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RESEARCH ARTICLE MICROBIOLOGY

COMPARATIVE STUDY OF EFFECT OF VARIOUS PARAMETERS AFFECTING GROWTH AND PHYSIOLOGY OF NORMAL FLORA OF HUMAN & ANIMAL GIT WITH COMMERCIAL PROBIOTICS

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ABSTRACT

Probiotics are the normal gut flora of human which may get affected by several growth conditions. These characteristics include the demonstration of bile tolerance, acid resistance and antibiotic resistance. The primary objective of this work is to compare commercially available products of probiotics with normal flora of human and animal origin as well as microbes in milk and milk products. Further study involves the study of colony and morphological characteristics of the selected organisms and study of their salt, pH and Bile tolerance. Effect of presence and absence of probiotics on the growth pattern of the organism is significant. All these characters were comparatively analysed to establish potent organism as a probiotics in GIT. 21 natural samples comprised of stool samples, vaginal swabs, cow dung and milk & milk products were the natural samples selected which were compared with 7 commercially available probiotics. Natural samples were having bacilli, cocci and yeast while commercial samples were having bacilli and cocci. The isolates from natural samples proved to be having higher growth and survival as compared to commercially available probiotics with the effect of salt, bile salt and pH. The uses of prebiotics enhanced the growth of both natural as well as commercial isolates.

KEY WORDS

GIT, Normal flora, Prebiotics, Probiotics.

INTRODUCTION

Microbial cultures have been used for thousands of years in food and alcoholic fermentations, and the past century have undergone scientific scrutiny for their ability to prevent and cure a variety of diseases. Elie Metchnikoff in 1907[1] first introduced the probiotics concept in 1908, when he observed the long life of Bulgarian peasants who consumed fermented milk foods. He suggested that *lactobacilli* might counteract the putrefactive effects of gastrointestinal metabolism. The concept of probiotics has evolved from the work of Metchnikoff (1908) although the term was probably first used by Lilly & Stillwell (1965)[2].

Probiotics is the term used to describe the substances "Secreted by one microorganism which stimulated the growth of another. It has the activity just opposite of Antibiotics. In 1971 probiotics was described as tissue extracts which stimulated microbial growth[3]. However, in 1974 probiotics was defined as "Organisms and substances which contribute to intestinal microbial balance[4]. Fullar in 1989[5] redefined probiotics as "A live microbial feed supplements or food ingredients that have a beneficial effect on the host by influencing the composition and or metabolic activity of the flora of the gastrointestinal tract (GI)".

Probiotics exert their effects on the host but mechanisms are still speculative. The mechanism of action of probiotics strain seems to be the manufacture of specific chemicals and from existing evidence, appears to be strain specific. Enhancement of colonization resistance and/or direct inhibitory effects against pathogen is likely to be an important mode of action in situations in which probiotics have reduced the occurrence and duration of gastrointestis. They may antagonize pathogen directly through production of antimicrobial and antibacterial compounds such as Cytokines and Butyric acid [6, 7] , reduce the gut pH by stimulating the Lactic acid to improve micro flora, compete with pathogens for the binding to receptors at various sites [7, 8].

Microorganisms important in probiotics must have characteristics like adherence to cells, must exclude or reduce pathogenic adherence, persistence and multiplicity, produces acids, peroxidases and bacteriocins antagonistic to pathogen growth, safe, non-invasic, non-carcinogenic, non-pathogenic, co- aggregate to form a normal balanced flora[9].

The Probiotics appears to improve immune function and stimulate immune modular cells [10, 11], produces lactose which aids in lactose digestion, compete for the nutrients and adhere to the sites on the gut wall and regulate colonocyte gene expression. (Eg. Expression of mucin genes)[12, 13].

MATERIALS AND METHOD

Isolation of normal flora of Human and Animal origin & From Commercially available sources

Isolation of normal flora was carried out from human origin (Baby stool sample), Animal origin (Cow dung) & Milk and Curd using De Man Rogasa & Sharpe (MRS) agar medium. MRS was also used to isolate commercially available probiotics products like, Aldolac, Sporlac, Neurobax, Ecoflora, Ampoxin, Suprimox

Characterization

Isolated microbes were primarily characterized on the basis of colony characteristics, cell morphology and Gram's staining.

