MINIREVIEW

Significance of V-Factor Dependency in the Taxonomy of Haemophilus Species and Related Organisms

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The family Pasteurellaceae comprises the genera Haemophilus, Pasteurella, and Actinobacillus (22). These three genera are closely related, with Pasteurella and Actinobacillus being particularly difficult to differentiate (3, 24, 27, 33, 40, 41); such difficulties have led to the suggestion that the genera Pasteurella and Pasteurellaceae should be considered to form one genus (40, 41). On the other hand, organisms of the genus Haemophilus have been distinguished, traditionally, by their requirement for V factor or for both (15). Recently, however, the results from DNA homology studies (35) have been used to challenge the use of V-factor requirement as a generic criterion (21, 35), and in subsequent studies (36), Haemophilus pleuropneumoniae, which requires V factor (16), and the closely related Pasteurella haemolytica-like organism of Bertschinger and Seift (H. U. Bertschinger and P. Seift, Abstr. 5th Int. Pig Vet. Soc. World Cong. Hyol. Hyiatr., abstr. no. M19, 1978) were transferred to the genus Actinobacillus as Actinobacillus pleuropneumoniae biotypes 1 and 2, respectively. There have also been suggestions and proposals for the transfer of organisms from the genus Haemophilus to the genus Pasteurella (26) and from the genus Actinobacillus to the genus Haemophilus (5, 14, 37, 38), as well as for the transfer of organisms from the genus Pasteurella to the genus Actinobacillus (7, 9, 24, 25, 28, 35). Indeed, it now appears to be generally accepted that genera belonging to the family Pasteurellaceae may comprise both V-factor-requiring and V-factor-nonrequiring species (10).

While the information described above suggests that the taxonomic significance of V-factor requirement or V-factor dependency has been overemphasized in the past, it is our contention that such a conclusion may be inappropriate as it relies on a somewhat superficial interpretation of the data. To lend credence to this view, the aims of this paper are to review, briefly, the nature of V factor and the biochemical basis for V-factor dependency and to present evidence suggesting that under appropriate conditions, V-factor-dependent growth may be a characteristic exhibited by all members of the family Pasteurellaceae, suggesting in turn that all of these organisms may be incapable of synthesizing V factor de novo. The available information also suggests that of the pyridine compounds involved in procaroytic pyridine nucleotide metabolism (8, 23, 48), only a few specific pyridine nucleotides and precursors can support the growth of these organisms. Such a feature may help to consolidate the family Pasteurellaceae and may prove to be useful in future decisions regarding the placement of organisms such as Haemophilus ducreyi, Haemophilus somnus, and other related organisms, whose taxonomic positions remain somewhat uncertain (2, 4, 34, 42).

HAEMOPHILUS: REQUIREMENTS FOR PYRIDINE NUCLEOTIDES OR PRECURSORS AND REASONS FOR SUCH REQUIREMENTS

Collectively, the results of several studies have demonstrated that NAD, nicotinamide mononucleotide (NMN), and nicotinamide riboside (NR) can function as V factor for the growth of various V-factor-dependent haemophili, whereas nicotinamide (NAM) and the (nicotinic) acid analogs of these compounds, as well as quinolinic acid (QA) and the α-anomer of NAD, are ineffectual (6, 11, 18, 19, 31, 39; N. R. Bachur and N. O. Kaplan, Bacteriol. Proc. 55:116, 1955). In effect, pyridine compounds that can serve as V factor can be characterized by possession of an intact pyridine-ribose bond, in the β-configuration, and a pyridine carboxamido group at position 3. In support of this conclusion, NAD analogs such as nicotinamide guanine dinucleotide, nicotinamide hypoxanthine dinucleotide, and dinitocinamidinucleotide can also function as V factor, whereas 3-acetylpyridine adenine dinucleotide and 3-pyridinealdehyde adenine dinucleotide cannot (11, 31; Bachur and Kaplan, Bacteriol. Proc. 55:116, 1955). On the other hand, while the definition given above also applies to NADP, and NADP is usually included among the compounds serving as V factor (15), we have demonstrated that of 30 strains of V-factor-dependent porcine haemophilii and actinobacci tested, only 1 (Haemophilus parasuis ATCC 19417T [T = type strain]) can use purified NADP as a pyridine nucleotide source (31). Hence, it appears that the use of NADP as V factor may be the exception rather than the rule, and in keeping with this suggestion and the finding that commercial NADP may contain both NMN and NR (31), it seems reasonable to suggest that the capacities of other V-factor-dependent organisms to use NADP should perhaps be reexamined by using purified compound. In this respect, it is interesting that while V factor was identified as NAD or NADP, the pyridine nucleotide preparations used by Lwoff and Lwoff (20) were, at best, almost pure ("fast rein" according to O. Warburg), while the NADP preparation used by Gingrich and Schlenk (11) was only 50% pure and contained 2% NAD.

