Abstract

The theory of casein and gluten-derived exorphins and their influence on autism via gut diabiosis is well known. This theory has given rise to a demand for foods and supplements, which are both casein- and gluten-free. However, as more test results have been reported on different products, there has been an appearance of often-spurious results. This has been especially true of probiotic supplements. Until now, the issues surrounding such wide-ranging results have not been addressed. This report discusses the technical issues involved in testing and presents a concluding commentary as to the relevance of such tests to the autistic community.

Currently, there is a demand in the autism community for foods and supplements, which are both casein and gluten free (CGF). There is also an increasing demand for probiotic organisms. Question has arisen regarding the ability to produce probiotic organisms which can qualify as CGF. The CGF issue presents several technical issues, which have remained un-addressed.

At present, the overwhelming majority of probiotics are produced in growth media, at some point contain at least one dairy product. However, it is generally agreed that through proper attention to growth conditions as well as processing methods, the state of the art is such that for all intents and purposes, the final products can be considered milk or casein free. This is because during normal growth and processing, the bacteria consume the dairy constituents of the growth media and residuals are separated during the concentration/purification of the probiotics. However, there are often problems demonstrating this property due to the inherent problems of current assay methods.

There are several types of assay methods currently employed for the detection of casein. The first is precipitation and quantification by "total protein" methods such as the Kjeldahl procedure. The second is by an enzyme-linked immunosorbent assay (ELISA). The ELISA method utilizes antibodies to detect the casein as a target antigen with subsequent reporter systems (e.g., colorimetric). The former method evolved out of the food processing industry as a way to test milk products for casein. The method relies on the fact that the vast majority of protein present in milk is casein and for that industry, the method proved useful. Additionally, the probiotics are living organisms producing a wide variety of proteins. These too can contribute to a false-positive signal from the reporter system due to similarity to casein in sequence. The chance of this happening when using a mAb is much less than when a pAb is employed, however, the standard "kit" used to detect casein contain pAbs not mAbs. It is worth emphasizing that different bacteria produce different levels of various proteins so there can be what appear as spurious results from species to species and even strain to strain. Because the probiotic organisms contain thousands of different proteins at any given time, the method is not appropriate for the determination of casein in any given culture. Similarly, the latter method has several drawbacks, which are discussed below.

First, in the ELISA, it is most desirable to use a monoclonal antibody (mAb) vs. a polyclonal antibody (pAb) for reasons of specificity. With a mAb, the chances of a false-positive are much less because the mAb is much more specific for the desired target (in this case casein) than the pAb is. The pAb by its very definition, is specific for several if not many different targets. The reason is that a pAb is not one single antibody, but consists of many different Abs and hence the "poly." For many purposes, a pAb is sufficient. The reasons for the use of a pAb over a mAb range from time to cost. A pAb can be produced much faster than a mAb and at much less cost.

Those are just the first problems associated with the ELISA assay. While ELISA is a very good assay technique, it is less than desirable for assaying casein in probiotics. The reason for this, in addition to those above, has to do with how the "reporter" portion of the ELISA functions. While there are different ways to perform the ELISA assay, they all can be generalized as follows. During the ELISA, when a target molecule binds to the Ab, a subsequent enzymatic reaction (typically the enzyme is "linked" to the antibody) is used to "report" that the binding occurred. This enzymatic reaction, more often than not, involves a peroxidase or phosphatase. Herein lies the problem, because probiotic produce prxidases and phosphatases. The problem is further confounded because different bacteria produce different levels of these enzymes. For instance, Fitzsimmon and Berry (1994, pp 125-33) showed that Lactobacillus acidophilus (LA) produce peroxidase. Not surprisingly, LA shows up as a positive using the ELISA even when produced using the very same procedure for other strains which show up as negatives. Further, there are probably other enzymes produced by the bacteria, which can similarly trigger the reporter system resulting in a false positive.
There is a third, though less-
common method for detecting casein, 
which utilizes gel (typically, SDS-
oligocyclamide) chromatography. 
Cells, cellular extracts are placed in 
a gel at one end of a gel 
with the smaller 
proteins to move through first and the 
smaller proteins to migrate more slowly 
right relative to the smaller 
proteins. This differential mobility in 
the gel affects a separation of proteins 
and protein fragments. The main 
problem with this method is that it is 
only possible, but probable, that 
very different proteins (based on 
molecular weight) can have the same mobility 
the gel. This limitation can be overcome, but only to a degree, using 
electric focusing gels. This process 
has similar problems associated 
with it.

Conclusion
As a final note, it should be kept 
that the reason for the 
ignorance over the casein in the first 
place is the fear of the production of 
probiotics from the casein. This is 
due to the fact that the probiotic 
organism is being subjected to 
incubation, themselves 
to enzymes capable of breaking 
the casein. Vamanen et al. 
(2005, pp. 146-64) recently showed 
that the probiotic organisms, currently 
used as health supplements, 
are analogues of the Dipeptide 
IV enzyme (e.g., PepX) 
and is known to be able to digest 
its. Noteworthy that the fact is that 
the higher the concentration of 
casein, the more likely it is to produce 
false positive for casein while 
colouring predominantly 
white or yellow amounts of the 
"amino acids."

Even as a whole, this information 
clarify several standing 
questions regarding both probiotic 
organisms in their effect on 
mental status in autism, as well as 
observed test discrepancies. In light 
of recent advances in understanding 
underlying enzymology of 
probiotics, it is prudent to 
look at casein testing in light of the 
biological significance that any 
new presence might have. 

The current state of the art 
of casein testing should be given 
proper consideration.

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