Selection of 5 isolates from 23 for further study:

For the further study of effect of NaCl, pH and Bile salt, out of total 23 isolates, following isolates were selected for further studies

- 1 yeast(From baby stool sample),
- 2 Gram Positive rods (From vaginal samples),

- 2 Gram Positive rods (From commercially available source) were selected.

Comparative study of Normal flora and commercially available probiotics

Effect of NaCl % (w/v), Bile Salt % (w/V) and pH on growth and physiology of microorganisms were investigated. The cultures were inoculated in respective concentrations and after 24 hrs. photoelectric quantitative measurement was performed to study turbidity by the use of colorimeter at 540nm. Comparative study of normal flora and commercially available probiotics at different NaCl concentration, %, (w/v), Bile Salt, %, (w/v), pH in (liquid-media) was performed.

Comparison with reference to NaCl concentration, %, (w/v)

Selected five cultures were studied with effect of different NaCl concentration %, (w/v) 0%, 1%, 3%, 5%, 7%. Activated culture of isolates were inoculated in the respective flasks of 150ml containing 100ml Sterile MRS broth with NaCl concentration %, (w/v) 0%, 1%, 3%, 5%, 7%. Inoculated broth was kept at 37˚C for 24 hrs. After incubation quantitative measurement turbidometry analysis proceeded via colorimeter at 540nm and reading were plotted in graph to know the growth and survival.

Comparison with reference to Bile Salt concentration, %, (w/v)

Selected five cultures were studied with effect of different Bile salt (Sodium taurocolate) concentration %, (w/v) 0%, 1%, 2%, 3%. Activated culture of isolates were inoculated in the respective flasks of 150ml containing 100ml Sterile MRS broth with Bile salt (Sodium taurocolate) concentration %, (w/v) 0%, 1%, 2%, 3%. Inoculated broth was kept at 37˚C for 24 hrs. After Incubation for quantitative measurement turbidometry analysis was proceeded via colorimeter at 540nm and reading were plotted in graph to know the growth and survival.

Comparison with reference to pH.

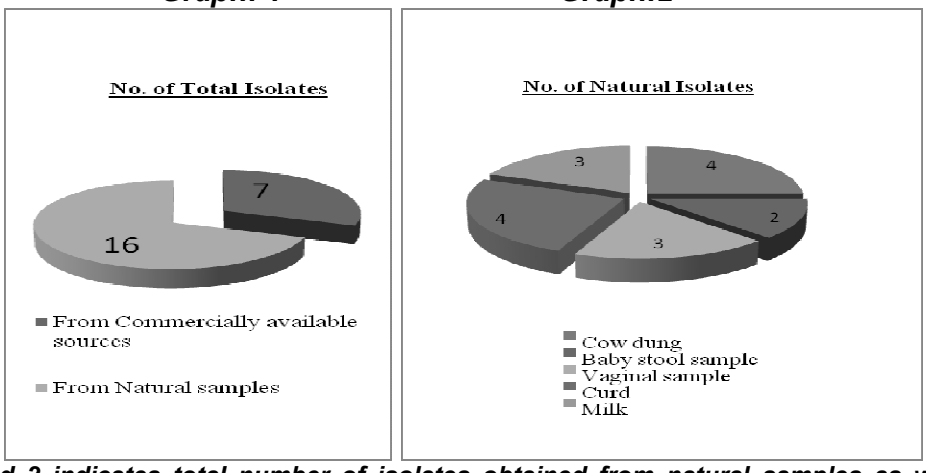
Selected five cultures were studied with effect of different pH 1, 2, 3, 4, 5, 6, 6.6 Activated culture of isolates were inoculated in the respective flasks of 150ml containing 100ml Sterile MRS broth with pH 1, 2, 3, 4, 5, 6, 6.6. (pH was adjusted by lactic Acid). Inoculated broth was kept at 37˚C for 24 hrs. After Incubation for quantitative measurement turbidometry analysis was proceeded via colorimeter at 540nm and reading were plotted in graph to know the growth and survival.

