a very complicated pathogenesis, which might involve several factors that connect to each other in order to cause this symptom which could have more than one clear possible cause. As previously noticed, autism occurred as a result of the interaction between gut microbiome and oxidative stress, which could explain the reason why, proposed treatments of this disorder take in consideration both gut microbiome imbalances and oxidative stress disturbances. Nevertheless, the majority of these studies have tried to treat each factor separately on the other one. For instance, nowadays, multiple proposed clinician's tools for carrying on intestinal imbalances exist such as: Antibiotics, probiotics, prebiotics, fecal microbiota transplantation (FMT) treatments, and dietary interventions. It has been reported that prebiotics could be beneficial for intestinal mucosa in addition to systemic immunity because of their ability to get at the large intestine nonhydrolyzed and stimulate the growth of wholesome intestinal microbiota (SJ LANGLANDS & al [65]). Besides, probiotics could restore intestinal permeability by enhancing the mucosal barrier function (BS RAMAKRISHNA [66]). In the same context, multiple antibiotics have been proposed for treating gut microbiome disturbances. Finegold has reported treating *Desulfovibrio* overgrowth with aztreonam and beta-lactamase inhibitor (SM FINEGOLD [67]), and Clostridial overgrowth with antibiotics vancomycin or metronidazole (SM FINEGOLD [68]). Recently, fecal microbiota transplantation (FMT) becomes a famous treatment of autism. The possible therapeutic uses of FMT have been supported by many published studies that have suggested that probiotics could affect brain function like those of the brain's emotional and pain centers (K TILLISCH [69]). Although, the success of any of the treatments cited above still limited and insignificant maybe due to the short-term effects of these interventions, then while stopping the treatment, we assess a gradual return to the pretreatment microbiome state (JB ADAMS & al [70]; BMD TIMOTHY [8]), or because of the high resistance of some bacteria to several antibiotics.

In the other hand, other proposed interventions to treat autism have focused on treating oxidative stress disturbances by using natural and/or synthetic antioxidants. For instance, James and his group have focused on treating patients with dietary supplements such as, betaine, folinic acid, and methyl vitamin B12. It seems that their combination restored transmethylation and transsulfuration metabolites to similar levels of that of controls, ensuing

in symptoms improvements (SJ JAMES & al [71]). Moreover, it has been reported recently that ascorbic acid (MC DOLSKE & al [72]), N-acetyl-cysteine (AY HARDAN & al [73]), or coenzyme Q10 (A GYOZDJAKOVA & al [74]) treatments ameliorate symptoms in the autistic patients. Nevertheless, some limitations have been noted for example, the co-administration of N-acetyl-cysteine and risperidone was able to decrease irritability in autistic patients, nonetheless did not change the core symptoms of autism like social withdrawal, stereotypic behavior, inappropriate speech (A GHANIZADEH & E MOGHIMI-SARANI [75]). All together, show that the available pharmaceutical treatments still limited. Therefore, more attempts are needed in order to find alternative approaches for better yielding. The main idea would be to offer an approach that could deal with both gut microbiome and oxidative stress disturbances, which is the case with Halophilic bacterial biomolecules that exhibit antagonistic and antioxidant effects. Perhaps, stopping autism pathogenesis at these two principal steps would stop autism at an earlier state. In this manner we may be able to target sulfur metabolic deficiencies, bacterial overgrowth and abnormal intestinal bacteria, in addition to increased gut permeability, altogether. It is true that the yield observed in animal models is not the same that will be found in clinical applications but the advantages and the great prospects of this natural approach are strong reasons that encourage its application.

Conclusion

Autism is a serious neurological disorder that has several factors and more than one clear cause. The main factors leading to autism spectrum disorders development are gut microbiome imbalances and oxidative stress disturbances. Because of the multiple limitations of available pharmaceutical treatments, Halophilic bacterial biomolecules with double effects on both oxidative stress and gut microbiota troubles could be a great treatment that would be able to stop autism at an earlier state.

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