The biosynthetic and salvage pathways involved in procaroytic pyridine nucleotide metabolism have been reviewed elsewhere (8, 23, 48), and it follows that if the growth of an organism requires the presence of QA or a pyridine compound based on nicotinic acid (NA) or NAM, the organism lacks a de novo pathway for the synthesis of NAD. Furthermore, when such a requirement is satisfied by only certain of

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these compounds, as is the case with V-factor-dependent haemophili, the conclusion is that in addition to lacking a de novo pathway for NAD biosynthesis, the organism possesses only a limited capacity for uptake or metabolism of both of pyridine nucleotides and precursors. Unfortunately, information regarding the assimilation of such compounds by haemophilii is scarce. In 1960, Wheat and Pittman (47) reported that Haemophilus influenzae (and probably Hae-
mophilus aegyptius) possesses NAD pyrophosphatase (EC 3.6.1.22), which cleaves NAD to yield NMN and AMP. This enzyme has since been isolated from H. influenzae and has been purified, and in addition to being orientated extrinsically, its activity is apparently required for growth when the factor is supplied in the form of NAD (12). Therefore, it appears that NAD is not transported intact by H. influenzae but rather is first hydrolyzed to yield NMN. In keeping with this conclusion, uptake studies indicate that the assimilation of NAD by Haemophilus parainfluenzae also involves the degradation of NAD by extracytoplasmic enzymes, with NMN or NR or both being produced for transport purposes (6). Note, however, that the extracellular production of NR is apparently not involved in the assimilation of NAD or NMN by H. parasuis although exogenous NAD probably is cleaved by an extrinsic pyrophosphatase (31); presumably NMN is the compound transported when NAD or NMN serves as V factor, and when growth is supported by NR, the NR is presumably transported per se. However, such a conclusion does not preclude the transport of NR by NAD- and NMN-grown organisms, and indeed, isolated, NAD-grown H. parainfluenzae cells are capable of rapid NR transport (6); interestingly, these same organisms fail to transport NAm. In summary, although the results described above allow some insight into the processes involved in the assimilation of V factor, they provide essentially no information on the capacities of haemophili to transport other pyridine nucleotide cycle intermediates (8, 23, 48) and hence no information that might help to explain V-factor dependency.

The intracellular metabolism of pyridine nucleotides and precursors by haemophilii has also been little studied. However, in 1973, Kasar and Moat (13) reported that cell extracts derived from Haemophilus haemoglobinophilus, a V-factor-independent organism (15) of uncertain genetic affiliation (34), can synthesize NAD from NAm with NMN as the only intermediate, and perhaps from NR (also via NMN), but not from QA, NA, or nicotinate adenine dinu-
cleotide. These authors concluded that H. haemoglobinophilus lacks the de novo and Preiss-Handler pathways for NAD biosynthesis and uses instead the nicotinamide pathway described for Lactobacillus fructosus (30). In effect, H. haemoglobinophilus requires a pyridine nucleotide precursor for growth, and this requirement can be satisfied by NAm (23). More recently, cell extracts derived from H. parasuis were shown to catalyze the ATP-dependent synthesis of NAD from NR and NMN, and with NR, NMN was detected as a probable intermediate (32); QA, NA, nicotinate riboside, nicotinate mononucleotide, nicotinate adenine di-
ucleotide, and also NAm were ineffectual. Analogous findings have also been reported for H. influenzae and H. parainfluenzae (6, 12; T. Sorg, M. Cynamon, and P. Tyson, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, D19, p. 69), indicating that in V-factor-dependent haemophilii, the bio-
synthetic pathway to NAD involves only two enzymes, NR kinase (ribosylnicotinamide kinase; EC 2.7.1.22) and NMN adenyllytransferase (EC 2.7.7.1), with the former enzyme catalyzing the synthesis of NMN from NR and the latter catalyzing the synthesis of NAD from NMN. Interestingly, it appears that both of these enzymes are also present in H. haemoglobinophilus (13), suggesting that with respect to the assimilation and metabolism of pyridine compounds, perhaps the only difference between H. haemoglobinophilus and the V-factor-dependent haemophilii is the possession by the former of NAm phosphoribosyltransferase (EC 2.4.2.12) and perhaps a NAm transport system. In summary, it now becomes clear that irrespective of the capacities of V-factor-dependent haemophilii to transport pyridine com-
pounds other than NR, NMN, and NAD, V-factor dependency can be explained entirely on the basis of the limited capacities of these organisms for pyridine compound metabol-
ism. Moreover, it appears that the differences between V-factor-dependent and V-factor-independent haemophilii may be more subtle than the terminology suggests.