RESULTS

- ***Isolation* of normal flora of Human and Animal origin & From Commercially available sources**

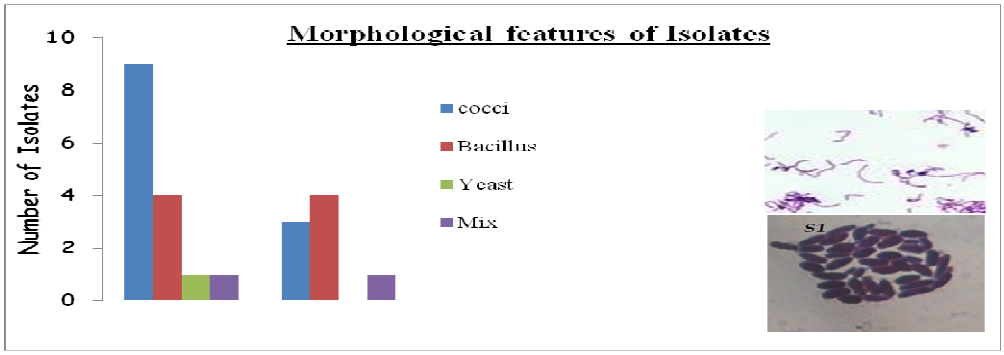
Graph 1 and 2- number isolates from natural samples

Graph: 1 ***Graph:2***



Graph 1 and 2 indicates total number of isolates obtained from natural samples as well as from commercially avilable sources.

Graph:3
Morphology of isolates



Graph 3 shows morphology of isolates; cocci, bacilli as well as yeast were obtained by isolation from natural as well as from commercially available sources.

Table 1
Cell morphology along with gram reaction

TABLE 1: cell morphology along with Gram reaction

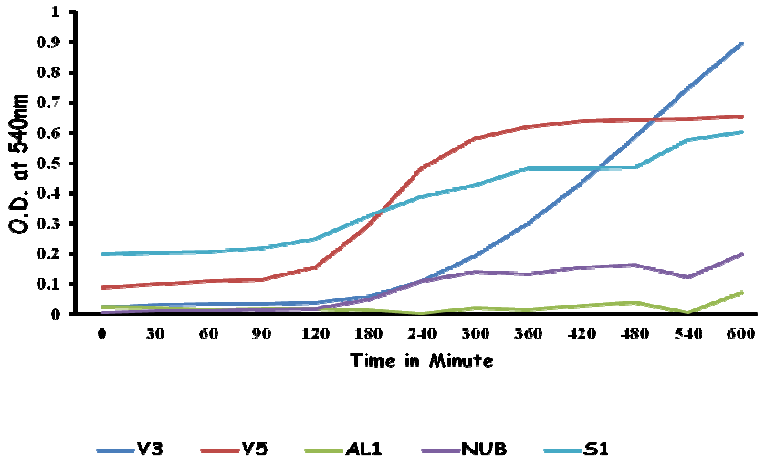
Characters	S1	AL1	NUB	V3	V5
Shape	Oval	Long Rod	Rod	Curved Rod	Thin Long Rod
Arrangement	Bunch	Singly and Bunch	singly	Bunch and singly	Bunch And singly
Color	Dark Violet	Violet	Violet	Violet	Violet
Gram's Reaction	Gram Positive	Gram Positive	Gram Positive	Gram Positive	Gram Positive

Table 2
Colony characterization

TABLE 2: COLONY CHARACTERIZATION					
Colony Characteristic	S1	AL1	NUB	V3	V5
Size	Small	Small	Very small	Small	Very small
Shape	Round	Round	Round	Round	Round
Margin	Regular	Regular	Regular	Regular	Regular
Texture	Smooth	Smooth	Smooth	Smooth	Smooth
Elevation	Convex	Convex	Convex	Convex	Convex
Opacity	Opaque	Opaque	Trans lucent	Opaque	Opaque
Pigmentation	Chreemish White	Chreemish White	-	Chreemish White	Chreemish White

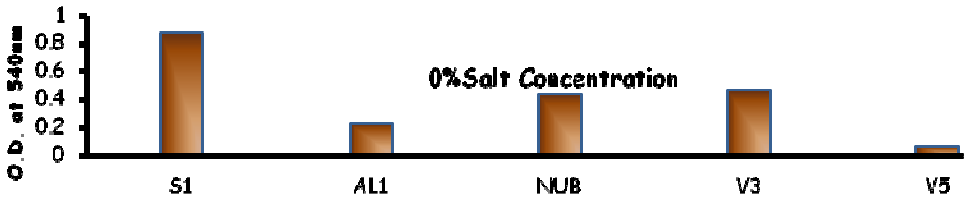
Table 1 shows size, shape and arrangement of isolates selected for further studies and that can be comparable with Bergy's manual.
Table 2 shows colony characteristics indicates resemblance of isolates as probiotics when compared with Bergy's manual.

