

## INTRODUCTION

Urea has long been regarded as a surrogate marker for measuring declining renal functions. Blood urea nitrogen and creatinine clearance are the measured parameters for renal health. Urea was considered non-toxic but has made a comeback as a toxic waste, "Urea and chronic kidney disease: the comeback of the century?" (Vanholder et al NDT, 2018)<sup>1</sup>. In addition, even in healthy persons "Higher blood urea nitrogen is associated with increased risk of incident diabetes mellitus" (Xie Yan. Kidney International 2018)<sup>2</sup> and, cardiovascular impact (Urea, a true uremic toxin: the empire strikes back. ND Vaziri. Clin Sci 2017)<sup>3</sup>. Urea is re-emerging as a dark force in pathophysiology of CKD and needs to be addressed in renal failure patients. Over 100 strains of *S. thermophilus* from dairy and plant sources prevail with varying properties. Renadyl™, a Pro/Prebiotic dietary supplement contains a proprietary *S. thermophilus* strain KB19 which has demonstrated its potential to reduce serum levels of urea in multiple clinical trials. For comparative analysis we tested various commercial probiotic supplements from US and India containing different strains of *S. thermophilus* for its in vitro urea metabolism.

## METHODS

An Artificial Intestinal Fluid (AIF-US Pharmacopeia) was prepared with urea at 230 mg/dL and incubated at 37°C with 9 *S. thermophilus* containing probiotic products from US including Renadyl™. After 24 hours residual urea in each sample was measured using the colorimetric diacetyl monoxime method. In addition Renadyl™ like products are being marketed by various pharmaceutical companies in India. Four of these products were also measured for their urea hydrolysis potential.

## RESULTS

All four commercially sold Indian probiotic products which are claimed to mimic Renadyl™ and contain strains of *S. thermophilus* strains other than KB19 showed very poor urea hydrolysis ranging from 2.0% to 3.5%. Renadyl™ which contains the strain KB19 hydrolyzed urea by 55% to 65%.

Sr No	PRODUCT	Manufactured by	Marketed By	STRAINS	Urea Hydrolysis(%) per cap
1	Cudce	Rivpra formulation Pvt Ltd, Haridwar	Celera Pharma Pvt Ltd.	<i>S. thermophilus</i> <i>L. acidophilus</i> <i>B. longum</i>	2.5
2	Lobun Forte	Sanzyme, Dehradun	Sanzyme, Dehradun	<i>S. thermophilus</i> <i>L. acidophilus</i> <i>B. longum</i> <i>B. coagulans</i>	3.3
3	Cudo Forte	Stanford Labs, Mehatpur	LaRennon Ahmedabad	<i>S. thermophilus</i> <i>L. acidophilus</i> <i>B. longum</i>	3.3
4	Auxipro	Pharose Remedies, Brahmana (J&K)	Alniche Life Sciences, New Delhi	<i>S. thermophilus</i> <i>L. acidophilus</i> <i>B. longum</i> <i>B. coagulans</i>	2.2
5	Renadyl™	Kibow Biotech	Kibow Biotech	<i>S. thermophilus</i> <i>L. acidophilus</i> <i>B. longum</i>	66

## RESULTS contd....

All nine commercially available multi strain probiotic samples in the US which contain strains of *S. thermophilus* strains other than KB19 showed very poor urea hydrolysis ranging from 0.0% to 15.0%. Renadyl™ which contains the strain KB19 hydrolyzed urea by 55% to 65%.

Sr No	PRODUCT	STRAINS	Urea Hydrolysis (%) per cap
1	Go Live	<i>S. thermophilus</i>	8.35
		Lactobacillus -9 species	
		Bifidobacteria-5 species	
2	Nutrabiotic	<i>S. thermophilus</i>	0
		Lactobacillus -4 species	
		Bifidobacteria-3 species	
3	Hyperbiotic Pro-15	<i>S. thermophilus</i>	0
		Lactobacillus -9 species	
		Bifidobacteria-5 species	
4	Multi Probiotic 4000	<i>S. thermophilus</i>	16.65
		Lactobacillus -4 species	
		Bifidobacteria-2 species	
5	iFLora Multiprobiotic	<i>S. thermophilus</i>	8.35
		Lactobacillus -10 species	
		Bifidobacteria-5 species	
6	Mightidophilus	<i>S. thermophilus</i>	0
		Lactobacillus -7 species	
		Bifidobacteria-4 species	
7	Probiotic 12	<i>S. thermophilus</i>	12.8
		Lactobacillus -8 species	
		Bifidobacteria-3 species	
8	Renadyl™	<i>S. thermophilus</i>	54.05
		Lactobacillus -1 species	
		Bifidobacteria-1 species	
9	FloraGenix	<i>S. thermophilus</i>	2
		Lactobacillus -3 species	
		Bifidobacteria-4 species	
10	Accuflora	<i>S. thermophilus</i>	2.8
		Lactobacillus -3 species	
		Bifidobacteria-1 species	

## DISCUSSION

Urea retention though regarded non-toxic can degrade to cyanate which is highly toxic. Cyanate is a free radical that is in equilibrium with urea. Probably due to the increased availability of urea, cyanate levels are also elevated in CKD<sup>4</sup>. Cyanate binds to proteins, including albumin, by carbamylation. High carbamylated serum albumin concentrations are a mortality risk<sup>5</sup>. Carbamylated compounds interfere with organ and body functions through multiple mechanisms. Carbamylated proteins activate mesangial cells into a profibrogenic prototype, with a potential to play a role in the progression of kidney failure<sup>6</sup>.

Excessive blood urea nitrogen concentrations have been shown to be associated with an increased risk for diabetes mellitus<sup>2</sup> as well as engendering multiple other toxicities<sup>1</sup>.

Urea itself induces molecular changes related to disruption of the protective intestinal barrier. Vaziri et al.<sup>7</sup> tested the impact of urea on the integrity of the intestinal epithelial barrier. They also showed that in CKD or in the presence of uremic plasma there is a disruption of intestinal barrier functions, potentially impairing the protection against leakage of intestinal content such as proinflammatory endotoxin into the body<sup>8,9</sup>. At the molecular level, this derangement was attributed to a decrease in expression of tight junction proteins.

Probiotics have shown their beneficial effects in renal failure patients. Amine production has been noted to be reduced by the use of probiotics<sup>10</sup>. A recent systemic review and meta-analysis of clinical trials showed that urea concentrations can be decreased by probiotic administration<sup>11</sup>.

## SUMMARY/CONCLUSIONS

*S. thermophilus* KB19 was selected from rigorous lab studies and evaluated further using the simulated human intestinal microbial ecosystem (SHIME) for its ability to metabolize urea and also to demonstrate the lack of ammonia toxicity. It was further evaluated in nephrectomized rats and Gottingen minipigs. Formulation with this strain was successfully tested in renal failure cats and dogs. Human trials further confirmed KB19's potential to reduce urea concentrations in CKD and dialysis patients. Multiple other probiotic-containing preparations, not always proprietary products, have been used in renal investigations and, many products in the market containing *S. thermophilus* are ineffective in metabolizing urea demonstrating the importance of strain specific health benefits in our product formulation Renadyl™ with proven safety and efficacy. A probiotic dietary supplement which can metabolize urea offers an inexpensive, safe and effective alternative to reduce uremia in CKD patients.

## REFERENCES

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