PASTEURELLA AND ACTINOBACILLUS: REQUIREMENTS FOR PYRIDINE NUCLEOTIDES OR PRECURSORS

As early as 1941, it was well known that Pasteurella multocida requires NAm for growth and that NA cannot substitute for the NAm (17). Note that most complex media probably contain NA and NAm, and for this reason such studies relied on the use of a basal growth medium containing hydrolyzed purified gelatin. Using such a medium, Berkman (1) then demonstrated that while QA was also ineffectual, NAD and also NADP could substitute for NAm; in effect, Berkman demonstrated that under appropriate growth conditions, P. multocida can exhibit V-factor-de-
dependent growth. Berkman (1) also studied a single strain of Pasteurella haemolytica and reported that unlike P. multo-
cida, this organism could grow in the absence of accessory growth factors. However, in subsequent studies, Wessman (43, 44) questioned the identity of the putative P. haemolytica strain used by Berkman (1) in that several strains of P. haemolytica were shown to require NAm for growth (43, 44), with NA again being ineffectual (43). Pasteurella ureae also requires NAm (45, 46), but to our knowledge it is not known whether other pyridine compounds, such as NA, can substitute for NAm; paradoxically, such information may have been available, but was not reported (46). More re-
cently, the exceptional similarities between V-factor-inde-
dendent A. pleuropneumoniae biotype 2 and V-factor-de-
dependent A. pleuropneumoniae biotype 1 (35, 36) prompted investigation of the requirements of the former for pyridine nucleotide precursors (29); with respect to pyridine compound requirements and metabolism, A. pleuropneumoniae biotype 1 resembles H. parasuis (31, 32). Whereas a chemically defined medium containing NAm supported the growth of A. pleuropneumoniae biotype 2, the same medium lacking NAm did not (29); interestingly, NAD, NMN, and NR could substitute for NAm, whereas nicotinate adenine dinucleotide, nicotinate mononucleotide, NA, and QA could not. Hence, it appears that with respect to requirements for pyridine nucleotides or precursors, the only difference be-
tween A. pleuropneumoniae biotype 1 and A. pleuropneumoniae biotype 2 is the capacity of the latter to use NAm (29, 31). In this way, A. pleuropneumoniae biotype 2 and also P. multocida, P. haemolytica, and P. ureae resemble H. haemoglobinophilus, suggesting in turn that these organisms possess similar pathways for the biosynthesis of NAD. Note, however, that while A. pleuropneumoniae biotype 2 and P. multocida can be shown to exhibit V-factor-de-
dependent growth in that NAD can substitute for NAm (1, 29),
it appears, on the basis of apparently unpublished data, that this is not the case for H. haemoglobinophilus (23); on the other hand, we do not know whether NMM or NR can support the growth of the latter organism.

CONCLUDING REMARKS

The requirements of members of the family Pasteurellaceae for pyridine nucleotides or precursors, as well as the assimilation and metabolism of such compounds by these organisms, have been little studied. Provided that the organisms discussed above are typical of other members of the family, the requirements for pyridine nucleotides or precursors indicate that none of these organisms is capable of synthesizing NAD de novo. More specifically, a nutrient requirement satisfied by only NAM or more complex NAM-containing nutrilite or both leads to the suggestion that while some members of the Pasteurellaceae are unable to catalyze the formation of the NAM-ribose bond, all others may be incapable of amidating the carboxyl group on a nicotinate residue. In effect, the family Pasteurellaceae may comprise V-factor-dependent and V-factor-independent members, with the sublety of these definitions reflecting the ability or inability to use NAM rather than the capacity to exhibit V-factor-dependent growth; furthermore, with respect to NAD biosynthesis, the presence or absence of a single enzyme (NAM phosphoribosyltransferase) may be the sole feature that distinguishes these two groups of organisms.

Clearly, additional studies are necessary, and in particular, the inference that all members of the family Pasteurellaceae require NAM or a more complex NAM-containing nutrilite for growth requires further investigation. Confirmation would help to consolidate the family Pasteurellaceae because to our knowledge, no other gram-negative bacterium exhibits such unique requirements. Furthermore, additional studies may well reveal that with respect to pyridine compound requirements, assimilation, and metabolism, the differences between V-factor-dependent and V-factor-independent members of the Pasteurellaceae are not sufficient to warrant continued use of V-factor terminology as it is currently understood. Indeed, as suggested previously (29), perhaps the time has already come to redefine V factor and to include NAM among the compounds that can serve as such. However, this could create considerable confusion in the literature, and it may be advisable, rather, to abandon V-factor terminology altogether and to introduce a new term circumscribing both V factor and NAM. In either case, a "V-factor-dependent" organism could then be defined as an organism that requires a pyridine nucleotide source for growth and can use at least one, but not necessarily all, of the newly defined "V-factor" compounds (NAM, NR, NMM, NAD, NADP) for this purpose, but not the comparable NA analogs. When this definition is used, and provided that NAD cannot substitute for NAM for the growth of H. haemoglobinophilus, it is conceivable that all members of the family Pasteurellaceae may eventually be shown to be "V factor" dependent, with some being able to use NAM but not NAD (e.g., H. haemoglobinophilus), others using NAD but not NAM (e.g., H. influenzae, A. pleuropneumoniae biotype 1), and still others being able to use NAM or NAD (e.g., P. multocida, A. pleuropneumoniae biotype 2). In effect, "V-factor" dependency has the potential to serve as a familial criterion of considerable taxonomic significance.

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LITERATURE CITED