Graph: 4
Comparative Study of the Growth pattern of selected organism

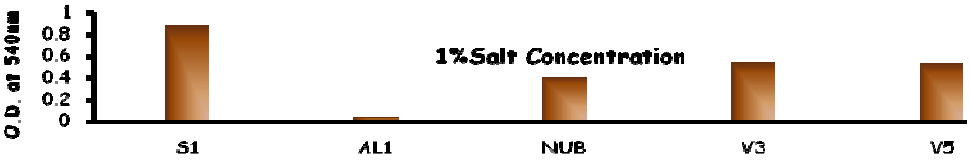


Graph 4 indicates growth pattern of V3, V5, S1 natural isolates as well as of AL1 and NUB commercially available isolates. Growth pattern reveals that natural isolates shows log phase starts at 120 hrs and after 300hrs stationary phase starts but this pattern of growth not remain there as with AL1 and NUB as graph shows only little increase in their growth.

Graph: 5
Effect of 0% salt on growth



Graph: 6
Effect of 1% salt on growth



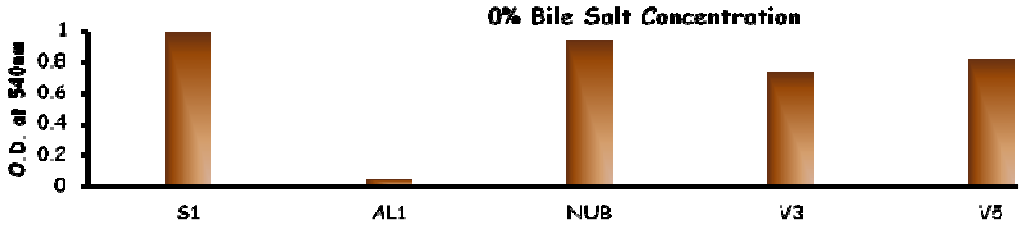
Graph: 7
Effect of 3% salt on growth

Graph: 8
Effect of 5% salt on growth

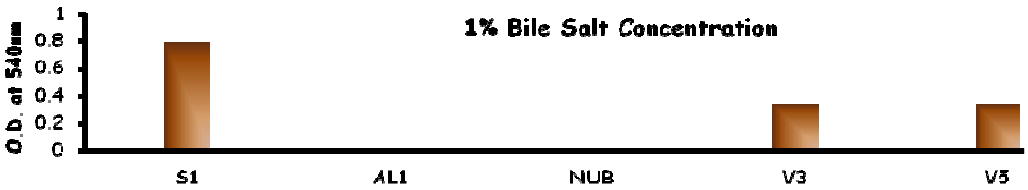
Graph: 9
Effect of 7% salt on growth

Graph 5, 6, 7, 8 and 9 shows growth of isolates at different Nacl concentrations (0%, 1%, 3%, 5% and 7%(w/v)). Graph indicates that isolates of natural sample can survive up to 5% and up to 7 % NaCl(w/v) but commercially available isolates can not survive and only gives growth at 0% NaCl (w/v).

Graph: 10
Effect of 0% bile salt on growth

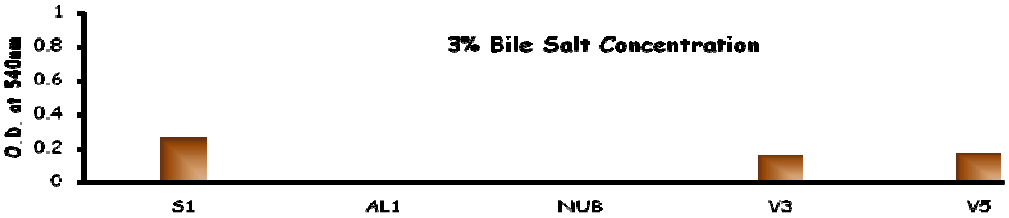


Graph: 11
Effect of 1% bile salt on growth



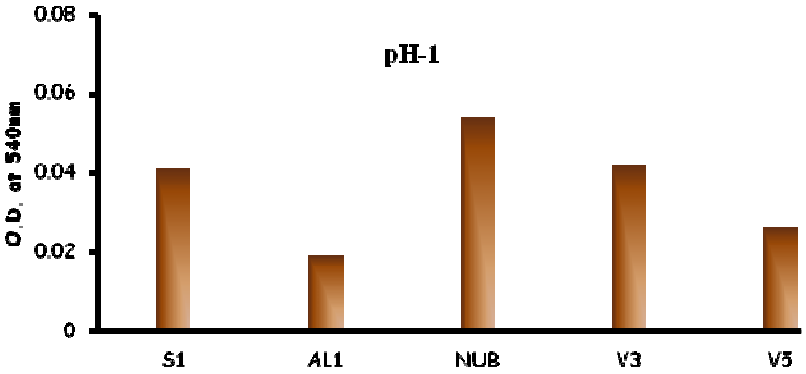
Graph: 12
Effect of 2% bile salt on growth

Graph: 13
Effect of 3% bile salt on growth

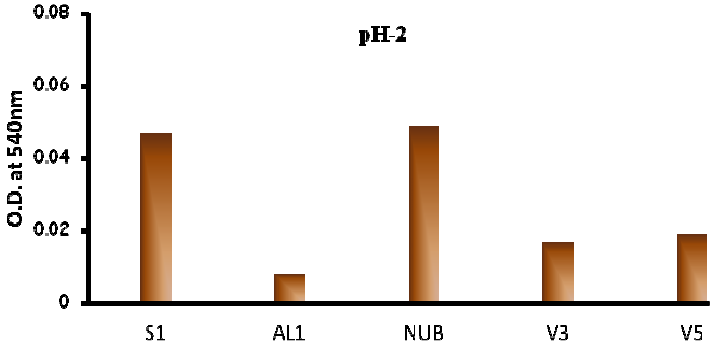


Graph 10,11,12 and 13 shows growth of isolates at different Bile salt concentrations (0%, 1%, 2% and 3%(w/v)). Graph indicates that isolates of natural sample can grow at differentbile salt concentrations but commercially available isolates can not grow.

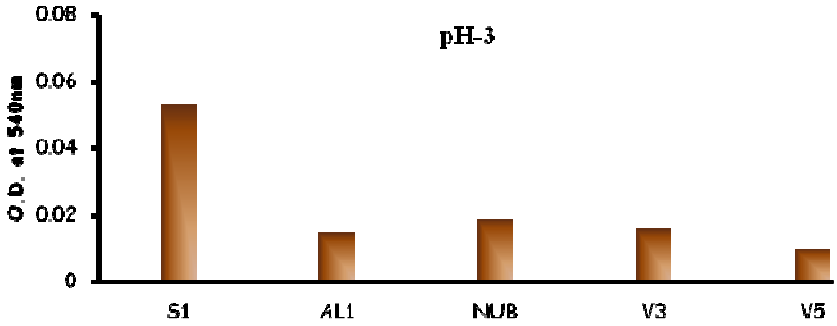
Graph: 14
Effect of pH 1 on growth



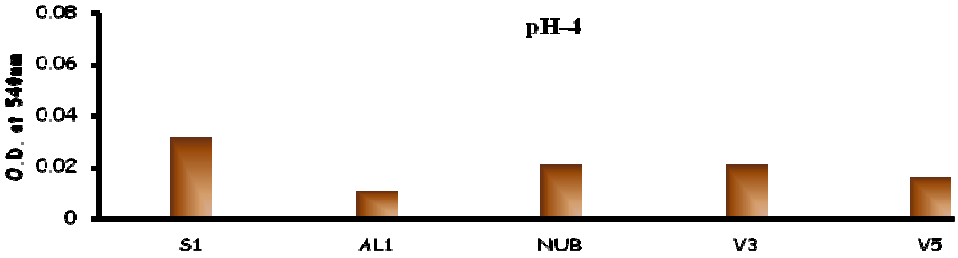
Graph: 15
Effect of pH 2 on growth



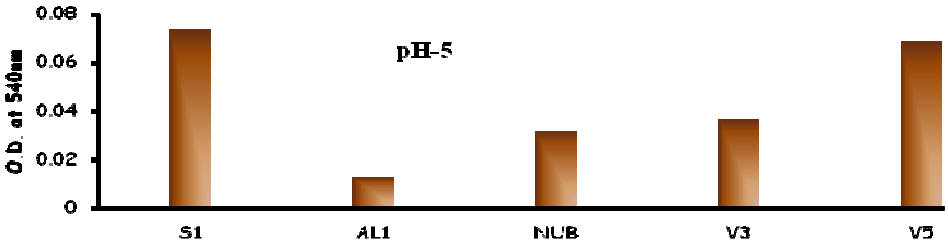
Graph: 16
Effect of pH 3 on growth



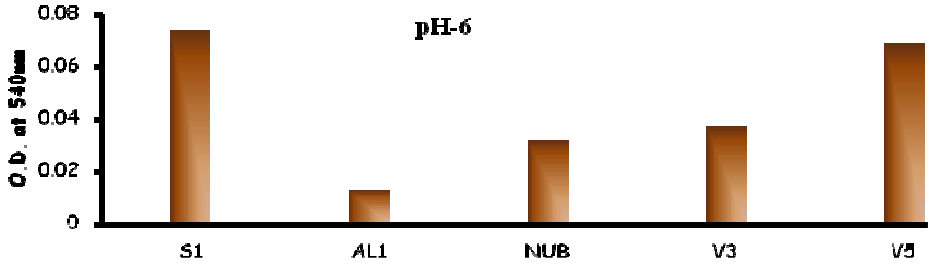
Graph: 17
Effect of pH 4 on growth



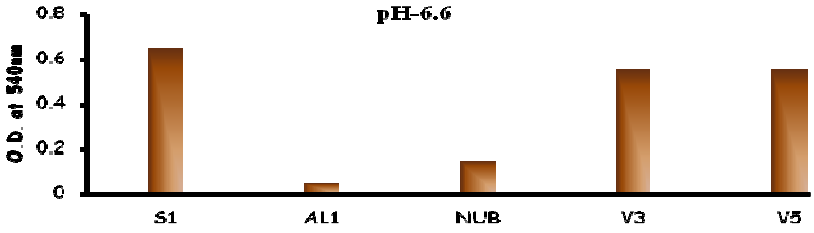
Graph: 18
Effect of pH 5 on growth



Graph: 19
Effect of pH 6 on growth



Graph: 20
Effect of pH 6.6 on growth



Graph 14, 15, 16, 17, 18, 19 and 20 shows growth of isolates at different pH: pH 1,2,3,4,5,6 and 6.6. At different pH natural as well as commercially available isolates can grow and can survive but growth of natural sample isolateswas comparatively high as compare to commercially available isolates.

DISCUSSION

Gram positive and catalase negative isolates were preserved on MRS agar plates. Probiotics are live microbial food supplements which beneficially affect the host by improving the intestinal microbial balance. Probiotic bacteria used as food adjuncts are commonly delivered in a food system and their journey starts from the mouth to lower intestinal tract. Therefore probiotic bacteria must overcome physical and chemical barriers such as bile and acid in the gastrointestinal tract. Hence, tolerances to bile and acid and growth in presence of NaCl are the most important properties for selection of potential probiotic strains.

NaCl is an inhibitory substance which may inhibit growth of certain types of bacteria. Current results show that *Lactobacillus* spp. isolated from yoghurts On the other hand, they are able to tolerate 1-9% of NaCl and good growth can be observed at 1% NaCl[14].

Effect of NaCl-tolerant lactic acid bacteria and NaCl on the fermentation characteristics and aerobic stability of silage was studied by Y. Cai[15]. Significant lactic acid bacteria which are able to tolerate NaCl concentration was studied and eventually that could be applied for dairy industry and food industry.

Bile tolerance is considered to be one of the important properties required for high survival and as a consequence for a probiotic activity. There is no consensus about the precise concentration to which the selected strain should be tolerant. Among the isolates, some grew well in the presence of different concentrations of the bile salt whereas growth was significantly reduced or completely inhibited for the rest.

Being resistant to low pH is one of the major selection criteria for probiotic strains [16, 17]. To reach the small intestine they have to pass through from the stressful conditions of stomach[17]. Although in the stomach, pH can be as low as 1.0, in most in vitro assays pH 3.0 has been preferred. Due to the fact that a significant decrease in the viability of strains is often observed at pH 2.0 and below [18].

SUMMARY

- Growth pattern of selected organism suggest that natural isolate growth was significantly high.
- S1, V3 and V5 isolates grow successfully with all selected parameters compared to AL1 and NUB.
- As compared to commercially available probiotics, natural isolates grow successfully with high salt concentration, high bile salt concentration &variation of pH
- Construction or development of a highly efficient and effective consortium suitable to function under stress and adverse condition can be administered along with commercial one for effective remedy.

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